Muneeb U. Rehman
Sabhiya Majid *Editors*

Therapeutic Applications of Honey and its Phytochemicals

Vol.1

Springer

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ISBN 978-981-15-6798-8 ISBN 978-981-15-6799-5 (eBook)
https://doi.org/10.1007/978-981-15-6799-5

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Dedicated to all Frontline COVID Warriors

Foreword

The book *Therapeutic Applications of Honey and its Phytochemicals* edited by Dr. Muneeb U. Rehman, a Faculty Member at King Saud University, Riyadh, Saudi Arabia, and Dr. Sabhiya Majid, Professor and Chairperson of Biochemistry Department, Govt. Medical College (GMC-Srinagar), University of Kashmir, India, is in my opinion the first volume in this series. The editors are specialised in biochemistry, toxicology, in particular cancer biology and natural product research, as well as pharmacogenomics. In this volume they have included 20 chapters on the theme with contributions mainly from India, Canada, USA, Taiwan, UAE, Saudi Arabia, and Egypt.

"Bee and Honey" are known as the symbols of productivity, even resurrection. Honey has been used as the material for embedding the dead because of its air proof features. Its nutritional value is associated with human immortality because it was known as a drink of the "gods". The wall paintings from 8000 to 9000 BC in Çatalhöyük (Turkey) depict that honey was known in Anatolia as an important part of nutrition. History reveals that honey and bee keeping were known in Mesopotamia during the middle period of 3000 BC. The King of Babylon, in the Sin Temple at Harran (Urfa-Turkey), poured honey on the walls as well as wooden structures with other materials.

Apitherapy or "treatment with bee products" has developed fast at a global scale. It is a complex natural food, monofloral or plurifloral type, varying in composition depending on the season, origin of nectar, method of production, species of honeybees, honeydew sources, flora and their origin, geographic region, manipulation, processing, packaging, and time of the storage. According to the FAO agricultural statistics 1.78 million metric tons of honey is produced globally. The average honey production per comb in the world is 20 kg. In the ancient Egyptian culture, honey was documented to have used for gynaecological issues and consumed to prevent pregnancy. A comparison of honey with other foods of the same calorific value reveals that it is more appropriate for diabetic patients than other sugar products, resulting in lesser insulin production. However, there is another type known as "Mad Honey". Its poisoning effect was reported by Xenophon (an Athenian military commander and author) for the first time in 401 BC. Moreover, mad honey was used by King Mithradates IV (Northeast Anatolia, Turkey) as a weapon in 67 BC against Pompey the great. Mad honey, which is contaminated with grayanotoxins, generally found in *Rhododendron* genus (family: Ericaceae), is different from natural or commercially available honey. It leads to poisoning upon consumption. Such types of honey have been commonly used as an aphrodisiac, in alternative therapy for peptic ulcer disease, dyspepsia, and gastritis, and for hypertension for a long time. Although fatalities are very rare, its ingestion may lead to arrhythmias, which can be life-threatening. It is mostly reported in Nepal, Korea, and Turkey whereas the consumption of honey containing tutin (a neurotoxin from *Coraria* species), which is termed tutin honey that also has poisoning effect, is reported from New Zealand.

The salient features of this volume are the historical and traditional usage of honey presented in Chap. 1, which focuses on the historical aspects of honey consumption, along with its uses in traditional folk medicine. Chapter 2 deals with "Honey: A Powerful Natural Antioxidant and Its Possible Mechanism of Action". It includes information on honey composition, type, antioxidant properties, and antioxidant mechanism. A detailed overview of the phytochemical characteristics of honey and pollen, the therapeutic ability of its biologically active ingredients, and their use in value-added food products are presented in Chap. 3. The data on "Honey: A Sweet Way to Health" provides readers a better understanding of complex composition, biological activities, adverse effects, and therapeutic benefits of honey; possibilities for its development as a natural therapeutic agent for many pathologies and extensive studies are summarised in Chap. 4. In Chap. 5 authors have reviewed how honey composition and its concentration influence the shelf life of the product and how ingredients of honey's quality and quantity fix the nutritive and medicinal value. The nectars as secretion of plants are the main component of honey and its properties are discussed in Chap. 6 presenting information on honey and its authenticity—an analytical approach. Chapters 7–9 cover data on the anti-microbial activities, the traditional and modern applications, and recent advances in the discovery of bioactive components from natural honey. In Chap. 10, researchers have focused their interest towards honey as a successful antioxidant and antimicrobial agent and the possibilities for its use against organisms with antimicrobial resistance, specifically the expansion in multi-drug resistance (MDR), the quantity of efficient antibiotics which has compelled scientists to think back to the pre-antibiotic period for creating solutions, directing their consideration towards the mechanisms of action of antimicrobial activity of honey. "Honey as Component of Diet: Importance and Scope" is the title of Chap. 11. It discusses the part played by honey in symbolism and religion. In Chap. 12 authors discuss the positive influence of honey on human health, as advocated by all religious and cultural beliefs as well as traditions whether ancient or modern. This chapter presents insights into the health benefits associated with the consumption of honey and a brief description about the composition and clinical trials of honey. "Different Types of Honey and Their Properties" is the topic of Chap. 13, followed by data on the "Pharmaceutical Applications of Honey" in Chap. 14. The authors provide information on the effectiveness of honey in the eradication of multidrug resistant pathogens such as methicillin resistant *Staphylococcus aureus* (MRSA), controlling blood sugar in diabetic patients, accelerating healing of wounds and chronic ulcers, improving cough and asthma, treatment of different types of cancers, and reducing symptoms associated with periodontal diseases.

Chapter 15 summarises the data on certain clinical attributes of honey and the active chemical ingredients responsible, together with some of its physicochemical properties. In Chap. 16 Chinese honey composition, production, trade, and health benefits have been reviewed as China is the top producer of honey in the world. "The Gut–Brain Axis, Cognition and Honey" is the topic of Chap. 17, which provides data on the use of honey as a prebiotic. Authors conclude that improvement in cognitive functions is a cumulative effect of the unique chemical composition of honey, and it may not be identical for all types of honey. More longitudinal research is required to establish honey as a brain tonic. Chapter 18 "Antiproliferative and Apoptotic Activities of Natural Honey" provides insights into the role of honey in regulating anti-proliferative and pro-apoptotic mechanisms in human cancers and also endorses honey as a promising candidate against cancer. Chapter 19 deals with the "Heath Benefits of Phenolic Compounds in Honey: An Essay" with a discussion on the classification, structural, medicinal, and health benefits of phenolic compounds. In Chap. 20, the role of honey for enhancing performance in endurance sports has been presented, because there are limited studies showing that honey improves the physical performance among endurance athletes.

Since times immemorial honey has been an integral part of the human diet, used in traditional medicines for ages and is considered as "health tonic". It is mentioned in the holy books including The Holy Quran: "And thy Lord taught the bee to build its cells in hills, on trees, and in men's habitation: Then to eat of all the produce (of the earth), and find with skill the spacious paths of its Lord: there issues from within their bodies a drink of varying colours, wherein is healing for humans: verily in this is a sign for those who give thought" illustrating the potential curative worth of honey.

The editors have put concerted efforts while compiling this volume with a very rich content. I am sure it will prove a very valuable document for researchers engaged in the field of natural products and for entrepreneurs, those involved in research and development in industries, medical practitioners and academicians, as well as graduates and undergraduates. The book is a thorough compilation of the latest literature regarding the chemistry and pharmacology of honey. The editors have painstakingly provided a solid foundation of the subject which can be of immense value for researchers involved in the therapeutic role of natural compounds, especially honey in various diseases and illnesses. In both Unani and Ayurveda, honey has been documented to possess the potential to treat various ailments. Recent studies report that honey is used as a natural remedy against respiratory disorders and nervousness. The indicator of COVID-19 infection suggests that increased inflammation, oxidation, and an overstressed immune response are the key contributor of COVID-19 pathology. This adds to the importance of this volume while humanity is passing through a pandemic. It will mainly prove helpful to pharmacologists, toxicologists, chemists, phytochemists, and pharmacognosists.

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About the Editors

Muneeb U. Rehman, Ph.D. is a faculty member at College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. He holds a doctorate in Toxicology (specialization in cancer biology and natural product research) from Jamia Hamdard, New Delhi, India. Dr. Rehman has more than 10 years of research and teaching experience in the field of toxicology, biochemistry, cancer biology, natural product research and pharmacogenomics. He is the recipient of several national and international fellowships and awards. He has published 90 research papers in peer-reviewed, international journals and 25 book chapters. Dr. Rehman is on the editorial boards and is a reviewer of several high-impact, international scientific journals. He is also a life member of various international societies and organizations. Currently, Dr. Rehman is engaged in studying the molecular mechanisms of cancer prevention by natural products and the role of pharmacogenomics and toxicogenomics in evaluating the effectiveness and safety of drugs.

Sabhiya Majid, Ph.D. is a Professor and Chairperson of the Department of Biochemistry, Govt. Medical College Srinagar (GMC Sgr.), J&K, India. Prof. Majid has 30 years of experience in teaching, research and diagnostic biochemistry, having published around 100 research papers in journals of repute, 3 books and 12 book chapters. She has been the recipient of several fellowships, awards and grants from various reputed funding agencies. She has been the nodal officer and implemented various research and infrastructure development schemes including Fund for Improvement of Science & Technology Infrastructure, Department of Science & Technology, Govt. of India. She is a member of several scientific associations and boards of undergraduate, postgraduate and research studies. She is on the reviewer panel of a number of online peer-reviewed journals. Having moved on from her doctoral work on nutritional modulation of carcinogenesis to understanding the molecular basis of various disease processes, her current research focuses on non-invasive cancer markers.

Brief History and Traditional Uses of Honey

1

Wajhul Qamar and Muneeb U. Rehman

Abstract

Honey, a thick, viscous, sugary food substance, produced by honeybees has a history of use since thousands of years. Due to its concentrated sweetness and energy condensed properties, it is used as food ingredient since millennia. Evidences of its consumption has been found dating back to Neolithic age. Various cultures including Vedic, Egyptian, Roman, Greek, Mayans, Babylonians, and Chinese have been consuming honey and beeswax since ancient time. Evidences suggest the advent of apiculture or beekeeping around 2500 BCE in Egypt and then in different civilizations worldwide. Various religious texts around the group mention honey for its beneficial effects on health. During the long historical course of its association with human feeding habits, it has also been endorsed for religious rituals. It has been used in traditional medicine as well for several medicinal purposes. Traditionally, it has been mainly used for healing injuries in the form of wounds and burns. Other therapeutic applications of honey includes against oral ailments, digestive troubles mainly diarrhea and constipation, skin disorders, eye ailments, lung diseases, etc. Beneficial effects of honey are known since the beginning of its consumption; now these claims have scientific support with evidences coming from multiple research studies conducted in past decades. This chapter focuses on the historical aspects of honey consumption, along with its uses in traditional folk medicine.

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_1

Keywords

Honey · Histroy · Religious · Folk Medicine

1.1 Introduction

Honey, a thick, viscous, sugary food substance, produced by honeybees has a history of use since thousands of years. Due to its concentrated sweetness and energy condensed properties, it is used as food ingredient since millennia. Evidences of its consumption has been found dating back to Neolithic age. Various cultures including Vedic, Egyptian, Roman, Greek, Mayans, Babylonians, and Chinese have been consuming honey and beeswax since ancient time. Evidences suggest the advent of apiculture or beekeeping around 2500 BCE in Egypt and then in different civilizations worldwide. Various religious texts around the group mention honey for its beneficial effects on health. During the long historical course of its association with human feeding habits, it has also been endorsed for religious rituals. It has been used in traditional medicine as well for several medicinal purposes. Traditionally, it has been mainly used for healing injuries in the form of wounds and burns. Other therapeutic applications of honey includes against oral ailments, digestive troubles mainly diarrhea and constipation, skin disorders, eye ailments, lung diseases, etc. Beneficial effects of honey are known since the beginning of its consumption; now these claims have scientific support with evidences coming from multiple research studies conducted in past decades. This chapter focuses on the historical aspects of honey consumption, along with its uses in traditional folk medicine.

1.2 Background

Honey is a well-known food substance worldwide for its unique taste, sweetness, and health benefits. It is produced by honeybees in wild and in beekeeping (apiculture) facilities. Other honeybee byproducts are also there including bee pollen, beeswax, royal jelly, propolis, and bee bread. Honey is the only natural sweetener that does not need any processing to yield its sweetness for culinary purposes. It contains 80–90% of sugar content in the form of glucose and fructose and can be stored for years without adding any preservative. In addition to sweetness, it bears a particular flavor and aroma that is adored by many. Moreover, it is not just sweetness and flavor that make honey a preferable food item since the beginning of human civilizations. It has been seen as a health-boosting food ingredient since ages and even today is valued by nutritionists and consumers. Even today, in modern culinary methods, honey has its own unique place as an ingredient of several delicacies. These may include, but not limited to, honey butter, salad dressings, sweetened soups, snack bars, cookies, cakes, desserts, bread spread, beverages, etc.

Similar to some other food substances, including fruits and vegetables, honey has been lauded as a healthy and nourishing food substance that fulfills body’s need

Fig. 1.1 Honeybees collecting nectar from flowers. (Image source: Biodiversity Heritage Library Public Domain)

of glucose, vitamins, and antioxidants. On the other hand, several recent research studies identified a large number phytochemicals in it and indicated their preventive role against certain diseases and conditions (Ahmad et al. 2017). These include bacterial infections, wounds, burns, atherosclerosis, gastrointestinal tract ailments, inflammation, oxidative damage, etc. Honey has been a much relished part of human diet since ages owing to its sweetness and particular taste. Uses of honey in folk medicine also root back to similar periods of time.

Based on bee species, geographical areas, and seasons, honey can be of different varieties, differing in taste, texture, and color. Honey is mainly found in golden yellow color; however, colorless, white, light yellow, and dark brown colored honey is also produced by different honeybees. There are over 300 types of honey that have been recognized worldwide (Samarghandian et al. 2017). The process starts with the collection of sugar-rich nectar of different flowers by a massive team of honeybees (Fig. 1.1). Types of flowers from different plant species determine the sweetness, taste, color, and phytochemical composition of the honey (Ahmad et al. 2017; Nguyen et al. 2019; Eteraf-Oskouei and Najafi 2013).

Honeybees are geographically distributed worldwide, and certain species are localized to a particular region (Al-Ghamdi et al. 2013; Cridland et al. 2017). *Apis mellifera* is the most widely distributed honeybee in the world. And this natural distribution along with apicultural activities makes this golden food item easily

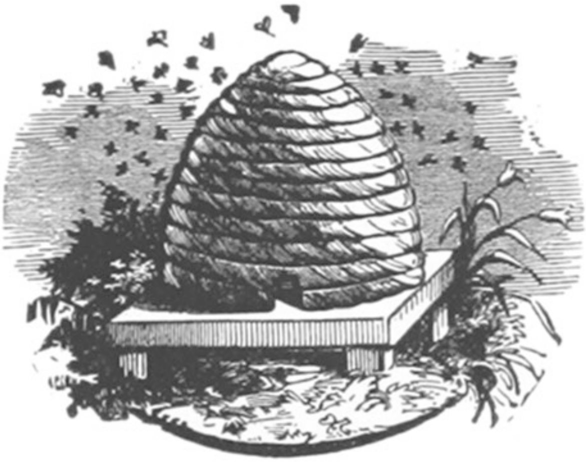

Fig. 1.2 Beehive for domestication of honeybees (beekeeping), circa 1881 (Image source: British Library Public domain)

available globally. It is known to contain around 200 natural substances (Escuredo et al. 2013; Cianciosi et al. 2018), including sugars, proteins, minerals, vitamins, organic acids, and volatile compounds (Escuredo et al. 2013). Honey is harvested from wild or domesticated beehives (Fig. 1.2). The part of beehive that contains the honey or the storage unit is called honey comb that is collected only instead of destroying the whole hive. It helps maintain a continuous existence of bee colony ensuring ample supply of the honey. Honey comb is cut into pieces to release the honey from cells, and collected honey is strain filtered before storage.

Throughout the history of human civilization, food and feeding habits have been one of the major aspects of cultural attributions. Their ingredients, way of preparation, etc. are not only a part of essential requirements but also a part of cultural heritage. Certain food items has become a part of daily essential needs while some are just included to enhance the yield of health befits from the food. Honey may be included in such category along with dozens of others with promising health benefits. The following section focuses on the history of consumption and uses of honey.

1.3 Historical Aspects of Consumption and Uses of Honey

Through the course of history of human civilization, feeding habits also evolved along with the development and advancements of civilization. Several staple food items including wheat, rice, corn, and different kinds of vegetables and fruits became a part of human food with the emergence of agricultural practices when groups of humans started settling down. A new era started when humans left their way of hunting and gathering and started agricultural activities including farming and animal breeding (mainly cattle, goats, etc.) for milk, meat, and transportation of goods. All these practices including settlements, farming, animal breeding mainly revolve around assurance of food and safety. The list of numerous food items and ingredients kept growing since the early era of human culinary history. New methods developed to process food with different ways and became a mark of identification for cultural communities. However, few food items appear to be unrelated with any particular community and remain in their original form for most of the feeding purposes. Honey is such a kind of major food that does not need any particular processing. It remains sweet, tasty, and energy rich even in its natural form. It can also be used as natural sweetener to make certain sweet dishes. It surly attracted humans due to its sweetness and energy-dense nature.

The use of honey as food by humans dates back to Stone Age (Crittenden 2011). Honey still remains a major source of energy for most of the tribal people around the world. The importance of honey diverted the focus of humans from honey-hunting to beekeeping (Kritsky 2016). Evidences suggest that the apiculture or beekeeping activities by humans dated back to around 9000 years (Fig. 1.3) (Roffet-Salque et al. 2015; Dams and Dams 1977). However, the evidences from 9000 years ago do not provide information regarding the methods followed to culture the bees. Other investigations provide such evidences that shed light on true beekeeping dates back to around 2450 BCE (Kritsky 2016).

Mesopotamian people used to offer honey to deities and consumed it as food. Another major product associated with honey, beeswax is also used as a burning fuel, for waterproofing of vessels, and in religious ceremonies. The earliest evidences date back to the seventh millennium BCE in Neolithic era in present day Anatolia, Turkey. During around 2500 BCE, Egyptians started beekeeping to produce honey and beeswax for various purposes. During excavations of pyramids and tombs, archaeologists have found world's oldest samples of honey in pots, approximately 3000 years old, interestingly in edible condition (Geiling 2013).

All major religious texts have little mentions of honey indicating its importance for human well-being.

1.3.1 Significance of Honey in Hinduism

In Hinduism, honey ("मधु"—Madhu) as a food has certain religious attributes. Honey is one of "the five Nectars" (Panchamrita), four others are milk, ghee, buttermilk, and sugar. In "Madhu Abhisheka" ritual, honey is poured on deities. Medicinal and nutritional values of honey has been mentioned in the Vedas. In Rigveda, one of the oldest sacred books, at several occasions, mentions bees and honey many times. The sacred book is believed to be compiled between 2000 and 3000 BCE. Following verses are from Rigveda about honey.

> *Rigveda (1:9:6-8)*
>
> *"This herb, born of honey, dripped in honey, sweetened by honey, is the remedy for all injuries."*

Fig. 1.3 'Man of Bicorp' cave painting found in Spain in 1921 depicts a honey hunter harvesting honey from a tree (circa around 8000 BCE) (Source: Public Domain)

> *"Let every wind that blows drop honey; let the rivers and streams recreate honey; let all our medicines turn into honey; let the dawn and the evening be full of honey; our nourisher, this sky above, be full of honey; let our trees be honey; let the sun be honey, let our cows secrete honey."*

Honey is a very significant part of Hindu birth ritual "Jatakarma," wherein the father touches the baby's lips with honey and ghee along with recitation of holy "Mantras."

1.3.2 Significance of Honey in Islam

In Islam, an entire chapter is entitled Al-Nahl (The Honeybee), which mentions nutritious, healthy, and healing nature of honey. Following are the verses (English translation of verses 68 and 69) from chapter 16 (Al-Nahl) mentioning about honeybees and healing nature of honey.

Holy Qur'an, 16th Chapter Al-Nahl

Verse 68. *And your Lord inspired to the bee, "Take for yourself among the mountains, houses, and among the trees and [in] that which they construct.*

Verse 69. *Then eat from all the fruits and follow the ways of your Lord laid down [for you]." There emerges from their bellies a drink, varying in colors, in which there is healing for people. Indeed in that is a sign for a people who give thought.*

1.3.3 Significance of Honey in Judaism

Jewish traditions relate honey with New Year, called Rosh Hashanah, and use honey-dipped apples as a traditional meal on the day. The use of honey in this case is associated with "Manna," the food from Heaven, described in the Torah as being "like honey wafers" or "a pastry fried in honey" (Book of Exodus, 16:31). Manna was provided by the God during the wandering of Israelites for 40 years in the desert (Bramen n.d.). The Hebrew Bible contains several mentions of honey in it.

Song of Songs (4:11)

"The sweetness of Torah drips from your lips, like honey and milk it lies under your tongue."

Ezekiel (3:2–4)

"Feed your stomach and fill your body with this scroll which I am giving you. Then I ate it, and it was sweet as honey in my mouth."

1.3.4 Significance of Honey in Christianity

In Christianity, there are several references in the Bible indicating the significance of bees and honey including in the Books of Exodus, Judges, Mathew, and Proverbs.

Proverbs 24:13

My son, eat honey, for it is good, and the drippings of the honeycomb are sweet to your taste.

Proverbs 16:24

Gracious words are like a honeycomb, sweetness to the soul and health to the body.

Matthew 3:4

Now John wore a garment of camel's hair and a leather belt around his waist, and his food was locusts and wild honey.

1.3.5 Significance of Honey in Buddhism

In Buddhism, there is an old legend that when the Buddha was seeking enlightenment in wilderness, a honeycomb was offered to him by a monkey. Acceptance of monkey's offer by Buddha led him to celebrate in joy. He began leaping from tree to tree and ultimately resulting in his death by falling from the tree. Monkey fell to his death from the trees but was reborn because of his generosity. This joy of the monkey is remembered in Buddhism by naming the month, that he died in, Madhu Purnima, meaning "Honey (Sweetened)-Full moon." On Madhu Purnima, mainly celebrated in India and Bangladesh, Buddhists remember this act by offering honey to monks .

Buddha says about the honey bees:

"As a bee gathers honey from the flower without injuring its color or fragrance, even so the sage goes on his alms-round in the village."

In Sikhism also, honey is used in certain religious ceremonies. Similarly, in various other minor religious groups worldwide, honey has been given certain importance in rituals.

1.4 Honey in Folk Medicine

In ancient times, in different civilizations, honey was preferred as a natural sweetener and a good source of carbohydrate. However, since the very same time period, honey found a place in various religious rituals and folk medicine. Several traditional medicine systems worldwide value honey as a natural medicine that is recommended to improve a number of health conditions.

In Indian system of medicine "Ayurveda," honey is mentioned as a gift of nature to mankind and improves weak digestive system. In addition, honey can help in soothing upper respiratory tract in case of irritating cough. Ayurvedic practitioners also recommend honey for keeping teeth and gums healthy; other ailments that can be improved by honey include insomnia, skin disorders, cardiac problems, lung issues, and eye disorders (Eteraf-Oskouei and Najafi 2013). According to the Ayurveda, there are several kinds of honey that are used to treat different ailments. These include Pauttika, Bhramara, Kshaudra, Makshika, Chatra, Ardhya, Auddalaka, and Dala. Based on their properties, they can be used for treating blood ailments, cough, asthma, leukoderma, nausea, and worm infestations (Arawwawala and Hewageegana 2017).

In ancient Egypt, honey was used as a wound healing medicine either alone or combined with other ingredients. Smith papyrus, in hieroglyphic text, between 2600 and 2200 BCE, provides medicinal recipe for wound healing (Arawwawala and Hewageegana 2017; Eteraf-Oskouei and Najafi 2013). Ancient Egyptians also used honey in embalming recipes for their dead (Flowers 2017). Several modern research investigations have supported the wound healing properties of honey (Layflurrie 2008; Molan and Rhodes 2015; Sivamani et al. 2012).

A part of the Islamic texts also deals with health and medicinal issues. Quranic text mentions that honey is “healing for men.” According to Hadith text, Prophet Muhammad (PBUH) recommended honey as a treatment for diarrhea (Molan 2001). Recent clinical findings support the health-boosting effects of honey as an enteral nutrition in critically ill patients probably by reducing incidences of diarrhea (Shariatpanahi et al. 2018). It showed similar effects against infantile diarrhea in another study (Elnady et al. 2011).

The biblical traditional diet including date honey in addition to figs, wheat, grapes, barley, olives, and pomegranates has been associated with low rates of cardiovascular diseases along with obesity and other non-communicable diseases (Berry et al. 2011).

Hippocrates, considered one of the founding father of Unani Medicine, recommended several combinations of honey with other ingredients for pain, dehydration, and acute fever. Other conditions for which he used honey include wound healing, constipation, cough, baldness, eye ailments, and skin disorders (Eteraf-Oskouei and Najafi 2013). Ancient Greeks also believed that honey consumption is associated with longer life. Romans also, like any other culture, relied heavily on the natural medicines including herbs and other ingredients. Romans believed that honey can cure pneumonia, pleurisy, mouth ailments, and snakebite.

In Traditional Chinese System of Medicine or Traditional Chinese Medicine (TCM), honey has been used to treat cough, bronchitis, burns, open wounds, scars, dry skin, dry mouth and throat, and constipation and for boosting immune system. In this system, the main function of honey is to strengthen lungs, stomach, large intestine, and spleen. It is also used as a common ingredient in capsules, pills, herbal preparations, and other formulas as a flavoring agent. Honey is also recommended as an antihypertensive food in TCM (Zou 2016).

Use of honey-based medicines by non-indigenous people in Argentina has been reported (Kujawska et al. 2012). These people employed 50 different plant species and eight animal products to make different honey-based remedies. The most commonly treated ailments by these natural mixtures include respiratory disorders, skin ailments, ophthalmic conditions, gastrointestinal issues, musculoskeletal, and circulatory problems (Zamudio et al. 2010; Kujawska et al. 2012).

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2 Honey: A Powerful Natural Antioxidant and Its Possible Mechanism of Action

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Abstract

Honey, a supersaturated concentrated solution with complex constituents, has been used as therapeutic agent since ancient times. Natural products have been used as a substitute for various conventional treatments and drug discoveries. Different in vivo and in vitro studies have shown properties of honey including antioxidant, antibacterial, anti-inflammatory, anti-cancerous, and much more. Therapeutic properties of honey greatly depend on its constituent composition

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_2

which may vary based on various factors like species of bee, environmental conditions, type of flower, and processing methods. Oxidative stress due to cellular metabolism and other physio-biochemical activities of the body demand the necessity of antioxidants in diet which can be fulfilled by honey. Antioxidant and other biological properties of honey are greatly determined by the polyphenol composition. This chapter comprises honey composition, type, antioxidant properties, and antioxidant mechanism of honey according to different research studies.

Keywords

Apitherapy · Antioxidant · Polyphenols · Reactive oxygen species (ROS) · Oxidative stress

Abbreviations

CAT	Catalase
COX-2	Cyclooxygenase-2
CZE	Capillary zone electrophoresis
DPPH	1, 1-Diphenyl-2-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
GPx	Glutathione peroxidase
GSH	Glutathione
H_2O_2	Hydrogen peroxide
HPLC	High-performance liquid chromatography
IL-6	Interleukin-6
LDL	Low-density lipoprotein
MEKC	Micellar electro-kinetic chromatography
MMP9	Matrix metallopeptidase 9
MRSA	Methicillin-resistant *Staphylococcus aureus*
ORAC	Oxygen radical absorbance capacity
PGE2	Prostaglandin E2
SOD	Super oxide dismutase
TLR	Toll-like receptor
TNF	Tumor necrosis factor
UPLC	Ultra-performance liquid chromatography
VREF	Vancomycin-resistant *Enterococcus faecium*

2.1 Introduction

Natural products being rich source of compounds are intended for various drug discoveries. Practical substitute by products from nature to lessen the escalating rebuke of diseases and their inevitable aftermath has clinched the consideration toward honey and has led toward an alternate medicine branch called apitherapy (Aggarwal and Shishodia 2006; Othman 2012). Honey remained the foundation of pharmacy from ancient period since classic civilization (Greeks and Romans) and of Middle Age Arab people. Honey as a balanced diet and folk medicine since ancient times from the history of human race has been used as a remedy in various fields like medicine, cosmetics, a preserving substance (Wieckiewicz et al. 2013), and its therapeutic value is even intensely specified in Qur'an (Eteraf-Oskouei and Najafi 2013). Honey being one of the ancient traditional medicine by *Apis mellifera* (*A. mellifera*) has various medicinal properties like antioxidant, antibacterial, hepatoprotective, hypoglycemic, reproductive, and antihypertensive, thus significantly used in human ailments. Meanwhile production of free radicals by various disease conditions especially in chronic cases cause potential damage at molecular level and further aggravate the conditions; incorporation of honey as an antioxidant in the diet neutralizes these free radicals either directly or indirectly and lessen the harm by these reactive species without having any adverse effects. Usage of present-day antibiotics has abandoned the use of honey, but in recent studies, several investigations are being carried out regarding the bioactive properties of honey and bee products against numerous diseases (Carter et al. 2016). Currently, honey with standardized antibacterial activity levels are present, the finest identified honey from *Leptospermum scoparium* (*L. scoparium*) is recognized to inhibit about 60 diverse species of microorganisms including gram-positives, gram-negatives, aerobes, and anaerobes (Babacan and Rand 2017). Honey has a water activity of 0.56–0.62 and 3.9 pH value (Escuredo et al. 2014). The main objective of this chapter is to understand and evaluate the properties of honey with the main focus on antioxidant properties and the possible mechanism of antioxidant action. This chapter also highlights the role of honey in ameliorating various disease conditions, antioxidant effects on GIT, pancreas, inflammation, reproductive organs, and other chronic and degenerative diseases. Honey either alone or in combination with conventional therapy acts as a novel antioxidant in regulation of various conditions associated with oxidative stress. The study regarding the therapeutic role of honey is still under different phases and may be used as a main antioxidant in near future.

2.2 Composition of Honey

Composition and properties like color, aroma, flavor, and antioxidant nature of honey greatly depend on

1. Honeybee species
2. Flowers
3. Geographical regions
4. Weather and climate
5. Processing and storage (Tornuk et al. 2013; Alvarez-Saurez et al. 2009)

Table 2.1 General composition of honey

Component	Value/100 g
Total carbohydrates	82.4 g
Fructose	38.5 g
Glucose	31.28 g
Moisture content	17.1 g
Maltose	7.31 g
Sucrose	1.31 g
Total acid as gluconic	0.57 g
Fiber	0.2 g
Amino acids/proteins	0.3 g
Ca	6.00 mg
Ash	0.169 g
P	4.00 mg
K	52 mg
Mg	2.00 mg
Fe	0.42 mg
Zn	0.22 mg
N	0.041 g
Cu	1–100 µg/g
Vitamin B2	0.038 mg
Vitamin B3	0.21 mg
Vitamin B5	0.068 mg
Vitamin B6	0.024 mg
Vitamin B9	2 µg
Vitamin C	0.5 mg
Miscellaneous groups	Bogdanov et al. (2008) and Gheldof et al. (2002)

Honey is a concentrated aqueous solution with >95% of its dry weight constituted by sugars followed by water (Sato and Miyata 2000). Of the sugars chiefly present are fructose and glucose (Gheldof et al. 2002) which determine its nutritional and physical features. Honey is a complex mixture whose constituents are mentioned in Table 2.1. Honey constituting less than 18% water can be stored without the risk of fermentation. Alcohols, aldehydes, ketones, acids, terpenes, and esters are the main volatile compounds present in honey (Molan 2002; Zhou et al. 2002). Organic acid predominantly in honey is gluconic acid and originates largely from glucose and water in the presence of glucose oxidase enzyme (Bastos and Alves 2003) and a minor amount from genus Gluconobacter bacteria (French et al. 2005). Another group of compounds contributing to the anti-oxidant capacity of honey and responsible for its geographical properties are the polyphenols (Davis 2005). Classifications of 501 polyphenols into six different classes and 31 subclasses have been done by "phenol explorer," as flavonoids, phenolic acids, non-phenolic metabolites, lignans, stilbenes, and other polyphenols (Tomás-Barberán et al. 2001).

2.2.1 Types of Honey

Variety of honey can be determined on the basis of time of nectar existence and the accessibility of individual nectar flows. Currently, beekeepers use melissopalynological method based on the microscopic quantitative identification of plant pollens present in the honey; it is the only laboratory method providing certainty about the variety of honey. On the basis of different characteristics, honey can be divided into various classes as given below in Fig. 2.1.

2.3 Antioxidants in Human Health

Antioxidants are the molecules that have the capability to accept or donate electrons in order to neutralize free radicals produced by various biological processes. Consequences of biological processes cause generation of free radicals called reactive oxygen, reactive sulfur, and reactive nitrogen species (ROS, RSS, RNS) such as hydroxyl radical (·OH), superoxide anion (O_2•), hydrogen peroxide (H_2O_2), nitric oxide (NO), and further other types like singlet oxygen, hypochlorous acid, and peroxynitrite (Vajragupta et al. 2004) with hydroxyl radical (.OH) being the

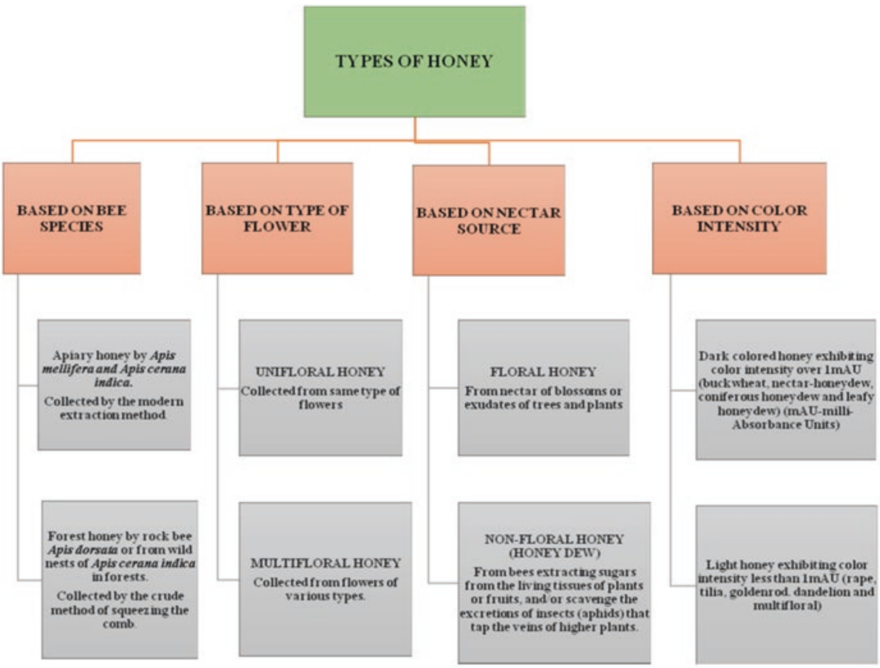

Fig. 2.1 Types of honey based on different features (Subrahmanyam 2007). Honey can be classified into different types based on different features, viz. bee species, type of flower, nectar source, and color intensity

Table 2.2 Different polyphenols present in honey

Phenolic acids	Flavonoids
4-Dimethylaminobenzoic acid	Apigenin
p-Coumaric acid	Pinocembrin
Caffeic acid	Genistein
Vallinic acid	Chrysin
Gallic acid	Tricetin
Chlorogenic acid	Luteolin
Syringic acid	Quercetin

strongest. Oxidative stress is the resultant stress that arise due to free radicals attacking on nucleic acids, unsaturated fats, and amino acid which play an important role in pathogenesis of various diseases like inflammatory diseases, cancer, Alzheimer's disease, aging, diabetes, cardiovascular diseases, and various other diseases (Giles and Jacob 2002; Geier et al. 2009). Our body has established an antioxidant defence system to tackle the oxidative damage brought by ROS which include chelation of metals, enzymatic activities, scavenging free radicals, oxidation shielding agents like catalase (CAT), peroxidase, superoxide dismutase (SOD), polyphenols, and vitamin E and C (Nagai et al. 2001). All these defences act to deactivate these radicals. Honey acts as a dietary antioxidant with polyphenols chiefly accountable for its strong antioxidant action (Hussein et al. 2011), and this characteristic of honey significantly reduces several acute and chronic disorders. The neutralization of free radicals by antioxidant molecules can occur either by directly reacting with them or they may become less active free radicals and less dangerous than those they have neutralized, though dietary intake of antioxidants maintains satisfactory antioxidant status in the body. According to a research, honey at the rate of 1.2 g/kg enhances the activity of beta-carotene, glutathione reductase, vitamin C, and uric acid as antioxidants (Tomás-Barberán et al. 2001). Polyphenol constituents of honey are potential biochemical markers as they are phytochemicals, i.e., plant-based molecules with antioxidant properties. Flavonoids and phenolic acids are the polyphenols accountable for antioxidant properties of honey and are cited in Table 2.2 (Davis 2005).

2.3.1 Mechanism of Antioxidant Effect

Flavonoids present in honey have been demonstrated as very effective scavengers of reactive oxygen (ROS) and reactive nitrogen species (RNS) (like peroxyl, alkyl peroxide, hydroxyl, superoxide radicals, nitric oxide, and peroxynitrite) to counter the oxidative damage induced by these molecules (Snow and Harris 2004). Flavonoid structure is attributed with three chemical features, presence of a 2, 3 double bond in the C-ring, B-ring having ortho-dihydroxy structure (Sekher Pannala et al. 2001; Burda and Oleszek 2001) and presence of a 4-oxo function (Heim et al. 2002). On the B-ring, hydroxyl groups donate an electron and hydrogen to stabilize peroxynitrite and peroxyl and hydroxyl radicals making comparatively stable flavonoid radicals. On the B-ring occurrence of catechol leads to oxidation of flavonoid (Van Acker et al. 1996a) facilitating electron delocalization (Arora et al. 1998) and forms relatively stable ortho-semiquinone radical (Mora et al. 1990). Presence of a free 3OH in some flavonoids impart them a heterocyclic character which allow conjugation of aromatic rings between them, although activity of flavonoids does not need closed C-ring itself (Matthiesen et al. 1997). Ebselen, a known RNS scavenger, is reported to be tenfold less potent than flavonoids with 3-OH and 3′,4′-catechol against peroxynitrite radical (Heim et al. 2002). Oxidative damage brought about by metal and nonmetal is regulated partially by free 3-OH substituent on quercetin (Heim et al. 2002; Arora et al. 1998), which enhances the stability of this flavonoid radical, in contrast its stability decreases on substitution by a methyl or glycosyl group at 3-OH position (Burda and Oleszek 2001). Conjugation between 4-oxo function and unsaturated 2–3 bond offers a characteristic feature among the structural classes of general flavonoids, and the lack of one or both of these features results in the reduction of antioxidant capacity (AOC). Presence of more number of hydroxyl groups leads to increased free radical scavenging capacity of flavanols than flavones (Lien et al. 1999). Honey avoids RBC oxidative damage most probably due to its integration into cell membrane and capability to enter and reach cytosol. Antioxidants protect key cell components from damage by neutralizing the free radicals. Antioxidants that occur naturally in the body or are consumed through the diet may block damage to cells. Various other constituents of honey responsible for reducing the oxidative stress are mentioned in Table 2.3.

2.3.2 Honey as Antioxidant from In Vitro Studies

The antioxidant properties contributed by honey can be assessed by the method of antiradical activity through different assays like ORAC, FRAP, and DPPH scavenging assay (Davis 2005; Hussein et al. 2011; Tomasin and Gomes-Marcondes 2011). Individual honey polyphenol contents in honey can be analyzed by HPLC

Table 2.3 Antioxidant mechanism of various constituents present in honey

Constituent	Mechanism of action to control the oxidative damage
Quercetin	Antiradical activity by scavenging, chelation of ion inhibition of lipid peroxidation inhibition of xanthine oxidase (Sekher et al. 2001; Burda and Oleszek 2001)
Caffeic acid	Reduction in lipid peroxidation, increase in plasma levels of vitamin E
Caffeic acid phenethyl ester (CAPE)	Free radical scavenging
Kaempferol in hippocampal cell line HT-22 of mouse	Hindering ROS generation (Heim et al. 2002) Blocks oxidative stress during apoptosis induced by low potassium in granule cells (van Acker et al. 1996b)
Apigenin	Lessens oxidative damage, prevents NO-induced vasorelaxation of aorta in male Sprague–Dawley rats (Arora et al. 1998)

with its modified form UPLC having advantage of more resolution, speed, and sensitivity over HPLC (Hussein et al. 2011). Another new technique, alternative method to analyze phytochemicals is called capillary electrophoresis (CE), done by two different types of methods: MEKC and CZE are gaining popularity (Hussein et al. 2011). Honey obtained from *Marchalina hellenica* (Turkish red pine honey) has been reported to scavenge DPPH due to its antiradical action (Kassim et al. 2010). Honey obtained from *Trigona carbonaria* (Australian stingless bees) show good antioxidant properties (Akbulut et al. 2009), similarly good antioxidant and antiradical activities in tualang honey of Malaysia by *Apis dorsata* the giant Asian bees and American buckwheat honey have been reported (Oddo et al. 2008; Mohamed et al. 2009). Pine honey from Greece showed antioxidant effect on human serum lipoproteins and LDL; similarly oxidative stress caused by cumen hydroperoxide (CuOOH) causing damage to membrane and intracellular levels was reversed by antioxidant and antiradical effect of honey by inhibiting the progression of oxidative cascade, free radical species, and increasing the cell longevity as compared to control cells. A protective effect shown by pre-incubated cells with honey when exposed to CuOOH stress showed little oxidative damage, increased GSH levels, fewer morphological changes, and an increase in cell survivability compared to control cells. The outcome of these studies showed antioxidant and anti-inflammatory role of honey on endothelial cells (Makedou et al. 2012).

2.3.3 Honey as Antioxidant from In Vivo Studies

Honey as an in vivo antioxidant has been studied with respect of its effects on various body parts to ameliorate oxidative stress as mentioned.

2.3.3.1 Effect of Honey on GIT

Besides antioxidant action, gastroprotective effect of honey in ethanol-, aspirin-, indomethacin-, or ammonia-administered rodents is reported (Beretta et al. 2007). The gastroprotective effect of honey studied by Kim revealed that gastric injury and duodenal ulcers induced by *Helicobacter pylori* is due to oxidative stress, and honey inhibits its growth (Gharzouli et al. 2002; Kim 2005). GIT in diseased conditions is prone to oxidative stress, disturbing fluidity of brush border membrane (BBM) (Bhor and Sivakami 2003). Honey having gastroprotective action improves glycemic control in diabetic rats stimulating the hypoglycemic drug bioavailability through alteration of intestinal oxidative state (Erejuwa et al. 2011a), and co-administration of honey with antibiotic (sulfasalazine) reduced oxidative damage, colonic inflammation, and mucosal malondialdehyde (MDA) level in ulcerative colitis induced by trinitrobenzosulfonic acid (TNBS) in rat model (Ali et al. 1991; Medhi et al. 2008).

2.3.3.2 Effect of Honey on Liver

The abnormalities in diabetes mellitus usually seen are increased susceptibility of liver to oxidative stress and elevated levels of serum aspartate aminotransferase, alkaline phosphatase, and alanine aminotransferase (Bilsel et al. 2002; Leeds et al. 2009). Studies showed that pine honey restored the activities of hepatic CAT, GPx, and SOD in the liver of young and middle-aged rats (Gumieniczek 2005) and reduced hepatic damage in trichlorfon-administered male BALB/c mice, hepato protective effect in sheep administered carbon tetrachloride (CCl_4) (Yao et al. 2011), STZ-induced diabetic rats and in common bile duct obstruction of rats (Erejuwa et al. 2012). Supplementation of honey-restored hepatic glutathione levels ameliorated the mononuclear cellular infiltration induced by NEM and congestion in liver (Erguder et al. 2008).

2.3.3.3 Effect of Honey on Diabetes Mellitus and Pancreas

Fall of glycemic control in diabetes mellitus is seen as β-cells of pancreas are susceptible to oxidative stress leading to reduction in the efficiency of insulin secretion by pancreas an outcome of oxidative stress (Korkmaz and Kolankaya 2009; Poitout and Robertson 2002). Honey improves total antioxidant status (TAS), glutathione reductase (GR), CAT, glutathione S-transferase (GST), and GPx enzyme activities (Evans et al. 2003). It also reduced the levels of lipid per-oxidation and restored SOD activity. On kidney, its antioxidant effect reduced the thickening of glomerular basement membrane and mesangial matrix expansion in the honey-treated diabetic rats. Combination of honey with hypoglycemic agents like glibenclamide and metformin distinctly protected the pancreas and kidney against oxidative damage and restored antioxidant enzymes much better than any of these agents when given alone (Grankvist et al. 1981).

2.3.3.4 Effect of Honey on Plasma/Serum

Elevated levels of plasma glucose are responsible for oxidative stress by generating ROS. Supplementation of honey reduced hyperglycemia in Sprague–Dawley rats with streptozotocin-induced diabetes (Evans et al. 2003; Erejuwa et al. 2010) rats with diabetes induced by alloxan (Erejuwa et al. 2009), Wistar–Kyoto rats with streptozotocin-induced diabetes (Fasanmade and Alabi 2008). Formation of advanced glycation end products (AGEs) on reaction of glucose (carbonyl group) and protein (amino group) results in the formation of a stable compound called fructosamine, a glycosylated protein formed as a consequence of diabetes (Erejuwa et al. 2011b), and consumption of honey reduces formation of fructosamine due to its antioxidant properties. A study on Nigella grains and honey against carcinogenesis and oxidative stress induced by methylnitrosourea showed that combination of *Nigella sativa* and honey stopped the increase in MDA and NO levels and exerted 100% protection against the effects of methylnitrosourea (Selvaraj et al. 2006). The antioxidant activity of honey in plasma is also revealed by an increase in the activity of GPx and NO in rats with alloxan-induced diabetes (Mabrouk et al. 2002).

2.3.3.5 Effect of Honey on Reproductive Organs

Cigarette smoking leading to cigarette smoke-induced testicular damage by causing apoptosis and damage in the testis in response to oxidative stress (Hassan and Bayoumi 2010; Rajpurkar et al. 2002). Honey brings higher Leydig cell count, larger diameter and epithelial height of seminiferous tubules and reduction in the percentage of tubules holding germ cell loss result in the amelioration of testicular damage (Mohamed et al. 2011). Honey caused an increase in epididymal sperm count and improvement in testicular marker enzyme activity owing to reduction in lactate dehydrogenase and elevation in sorbitol dehydrogenase a study in rats conducted by Abdul-Ghani and colleagues (Mohamed et al. 2011). Another study on ovariectomized female rats also suggested advantageous effects of honey on reproductive organs of female (Abdul-Ghani et al. 2008) (Fig. 2.2).

2.3.4 Advantages of Honey as an Antioxidant

Different antioxidants with valuable effects have been documented in various disease models (rodents as well as humans) (Köhler et al. 2011; Shargorodsky et al. 2010; Rodrigo et al. 2008). However, shortcomings of these antioxidants or vitamins have been reported due to their complex mechanism by acting as

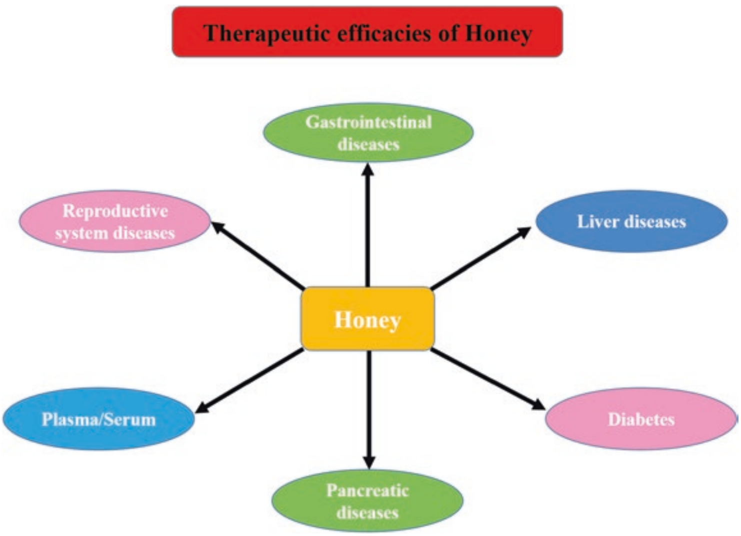

Fig. 2.2 Various pathways regulating antioxidant mechanism of honey. Honey acts as potent antioxidant and can be used in different diseases. The antioxidant property of honey is by elevation of total anti-oxidant defense of the body, donation of hydrogen, free radical sequestration, chelation of metallic ions, and acts as substrate for radicals

pro-oxidants that need antioxidants for their activation (Bowry et al. 1992). Vitamins C and E considered as first-choice antioxidants when used in trials had a disadvantage of undefined dose selection and with supplementation of large doses of α-tocopherol in the diet, and they interfere with the plasma bioavailability of γ-tocopherol (Handelman et al. 1985) or may increase tumor formation (Mitchel and McCann 1993). α-Tocopherol is less effective as inhibitor of nitrogen dioxide-mediated nitrosation than γ-tocopherol (Cooney et al. 1993). Study on smokers supplemented with β-carotene is reported to exaggerate cancer risk (Heinonen and Albanes 1994). Beneficial effects of honey over other vitamins are that honey is devoid of pro-oxidant properties, comprises several bioactive constituents which may produce synergistic antioxidant effects, and does not require regeneration into active form (Köhler et al. 2011; Rodrigo et al. 2008), and honey can scavenge both free radicals like OONO−, O2•− and non-free radicals like NO (Estevinho et al. 2008; Bilsel et al. 2002), upregulates intracellular transcription factor Nrf2 moderately, and is capable of reducing inflammation by inhibiting the production of NO and prostaglandin E (2) (Kassim et al. 2011; Bilsel et al. 2002). In view of the few above-mentioned advantages, honey might be more advantageous in preventing complications of various acute and chronic coursed diseases (Fig. 2.3).

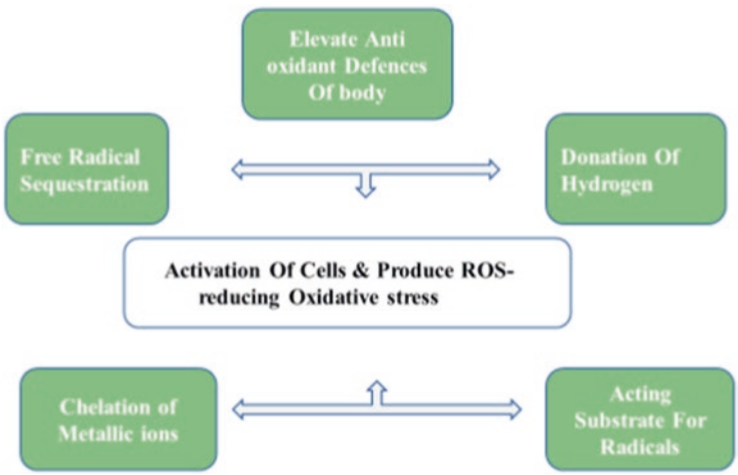

Fig. 2.3 Various anti-inflammatory pathways through which honey acts (Swartz 2005; Larocca et al. 1995). Honey inhibits release of various cells like macrophages, neutrophils, monocytes which have anti-inflammatory effects. It also increases the production of H_2O_2 which also accounts for anti-inflammatory effects. It is known to decrease the levels of IL-6, PGE2, COX-2, etc., which acts as proinflammatory molecules and inhibits expression of MMP9

2.4 Other Biological Activities of Honey

In addition to the antioxidant activity, honey also exhibits some other beneficial health roles as follows.

2.4.1 Antimicrobial Activity

Various constituents present in honey like H_2O_2, methylglyoxal, NO-metabolites, flavonoids, defensins, and phenolic acids along with certain other properties of honey like high osmolarity and acidity make it an effective antibacterial against various bacteria like MRSA, VREF, and ciprofloxacin-resistant *Pseudomonas aeruginosa* (Ishige et al. 2001; Samhan-Arias et al. 2004; Kwakman and Zaat 2012). Antibody production, lymphocytic and phagocytic activities may also increase by using honey (Alvarez-Suarez et al. 2010). Level of H_2O_2 produced determines the antibacterial action of honey due to increased activity of two enzymes, i.e., catalase and glucose oxidase. Respective levels of these two enzymes determine the level of H_2O_2 in the honey (Weston 2000). In the presence of enzyme catalase, hydrogen peroxide produces oxygen and water and shows inverse relationship between hydrogen peroxide and catalase activity which is used to assess the "inhibine number" of honey. Therapeutic effects of honey are very important especially in immunocompromised individuals, effective against a range of microbes including both pathogenic and non-pathogenic micro-organisms (Zaghloul et al. 2001). Honey can act as bacteriostatic agent or bactericidal depending upon the concentration used. Hydrogen peroxide can destroy microbes by the generation of strong free radicals on decomposition, but catalase enzyme or heat can easily destroy its activity. Catalase has no effect on antibacterial action of manuka honey (from New Zealand) and jelly bush (from Australia); both are examples of non-peroxide honey (Snow and Harris 2004: Weston 2000). Flavonoids and cinnamic and benzoic acid are the constituents of nonperoxide honey (Weston 2000) due to which they show more stable and persistent antibacterial action (Alvarez-Saurez et al. 2009). Upon reaction of hydrogen peroxide with benzoic acids, more stable and more powerful peroxyacids exhibiting antimicrobial properties are formed. Manuka honey has the highest level of non-peroxide activity (Cushnie and Lamb 2005). Infections caused by *E. coli* and *S. aureus* can be prevented by manuka honey (Lusby et al. 2005).

2.4.2 Beneficial Role of Honey on Immune System

In cell culture, honey has shown immunostimulatory effect by activating T- and B-immune cells. Besides showing potent antibacterial activity, honey also helps in clearing infection by activating immune system. It stimulates multiplication and activation of neutrophils (Tonks et al. 2003). It also stimulates monocytes where from cytokines (IL-1, IL-6 and TNF-alpha) are released, which further activates immune system thus clearing the infection. An active component in manuka honey has been found to

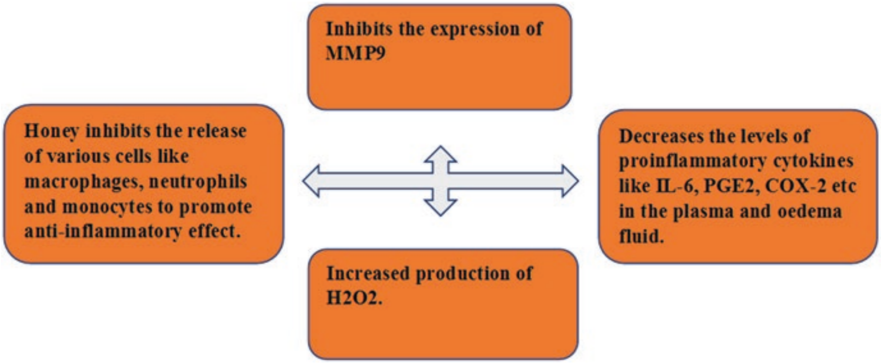

Fig. 2.4 Overall therapeutic efficacy of honey: Honey is known to have therapeutic efficacy in different disorders. It has been known to prevent disorders related to GIT, liver, reproductive system, diabetes, pancreas, plasma, and serum. This efficacy could be attributed to different mechanisms discussed in the chapter

stimulate the release of TNF from macrophages by the activation of TLR (Tonks et al. 2007). Respiratory burst in macrophages requires supply of glucose that produce hydrogen peroxide to destroy the bacteria (Molan 2001), and such substrates of glycolysis in the macrophages for energy production to perform its action in injured tissue with low oxygen supply and exudates are provided by honey. Honey has suitable pH at which macrophages show enhanced phagocytic activity (Molan 2001) (Fig. 2.4).

2.4.3 Honey in Wound Healing

Honey from different sources has been used for a broad spectrum of wounds (Al-Waili 2004). Presently a blend of jelly bush and manuka honey (Medihoney) is first certified and medically licensed for wound care in many European countries (Molan and Betts 2004; Molan 2006). It stimulates regrowth of tissues, angiogenesis, and fibroblast growth to produce collagen fibers and replace connective tissue. Honey facilitates formation of new skin by stimulating the regrowth of epithelial cells over healed wounds, prevents scar and keloid formation, and eliminates the necessity of skin grafting (Rozaini et al. 2004). Honey facilitates autolytic debridement due to its high osmotic pressure, it takes lymph from deeper tissue and immerses the wound bed constantly, and protease activity of lymph is responsible for debriding activity (Molan and Rhodes 2015).

2.4.4 Apoptotic Activity of Honey

Honey because of its apoptotic activity is considered as natural substance with anticancer property. Apoptosis inducers are the chemicals used in the treatment of cancer, as uncontrolled cellular proliferation and inadequate apoptotic turnover occur in cancer (Rozaini et al. 2004; Boukraa and Niar 2007). Honey is responsible for apoptosis through depolarization of mitochondrial membrane (Boukraa and Niar 2007) by changing the expression of pro- and anti-apoptotic proteins in neoplastic cells. Honey having more phenolic constituents increases cleavage of poly(ADP-ribose) polymerase (PARP) and caspase-3 activation in the human colon neoplastic cells (Earnshaw 1995). It causes upregulation of proapoptotic proteins (Bax, caspase-3, p53) and downregulation of Bcl2 anti-apoptotic factor (Earnshaw 1995). Manuka honey through intravenous injection exhibits its apoptotic effect through the involvement of caspase-9 which then causes caspase-3 activation, PARP activation, DNA fragmentation, and inexpression of Bcl2 factor in neoplastic cell lines (Tomasin and Gomes-Marcondes 2011). Cancerous tissue of Wistar rats on oral administration of honey showed increased expression of pro-apoptotic protein (Bax) and reduced expression of anti-apoptotic protein Bcl-2 (Park et al. 2005). Presence of Quercetin in honey reduces transcriptional activity and signaling of β-catenin/Tcf in cell lines of SW480 (Gulati et al. 2006), and inhibition of the PI3K-Akt/PKB by Quercetin present in honey also exhibits an anticancer effect.

2.5 Conclusion

Changing lifestyle and food habits have exposed humans to various stress conditions leading to the enhanced incidence of different diseases like hypertension, cancer, atherosclerosis, and diabetes mellitus resulted in decreased lifespan of humans and increased mortality. Oxidative stress having vital role in pathogenesis of these diseases demands the incorporation of dietary antioxidants beneficial for bringing down such conditions to a low level. But the antioxidants to be selected should be effective without any harmful effects. Honey being a natural product have plentiful benefits which in combination with conventional therapy can produce synergistic effects to ameliorate the oxidative stress in different body parts and produce positive effects in the management of several disease conditions suggesting that honey can be used both as nutrient and as medicine.

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Honey and Its Phyto-Constituents: From Chemistry to Medicine

3

Adil Farooq Wali, Jayachithra Ramakrishna Pillai, Maryam Razmpoor, Salma Jabnoun, Imra Akbar, Saiema Rasool, Azher Arafah, Andleeb Khan, Rukhsana Akhter, and Sabhiya Majid

Abstract

Honeybees depend upon plants for everything they want to maintain the colony running; nectar and pollen that is their only carbohydrate and protein essential nutrients. In order to achieve their necessary nutritional requirement, honey bees eventually collect essential plant metabolites when component of nectar and pollen. In addition, several molecules exhibit biological activity which may become significant in the battle against pests and pathogens in the hive. Flavonoids, ter-

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_3

penoids, and polyphenols are essential biologically active ingredients found in honey and also have antioxidant properties. Nonetheless, for reasons of room, it is practically impossible to give a detailed overview of the phytochemical characteristics of honey and pollen in a literature review of this scope. In addition, the therapeutic ability of biologically active ingredients and their use in value-added food products are also at the core of this chapter.

Keywords

Honey · Apis mellifera · Flavonoids · Terpenoids · Polyphenols

3.1 Introduction

Honey is a natural organic material produced by a particular species of bees of the genus Apis from flower nectars (Alvarez-Suarez et al. 2014). It is a sweet, viscous, and flavorful liquid that is high in nutritious content (Havsteen 2002). The transfer of nectar to honey is a process of spewing and evaporation. This is then preserved in honeycomb wax as the primary source of food for honey bee (Adebiyi et al. 2004). The honeycomb consists of hexagonal waxy cells manufactured by the bees to shelter their larvae and store the honey. Beekeepers will take the entire honeycomb out for the honey harvest. The chemical composition of the honey varies depending on the nature of the flora from which the nectar was collected, geographic origin, seasonal and environmental conditions. There are raw honey and pasteurized honey. Raw honey is extracted right out of the hive. It can contain traces of wax and pollen since it is not purified. Pasteurized honey is stored without any impurities. Consumption of fresh honey increases immunity to seasonal allergies. Raw honey is rich in nutrients (Olaitan et al. 2007).

Pollen and nectar are the primary food sources for honey bees. And they are the primary source of proteins and carbohydrates.

Three types of bees are present in the bee hive: the bees of the workers, the bees of the drone, and the bees of the queen. Females that do not breed bees are referred to as worker bees. Queen bee and drone bees develop eggs, and these eggs live in bee hives and form larvae after 3–4 days. The female worker bees are produced from the larva. Mainly sterile female worker bees are found in a typical hive colony. The queen bee will live for up to 3–4 years (Bishop 2005).

3.2 Physical Properties of Raw Honey

Freshly extracted raw honey is very viscous in nature. Depending on the composition and water content, the viscosity of honey varies. It can absorb moisture from the environment. Presence of colloidal particles are responsible for the difference in the surface tension. The surface tension as well as viscosity are accountable for the frothing nature of raw honey (Rueppell et al. 2007).

Liquid honey has different colors from colorless to amber color. The variation in the color depends on the botanical origin, age, and storage. If suspended particles like pollens are present, then the clarity varies. Crystallized form of honey has a light color because of the glucose crystals present in it. Presence of fructose and glucose makes the honey sweet. It has almost same sweetness as that of sucrose. Since the microorganisms do not grow in honey, it can be stored for many years.

3.2.1 Types of Honey Based on the Production Procedures

Based on the production procedures, honey can be classified as extracted honey (made by centrifuging the broodless honey combs), pressed honey (made by pressing the honey combs), drained honey (made by slow draining of the broodless honey combs), and organic honey (made by organic beekeeping). Extracted honey is the most widely marketed honey. Organic honey and natural honey have the same composition. The difference between organic honey and natural honey is that the latter contains no traces of beekeeping pesticides.

3.2.2 Types of Honey Based on the Processing Procedure

On the basis of processing procedures, honey may be classified as normal honey, comb honey, and cut comb honey. Normal honey appears as crystalline form or as liquid form or as a mixture of both. Comb honey usually retailed in the broodless combs itself. Cut comb honey contains small pieces of honey comb in it.

3.2.3 Types of Honey Based on the Origin

According to the Codex Alimenterius

1. Based on the topographical region of the honey production, it can be named, if it is produced within the area.
2. Honey may be named based on the plant or floral source if it is produced mainly from that specific source. It will have the organoleptic, physicochemical, and microscopical characteristics equivalent to that origin.
3. Honey may be named based on the geographical or botanical origin (Bogdanov 2011a).

3.2.4 Types of Honey According to the Botanical Source

Depending on the botanical source, honey may be divided as blossom and honey dew. Each honey type is different from another because of the different sources and different proportions. It can be unifloral or multifloral honey. If the pollen grain is initiated from only one particular plant, it is known as unifloral honey. If there is no dominant pollen type, it is known as multifloral honey. Unifloral honey is more valuable.

3.3 Different Species of Honey Bees

Honey bees are known as one of the primogenital forms of animal life. Honey bees are eusocial, flying creature from the genus *Apis* of the bee clade. They construct colonial nests using wax for their colonies. In these waxy nests, they produce and store honey. The practice of collecting honey from the wild bee colonies is called beekeeping or apiculture. Seven species of honey bees and 44 subspecies of this were identified in the early twenty-first century. Western honey bee is the best known among these and had been used for the honey making and crop pollination. The bee wax has been used for candle making, soap making, lip balms, and other crafts. The scientific study of honey bees is called as melittology.

The main two species of honey bees have been named as *Apis mellifera* and *Apis cerana.*

The *Apis mellifera* or the European honeybee species are the most widely spread all around the world and utmost commonly collected and sold in the world.

In tropical Asia*, Apis cerana* is used for making honey most commonly. This honey is almost similar to the *mellifera* honey in composition and taste. Other common species are *Apis dorsata* and *Apis florea*. These honeys are marketed locally not worldwide.

Small honey bees like *Apis florea* and *Apis andreniformis* can be seen in southern and southeastern Asia. They make their hives in trees and shrubs, and they are relatively small. Their stings are usually unable to penetrate through the human skin. Therefore, these hives and swarms can be picked up with marginal protection (Arias and Sheppard 2005). *Apis florea* is completely yellow in color (Wongsiri 1997).

The subgenus *Megapis* can be very dangerous. They build their hives on tall tree branches, on cliffs, and sometimes on buildings. Honey hunters sometimes robbed their honey and may get stinging from it, and it can be fatal (Nathan et al. 2009).

Africanized bees or killer bees are the crosses of European stock and the subspecies *A. m. scutellata* which is East African lowland species. These bees do not produce excess honey and are more violent than European bees. These honey bees are known to be more resistant to disease and are very good hunters (Wongsiri 1997).

3.4 Folklore Uses

Honey has long been recognized to possess various medicinal properties, and it is used as a wound dressing and as an antiseptic since ancient times. Honey is being used in the traditional medicine since stone age (Needham 2008). Honey is considered as the oldest traditional medicine which has been used for various human diseases worldwide (Fig. 3.1). Some are listed below.

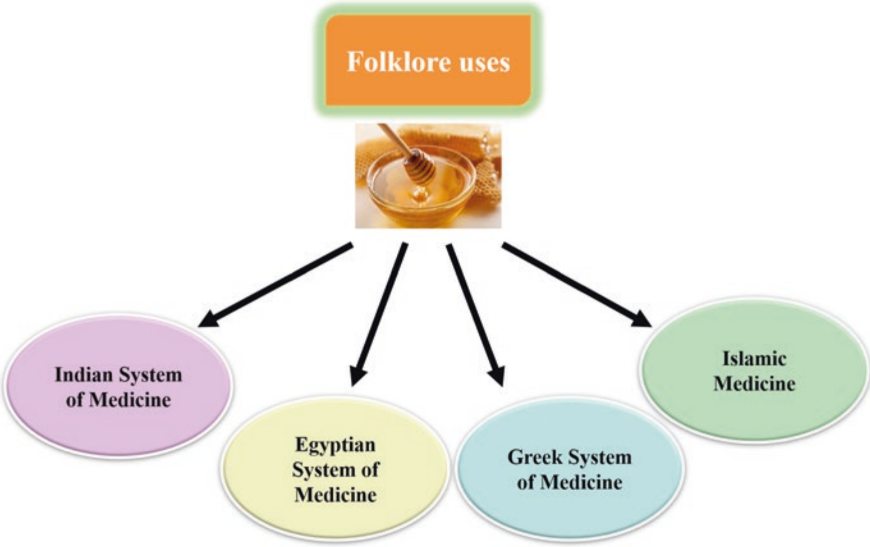

Fig. 3.1 Folklore use of honey used in different medicine systems

3.4.1 Honey in Indian System

According to *Ayurveda,* the ancient system of Indian medicine, honey, without changing its own properties, stimulates the activities of the substances to which it binds. In Ayurveda, honey was used both internally and externally for nutritional and therapeutic uses since hundreds of years. Externally honey was applied for ophthalmic ailments and for wound healing. Internally it was used mainly for cough and asthmatic problems. Honey was used as a base for these cough preparations along with other herbs. It is being used to treat sleep disorders since it has hypnotic action (Murty 2001). Honey has been used in Ayurveda to improve the oral hygiene and to keep the gums healthy (Vaidya et al. 2002). Alvarez-Suarez et al. reported that the consumption of newly formed and collected honey may increase the body weight and stored or old honey can decrease the fat of the body and therefore reduces the body weight (Alvarez-Suarez et al. 2012). In Ayurveda, honey is used as a medicine for the eyes and for the vision, and it reduces thirst, poises hemostasis, and decreases the toxicity. It is used for urinary tract disorders and also for diabetes. Honey is used to stop hiccups, worm infestations, skin disorders, diarrhea, nausea, and vomiting and also for bleeding complaints. Honey speeds up the healing process, and it has been used for the wound healing since long and also for cleaning the wounds (Eteraf-Oskouei and Najafi 2013). According to this system, hot honey can cause toxic effect so it should not be heated or consumed when it is warm (Megan Ware 2015). In Ayurvedic preparations, honey is used as a vehicle or as a preservative (Zumla and Lulat 1989).

3.4.2 Honey in Egyptian System

Ancient Egyptian medicines utilized the medicinal properties of honey. They combined honey with wine and milk and used for many ailments. They offered honey as a sacrifice to their deities in older times (Dash 1972). For embalming the dead bodies, they used honey. Honey was used to heal the infected wounds because of its antibacterial properties. For the topical application, they used honey (Molan 1999).

3.4.3 Honey in Greek System

Ancient Greek people used honey in a drink called Oenomel along with unfermented grape juice. It has been used to treat gout and certain nervous disorders (Dash 1972). Also they used honey for topical antisepsis, contraception, eye diseases, wound healing, cough and sore throat, laxative action, baldness, prevention, and management of blemishes (Molan 1999).

3.4.4 Honey in Islamic Medicine

According to Islamic medicine, honey is used as a healthy drink. The holy Qur'an intensely demonstrates the possible therapeutic values of honey. They used honey for a variety of medical problems, including stomach ailments. They used the beeswax to prevent from cold during winter (Molan 1999).

According to Unani Medicine, honey is used as a natural food supplement, as a nutritive agent, and as antibacterial and anti-inflammatory agent, and it also has wound healing properties (Molan 1999).

3.5 Phyto-Constituents

Pure honey is one of the most essential classes of composites found in plants, containing different identified varieties of primary and secondary metabolites (Fig. 3.2). Some of them are described below.

3.5.1 Carbohydrates

The general chemical formula of simple sugars is $C_nH_{2n}O_n$. It is the fundamental source of energy for cells and among the four major classes of biomolecules (Dilworth et al. 2017; Lee 2007). Carbohydrates are expressed as monosacharaides, disaccharides, polysaccharides, oligosaccharides, as well as glycoconjugates (Wong and Bryan 2003). Honey carries an array of carbohydrates including monosaccharides which makes up 75% of the carbohydrate content, like glucose and fructose, disaccharides such as maltose,

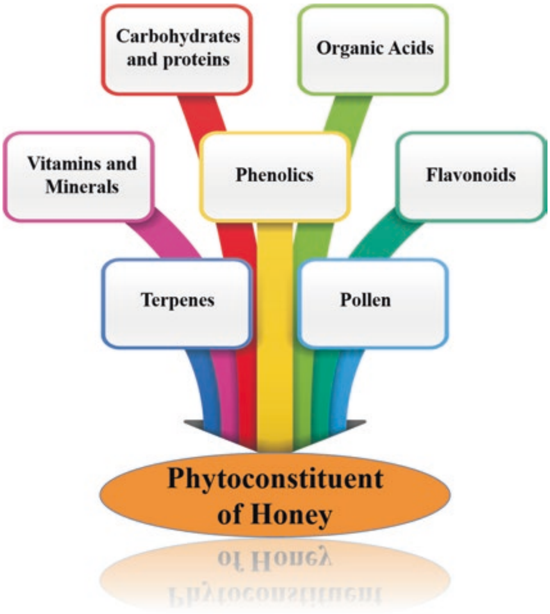

Fig. 3.2 Phyto-constituent profile of honey

sucrose, and palatinose making up 10–15%. Honey owes it sweet flavor to the high concentration of carbohydrates present, and there are nearly 30 complex sugars, taking up 80–83%, therefore making honey an excellent energy source (304 Kcal/100 g) (Sarfraz et al. 2018; Celestino Santos and González-Paramás 2017). Fructose is the superior sugar constituent in the majority of honeys although some exceptions are present in which glucose is the dominant monosaccharide, such as in uniflower honey like rape honey and dandelion honey. About 8–10% of disaccharides present constitute maltose, isomaltose, kojibiose, and turanose. Due to invertase enzyme action, sucrose is present in less than 30% of the total sugar content. Melezitose, erlose, and raffinose are trisaccharides that are present in relatively high amounts in honeydew honey, nevertheless origin/botanical sources of honey can influence its sugar content (Celestino Santos and González-Paramás 2017).

Fructose being the major constituent contains many benefits including evidence in aiding in diabetes, reducing hyperglycemia in rodents, diabetic patients, and healthy subjects (Vaisman et al. 2006; Kwon et al. 2008). Fructose was found to slow down gastric emptying time and absorption; furthermore, studies show that fructose decreases food ingestion which in turn causes the gastric emptying delay (Kashimura and Nagai 2007; Lina et al. 2002; Thibault et al. 1997). Decreased food intake due to fructose has further shown an impact on the selection of macronutrients for absorption (Gregory et al. 1989; Henry et al. 1991). With decreased food intake comes the suggestion that fructose aids in weight loss; a recent study shows that administering supplements of fructose in low or moderate concentrations to obese subjects shows effective weight loss (Madero et al. 2011). Albeit some studies suggest that fructose intake causes an increase in weight hence the results are inconclusive (Bocarsly et al. 2010; Meirelles et al. 2011; Lavin et al. 1998; Anderson and Woodend 2003).

The second major constituent after fructose is glucose. Although it does not have as many effects as fructose, it aids in the absorption of fructose, and the best results were found when equal amounts of glucose and fructose are given as glucose has a synergistic effect however fructose does not enhance the absorption of glucose (Jones et al. 2011; Fujisawa et al. 1991).

Several studies reported that high-fat-fed rats exhibited a decrease in the amount of intestinal bifidobacteria, and those treated with oligofructose present in honey showed an increase in bifidobacteria with enhanced glucose tolerance in addition to glucose-induced insulin secretions (Cani et al. 2007). Monosaccharides join together to form oligosaccharides (Bogdanov 2008; Erejuwa et al. 2012). Several research studies on honey have revealed that it can multiply the amount of Lactobacillus, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*. Evidence has shown that large amounts of fructose and glucose present in honey can increase the development of gastric microflora (Shamala et al. 2000; Chick et al. 2001). Finally, honey varieties that are fructose-rich are considered as a beneficial alternative to high GI sweeteners in the management of diabetes as well as cardiovascular ailments (Bogdanov 2011a; Deibert et al. 2010;). The chemical structure of these compounds are summarized in Fig. 3.3 (Celestino Santos and González-Paramás 2017).

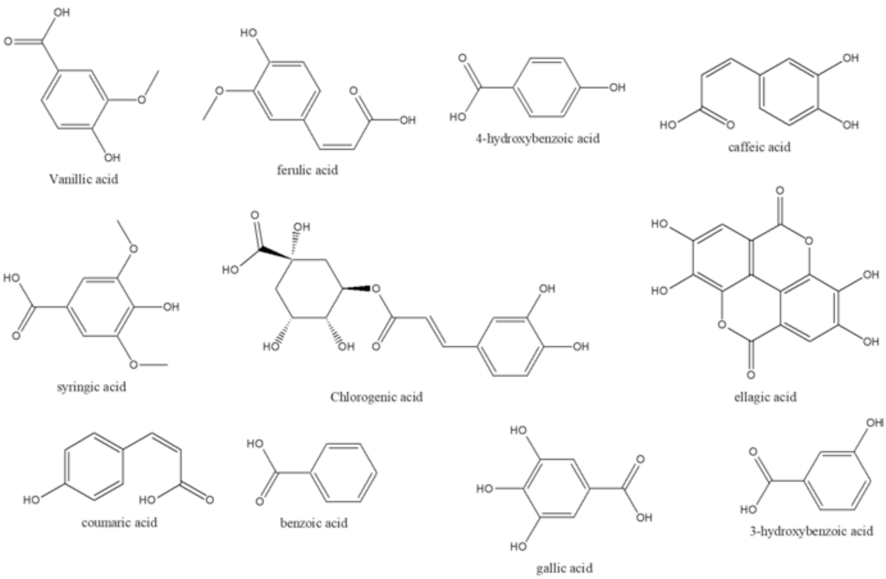

Fig. 3.3 Chemical structures of few carbohydrate derivatives present in different types of honey

3.5.2 Proteins

Honey contains 20 non-enzymatic proteins (0.1–0.5%), comprising albumins, globulins, proteases, and nucleoproteins common to all honey forms. The amino acid content is one fifth of the total content (Gonzalez-Paramas et al. 2006; Sajid and Azim 2012; Hermosın et al. 2003). A free amino acid namely proline is found in honey very commonly (50–85%) (Belitz et al. 2009; Hermosın et al. 2003). Proline is an indicator for botanical origin of honey, and it originates from the salivary secretions of honey bee (Biino 1971). Proline shows a significant role in regulating the nectar enzymatic transfer, in the process of converting the flower nectar into honey (Ortiz-Valbuena and Silva-Losada 1991). Furthermore, a few researchers analyze proline as an indicator for the adulteration of honey with sugar (Bogdanov et al. 1999). Table 3.1 shows the summary of various amino acids present in honey (Doner 2003).

The enzymes such as glucose-oxidase, diastase, and invertase are mostly found in honey along with other enzymes like β-glucosidase (White Jr 1979). Invertase causes honey to be a high energetic food (Crane 1980; Sancho et al. 1991). It hydrolyzes sucrose into fructose and glucose, and it also produces some oligosaccharides in an intermediate step therefore making it an important enzyme which keeps its activity after extraction and during storage (White Jr and Maher 1953).

Diastase value is regulated by many legislations as it is utilized as an indicator for the freshness of honey as it is resistant to heat and provide accurate results. Moreover, diastase produces smaller carbohydrates by hydrolyzing starch and dextrin (Crane 1980; White Jr 1978). The presence of an enzyme glucose oxidase causes increased acidity of honey. Conversion of glucose to gluconolactone also

Table 3.1 List of various chemical components present in various types of honey

S. no.	Amino acids (proteins)	Non-aromatic organic acids	Vitamins
1.	Proline	Butyric	Vitamin B1 (thiamine),
2.	Glutamic acid	Citric	Vitamin C
3.	Alanine	Acetic	Vitamin B6
4.	Phenylalanine	Formic	Vitamin B3 (niacin)
5.	Tyrosine	Fumaric	Vitamin B2 (riboflavin)
6.	Leucine	Galacturonic	Pantothenic acid
7.	Isoleucine	Gluconic	Nicotinic acid
8.	Lysine	Lactic	Folic acid
9.	Histidine	Isocitric	–
10.	Arginine	Methylmalonic	–
11.	Aspartic acid	2-Oxopentanoic	–
12.	Tryptophan	Tartaric	–
13.	Serine	Oxalic	–
14.	Valine	Quinic	–
15.	Methionine	Pyruvic	–
16.	Trypsin	Propionic	–
17.	Threonine	Malonic	–

facilitate by this enzyme, which results in the formation of gluconic acid along with minor quantity of hydrogen peroxide which accounts for honey's microbial resistance (White Jr et al. 1963). Glucose oxidase is inactivated at 60 °C and light sensitive (425–525 nm) (Gonzalez 2002; Ortiz-Valbuena and Silva-Losada 1991).

Some other enzymes found in honey though at lower concentrations include B-glucosidase, an enzyme added by bee secretions that hydrolyzes glycosidic toxins ingested by honey bee and transforms β-glucans into oligosaccharides and glucose (Labropoulos and Anestis 2012). In addition to B-glycosides catalase and phosphates and proteases are present. Catalase produces water and oxygen by converting the hydrogen peroxide produced (Huidobro et al. 2005); acid phosphatase can also be used as an indicator, it produces inorganic phosphate from organic phosphate although phosphatase is used as an indicator for fermentation, the optimum pH for its action is between 4.5 and 6.5 (Alonso-Torre et al. 2006). Finally, there are proteases that yield peptides of lower molecular weight by hydrolyzing polypeptides and proteins as well as esterases that breakdown esters (Labropoulos and Anestis 2012).

3.5.3 Organic Acids

These are essential for the preservation of honey, odor, color, and taste, making it difficult for microorganisms to grow therefore preserving it. Organic acid constitutes less than 0.5% of total solids although they also contribute in electric conductivity and honey acidity (Ananias et al. 2013; Bogdanov 2011b). Organic acid is in equilibrium along with the respective lactone (Gomes et al. 2010; White Jr 1979), and it represents 70–90% of the total organic acid. These lactones are produced with the help of an enzyme called glucose-oxidase from glucose (Bogdanov 2011b; Mato et al. 2003). Various organic acids present in honey are listed in Table 3.1 (Bogdanov 2011c).

The value of citric acid compared to gluconic acid indicates if the honey is from floral or honeydew sources (Selvaraj et al. 2006). The malic, gluconic, and citric acids present in honey can chelate with metal ions and strengthen the antioxidant activity of flavonoids (Aazza et al. 2013). A study directed by Cavie et al. tested the free acidity of 35 Spanish honeys for 30 months with no heat and analyzed every 5 months. During the first 5 months, the free acidity remained the same with a very slight increase. The sample started to show a constant increase in free acid after 20 months although it may vary widely. Increased acidity of honey shows the fermentation due to the conversion of alcohol and sugars by honey yeast into acids (Hemadi et al. 2013).

3.5.4 Vitamins

Trace the amount of vitamins found in honey, and it comprises more water-soluble vitamins than fat-soluble vitamins as well as contains very small amounts of lipid substances (Hemadi et al. 2013; Rahman et al. 2014). The various vitamins present in honey are listed in Table 3.1 (Hemadi et al. 2013). Vitamins C and E are known to have antioxidant activity (Bogdanov et al. 2008). Vitamin E, also known as an antioxidant, is reported to increase antioxidant activity and decrease protein oxidation and lipid peroxidation throughout the small intestine (Shirpoor et al. 2007) and reduces glycosylated hemoglobin and fructosamine (Selvaraj et al. 2006; Ceriello et al. 1991; Vinson and Howard 1996). For the regeneration into their antioxidant form as they are pro-oxidants, these vitamins need anti-oxidants (Halliwell 1996; Bowry et al. 1992). It was reported that pure natural honey may cause healing effects and an induction in its cardio protective (Rakha et al. 2008; Khalil and Sulaiman 2010). All the complex B vitamins and vitamin C are mainly derived from pollen; these vitamins can be influenced by filtration as well as by oxidation reactions carried out by glucose oxidase (Ciulu et al. 2011; Rahman et al. 2014). High-performance liquid chromatography-reverse phase (HPLC-RP) is used for the determination of five water-soluble vitamins in honey (Hemadi et al. 2013). Vitamin E has been reported to be successful in reducing programmed cell death and necrosis in noise-affected cells (Leon-Ruiz et al. 2013).

3.5.5 Phenolic

Phenolics are the groups of compounds that are present in plants. Over 8000 diverse structures of phenolics have been found (Estevinho et al. 2008; Bravo 1998). Phenolic compounds found in plants have been reported to be responsible for various therapeutic activities such as anti-inflammatory and anti-atherogenic (Vinson et al. 1998). Phenolic compounds can indeed be divided into flavonoids and phenolic acids, and honey is rich in both flavonoids and phenolic acids (Fig. 3.4), serving as a reference to the biological source of honey (Yao et al. 2003). Honey possesses strong antioxidant activity due to the presence of phenolic compounds or polyphenols generated as secondary metabolic components, which may differ with floral source (Kucuk et al. 2007; Pandey and Rizvi 2009). For instance, specific phytochemicals such as hesperetin and quercetin have already been discovered in citrus and sunflower honey (Anklam 1998; Ferreres et al. 1993; Tomás-Barberán et al. 2001; Aljadi and Kamaruddin 2004). The total phenolic content could be measured as gallic acid equivalent, and the total phenolic content in Indian honey is approximately 65.06 GAE/100 g and in Rhododendron honey is between 0.24 and 141.83 mg GAE/100 g (Bertoncelj et al. 2007; Jaganathan et al. 2010; Silici et al. 2010; Pontis et al. 2014). Different studies indicate that somehow the phenolic compounds found in honey are accountable for different beneficial effects (Turkmen et al. 2005), and techniques such as TLC, HPLC, GC, CE, and colorimetric assays were also used to evaluate polyphenols in honey and propolis and are separated according to environmental conditions (Alvarez-Suarez et al. 2009; Alvarez-Suarez et al. 2012; Trautvetter et al. 2009). Phenolic compounds which are present in Spanish honey for industrialized thermal processing as well as further liquefaction change to caffeic acid and for liquefaction, and further pasteurization contribute to β-coumaric acids (Escriche et al. 2014). Depending on molecular properties,

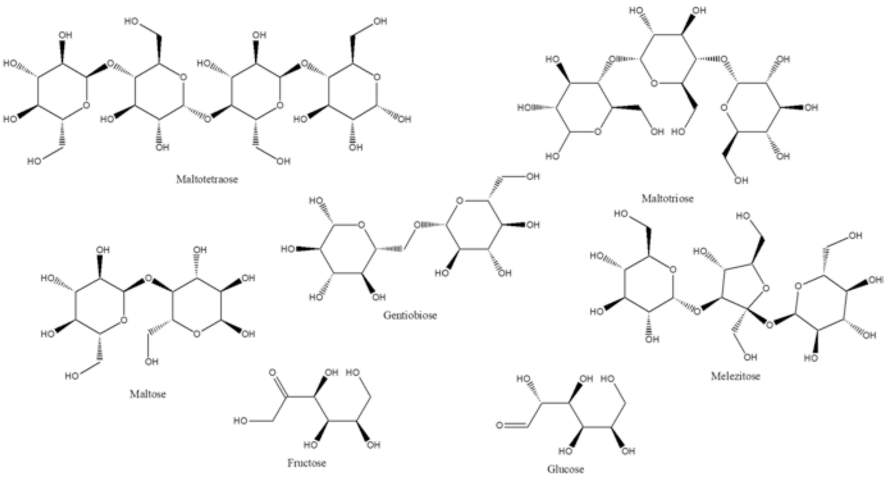

Fig. 3.4 Chemical structures of few phenolic compounds present in various types of honey

polyphenols can be classified into different categories (Grassi et al. 2010; Tomás-Barberán et al. 2001), phenolic acids comprise only one phenolic ring in their molecules and are also known as non-flavonoid polyphenolic compounds (Grassi et al. 2010; Amiot et al. 1989; Ciulu et al. 2016). Phenolic compounds acquire several safe and effective actions like those of antioxidant, antibacterial and antiviral activities, etc. (Zhang et al. 2016; Lampe 1999; Liu 2013). Many various studies also investigated the phenolic profiles in honey and reported a high correlation of phenolic content with antioxidant (Anand et al. 2018; Saxena et al. 2010).

3.5.6 Flavonoids

Flavonoids are present in honey in high amounts and constitute a few thousand compounds making up to 50% of the total phenolic compounds, with a prevalent C6-C3-C6 phenylchromane skeleton, and are known for their antioxidant effect. Various categories of flavonoids are present in honey, such as flavanes, flavonols, and dihydroflavonols, based on oxidation levels; the components of flavonoids differ among honeys from different parts of the world or botanical origins (Tomás-Barberán et al. 2001). Table 3.2 summarizes some of the phenolic and flavonoid compounds present in different types of honey (Kassim et al. 2010; Hussein et al. 2011; Eraslan et al. 2010; Petrus et al. 2011). In most of those varieties of honey, hesperetin and naringenin have been identified. However, flavonoids, such as isorhamnetin, alangin, kaempferol, quercetin, and luteolin, have been reported in most honey varieties (Petrus et al. 2011), and catechin has been identified as a prevalent flavonoid in some of Malaysia's honey that has already been explored (Khalil et al. 2011). Several findings have documented that honey inhibits cellular damage and prevents cell oxidation of cell membrane (Beretta et al. 2007). Antioxidant activity

Table 3.2 Various flavonoids present in different types of honey

Name of the compound	Subclasses	Molecular formula	Molecular weight, g/mol
Apigenin	Flavone	$C_{15}H_{10}O_5$	270.02
Catechin	Flavanol	$C_{15}H_{14}O_6$	290.26
Chrysin	Flavone	$C_{15}H_{10}O_4$	254.20
Galangin	Flavonol	$C_{15}H_{10}O_5$	270.24
Genistein	Isoflavone	$C_{15}H_{10}O_5$	270.24
Isorhamnetin	Flavonol	$C_{16}H_{12}O_7$	316.26
Kaempferol	Flavonol	$C_{15}H_{10}O_6$	286.23
Luteolin	Flavone	$C_{15}H_{10}O_6$	286.24
Myricetin	Flavonol	$C_{15}H_{10}O_8$	318.23
Pinobanksin	Flavonol	$C_{15}H_{12}O_5$	272.25
Pinocembrin	Flavanone	$C_{15}H_{12}O_4$	256.25
Quercetin	Flavonol	$C_{15}H_{10}O_7$	302.23
Rutin	Flavonols	$C_{27}H_{30}O_{16}$	610.52
Naringenin	Flavanone	$C_{15}H_{12}O_5$	272.25
Hesperetin	Flavanone	$C_{16}H_{14}O_6$	302.27

might also be linked to certain other actions, such as increased lipid metabolism and weight loss in human or rat subjects treated with honey (Busserolles et al. 2002; Razquin et al. 2009). The number and orientation of the hydroxyl group and perhaps even the substituents and glycosylation of that same compound determine the antioxidant function of the flavonoid. Glycolysation reduces antioxidant activity particularly in comparison to aglycones.

Quercetin and kaempferol are flavonoids that have a statistically significant effect on heart disease and those whose amount relies on its geographic origin (Alvarez-Suarez et al. 2013). Flavonoids are suggested to minimize the risk of cardiovascular disease through three main mechanisms of action: enhancing vasodilatation, the ability of blood platelets to coagulate, and preventing low-density lipoprotein oxidation (Khalil et al. 2011).

According to a study done by Viuda-Martos et al., it has been shown that galangin is effective against herpes simplex virus and coxsackie B virus, while quercetin and rutin show antiviral activity against herpes simplex virus, syncytial virus, poliovirus, and sindbis virus. Never before has the less unambiguous relation among honey and its chemical compounds been formally reported for its antiviral properties (Viuda-Martos et al. 2008). Studies have shown that certain flavonoids are effective of suppressing sodium-dependent, stimulated migration of monosaccharides into intestinal epithelial cells (Kimmich and Randles 1978). Flavonoids, such as quercetin, chrysin, and galangin, have been shown to minimize the activity of pro-inflammatory enzymes such as cyclooxygenase-2 and prostaglandin, and inducible nitric oxide synthase (Murtaza et al. 2014). Flavonoid content in honey has been shown to reduce matrix metallopeptidase-9, which is an inflammatory mediator leading to chronic inflammation (Candiracci et al. 2012). The first and most important activities of flavonoids are their cytotoxic activity. Standard flavonoid Chrysin has been shown to induce apoptosis (Kasala et al. 2015) in rectal and hepatocellular cancer cell lines used levels ranging from 40 to 100 μm (Ronnekleiv-Kelly et al. 2016; Zhang et al. 2016; Li et al. 2011).

In breast, prostate, and lung cancer cell lines, low levels (10 μm) were successful (Samarghandian et al. 2011; Huang et al. 2016), Chrysin induces apoptosis by caspase activation and Akt inactivation in U937 leukemia cells (Woo et al. 2004). Quercetin is reported to generate apoptosis in cancer cell lines including such human bladder, cervical, ovarian, and breast (Su et al. 2016; Ranganathan et al. 2015). Ellagic acid, another flavonoid observed in honey, generates apoptosis in cancer cells (Ranganathan et al. 2015; Mishra and Vinayak 2014). A mouse study found that Kaempferol had a significant effect on apoptosis in bladder cancer (Umesalma et al. 2015; Dang et al. 2015), colon cancer, ovarian cancer, human cervical cancer, and breast cancer cells (Xie et al. 2013; Lee et al. 2014).

3.5.7 Terpenes

Terpenes are organic and volatile compounds naturally synthesized by honey. Very small amount of terpenes are reported from honey (Kaskoniene and Venskutonis 2010). The characterization of botanical sources of honey has been done by terpenes present in it (Kaskoniene and Venskutonis 2010; Bogdanov et al. 2004). These compounds are aromatic and reported to be active against a wide range of microorganisms such as Gram-negative and Gram-positive bacteria, fungi, and viruses. Different terpenes and their derivatives such as linalool, a-pinene, b-pinene, limonene, camphene, myrtenol, trans-anethol, p-cymene, nerol, and cumene are present in honey (Mato et al. 2003). The flavor, odor, and biomedical properties in honey vary due to the presence of terpenes and their derivatives (Labropoulos and Anestis 2012; Ananias et al. 2013). Norisoprenoids are products of carotenoids (White Jr 1979; Bogdanov 2011b), which influence honey odor (Bogdanov 2011b), and they are known to be anticarcinogenic (Gomes et al. 2010). Terpenes can be identified by gas chromatography quadrupole mass spectrometry which provides qualitative and quantitative data for the identification (Anklam 1998; Cuevas-Glory et al. 2007). These terpenes are found to possess antimicrobial, anti-oxidant, and anti-cancer effects (Manyi-Loh et al. 2011). Several techniques are used for the isolation of terpenes like static headspace extraction, solvent extraction, ultrasound-assisted solvent extraction, etc. (Anklam 1998; Cuevas-Glory et al. 2007; Piasenzotto et al. 2003; Alissandrakis et al. 2003). The oxygenated terpenes can be water-soluble; therefore, heat should not be applied to honey during the isolation technique (Jerkovic et al. 2007). All terpenes are produced from the dimethyl allyl pyrophosphate and its isomer 3-isopentenyl pyro phosphate (Maffei et al. 2011; Dewick 2009). The mostly found terpenes in honey are monoterpenes which are derived from geranyl pyrophosphate (GPP) (Alissandrakis et al. 2007; Jerkovic et al. 2009, 2013).

3.5.8 Pollen

The clearness of honey lies on the level of suspended components like pollens (Busserolles et al. 2002). The pollen and the flower nectar are the key sources of carbohydrate and protein of the honeybees. They also contain fat, vitamins, micro-elements, etc. (Razquin et al. 2009). Hypersensitive responses from nectar are very uncommon, it could be because of pollen (Petrus et al. 2011). Pollen delivers anti-bacterial and antimicrobial properties to the honey (Khalil et al. 2011; Beretta et al. 2007). It is easy to describe the environmental conditions and the flora around the beehive using the pollen analysis. The flora of the origin reflects the pollen content (Alvarez-Suarez et al. 2013). Honey can be classified as monofloral or multifloral with the dominating pollen grain arising from one particular plant (Khalil and Sulaiman 2010; Viuda-Martos et al. 2008; Kimmich and Randles 1978). Acid phosphatase can be used as a parameter for honey characterization, and it mainly originates from nectar and pollen (Murtaza et al. 2014). The geographical region from which the honey is collected affects its phenolic, flavonoid concentrations and its pollen distribution (Candiracci et al. 2012; Kasala et al. 2015). The presence of vitamins, iron, other minerals, and immune enhancing properties has shown that honey bee pollen improves egg quantity, general fertility, and fecundity (Ronnekleiv-Kelly et al. 2016).

3.5.9 Minerals

Honey contains minerals which are classified as major and minor elements (**35,** Bogdanov et al. 2008). The major elements are potassium, chlorine, sulfur, sodium, calcium, phosphorus, magnesium, silicon, iron, zinc, and manganese, and the minor elements are copper, chromium, lithium, nickel, lead, tin, osmium, beryllium, vanadium, zirconium, silver, barium, gallium, bismuth, gold, germanium, and strontium (Solayman et al. 2016; Anderson et al. 1997). The elements like copper and zinc can increase the insulin sensitivity (Sitasawad et al. 2001; Song et al. 2003). These minerals are present in honey in a very low amount (Oh and Yoon 2008; Bogdanov et al. 2008), and a daily consumption of honey may give an adequate concentration of these minerals (Erejuwa et al. 2011). An evidence has shown that after the supplementation of honey, there is an increase in serum concentrations of these minerals (Al-Waili 2003), and these ions also promote the antidiabetic effect of honey (Sitasawad et al. 2001; Oh and Yoon 2008). In light and dark honey, the mineral content varies (Alqarni et al. 2012). Minerals in the soil transported to the flowers and get into honey by the honeydew or nectar (Anklam 1998), and they also come from anthropogenic sources or by beekeeping practices and honey processing methods (Pohl 2009). The mineral content in honey can be analyzed by acid digestion followed by the spectral analysis such as flame atomic absorption (FAAS), graphite furnace atomic absorption (GF-AAS), electro thermal atomic absorption (ET-AAS), inductively coupled plasma optical emission (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) (Pohl et al. 2012). Minerals are indestructible (Damodaran et al. 2010) and play an important role in body function (Pohl et al. 2012).

3.6 Conclusion

In conclusion, honeybee phytochemistry seems to be an interesting field of research with the prospects to explore new environmental relations between plants and bees, new chemical moieties, and new pharmacologically active molecules.

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Honey: A Sweet Way to Health

4

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Abstract

Honey is one of the most nutritional natural products that not only provides us healthy nutrition but also has a potential to be an alternative treatment option for different pathologies from microbial infection to metabolic disease. Honey is a byproduct of flower syrup produced by honeybees and possesses an intricate chemical composition that varies with botanical sources and geographical locations. This chapter is aimed to provide readers an understanding of complex composition, biological activities, adverse effect, and therapeutic benefits of honey. Honey possesses many biological activities, such as antioxidant, antimicrobial, anti-inflammatory, anti-proliferative, anti-cancer, and anti-metastatic effects, suggesting potential therapeutic roles in many human pathologies. Flavonoids and polyphenols in honey are the two active ingredients, which are of therapeutic importance in many diseases. In conclusion, honey may be developed as a natural therapeutic agent for many pathologies, and extensive studies are therefore recommended.

Keywords

Honey · Composition · Biological activity · Health · Flavonoids · Polyphenols · Natural therapeutic agent · Antimicrobial · Wound healing · Antioxidant · Immunomodulatory · Prebiotic · Preservative

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_4

4.1 Introduction

Honey is a nutritional natural product obtained by honey bees from flower nectars (Dashora et al. 2011) and is known for its cosmetic, therapeutic, and industrial values (James 1906; Bansal et al. 2005; Samarghandian et al. 2017). Since ancient times, Indians, Greeks, Romans, Egyptian, Babylonians, Chinese, and Mayans were cognizant of the nutritional and medicinal properties of honey (Adebolu 2005; Ashrafi et al. 2005; Samarghandian et al. 2017). Honey, which possess a long shelf life, is regarded as a balanced diet for all ages (Babacan and Rand 2007; Hassapidou et al. 2006; Bell 2007). Honey contains high content of sucrose and is used as sweetener since ancient time (Babacan and Rand 2007; Pataca et al. 2007). Besides fructose, other ingredients of honey are glucose, sucrose, oligosaccharides, proteins, vitamins, and minerals (Chow 2002; White and Crane 1975). Honey varies in overall composition depending on the surroundings flora on which bees feed. Natural honey also contains potent bioactive compounds such as antioxidants which include flavonoids (e.g., apigenin, pinocembrin, kaempferol, quercetin, and galangin), polyphenolics (e.g., ellagic acid, caffeic acid, and ferulic acids), vitamins (e.g., ascorbic acid and tocopherols), and antioxidant enzymes (e.g., glucose oxidase, catalase, superoxide dismutase, and peptides like glutathione) (Alvarez-Suarez et al. 2010; Johnston et al. 2005; Turkmen et al. 2006; Rakha et al. 2008; Al-Mamary et al. 2002).

Honey provided a natural cure for many human diseases (Inglett 1976; Samarghandian et al. 2017), in traditional medicine for centuries (Zumla and Lulat 1989; Chowdhury 1999; Ali et al. 1991), due to documented health-beneficial effects including antioxidant (Ahmed and Othman 2013), anti-inflammatory (Khalil et al. 2012), antibacterial (Attia et al. 2008), antidiabetic (Estevinho et al. 2008), anticancer (Swellam et al. 2003), and for the treatment of respiratory, gastrointestinal, cardiovascular, and neurological diseases (Ghosh and Playford 2003; Abdulrhman et al. 2011; Ezz El-Arab et al. 2006). Ancient people from Egypt, China, Greece, and Rome also utilized honey for wound healing and gastrointestinal diseases (Al-Jabri 2005). The most outstanding finding was potent anti-microbial property of natural honey (Al-Waili and Haq 2004a; Emsen 2007), against many microorganisms including *Salmonella* species*, Shigella* species*, Escherichia coli (E. coli)* (Alvarez-Suarez et al. 2010; Jeffrey and Echazarreta 1996), and *Helicobacter pylori (H. pylori)* (Chowdhury 1999). In addition to antimicrobial activity, honey may also possess immune-modulatory activity (Al-Waili and Boni 2003a) and thus helps in the recovery of wound (Medhi et al. 2008; Tonks et al. 2003) and colitis (Bilsel et al. 2002a). Honey, interestingly, may protect cardiovascular system as it could readily attenuate reactive oxygen species (ROS)-mediated lipid oxidation in vitro (Ahmad et al. 2009; Hegazi and Abd El-Hady 2009). Though honey has a vast history of its benefits in traditional medicine, a lack of documented scientific support has limited more widespread utility in modern medicine. This chapter provides a review of current literature and will highlight the therapeutic abilities of honey in various diseases.

4.2 Physicochemical Properties of Honey

Approximately 300 types of honey are currently recognized (Lay-flurrie 2008). These varieties differ widely in the composition, taste, and physical attributes. Freshly isolated honey is a hygroscopic viscous liquid that exhibits a yellow or amber color. The viscosity of honey varies by composition and particularly, by water content. Presence of colloidal substances in honey imparts its characteristic surface tension and, along with viscosity, creates foaming in honey (Olaitan et al. 2007). Color and clarity of honey differ with variations in surroundings, flora, age of beehives, storage conditions, and the amount of colloidal substances and pollens (Olaitan et al. 2007). Once crystallized, glucose present in honey crystallizes into monohydrate white crystals and turns honey lighter in color. This crystallization rate is faster with lower water content, but higher glucose amount (Olaitan et al. 2007).

Natural honey is composed of approximately 200 substances, including carbohydrates, proteins, amino acids, vitamins, minerals, organic acid, and enzymes. However, honey from different geographical locations vary with difference in honeybee species, climatic conditions, and availability of floral nectar. The components of honey are illustrated given in Tables 4.1 and 4.2 and Figs. 4.1, 4.2 and 4.3. Carbohydrate is a central component of honey and accounts for approximately

Table 4.1 Components and nutrients of honey

Components and nutrients	Amount (in g 100 g^{-1})
Carbohydrate	80–85
Fructose	36.2–47.11
Glucose (dextrose)	30.31–40.56
Maltose	0–3
Galactose	0–3.1
Fructooligisaccharides	4–5
Sucrose	0–2.4
Other sugars	11.9–12.9
Water	15–17.1
Protein	0.3–0.5
Ashes	0.2– <0.6
Dietary fibers	0–0.2
Polyphenols	0.04–0.103
Vitamins	0.0008–0.027
Elements	0.06864–0.126015
Other essential nutrients	0.23

Honey contains a wide number of nutrients that vary greatly with geographical location, climate, flora, and honeybee species. Below is the summary of nutrients and their compositions provided on the basis of published literature (Pasupuleti et al. 2017; Samarghandian et al. 2017; Eteraf-Oskouei and Najafi 2013; Khan et al. 2018) and USDA database (FDC 2019)

Table 4.2 Variation in trace elements in honey

Elements	Range (mg 100 g^{-1})
Potassium, K	9.092–195.57
Calcium, Ca	1.86–13.614
Phosphorus, P	0.117–10.07
Sodium, Na	0.61–8.998
Magnesium, Mg	0.601–4.657
Aluminum, Al	0.002–1.304
Iron, Fe	0.113–1.032
Zinc, Zn	0.014–0.387
Lead, Pb	0.0007–0.121
Manganese, Mn	0.007–0.068
Copper, Cu	0.005–0.068
Chromium, Cr	0–0.05
Fluoride, F	0.007
Selenium, Se	0.0008

Honey contains several trace elements that are essential for human health that vary greatly with geographical location, climate, flora, and honeybee species. Below is the summary of various elements present in the honey globally as cited in published literature (Eteraf-Oskouei and Najafi 2013; Khan et al. 2018) and USDA database (FDC 2019)

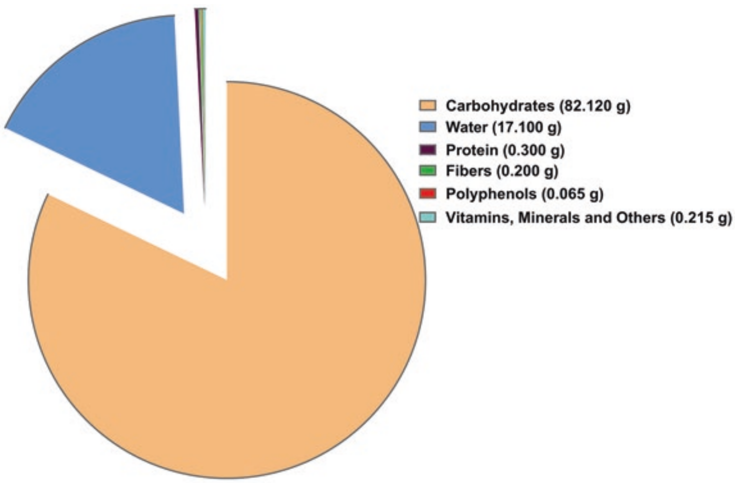

Fig. 4.1 Average compositions of honey. Honey contains many bioactive compounds from carbohydrate to essential elements and vitamins. The above pie chart depicts the average concentration of major group of components in honey (FDC 2019). The components are shown as amount in g 100 g^{-1} honey

95–97% of dry weight of honey. The principal carbohydrates in honey are monosaccharides, such as fructose (32.6 to 38.2%) and glucose (28.5 to 31.3%), which represent 85–95% of total sugars in honey (Ezz El-Arab et al. 2006; Moundoi et al. 2001; Betts 2008; El-Soud 2012; Clarke and Ndip 2011). In addition, smaller

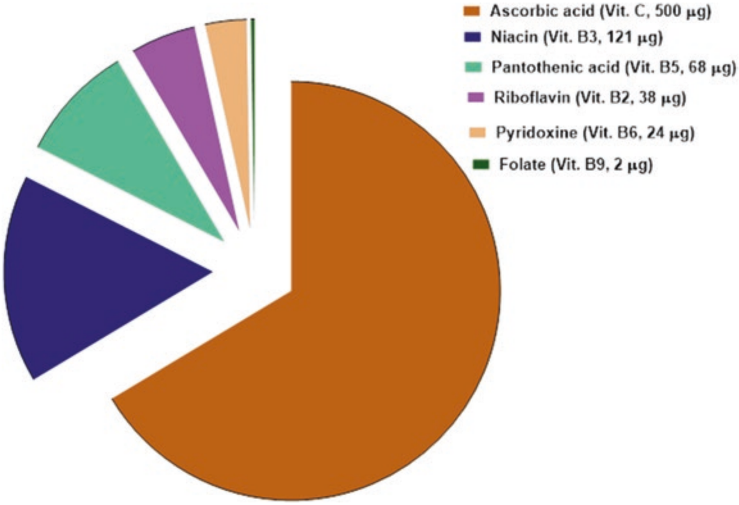

Fig. 4.2 Major vitamins present in honey. Honey contains most water-soluble vitamins, with vitamin C being the abundant one. The pie chart depicts the average concentration of major group of vitamins in honey (FDC 2019; Khan et al. 2007). The components are shown as µg 100 g^{-1} honey

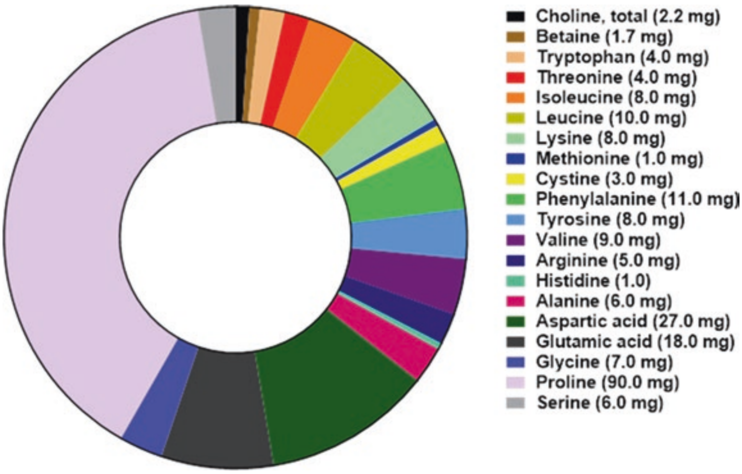

Fig. 4.3 Composition of essential nutrients and amino acids in honey. Honey provides essential nutrients to human in addition to carbohydrates, proteins, vitamins, and minerals. The above pie chart depicts the average concentration of reported nutrients in honey (FDC 2019). The components are shown as amount present in honey (mg 100 g^{-1})

quantities of disaccharides (such as maltose, sucrose, and galactose), trisaccharides (such as melezitose, maltotriose, and 1-ketose), and oligosaccharides are also observed in honey (Sato and Miyata 2000; Siddiqui and Furgala 1967; Ezz El-Arab et al. 2006; Chow 2002). Another type of oligosaccharides, fructo-oligosaccharides, comprises 4–5% of carbohydrates and may serve as probiotic agents (Ezz El-Arab et al. 2006; Chow 2002). Thus, being a natural source of carbohydrates, honey works as an effective nutritional supplements for athletes after strength exercise (Bansal et al. 2005).

Water constitutes about 15–17.1% of honey and essential for viscosity of honey (Bogdanov et al. 2008; Khan et al. 2007; Khan et al. 2018). Organic acids comprise of approximately 0.57% of honey and are responsible for its acidic nature (pH = 3.2–4.5) (Olaitan et al. 2007; Mato et al. 2003; Siddiqui and Furgala 1967). These acids are gluconic acid, acetic acid, formic acid, and citric acid. Gluconic acid is a main organic acid in honey and is derived from enzymatic oxidation of glucose (Siddiqui and Furgala 1967; Olaitan et al. 2007). Other acids found in honey are benzene derivatives and are present in minimum amount. To name few are syringic acid, 2-hydroxy-3-phenylpropionic acid, 2-hydroxybenzoic acid, 3,4,5-trimethoxybenzoic acid, and 1,4-dihydroxybenzene (Obi et al. 1994).

Protein content of honey is approximately 0.1–0.5% and mostly comprises enzymes (Lee et al. 1998; Jagdish and Joseph 2004; Won et al. 2009; French et al. 2005; Iglesias et al. 2004; Bansal et al. 2005; Olaitan et al. 2007). Diastase, invertase, and glucose oxidase are three prominent enzymes in honey. While, amylase and invertase provide fructose and glucose from complex starch, glucose oxidase oxidizes glucose to produce hydrogen peroxide and gluconic acid. Hydrogen peroxide provides antimicrobial properties, while gluconic acid facilitates absorption of calcium. Excessive hydrogen peroxide is neutralized by the activity of catalase and produces oxygen and water. (Bansal et al. 2005; Olaitan et al. 2007). Honey also contains all amino acids except asparagine and glutamine. Among these, proline, lysine, phenylalanine, aspartic acid, and glutamic acid are the most abundant amino acids found in honey (Khan et al. 2018; Samarghandian et al. 2017).

Approximately 600 volatile compounds were reported in honey (Ajibola et al. 2012). The volatile compounds of honey are low in abundance and include aldehydes, alcohols, hydrocarbons, ketones, acid esters, benzene, furan, pyran, terpene, isoprenoids, as well as other cyclic compounds (Manyi-Loh et al. 2011; Barra et al. 2010). Other bioactive compounds in honey are flavonoids, polyphenols, alkaloids, glycosides, and anthraquinone (White 1962, 1980; Islam et al. 2012). Flavonoids and polyphenols are particularly important molecules, which impart bioactive properties of honey (Nurul Syazana et al. 2012; Carlos et al. 2011). Honey has been found to have nearly 30 types of polyphenols (Nurul Syazana et al. 2012; Carlos et al. 2011). In general, the most frequent polyphenolics are gallic acid, ellagic acid, benzoic acid, cinnamic acid, chlorogenic acid, caffeic acid, isorhamnetin, ferulic acids, myricetin, coumaric acid, and flavonoids like chrysin, naringenin, luteolin, quercetin, and apigenin (Nurul Syazana et al. 2012; Carlos et al. 2011).

Flavonoids are active natural polyphenolic compounds with a 15-carbon structure, comprising two benzene rings joined by a heterocyclic pyran ring (Petrus et al. 2011). They are generally classified into four categories: flavonols (quercetin, kaempferol, and pinobanksin), flavones (luteolin, apigenin, and chrysin), flavanones (naringenin, pinocembrin, and hesperetin), isoflavones (genistein), and anthocyanidins (Zand et al. 2000; Nurul Syazana et al. 2012; Carlos et al. 2011). Common flavonoids galangin, luteolin, quercetin, isorhamnetin, and kaempferol are commonly found in honey, whereas naringenin and hesperetin are occasionally present in few varieties (Khalil et al. 2011; Khalil et al. 2012). Few flavonoids like genistein, chrysin, luteolin, and naringenin mimic estrogenic activity and are also known as phytoestrogens (Kyselova 2011).

Honey encompasses approximately 31 variable minerals, collectively comprises 0.1–1.0% of honey dry weight. Potassium is the major element in honey, followed by calcium, magnesium, sodium, sulfur, and phosphorus. Honey also contains essential trace elements like, iron, copper, zinc and manganese, and minor amount of trace elements like silicon, rubidium, vanadium, zirconium, lithium, and strontium (Kumar et al. 2010; Rashed and Soltan 2004; Lachman et al. 2007; Khan et al. 2018). However, it includes few heavy metals like lead (Pb), cadmium (Cd), and arsenic (As) as pollutants (Vorlova and Pridal 2002; Khan et al. 2018). Honey from countries like Nigeria, Bangladesh, Argentina, Spain, and Turkey were also reported to have ash content less than 0.6 g 100 g^{-1} (Khan et al. 2018; Gheldof et al. 2002; Gheldof and Engeseth 2002).

The vitamin level in honey is lower than recommended daily intake. Among all, vitamin C is present in higher amount. Other vitamins are B1 (thiamine), riboflavin, nicotinic acid, B6, and panthothenic acid (Olaitan et al. 2007).

4.3 Biological Activities of Honey

Natural honey possesses several bioactive compounds that provide many nutritional and medicinal properties. In the following section, we discuss different bioactivities of honey from antioxidant to prebiotic use (Fig. 4.4).

Fig. 4.4 Biological properties of honey. Honey provides polyphenols, vitamins, and flavonoids to exert different activities. The above pictorial presentation summarizes the different reported bioactivities of honey which are also utilized to cure different ailments

4.3.1 Antioxidant Activity

Natural honey contains many flavonoids, polyphenols, vitamins, and antioxidant enzymes which make it a potent natural antioxidant (Johnston et al. 2005; Turkmen et al. 2006; Rakha et al. 2008).

The phenolic compounds of honey, flavonoids and polyphenolics, are the major antioxidants (Bravo 1998; Estevinho et al. 2008; Gheldof et al. 2003; Yao et al. 2003; Al-Mamary et al. 2002). Polyphenolics scavenge free radicals, chelate metal ions (Küçük et al. 2007; Vinson et al. 1998; Estevinho et al. 2008), and may easily be distributed into body compartments to exert physiological effects (Blasa et al. 2007). Thus, honey either itself or in combination with other antioxidants might be utilized to reduce oxidative stress (Gheldof et al. 2003).

The antioxidant activity of honey differs greatly with variation in the surrounding flora, while extraction or storage affects honey minutely (Al-Mamary et al. 2002; Frankel et al. 1998; Gheldof and Engeseth 2002; Gheldof et al. 2002; Beretta et al. 2005). As floral variations affects polyphenolics content of honey, dark honey having higher content of total phenolics consequently has a higher antioxidant capacity (Frankel et al. 1998; Beretta et al. 2005; Beretta et al. 2007; Bertoncelj et al. 2007).

4.3.2 Antimicrobial Activity

Honey is known as antimicrobial in wound healing since 2100–2000 BC when Sumerian were using it as an ointment (Mandal and Mandal 2011). Honey works as a physical barrier to the wound when applied on it and maintains the moisture (Lusby et al. 2005). In modern era, honey was first recognized as antimicrobial by van Ketel and was rediscovered as antibacterial in 1892 (Dustmann 1979). Various results confirmed its activity against approximately 60 bacterial species, to name few important ones, are *Bacillus anthracis, Staphylococcus aureus, Corynebacterium diptheriae, Haemophilus influenzae, Klebsiella pneumoniae, Shigella dysentery, Mycobacterium tuberculosis, Yersinia enterocolitica, Salmonella diarrhoea, Salmonella typhi, Streptococcus pneumoniae,* and *Vibrio cholerae* (Jeffrey and Echazarreta 1996; Bansal et al. 2005; Asadi-Pooya et al. 2003; Olaitan et al. 2007; Al-Waili 2004b). Further, natural honey was also found effective against the methicillin-resistant *Staphylococcus aureus* (MRSA) (Natarajan et al. 2001; Chambers 2006; Maeda et al. 2008).

Honey has lower minimum inhibitory concentration (MIC= 1.8–10.8% v/v), which means that it can provide enough inhibition on bacteria if diluted nine times. It has also been implied that honey sufficiently inhibited most common wound pathogen, *Staphylococcus aureus* even if diluted by 56 times (Molan 2001c; English et al. 2004). Further, high dilution pasture (4–8% v/v) and manuka (5–11% v/v) honey inhibited the bacterial growth, while lower dilutions of same [pasture (5–10%) and manuka (8–15%) honey] killed bacteria when applied (Bansal et al. 2005).

Further, 20% (v/v) solution of honey inhibited *H. pylori* in gastric isolates in vitro (Ali et al. 1991; Jeffrey and Echazarreta 1996). Unlike most conventional antibiotics, chronic use of honey may not yield to treatment-resistant bacteria (Emsen 2007).

The anti-bacterial activity of honey is credited to four properties of honey. First, high amount of sugar in honey inhibits microbial growths and dehydrates bacteria; second, the acidic pH of honey restricts growth of many microorganisms; third and most important, hydrogen peroxide formed due to glucose oxidation acts as potent antibacterial factor, although nonperoxide activity (e.g., catalase) of honey might also be attributed to its antimicrobial property and lastly several other components like lysozyme, beeswax, nectar, pollen, propolis, polyphenols, terpenes, organic acid, and redox enzymes were also reported to add into antibacterial activity of honey (Emsen 2007; Al-Waili and Haq 2004b; Beretta et al. 2007; Cushnie and Lamb 2005; Simon et al. 2009; Olaitan et al. 2007; Bansal et al. 2005; Küçük et al. 2007; Estevinho et al. 2008). Other factors such as high osmotic pressure, low protein, high antioxidants, moderate viscosity, and low dissolved oxygen do not allow the growth of microorganisms like bacteria, yeast, and fungi (Patton et al. 2006; Badawy et al. 2004; Wilkinson and Cavanagh 2005). Thus, unique physiochemical properties of honey therefore enable it to be used as wound dressing to suppress infection (Basualdo et al. 2007; Molan 2002).

Antibacterial activities of honey vary in accordance to different amounts of compounds and enzymes present. Among all natural honey, manuka honey showed the highest efficiency against *E. coli* and *S. aureus* (Cushnie and Lamb 2005; Snowdon and Cliver 1996; Molan 2001a). Varied antibacterial activity of honey might be attributed to different components of flower and nectar available to bees (Küçük et al. 2007). In agreement to this, sugar contents in different types of honey were found to be correlated to their inhibition efficiency on growth of various intestinal bacteria (Shin and Ustunol 2005; Basualdo et al. 2007).

4.3.3 Apoptotic Activity

Honey induces intrinsic (mitochondrial) apoptotic events in cancerous cells which include mitochondrial membrane depolarization and rupture (Nicholson 2000; Earnshaw 1995), downregulation of antiapoptotic protein Bcl-2 (Jaganathan and Mandal 2009; Tomasin and Gomes-Marcondes 2011), activation, and cleavage of caspase-3 and poly (ADP-ribose) polymerase (Fauzi et al. 2011). In addition, honey also upregulated p53 and pro-apoptotic protein Bax in colon cancer cell lines in vitro (Fauzi et al. 2011). Similarly, oral gavage of honey inhibited tumor growth by upregulating Bax, activating caspase-9/3 and initiating DNA fragmentation in tumor tissue of Wistar rats (Jaganathan and Mandal 2009; Tomasin and Gomes-Marcondes 2011). According to a report by Fauzi et al., high phenolic contents of honey attribute to its anticarcinogenic activity (Fauzi et al. 2011). However, in view of lack of robust experimental findings, more experimental results are needed to ascertain therapeutic ability of honey in treating cancer.

4.3.4 Immunomodulation by Honey

Honey has shown anti-inflammatory activities in preclinical and in vitro experiments (Fernandez-Cabezudo et al. 2013; Candiracci et al. 2012; Bilsel et al. 2002b), and in clinical trials as well (Leong et al. 2012). Honey or its constituents have shown to regulate cytokines (Cho et al. 2004; Araujo et al. 2011; Hussein et al. 2012) and to activate myeloid/lymphoid immune cells (Timm et al. 2008a). Honey inhibited COX-1 and 2 activities, (Markelov and Trushin 2006), and reduced prostaglandins such as PGE2, PGF2α, and thromboxane B2 in normal human plasma (Al-Waili and Boni 2003b). The immunomodulation property of honey might be attributed to polyphenolic and flavonoid compounds which have shown to inhibit cyclooxygenase-2 and inducible nitric oxide synthase (Viuda-Martos et al. 2008; Al-Waili and Boni 2003b; Al-Waili and Haq 2004b; Cho et al. 2004; Araujo et al. 2011; Hussein et al. 2012).

Anti-inflammatory steroidal drugs, e.g., corticosteroids, dampen tissue regeneration and immune response, while non-steroidal drugs harm stomach linings. Honey is a good anti-inflammatory devoid of adverse side effects (Molan 2001c) and is as effective as other steroidal/non-steroidal drugs. For example, honey showed equivalent efficiency to treat experimental colitis as compared to prednisolone, a glucocorticoid drug (Bilsel et al. 2002b). Honey efficiently treated eczema, psoriasis, and dandruff, and efficiently reduced scar formation and exudation to promote wound repair and tissue regeneration (Al-Waili and Boni 2003b; Al-Waili 2003). Earlier, honey-stimulated release of cytokines from monocytes was believed to be partly associated with wound healing ability (Tonks et al. 2001b, 2003). Later, it was reported that honey's endotoxin contents (>30 kDa) might be responsible for wound healing (Timm et al. 2008b; Gannabathula et al. 2012). Evidently, Kanuka flowers' honey contains substantial amount of high molecular weight (>30 kDa) endotoxins, e.g., lipopolysaccharides, apalbumins, and arabinogalactan proteins, and thus, effectively stimulated the release of TNFα from monocytic cell lines. Similarly, deproteinized natural acacia honey which lacked high molecular weight molecules did not stimulate release of TNF-α. Therefore, high molecular weight components of honey might be argued to be responsible for immunomodulatory effects (Majtán et al. 2006). However, natural protein and peptide can cause nonspecific immune response (Dutta 2002); therefore, a cautious approach is always needed when applying natural products like honey to stimulate immune response.

4.3.5 Honey in Food Preservation

Hydrogen peroxide and non-peroxide components, e.g., antioxidants inhibit microorganisms, provide preservative ability to honey. As a result, honey has been shown to prevent *Shigella species, Listeria monocytogenes,* and *S. aureus* in food, in addition, honey being potent antioxidant, prevented polyphenol oxidation and thus reduced browning of fruits and vegetables during processing and storage (Bansal et al. 2005; Chen et al. 2000).

4.3.6 Honey as Prebiotic

Honey suppresses potentially harmful intestinal bacteria, while promotes the growth of beneficial gut flora (Bansal et al. 2005; Chow 2002; Ezz El-Arab et al. 2006). Honey supported the growth of microbiota like *S. thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus delbruekii*, and *Bifidobacterium bifidum* (Bansal et al. 2005; Sanz et al. 2005). Being rich in oligosaccharides, honey can be added as a dietary supplement to improve gut microflora health.

4.4 Honey in Traditional Medicinal History

Honey is known to mankind as natural food and medicines since stone age (approximately 8000 years ago). Other civilizations later also recognized the benefits of honey and were documented in various ancient scrolls, books, and tablets, e.g., Sumerian clay tablets (6200 BC), Veda (Hindu scripture) [5000 years ago] Egyptian papyri (1900–1250 BC), Hippocrates (460–357 BC), Koran, and Bible (Molan 2001b; Bergman et al. 1983; Mijanur Rahman et al. 2014; Newman 1983; Bansal et al. 2005; Samarghandian et al. 2017; Eteraf-Oskouei and Najafi 2013). These ancient civilizations (Egyptian, Roman, Greek, and Chinese) were fully aware of honey efficacy in treating wounds and diseases of the gut (Al-Jabri 2005; Eteraf-Oskouei and Najafi 2013; Zumla and Lulat 1989). The ancient *Vedic* people regarded honey the most valuable product of nature and emphasized its role in digestion, oral health, and eyesight. In addition, Ayurvedic medicine uses honey to treat herpes, cough, cold, insomnia, skin wounds/burns, lung disease, anemia, and cardiac disease (Bansal et al. 2005; Telles et al. 2007; Eteraf-Oskouei and Najafi 2013). Honey was also popular for embalming of dead (Bansal et al. 2005). “Oenomel,” an ancient Greek beverage, made up of honey and grape juice, was used to treat gout and neuro-disorders (Zumla and Lulat 1989; Eteraf-Oskouei and Najafi 2013). Hippocrates recognized the healing and nutritional properties of honey and prescribed different honey mixtures for different ailments such as oxymel (honey with vinegar) for pain, hydromel (honey mixed in water) for thirst, and a hydromel with antipyretic for fever (Zumla and Lulat 1989; Eteraf-Oskouei and Najafi 2013). In addition, he favored honey for conditions like wound, scar, baldness, cough, sore throat, and eye diseases (Bansal et al. 2005). Ancient Muslims treated diarrhea patients with honey (Molan 1999; Molan 2001c; Eteraf-Oskouei and Najafi 2013), while the Iranian physician, Avicenna (almost 1000 years ago), suggested honey for the treatment of tuberculosis (Asadi-Pooya et al. 2003).

4.4.1 Medicinal properties

Natural honey is being used for its medicinal properties since ancient times (Ahmed et al. 2003) and found to be useful in different disease conditions.

4.4.1.1 Honey in Wound Therapy

Honey has been known for its antibacterial, antiviral, anti-inflammatory, and antioxidant properties and therefore is regarded as the oldest wound-healing agent by us (Snowdon and Cliver 1996; Murosak et al. 2002; Medhi et al. 2008). The Germans and Russians utilized honey in combination with cod oil or alone for treating burns, boils, fistula, or battle wounds (Bansal et al. 2005). Wound pads permeated with honey act as non-sticky dressing and promotes wound healing faster (Bansal et al. 2005; Efem 1988; Al-Waili 2005).

Honey reduced ulcerations and inhibit bacterial infection after surgery of breast carcinoma, vulva and varicose veins, and in cesarean section or hysterectomies successfully. Therefore, it minimized the prolonged use of antibiotics, resulted in minimum scar formations, and thus minimized the hospital care (Cavanagh et al. 1970; Al-Waili 2005). Similarly, honey was effective in reducing bed eruptions, ulcers, or sores (van der Weyden 2003, 2005; Meda et al. 2004). Interestingly, sterilized manuka honey permeated dressing pads led to complete healing of conventional treatment-resistant amputated knee wound, which was also heavily infected with *P.* and *S. aureus* (Dunford et al. 2000). Honey showed expedited recovery in radiation-induced mucus inflammation and in Fournier's gangrene with rapid regeneration of tissue and minimal scar (Motallebnejad et al. 2008; Gürdal et al. 2003).

Honey acts as a good histological preservative for skin grafts (Subrahmanyam 1993) and has also been implemented as a cure for superficial to moderate burns (Simon et al. 2009). Initially, honey provides comforting effect, reduces pain, prevents infection, and later accelerates the healing of wound with less scar and contracture (Subrahmanyam 1991, 1993). Clinical trials showcased the higher efficacy of honey dressing pads in burn patients as compared to other commercially available dressings, for example, amniotic membrane, silver sulfadiazine, or potato peel dressings (Bansal et al. 2005; Meda et al. 2004). Thus, honey dressing provides an economical option to manage wounds by minimizing medical and hospital costs to patients (Zumla and Lulat 1989). However, honey dressing provides non-sticky and pain-free pads which are easy to change, the acidity of honey may cause discomfort in patients with naked nerve endings (Bansal et al. 2005).

Honey is remarkably effective in wound healing as a result of its three important properties: the osmosis gradient, generation of hydrogen peroxide, and immune-modulation. Osmotic outflow of fluid assists in removing debris from bed of the wound, thus provides space and nutrient supply for tissue regeneration (Bansal et al. 2005). Honey contains large amount of glucose which can be utilized by internal glucose oxidase or by leukocytes in respiration to yield hydrogen peroxide, a major antibacterial compound in honey (Efem 1988; Wilkinson and Cavanagh 2005; Dustmann 1979; Basualdo et al. 2007; Al-Waili 2001; Bansal et al. 2005). Further, honey has shown to stimulate phagocytic cells and to regulate the production of cytokines; therefore, its wound healing property might partially be attributed to its immune modulation property (Olaitan et al. 2007; Abuharfeil et al. 1999; Tonks et al. 2001a, 2003; Kumar et al. 2010).

4.4.1.2 Honey in Diabetes

Low glycemic index of honey was found effective in controlling blood sugar level diabetes as well as normal subjects as compared to sucrose and glucose (Erejuwa 2014; Samanta et al. 1985). However, either honey or glucose/sucrose did not show difference in blood sugar level in type 2 diabetes patients (Erejuwa 2014). In addition, management of metabolic complications is another advantage of honey administration (Yapucu Güneş and Eşer 2007) as it improved blood C-reactive protein (CRP) level, homocysteine contents, and lipid profile (Al-Waili 2004b; Bansal et al. 2005) and minimized the rise of plasma glucose in diabetic patients (Bogdanov et al. 2008; Cianciosi et al. 2018; Kamaruzaman et al. 2014; Khalil et al. 2011, 2012; Khalil and Sulaiman 2010). It was argued that honey stimulated insulin secretion that regulated blood glucose level and also improved hemoglobin concentration and lipid profile (Al-Waili and Haq 2004a). Therefore, honey may be utilized as supplement with standard antidiabetic drugs for different types of diabetes. However, in view of small experimental proof and lack of clinical trials, several questions about efficacy and mechanism remained unanswered and warrant further investigation.

4.4.1.3 Honey in Cancer

Honey possesses antiproliferative, apoptotic, mitochondrial membrane depolarization, anti-mutagenic, and immunomodulatory properties that might be attributed to its anticarcinogenic effect (Bansal et al. 2005; Sela et al. 1998; Molan 2001a, b; Eddy et al. 2008). Several reports stated its effectiveness in treating many tumors, e.g., hepatocellular, colorectal, renal, prostate, cervical, uterine cancers, and leukemia (Erejuwa et al. 2014; Fauzi et al. 2011; Yaacob et al. 2013; Samarghandian et al. 2010, 2011a, b, 2014a, b; Baiomy et al. 2009; Swellam et al. 2003; Aliyu et al. 2013). Exploration of honey in therapeutic treatment in cancer is relatively in infancy stage; therefore, more studies are warranted to investigate its mechanistic role as anticancerous mixture.

4.4.1.4 Honey in Asthma

Honey is very effective to treat cough and fever and has been recorded as common cough and fever medicines in folk medicines (Bâcvarov 1970; Ghashm et al. 2010). Honey showed the ability to prevent induction of asthma and reduced chronic asthma-related symptoms in experimental animal models (Ghashm et al. 2010). The beneficial effect of honey is related to its ability to curb inflammation and remodel the airway (Kamaruzaman et al. 2014).

4.4.1.5 Honey in Cardiovascular Diseases

Previously, honey is an excellent antioxidant, reduced cardiovascular risk by lowering hyperlipidemia and oxidative stress (Yaghoobi et al. 2008; Bahrami et al. 2008; Chepulis 2007; Schramm et al. 2003). Honey has shown these three distinct properties which might be attributed to its cardiovascular protection in various diseases: (a) Honey is a good vasodilator, (b) it inhibits platelet aggregation to make a clot, and (c) it prevents oxidation of low-density lipoproteins (LDL) (Kamaruzaman et al. 2014; Cianciosi et al. 2018; Bravo 1998; Khalil and Sulaiman 2010).

Ingestion of 70 g honey daily for a month in 38 overweight individuals significantly reduced total cholesterol, LDL-cholesterol (LDL-C), triacylglycerol (TG), and C-reactive proteins (CRP), without increasing the body weight (Yaghoobi et al. 2008). Similarly, natural honey improved blood lipid profile in hypertriglyceridemia and hyperlipidemia patients, while the exact amount of artificial honey aggravated LDL-C and CRP (Bogdanov et al. 2008; Cianciosi et al. 2018; Kamaruzaman et al. 2014; Khalil et al. 2011, 2012; Khalil and Sulaiman 2010). Abundance of nitric oxide (NO) and its metabolite in honey might be attributed to its cardiovascular activity (Bogdanov et al. 2008) and thus helped to clear venous congestion and to reduce cardiac burden (Rakha et al. 2008).

Several reports depicted protective effect in ischemia/reperfusion-induced injuries (Najafi et al. 2008, 2011; Eteraf-Oskouei and Najafi 2013). Langendorff's heart was protected from ischemia/reperfusion injury either by treating rats with natural honey for 45 days prior to isolation of heart or by perfusing isolated heart by honey-enriched Kreb's solution ex vivo (Najafi et al. 2008, 2011; Eteraf-Oskouei and Najafi 2013). In addition, honey attenuated oxidative stress and thus protected neurons from ischemia-induced cell death (Shimazawa et al. 2005). Thus, antioxidants in honey may serve as potential natural nutrients for improving cardiovascular health directly (Zalibera et al. 2008; Rakha et al. 2008). In addition, cardiovascular ability of natural honey might also be attributed to vitamin C-influenced release of NO from endothelium as administration of honey 1 h prior to adrenaline test in rats reduced vasomotor and cardiac function (Rakha et al. 2008).

4.4.1.6 Honey in Neurological Diseases

Several studies have proposed nootropic and neuroprotective properties of honey-derived polyphenols. Honey has shown anxiolytic, antidepressant, anticonvulsant, antinociceptive, and antioxidative effects (Khalil and Sulaiman 2010; Ghosh and Playford 2003). Honey polyphenols were effective in quenching ROS, inhibited pathological deposition of misfolded proteins and amyloid beta, prevented apoptosis, reduced oxidative stress, and excitotoxicity (Akanmu et al. 2011; Schmitt-Schillig et al. 2005; Shimazawa et al. 2005; Zalibera et al. 2008).

Raw honey as well as honey polyphenols inhibited microgliosis and thus attenuated neurotoxin or ischemia-induced neuroinflammation (Li et al. 2008). Most significantly, honey polyphenols prevented cognitive and memory impairment possibly through suppressing of hippocampal inflammation (Akanmu et al. 2011; Samarghandian et al. 2017) and via modifications of neural connections and synapses (Ghosh and Playford 2003).

4.4.1.7 Honey in Gastrointestinal Diseases

Ingestion of honey was reported to inhibit gastrointestinal diseases, e.g., gastritis, duodenitis, and gastric ulceration caused by *Helicobacter pylori* (Tallett et al. 1977; Haffejee and Moosa 1985; al Somal et al. 1994; Topham 2002; Alnaqdy et al. 2005; Oyefuga et al. 2012). Honey not only prevented gastrointestinal bacterial growth (Alnaqdy et al. 2005), it also limits the bacterial attachment to host intestinal epithelia by altering electrostatic charge and hydrophobicity of bacterial wall (Alnaqdy et al. 2005; Edebo et al. 1980; Sakai 1987). Although manuka honey-based therapy failed to indicate a beneficial treatment against *H. pylori* in earlier clinical trial (al Somal et al. 1994), honey solution with lower peroxide (20%) activity was found to be effective in vitro (Bansal et al. 2005; al Somal et al. 1994). It can be argued that in vitro study is more controlled study and is very different from complex in vivo system with many confounding factors. Further, gastrointestinal discomforts get resolved quickly with honey ingestion. Though, honey did not exert any effect in viral gastroenteritis (Bansal et al. 2005; Haffejee and Moosa 1985; Obi et al. 1994) but was effective in the management of peptic and antral ulcer (Ali 1995). Honey being rich in potassium and water worked as better rehydration fluid and did not increase sodium uptake (McGovern et al. 1999; Haffejee and Moosa 1985). It also helped to reduce inflammation and to repair the inner mucosa layer (Bansal et al. 2005). Further, in indomethacin-induced experimental gastric lesion, oral gavage of honey (2 g/kg) prevented microvascular permeability and myeloperoxidase activity in the stomach (Nasuti et al. 2006). Similarly, perfusion with isotonic solution of honey reduced ethanol-induced lesions in stomach significantly (Gharzouli et al. 2002).

4.4.1.8 Honey in Infectious Diseases

Besides the aforementioned antimicrobial activity (Brady et al. 1996; Kumar et al. 2010; Dunford et al. 2000; English et al. 2004; Molan 2001b; Patton et al. 2006; Al-Waili 2004a), honey also inhibits the fungal (including Aspergillus, Penicillium, as well as all the common *dermatophytes)* and yeast infections (Brady et al. 1996; Kumar et al. 2010). The pure honey was reported to be fungicidal, while its dilution abolished production of toxin (Al-Waili and Haq 2004a; Bansal et al. 2005; Obaseiki-Ebor and Afonya 1984). Honey effectively treated several skin diseases such as candidiasis, athlete foot, ringworm, and dermatitis (Bansal et al. 2005; Obaseiki-Ebor and Afonya 1984; Al-Waili 2001, 2005).

Honey prevented recurrent genital and labial lesions from herpes infection effectively as compared to conventional acyclovir application (Al-Waili 2004a) and inhibited rubella-induced rash (Al-Waili and Haq 2004a). Honey was found effective in various ophthalmological infections such as blepharitis and conjunctivitis and also helped in recovery from corneal injuries (Shenoy et al. 2009; Meda et al. 2004). Topical honey improved eye conditions in more than 85 patients while stopped disease progression in remaining patients in a study in 102 patients with nonresponsive eye disorders (Bansal et al. 2005; Obaseiki-Ebor and Afonya 1984; Al-Waili 2004a). Honey also ameliorated gingivitis and periodontitis (Khan et al. 2007) and prevented leishmaniasis in vitro (Al-Waili and Haq 2004b).

4.4.2 Nonmedicinal Uses of Honey

4.4.2.1 Nanoparticles Synthesis

Nanoparticles showed promises in various processes such as biomedical, optical, biosensor, catalytic, and energy application (Salata 2004). One of the important application of nanoparticle is in drug delivery and molecular doping (Wang et al.

2015). Copper nanoparticles (Cu-NPs) are being good antibacterial and are much advantageous over others in drug delivery (Grass et al. 2011). However, process of making Cu-NPs demands high cost and release toxic byproducts (Cerchier et al. 2017; Kumar et al. 2015). Moreover, copper is a highly unstable metal, and therefore, a stabilizing agent is always incorporated during Cu-NP formation (Sierra-Ávila et al. 2015). Owing to the viscosity and protein and carbohydrate constituents of honey, it provides green and nontoxic alternative to stabilizing agent in the nanoparticles synthesis (Oskuee et al. 2016). In addition, being potent antioxidant, honey could also regulate redox reactions in production of platinum nanoparticles (Venu et al. 2011).

4.4.2.2 Cryoprotective Agent

Saccharides being low toxic and having ability to interact with lipid bilayer and inhibiting crystal formations during freezing phase are increasingly used as stabilizers in cryo-preservative media (Leekumjorn and Sum 2008; Herrick et al. 2016). Honey provides multiple monosaccharides, mostly glucose and fructose, two most frequently utilized sugars in cryopreservation (Lazarević et al. 2012). Considering its antioxidant ability and abundance of monosaccharides, it was found to be effective in semen cryopreservation as alone/in combination with other natural cryopreservative agents (Fakhrildin and Alsaadi 2014; Jerez-Ebensperger et al. 2015) and acted as non-penetrating cryoprotective agent for mammalian embryo more effective than conventional sucrose (Sarmadi et al. 2019).

4.4.2.3 Other Effects of Honey

Natural honey contains high nutritional components and thus provides immediate calories to exhausted tissue in case of fatigue and starvation (Meda et al. 2004). Honey is also capable of immunomodulation (Al-Waili and Haq 2004b) and therefore has been a very effective agent for fixation of skin graft (Emsen 2007). Daily intake of honey improved hematological indices and endocrine system (Al-Waili 2003). Honey further reduced obesity, uterine atrophy, and loss of bone density in menopausal rats. The benefits of honey could be attributed to its influence on endocrine system. (Zaid et al. 2010). Honey was also reported to be beneficial in measles, period pain, toothache, dry mouth, male impotency, and pharyngitis (Meda et al. 2004; Bansal et al. 2005; Sela et al. 1998; Molan 2001a, b). Honey being excellent antioxidant prevented DNA damage (Guerrini et al. 2009) and apoptosis in animal model of obstructive jaundice (Kilicoglu et al. 2008) and augmented glutathione-based redox system to attenuate N-ethylmaleimide-induced hepatic injury (Korkmaz and Kolankaya 2009).

4.4.2.4 Undesirable Effects of Honey

Sometimes, a person having naked nerve endings in skin may experience a stinging sensation after applying honey topically. Honey rarely gives hypersensitive reactions, which is probably arisen due to presence of allergens, such as, pollen or bee proteins. Excess honey on wound or skin may dehydrate the applied region and may need saline treatment to restore hydration. Ingestion of honey infected with clostridia, can pose serious risk of botulism. However, honey irradiated with gamma radiation does not show any clostridia spores without losing its other biological activity (Bansal et al. 2005; Molan and Allen 1996). It is a known fact that not only pesticides have the capacity to eliminate target organism, they also affect the contamination of non-target species. One of the disadvantages of high pesticide use is contamination of food (Souza Tette et al. 2016). According to a global survey, 75% of all honey samples have nicotinoid contamination (Mitchell et al. 2017). More than 95 pesticides and their metabolites were observed in bee pollen, and the level was alarmingly high up to 214 ppm (Mullin et al. 2010). Therefore, pesticides in honey poses a great risk to health of human and bee as well and urge to take serious measures to reduce environmental contamination (Souza Tette et al. 2016).

4.5 Future Direction and Concluding Remarks

To date, with growing interest and notion, alternative medicines are receiving increased interest from the general public, fueling research into natural products as safe and efficacious alternatives to pharmaceuticals and drugs. Honey, an age-old natural medicine and nutritional product, is known for wound healing, as an antimicrobial and as a remedy for cough and throat infection. Honey contains number of compounds including antioxidants, phenolics, dextrose, and enzymes, which can be therapeutically useful in different diseases. However, like other natural mixture, honey varies in composition according to geographical locations, climatic conditions, floral species and bee populations, and thus, complicating the design and interpretation of experimental studies between different centers. Future research focused on identifying the bioactive ingredients and pharmacological components in honey may overcome this potential obstacle. Another problem is decline of bee population due to environmental change and pollution, which has declined the production of natural honey. Moreover, pollutants and pesticides present in floral nectar are accumulating in honey, posing a health threat to both beehives and humans. In conclusion, preservation of the environment is paramount to ensuring a supply of pure honey, a potentially safe and efficacious natural remedy with widespread application.

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5 Validation, Chemical Composition, and Stability of Honey from Indian Himalayas

Mohamad Taleuzzaman, Chandra Kala, and Sadaf Jamal Gilani

Abstract

The economy of hilly areas is dependent upon the apiculture, one of the sources which does not affect the ecological balance. Honey is a natural sweetener that is used globally. The content of honey is very unique; it balances the health of the human being as well as the role of both preventive and curative for several diseases. This natural food is also discussed in the holy books *Quran* and *Bible*. Physically honey is a viscous solution and has very large content of fructose and glucose followed by water and other kinds of ingredients, ash (0.2%); proteins and amino acids (0.1–0.4%); and trace amounts of enzymes, vitamins, and other substances such as phenolic compounds. The composition of honey depends upon many factors like geographical area, environmental condition, types of bees, and condition of the collection. Different types of analytical techniques are used to find out about the composition and strength of the content which include both qualitative and quantitative analysis.

Keywords

Honey · Apiculture · Natural food · Analytical technique · Qualitative and quantitative

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_5

5.1 Introduction

A substance having a sweet taste, unfermented, and developed from the nectar of floweret, living portion of plants or secretion by honey bees, then after it is transformed, further added with certain substances, and is stored in honeycombs is called "Honey." The definition of biological honey is the one prepared by bees and other social insects from nectar or honeydew, composed from living plants, followed by the process of evaporation and action of the enzyme they secrete to remove water. It is also defined as "an organic aromatic sweet mucilaginous material collected from the nectars of plants through the honey-bees, modified and keeps it by them as a denser liquid." It is one of the most complex foodstuffs, sources of vitamins, enzymes, organic acid, and minerals. According to the Codex Alimentarius Commission (2001) and FAO (1984), honey exhibits nourishing, healing, and prophylactic properties (Iglesias et al. 2004; Council of European Union 2002; Pereira et al. 1998). The total nine known species of honeybees are indigenous, and in Hindu Kush Himalayan (HKH), only five out of nine (*Apis cerana, A. dorsata, A. laboriosa, A. florea*, and *A. reniformis*) are involved, and among the ingenious species, only A. cerana can be managed in hives. These bees feed on the areas with the great diversity of flowering plants. In hilly areas, the economy grows up without harming the environment by apiculture is crucial, and productions of chief hive products such as honey, beeswax, royal jelly, bee venom, and propolis, etc. provide a valuable grant (Crane 1975).

The objective of this review study is how honey composition and its concentration influence the shelf life of the product. The quality and quantity of honey fix the nutritive and medicinal values.

Honey is a popular healthcare food with high nutrition value, provides better physical performance, and is used as a medicine for the treatment of disease like cough, cold, wounds, cuts, diarrhea, and other diseases. In some medicine, it is used as an ingredient to confirm the antibiotic and healing properties (Molan 1992). Polyphenol content of honey shows the antimicrobial activity according to M. Bucekova and coauthors, due to autoxidation-produced H_2O_2 and by influencing the Fenton reaction to produce the reactive hydroxyl radicals. Honey is the sweetest and the most nutritious natural food produced by the honeybees, other insects like few species of wasp, pouched ants, and innumerable other species of bees also produce honey (Bucekova et al. 2018). Subsidiary definition of honey is presented in Fig. 5.1. The production of honey is very interesting; the whole phenomenon is a teamwork by honeybees with the continuation of their young-ones. Society of honeybee consists of (queen, worker, and male) non-self-sufficient hatch. The members of the honeybee society coordinate all activities and communicate with each other by a chemical substance called pheromones (Trhlin and Rajchard 2011). A natural characteristic of honeybees is a perfect informative culture; with this behavior, the energetic workers of the team are operative every day with several trips for collecting the sugary secretions little by little from flowers on rainy days. The watery solution containing sugars that originate from floral and extrafloral nectarines of plants are called "nectar". Top ten plant species that produced nectar are yellow water iris (*Iris*

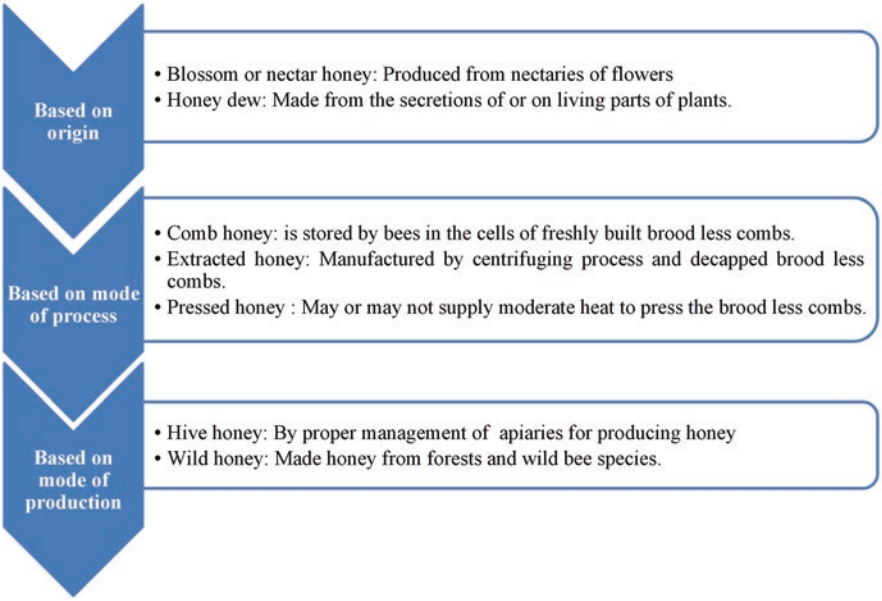

Fig. 5.1 Subsidiary definitions of honey, in Bucekova et al. 2018

pseudacorus), Himalayan balsam (*Impatiens glandulifera*), Gladioli (*Gladiolus spp*.), Blackberry (*Rubus fruticosus* agg.), common comfrey (*Symphytum officinale*), hedge bindweed (*Calystegia sepium*), Honeysuckle (*Lonicera periclymenum*), sweet pea (*Lathyrus latifolius*), foxglove (*Digitalis purpurea*), and Rhododendron (*Rhododendron panicum*). Aside from nectar, pollen is another important hive product collected by honeybees from flowers, which provide proteins, lipids, minerals, and vitamins that are the needed for the development of newly merged bees (Gray 1963). Nectar from the flowers is sucked by the worker bees and is stored in the honey sac, where it interacts with the saliva of bees, and when a chemical change takes place, the invertase enzyme of saliva modified dextrose/glucose (grape sugar) and laevulose (fruit sugar). Honey bees vomit the honey, but some of the ingredients in the honey are of their own and not only the plant nectar (Thakur 1991). The average distance covered by the honeybees is around 1.6–2.4 km, and they collect about a pond of nectar, for that, they need 40,000–80,000 trips and number of visit to the flowers (Metcalf and Flint 1979). Besides sugar and water contents, other components in honey may be organic or inorganic substances. Two factors that influence the composition of honey are geographical and botanical origin. Presence of minerals in honey with quantity and quality wise indicate the presence of elements in the soil and the geographical area of plants where honey was gathered (Chudzinska and Baralkiewicz 2010).

In honey, quantity of water influences its organoleptic and physical properties like color, crystallization, viscosity, flavor, and density. Because of its hygroscopic nature, precaution must be taken to avoid uptake of moisture from the environment during processing and packaging (White 1975). Water activity (Aw) value indicates water content in honey, and it is explained by the amount of water available for microorganism growth. Microorganism growth takes place in the presence of water; sugar binds with water and makes it unavailable. Water activity of pure water is 1, at standard temperature water vapor pressure of the food (p) to the vapor pressure of pure water ($p0$). The addition of water fixing substance causes $p < p0$, the water activity is always less than 1 (Gleiter et al. 2006). The general range of water activity is between 0.49 and 0.65, some kinds of honey have 0.75. In honey, due to the growth of microorganisms like bacteria, yeast, and molds, the water activity is about 0.90, 0.80, and 0.70, respectively. Aw value below 0.60 will inhibit the growth of osmophilic yeast (Costa et al. 2013). Honey is a very important nutraceutical product and has both nutritive and medicinal values. This chapter reviews the different analytical methods used in quality control and their validation. The parameters that affect the stability of the honey such as water, sugar, viscosity, and color are discussed below.

5.2 Analytical Methods and Validation

All the analytical method developed must be validated for honey characterization and quantification of different ingredients in the sample. Whenever a new method using different analytical techniques like titration method, chromatographic method, electro-analytical method, instrumental method, or hyphened techniques is developed for the characterization or estimation, it is very important to understand the application and limitations of sample information for accurate analysis. Always, validation is performed as per the guidelines, if it is not performed properly or some steps are skipped, the method is not considered to give authentic data.

The analytical procedure must follow the US Food and Drug Administration (FDA) regulations. The method or procedure employed for testing the substances must be fixed with proper standard of accuracy and reliability. To work on validation, the terminology and understanding of required elements in ICH guideline Q2 (R1), "Validation of Analytical, Text, and Methodology." The procedures have been accepted and addressed by the International Conference on Harmonisation (ICH) [Validation parameter] (Fig. 5.2). In the laboratory, perform different types of experiments for impurity determination like identification and quantitative tests, limit test of some elements of impurities, and assay for the active component in drug substances and finished product. In quality control, honey is analyzed to confirm the quality and authenticity, as well as to establish its geographical and botanical origin.

5.2.1 Moisture

Presence of water in honey determines the stability during storage and prevents granulation and fermentation (Nanda et al. 2003). Various methods are used for the estimation of moisture: (a) Refractometric method: Frequently, this method is used

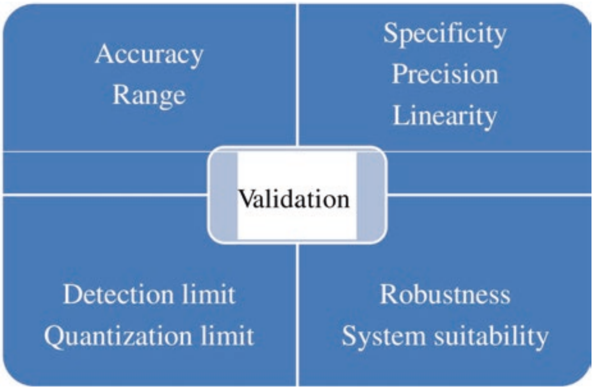

Fig. 5.2 Validation parameters

for schedule analysis, because of its easy handling and reproducibility of data. (b) Direct drying: This method is based on gravimetric drying the sample in oven at <70 °C under pressure ≤ 50 mmHg. (c) Other methods: Vibrational spectroscopy methods used are infrared spectrometer (IR), Fourier transform near-infrared spectrometry (FT-NIR), and Fourier transform mid-infrared spectrometry with attenuated total reflectance (FT-MIR-ATR). The drawback of the infrared spectroscopy method is the development of a calibration curve. Experiment for the quantification of water by Karl Fisher titration in honey samples employed an automatic potentiometric titrator (Association of Official Analytical Chemists (AOAC) 2012). Simultaneous determination of water and ash can be done by thermogravimetry in a honey sample (Felsner et al. 2004b).

5.2.2 Sugar

The content of sugar in honey is about 95 g/100 g dry matter. Different analytical techniques such as column chromatography, thin-layer chromatography (TLC), and high-performance liquid chromatography with infrared spectroscopy detector (HPLC-IR), HPLC with a pulsed amperometric detector (HPLC-PAD), and gas chromatography with flame ionization detector (GC-FID) are used for the determination of reducing sugars and apparent sucrose in honey. Column chromatography and thin-layer chromatography (TLC) are frequently employed for the estimation of glucose. Other analytical techniques like spectroscopy, enzymatic, and capillary electrophoresis are employed for the analysis of honey (AOAC 2012).

The qualitative analysis of the sugar is performed by Soxhlet's modification of Fehling's method, both types of reducing sugar (fructose and glucose) and apparent sucrose can be identified. Presently volumetric technique is employed for the estimation of reducing sugar and apparent sucrose (Official Journal of the European Communities (OJEC) 2002). The high-performance liquid chromatography (HPLC) instrumental technique has an advantage over the gas chromatography because sample derivatization is normally avoided. Sugar determination in HPLC with a refractive index detector (RID) is very common, but such detector has several drawbacks like deficiency of sensitivity and selectivity of signal, influenced by temperature and incompatibility of a flow rate of mobile phase with gradient elution. Required columns are silica-based polar aminopropyl silane (-NH2) with acetonitrile:water (80:20 v/v) as a solvent system. A chromatogram of the standard is produced and compared with samples for quantification by external calibration method (Almedia-Muradian et al. 2014; Bentabol-Manzanares et al. 2014). Nowadays, a pulsed amperometric detector (PAD) is the choice for the analysis of carbohydrates and is advantageous over RID for its lower detection limit. In nonderivatized sugar analysis by PAD detector, the advantages are the analysis is performed at alkaline pH, high resolution, and highly selective separation (Bogdanov 2009) and the disadvantage is that hydrocarbon chain with a high degree of polymerization is very less and is difficult to perform (Corradini et al. 2012). The most commonly used column for the analyses is "Carb Pac" manufactured by Dionex (Thermo Fisher Scientific Inc., USA). Mode of elution is generally kept isocratic using a different dilution of sodium hydroxide solutions. Gradient modes of elution have been experimented with a solvent system (Water/NaOH or Water/NaOH/NaOAc). Identification and estimation are done by comparing the retention time with standard chromatogram and with the external chromatogram method, respectively (Escuredo et al. 2014). The quantification of sugar is also performed by the detector "evaporative light scattering detector (ELSD)" with HPLC (Zhou et al. 2014). Gas chromatography (GC) is also employed for the analysis and gives better resolution and sensitivity than HPLC. The most common detector in GC is "flame ionization detector (FID)." The retention time of standard chromatogram is matched with a sample for qualitative analysis and is estimated with the internal standard method (Mannitol or Phenyl-β-D-glucoside) (Bogdanov 2009). Mass spectrometry (MS) detector is also employed for characterization and quantification of sugar with a high degree of polymerization (de la Fuente et al. 2011) and disadvantage of this detector is co-elution of compounds. Fragmentation pattern is for carbohydrates of a similar molecule and the interferences of other matrix compounds (Sanz et al. 2004). Qualitative analysis is performed by comparing the MS spectrum of a standard with the sample spectrum, and internal standard procedures (such as xylose) are for quantification (Terrab et al. 2002). Element analysis is performed based on the method of Kovacs et al. (1996). Procedure of this method, 3 g of the sample dissolve in 10 mL HNO_3 (69%) and allowed to stand overnight followed by predigestion at 60 °C for 30 min. After cooling, 3 mL of H_2O_2 (30%) is added followed by heating of the sample at 120 °C for 90 min. The volume is made up to 50 ml with ultrapure water. Afterward, the sample is homogenized and filtered using qualitative filter papers. For elements such as boron, potassium, magnesium, sodium, phosphorus, and sulfur, the quantitative analysis is performed by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer). The determination of arsenic, cadmium, chromium, iron, and zinc was carried out using ICP-MS (Inductively Coupled Plasma Mass spectrometer).

The qualitative and quantitative analysis with different analytical techniques is employed to determine the purity of the honey. Instrumental technique preferred hyphened which gives a very authentic result and performed the validation according to ICH guidelines. Several experiments have been done and confirmed the stability of the honey, analyzed the contents of honey whether it is inorganic or organic, and found out the source of impurity, degradation, and environmental effects. Several validated facts are summarized in Table 5.1.

Inductively coupled plasma mass spectrometer (ICP-MS) is used for the analysis of 13 elements in honey samples. A method for the analysis has been developed and validated. The analytical procedure has to be selective, linear, accurate, and precise. Elements analyzed are K, Mg, Al, B, Ba, Ca, Cd, Cu, Mn, Na, Ni, Zn, and Pb. The procedure followed the ICH guideline for validation, the experiments are performed on the following parameters: range of linearity, LOD, LOQ, and precision. Fulfill the acceptance criteria of the ICH guideline (Chudzinska et al. 2012). LC-MS/MS is used for the validation of a method for the multiclass component and determination of antibiotic residues such as tetracyclines, lincosamides, sulfonamides, macrolides, and aminoglycosides. Analyte recoveries in the range of 85%–111%, repeatability, and intra-laboratory (20.6% and 26.8%) indicate a good result, which is used in the routine analysis (El Hawari et al. 2017a). Several volatile substances such as furanic derivatives are generated during the manufacturing process and storage of sugarcane honey for which the Quality-By-Design (QbD) approach is followed. A method has been developed and validated for quantification by semi-automatic microextraction by packed sorbent (MEPS) combined with ultra-high performance liquid chromatography (UHPLC). The analytical technique is optimized based on QbD. All FDs were explained by polynomial function models and confirmed by the Fisher variance (F-test). The limit of quantification (30.6–737.7 μg kg^{-1}), recovery of analytes (91.9–112.1%), and the result of precision were for repeatability relative standard deviations (RSDs) less than 4.9% and 8.8% for intermediate precision (Silva et al. 2017). By liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI) quantification of sulforaphane (SFN) in sample of honey was performed, extraction by solid phase with a polymeric sorbent using analytical column (Synergi Hydro), and mobile phase 0.02 M ammonium formate in water and acetonitrile, at a flow rate of 0.5 mL min^{-1}. Follow the ICH guideline for validation. The limit of detection and limit of quantification values were 0.8 μg kg^{-1} and 2.6 μg kg^{-1}, respectively (Ares et al. 2015). Honey which is often contaminated with neonicotinoid insecticides and their metabolites. By liquid chromatography mass spectrometer, the quantification has been done. Neonicotinoids insecticides (acetamiprid, imidacloprid, thiametoxam, clothianidin, thiacloprid, nitenpyram, and dinotefuran), some of their metabolites (imidacloprid olefin, imidacloprid guanidine, imidacloprid urea, desnitro-imidacloprid hydrochloride, thiacloprid-amid, and acetamiprid-N-desmethyl), and 0.1% mixture of acetonitrile and ethyl acetate solvent system used for the extraction with the addition of TEA and Strata X-CW cartridges are used for cleaning the extracts. The developed method results were validated and were in an acceptable range. The result of the experiment depends upon the analytes and matrices, the range for recoveries (85.3%–112.0%), repeatabilities (2.8–11.2%) within-laboratory reproducibility (3.3–14.6%), and quantification (0.1–0.5 μg kg^{-1}) (Gbylik-Sikorska et al. 2015). The contamination of antimicrobial residues in

Table 5.1 Analytical validated facts

S. no.	Methods	Revealed	References
1.	Chemometric analysis for the identification of honey freshness and adulteration	A chemometric analysis for the determination of diastase activity and hydroxymethylfurfural (HMF) content in honey. Performed analysis in a real honey sample, conclusion about the correlation between the composition and quality criteria, calculated accuracy, precision, and uncertainty in the kinetics, using spectrometric techniques using the certified reference material and the determined values. Easily differentiated the diastase activity, sucrose, and hydroxymethylfurfural content, and these parameters were used for the indication of the adulteration of honey	Pasias et al. (2017)
2.	Determine pyrrolizidine and tropane alkaloids in honey, QuEChERS method coupled to LCMS	Liquid chromatography combined with mass spectrometry analytical technique used QuEChERS sample treatment for the validation of pyrrolizidine alkaloids (PAs) and tropane alkaloids (TAs) in food. The result showed good linearity ($R2 > 0.99$), percentage of recoveries 92.3–114.8%, repeatability and reproducibility 0.9 and 15.1% and 1.1 and 15.6%, respectively. Limit of detection and quantification 0.04–0.2 μg kg^{-1} and 0.1–0.7 μg kg^{-1}, respectively	Martinello et al. (2017)
3.	Analysis of chloramphenicol residues in honey samples by chiral LC-MS/MS	Honey was collected from different geographical regions and found a residue chloramphenicol (CAP); it has two asymmetric carbons, a total of four Para-CAP stereoisomers exist. RR-CAP enantiomer is bioactive, has significant antimicrobial activity. Developed a method and validated by LC–MS/MS to identify and quantify the four CAP enantiomers at residue levels in honey samples. For all four enantiomers, the decision limits (CCα) and detection capabilities (CCß) were well below 0.3 μg kg^{-1}, with limits of quantification (LOQs) between 0.08 and 0.12 μg kg^{-1}. A minimum required performance level (MRPL) of 0.3 μg kg^{-1} was established in 2003	Rimkus and Hoffmann (2017)
4	Determination of several antibiotic groups in honey by LC-MS/MS	A method developed by liquid chromatography–tandem mass spectrometry in ESI+ mode for the confirmation of 37 antibiotic substances from the six antibiotic groups: Macrolides, lincosamides, quinolones, tetracyclines, pleuromutilines, and diamino-pyrimidine derivatives. The developed method was validated based on an in-house validation concept with factorial design by a combination of seven factors to check the robustness in a concentration range of 5–50 μg kg^{-1}. The parameters performed detection decision (CCα) and detection limit (CCβ) in the range 7.5–12.9 μg kg^{-1} and 9.4–19.9 μg kg^{-1} respectively. Relative standard deviation within-laboratory reproducibility (<20% except for tulathromycin with 23.5% and tylvalosin with 21.4%), repeatability (<20% except for tylvalosin with 21.1%), and percentage of recovery (92–106%)	Bohm et al. (2012)

5.	Determination of adulteration using infrared spectroscopy and genetic-algorithm-based multivariate calibration	The determination of adulteration in honey by the instrument Fourier transform infrared spectroscopy (FTIR) equipped with attenuated total reflectance. Prepared adulterated honey samples by adding corn syrup, beet sugar, and water adulterant. Recorded spectra honey sample (n = 209) in various amounts between 4000 and 600 cm^{-1} wavenumber range. Content of honey calculated by genetic-algorithm-based inverse least squares (GILS) and partial least squares (PLS) methods. Results revealed that the multivariate calibration generated with GILS could produce successful models with a standard error of cross-validation in the range 0.97–2.52% and standard error of prediction between 0.90 and 2.19% (% w/w) for all the components contained in the adulterated samples. Similar results are produced by PLS generating the slightly larger standard error of cross-validation and standard error of prediction values. Quite a very simple method for the determination of adulterants in honey samples	Başar and Özdemir (2018)
6.	Validation of analytical method by LC-MS	An analytical method for the estimation of erythromycin A, widely used to treat and control foulbrood disease in honey bees. A method based on dispersive liquid–liquid microextraction and liquid chromatography coupled with tandem mass spectrometry with advanced i-funnel technology. The developed method has validated recoveries of erythromycin A and its degradation products from spiked honey samples were 76.1–102.1%, with reproducibility rates of 7.1–13.1% and correlation coefficients >0.99. The decision limit and detection capabilities were 0.02–0.07 and 0.03–0.10 ng/g, respectively	Zhao et al. (2017)
7.	HPLC method with enzyme-linked immunosorbent assay (ELISA)	High-performance liquid chromatography (HPLC) method developed and validated for the antigen in manuka honey, confirmed as leptosperin by HPLC fractionation with quantitation by an enzyme-linked immunosorbent assay (ELISA). It is a novel glycoside of methyl syringate. Established a monoclonal antibody to leptosperin and characterized the antibody in detail by a competitive ELISA	Kato et al. (2014)
8.	Quantification of 4′-geranyloxyferulic acid (GOFA) by validated RP-HPLC-UV method	A precise and accurate method RP HPLC-UV for the separation and quantification of 4′-geranyloxyferulic acid (GOFA) in four honey samples of different origins developed. The concentration values of four samples have a great variation. Honey samples GOFA concentration values (mg/g ± SD and % RSD), chestnut (7.87 ± 0.24 and 3.0), Forest (5.36 ± 0.12 and 3.9), Acacia (90.013 ± 0.002 and 2.9) and Orange (1.29 ± 0.05 and 2.1)	Genovese et al. (2016)

(continued)

Table 5.1 (continued)

S. no.	Methods	Revealed	References
9.	Determination of triazine herbicides in honey by HPLC	Herbicides of triazine in honey are quantified by the high-performance liquid chromatography (HPLC) and validated. First, it is extracted by the solvent floatation (SF) method. Factors affecting the extraction, such as type and volume of extraction solvent, type of salt, amount of $(NH_4)_2SO_4$, pH value of sample solution, gas flow rate, and floatation time, were investigated and optimized. The limit of detection in the range of 0.16–0.56 μg kg^{-1}. Analyzed five samples, the result of the recoveries and relative standard deviations for triazines found in the range of 78.2–112.9 and 0.2–9.2%, respectively	Wang et al. (2018)
10.	LC–MS/MS method for pyrrolizidine alkaloids and their N-oxides in honey and feed	Liquid or gas chromatography methods are used to find out the pyrrolizidine alkaloids (PAs) in honey. The developed method was validated. PAs are a group of secondary metabolites, four groups senecionine type, lycopsamine type, heliotrine type, and monocrotaline type that are carcinogenic and hepatotoxic properties. The method was validated, detection capability was less than 25 μg/kg for jacobine, lycopsamine, heliotrine, and senecionine. By zinc reduction, step for the additional detection of the presence of N-oxides of PAs. Analyzed (n = 146) samples from the various origins for the determination of PAs. Six samples were determined to contain measurable PAs >25 μg/kg by ELISA which correlated to >10 μg/kg by LC–MS/MS	Oplatowska et al. (2014)

sample of honey was indicated. Liquid chromatography mass spectrometer (LC-MS) analytical technique was employed for the estimation of residues, developed method has been validated according to ICH guideline. Different classes of residues are quantified. Generally, tetracycline, sulfonamide, macrolide, and aminoglycoside antimicrobial residues are present in honey. These residues are examined by combined chromatography techniques, for this perfluorinated carboxylic acid is used as an ion-pairing reagent for the separation. Results revealed that heptafluorobutanoic acid was more efficient as compared to pentafluoropentanoic acid. Validation was performed; the results showed that mean recoveries of analytes ranged between 93% and 104% and intermediate precision was below <21%. The values of detection limit and detection capability were in the range of 5–25 and 7–33 μg kg^{-1}, respectively (El Hawari et al. 2017b). Several classes of pharmacologically active substances like antibacterials, nonsteroidal, anti-inflammatories, antiseptics, antiepileptics, lipid regulators, β-blockers, and hormones are present in honey, which are estimated by gas chromatography-mass spectrometry (GC-MS) and the developed method validated. Precipitation of the sample was done by the solvent system acetonitrile: water (3:2) for proteins and lipids and further for cleanup and preconcentration centrifugation and continuous solid-phase extraction performed. Quite good result is obtained: limits of detection value are 0.4–3.3 ng kg^{-1} for 2 g of the sample with linearity $r^2 > 0.995$, recoveries in the range of 87–102%, with relative standard deviation (RSD) from 2.6% to 7.0%. In quality control, this method was used for routine analysis (Azzouz and Ballesteros 2015). Spectroscopic methods are used for the analysis of honey, e.g., the estimation of carbohydrates in complex mixtures was done by nuclear magnetic resonance (NMR). This analytical technique is helpful for the separation of multiple isomeric forms of carbohydrates in the honey. For this, the NMR analytical technique, which is highly selective chemical shift filters followed by TOCS, was done. This method is applied for the analysis of honey samples, and specific background free-signals for each sugar are acquired. In honey, there are a total of 22 sugars, 4 monosaccharides (glucose, fructose, mannose, and rhamnose) + 11 disaccharides (sucrose, maltose, maltulose, palatinose trehalose, turanose, melibiose, isomaltose, melezitose, gentiobiose nigerose, and kojibiose) + 7 trisaccharides (isomaltotriose, erlose, raffinose, melezitose, maltotriose, panose, and 1-kestose). Results are very satisfactory in terms of limit of quantification (0.03–0.4 g 100 g^{-1} honey), precision (% RSD: 0.99–4.03), trueness (% bias 0.4–4.2), and recovery (97–104%). The reproducibility of optimal chemical shifts was provided at the controlled temperature and pH of the sample. This is a very unique and innovative approach, used in the routine analysis for authentication of honey samples (Schievano et al. 2017).

5.3 Chemical Composition

Chemical composition of honey varies; it depends upon several factors like soil, air, and water. Quality and quantity of minerals in honey indicate the region from where it is collected (Hack-Gil et al. 1988). A major chemical portion of honey is sugar

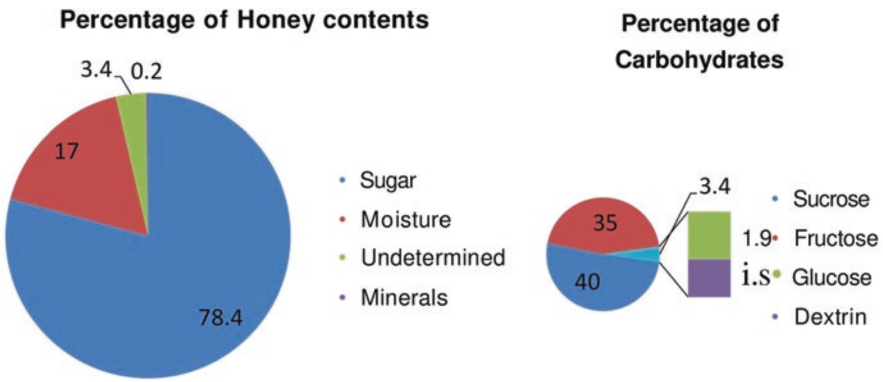

Fig. 5.3 Contents of honey and carbohydrates

(glucose, fructose, and sucrose) which is almost 82%, and water 17–20%. Around 181 different substances are identified; out of these, some are not quantified, and several are unique and are not found anywhere else. Quantity of these substances is very less, in the range of nanogram as compared to the total composition of the honey. Very less quantity of minerals, enzymes, lipids, amino acids, organic acids, vitamins, etc. are available as compared to sugar and water but are very important as these give aroma, flavor, and color to honey. The nutritional and medicinal values of honey are determined by the composition of major and minor elements in it. Chemical composition of honey also depends upon the variety of flowers visited by bees and environmental conditions where the plants grow and mature. Bees organize the hive, move around 7 km^2 for the collection of nectar, and come in contact with soil, air, and water. The physiochemical properties of honey are important for the industry to fix parameters like storage quality, granulation, texture, flavor, nutritional, and medicinal qualities of honey (Przybylowski and Wilczynska 2001; Atrouse et al. 2004).

A typical honey has a density 1.52 g mL^{-1} at 15 °C, Sugar (40% fructose, 35% glucose, 1.9% sucrose and 0.2%, dextrin minerals, 17% moisture, and 3.4% undetermined (Fig. 5.3). These figures, however, vary within certain limits and depend upon the season, geographical position, and the floral composition of a locality. Optically, it is levorotatory in nature because it contains more fructose. The caloric value is about (on an average) 3500 calories kg^{-1} (Czipa et al. 2019).

Specific gravity of honey is always more than water, and it depends upon the water contents. A general range is between 1.3648 and 1.4101 g mL^{-1} observed in the Garhwal Himalayan region in India (Gairola et al. 2013). Self-life of honey is related to its water content, a general range of moisture content from 13 to 25%. Optimum moisture content is very important; honey with a less percentage of water is difficult to handle and process, and more content of water favors fermentation due to the development of strong osmotic pressure, which makes it problematic to keep it away from osmophilic (sugar-tolerant) yeast growth (Bogdanov and Martin 2002).

5.3.1 Viscosity

It is a bulk property and indicates its thickness; the value varies from 10 to 30 poise. Usually, the good quality of honey has high viscosity. Its value depends on the water content and other variety of substances. In industry, during honey processing, it is an important technical property that influences flow during extraction, pumping, settling, filtration, mixing, and bottling. Colloid content and protein increase its viscosity. Fructose content also affects its viscosity and rheological properties inversely (James et al. 2009). Content of ash in honey is very less, and its quantity depends upon the nectar composition of predominant plants in their formulation and is related to honey's geographical and botanical origins. In Indian Himalayas honey, its range is 0.09–0.18% (Al-Khalifa and Al-Arify 1999).

5.3.2 Nitrogen Compounds

Important nitrogen compounds in honey are colloids, proteins, free amino acids, and enzymes, and its percentage is about 0.1–0.5%. Source of proteins in honey is bee's salivary glands and nectar, pollen, and honeydew of plants. Higher content of protein has thixotropic behavior and lower surface tension, resulting to entrap of air bubbles (Doner 2003). Twenty amino acids are present in honey; further, it is classified into three categories: essential amino acids (phenylalanine, leucine, isoleucine, lysine, methionine, histidine, tryptophan, valine, and threonine), conditionally amino acids (arginine, glycine, glutamic acid, proline, tyrosine, and cysteine), and nonessential amino acids (alanine, aspartic acid, serine, methionine, and trypsin). The main sources of amino acids are pollen. All these amino acids are a very good source of antioxidants (Hermosín et al. 2003).

5.3.3 Enzymes

Diastase, invertase, glucose-oxidase, phosphatase, catalase, and β-glucosidase are present (White 1979). Hypopharyngeal glands of the bees produce invertase enzyme which is required for glucose to complete the nectar for honey ripening process (Doner 2003). Catalase and acid phosphatase are produced from the vegetal origin (nectar, honeydew, or pollen), and these enzymes as an indicator of aging and/or overheating because of its thermolabile nature. Content of enzymes depends upon several factors like temperature, seasonal activity, botanical origin, the activity of the bee, hypopharyngeal glands, diet, and physiological stage of the bee (Persano-Oddo et al. 1999; White 1979). Diastase, an indicator of freshness of honey, hydrolyzes the starch and dextrin and gives smaller carbohydrates. However, its exact function is not known, maybe it participates in the pollen digestion by bees (Crane 1980).

5.3.4 Organic Acids

The source of organic acid (citric acid, oxalic acid, and malic acid) is nectar or honeydew, which gives taste, aroma, color, flavor, and preservation to honey. It is also responsible for the acidity and electrical conductivity of honey. It is 0.5% of the total solids and prevents microbial growth during storage (Ananias et al. 2013). Chief component of organic acids is gluconic acid which makes 70–90% of the total acid contents. Glucose oxidase converts glucose to gluconic acid, and it maintains equilibrium with gluconolactone in honey. Acidity is dependent upon free acids. Free acid contents in honey depends on factors like the source of nectar, bee species, and the action of enzymes or bacteria. The pH value of the acids is related to quality control regulation. Several nonaromatic acids are available in honey; these are acetic, butyric, citric, maleic, formic, lactic, malic, oxalic, fumaric, pyroglutamic, succinic, pyruvic, and tartaric acids (Mato et al. 2003). Acid content in respect to quality as well as quantity is a characteristic of botanical origin. Some components have buffer behavior, so the pH of the honey is not directly related to its acidity. The pH ranges from 3.4 to 6.4 and inhibits the growth of microorganisms (Gomes et al. 2010).

5.3.5 Minerals

Its percentage is very low around 0.02–0.3%, the contents of minerals depend upon climate soil and chemical composition of nectar (Crane 1980; Felsner et al. 2004a). Variation in content is also influenced by harvesting, beekeeping techniques, and material collected by the bees foraging flowers. Elements such as potassium, sodium, calcium, and magnesium are abundantly present in honey. Other elements like iron, copper, manganese, and chlorine are less. Whereas, boron, phosphorus, sulfur, silicon, and bare nickel are trace elements present in honey (Doner 2003). Ash percentage and electrical conductivity of honey depend upon the presence of the mineral. The dark color of honey indicates the presence of high mineral content as compared to light honey. Generally, nectar honey has less ash content and electrical conductivity as compared to honeydew kinds of honey (Felsner et al. 2004a).

Vitamins: The quantity of vitamins in honey is very less, and hence, it is not a good source of vitamins. Amount of water-soluble vitamins is more as compared to fat-soluble vitamins. It is a source of vitamin C which has potent antioxidant effects. Vitamin B is also present but in low quantities (Leon-Ruiz et al. 2013).

5.3.6 Phenolic Compounds

Phenolics are the bioactive compound present in honey responsible for its pharmacological effects such as antioxidant, antibacterial, and anti-inflammatory (Chen et al. 2000). Most dominant phenolic compounds present in honey are gallic acid (phenolic acid) and chrysin (flavonoid). Protocatechualdehyde and p-hydroxybenzoic acid are the second most and third most dominant phenolic acid present, respectively. Honey also contains quercetin and luteolin flavonoids. Honey contains 16 types of phenolic compounds and 14 types of flavonoids (Cheung et al. 2019). It is an abundant source of the hydroxybenzoic derivative.

5.4 Stability of Honey

Quality of honey generally refers to its genuineness, natural origin, the absence of adulteration, residues, thermostability, damage during storage, and other unwanted qualities. Quality control measures in honey are limiting or banning the presence of residues from antibiotics and pesticides; minimizing the levels of hydroxymethylfurfural (HMF—the high presence of which indicates that honey has been heated); setting limits for moisture content, diastase, pollen, sugars, acidity, and the amino acid proline; and defining required sensory values (taste, odor, and appearance) and handling processes.

5.4.1 Determination of Water

Honey absorbs moisture from the atmosphere because of its hygroscopic nature. When atmospheric humidity is high, it absorbs more moisture. At the time of harvesting, it is very important to monitor the moisture content; if precaution is not taken, there are chances of fermentation, especially in the tropics. Water content in honey affects the shelf-life; high water content enhances the crystallization, increases the activity of water to ferment, and deteriorates its quality (Gomes et al. 2010; Codex 2001). Standard water content should not be more than 20% to ensure its stability. The ratio of glucose/water (G/W) is another indicator for crystallization; it should not be greater than 2.1 (Alves et al. 2013). Water content by the legislation is established by the codex.

5.4.2 Electrical Conductivity (EC)

It is an important parameter; standard value should not be greater than 0.8 mS/cm as per The Codex Alimentarius. EC is affected by factors such as temperature, water amount, storage condition, minerals, and ion quantity. The values vary depending upon geographical region and source (animal or vegetable) (Kowalski et al. 2013).

Color: It is an indicator of its stability. The light color of honey is preferred over a dark color. Color intensity depends upon floral origin and nectar source. Carotin, xanthophyl, anthocyanin, and plant pigments are the main constituents responsible for color, and some bright yellow and dark green pigments are of unknown composition. Original color is contributed by colloidal particles and tannin bodies or chlorophyll derivative or decomposition products. The color was analyzed as follows:

luminosity, red (+) to green (−), and yellow (+) to blue (−). The saturation and hue angle are also identified. Luminosity indicates the lightness of honey, which ranges between 19.67 and 52.61 in honeydew.

5.4.3 Viscosity

This parameter is employed for the evaluation of state, fluidity, and crystallization of honey. Its normal range is 14.73 Pa.s to 4.17 Pa.s at 25 °C. Physico-chemical and sensory properties are affected by viscosity. A rheological property of honey is useful in its processing, handling, and storage. This physical property of honey depends upon several factors like composition, amount, and size of crystals present and temperature. Viscosity decreases as temperature increases and molecular friction and hydrodynamic forces decrease. On heating, as temperature gradually increases up to 30 °C, viscosity decreases rapidly, but after that, the change is very slow. The simplest equation to describe the temperature dependence of viscosity is the Arrhenius equation. Quantity of water is one of the main factor which influences the preservation (Gómez-Díaz et al. 2009).

5.4.4 Sugars

Glucose and fructose are predominant in honey. The ratio glucose/fructose (G/F) should be less than 1.2 for stability; this value favors the granulation as glucose is less soluble than fructose in water. The higher value indicates that the glucose is free and would remain liquid for a longer time. Glucose and fructose concentration range from 23.63 g/100 g to 42.55 g/100 g and 28.58 g/100 g to 45.98 g/100 g, respectively. Apart from glucose and fructose, other sugars that are present in honey are sucrose, trehalose, maltose, melezitose, ranose, erlose, and turanose which are very low in concentration. The content of sucrose ranged from 1.94 g/100 g to 3.74 g/100 g, which determines the authenticity of honey. The high content of sucrose indicates adulteration with different syrup by harvesting the product before maturation. This content can be reduced by the action of the enzyme invertase. Trehalose is found in the lowest concentration (0.83 g/100 g honey). Some sugars are not detected. During production, some sugars are added to increase the sweetness which changes some and/or biochemical properties such as enzymatic activity, electrical conductivity, and contents of specific compounds (HMF, glucose, fructose, sucrose, maltose, isomaltose, proline, ash) when compared to a control (Scripca et al. 2019).

5.5 Conclusion

Honey has been obtained from both animal and vegetable origins; its composition depends upon the source from which it is obtained. In animal origin, the type of bee involved and its saliva contribute to its composition. In vegetable origin, different types of flower and its nectar composition and the geographical factors are also very important. Different validated analytical methods are discussed like spectrophotometric, chromatography, titration, and electroanalytical for the quantification, which summarize the composition of honey in percentage that varies and its dependency on several factors, study the stability of honey, explain the parameters that affect the stability of the product. The contents of honey are determined by different analytical techniques; the objective of the method developed for quantification is to establish the limit of detection (LOD) and limit of quantification (LOQ). These methods validate the developed method according to ICH guidelines. Advanced analytical techniques are used for the analysis. Honey is very beneficial for health and effective against many diseases. It boosts up the immune system to fight against allergy and microorganism infections. Production of honey in hilly geographical areas builds up the economy. Shelf-life of the product is very important, and it is determined by performing the stability studies to know the factors and limitation which influence the shelf-life of the product.

Acknowledgment The authors would like to thank the Faculty of Pharmacy, Maulna Azad University, Jodhpur, Rajasthan, INDIA, for their valuable support.

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Honey of Authenticity: An Analytical Approach

6

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Abstract

The safety and quality of any food product is a primary concern. Adulteration of honey increases day by day in the market. Authentication is very important to confirm purity. Honey is a natural food product that is ready to eat with a high nutritional value which provides several health advantages. Adulterations of honey with sugar or syrups are common practice. Chemical tests and different analytical techniques are used to detect the adulterant in honey. Diverse ranges of the analytical techniques are employed for the analysis of honey-like chromatography, electro-analytical methods, and spectrophotometer technique. Estimation of adulterants even in low quantity can be detected by sophisticated instrument. Analysis in every step is required—part of preliminary screening, processing, and product standards. Most of the analytical methods provide information of pollen distribution, physicochemical parameters, and profile analysis of phenolic, flavonoid, carbohydrate, amino acids, aroma, and individual marker components.

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Keywords

Honey · Adulterants · Authentication · Analytical technique · Chromatography

6.1 Introduction

Honey has been used by human beings since ancient times. Sumerian tablet was a written evidence found in 2100–2000 BC (Crane 1975) "A natural sweet substance formed by bees (Apis mellifera) from a different source of plants like nectar, living parts, and excretion, collect it and transform by combining with specific substances of their own" defined by the European Union (European Commission 2002; Zielinski et al. 2014). Classified honey based on origin, harvest, and process. Further, based on origin, it is categorized into blossom, honeydew, monofloral, and multifloral kinds of honey. Honey obtained through the nectar of flowers was named blossom honey, and honeydew is formed by bees from plant saps. Monofloral honey has more than 45% of the total pollen content of single species of plant. Honey is also classified on the basis of sources such as plant-like citrus, manuka, and acacia honey (Alvarez-Suarez et al. 2010b). The botanical source of multifloral or polyfloral honey is meadow and forest. Based on the secretion of plants, honey can be categorized in to blossom honey produced from nectar of flower and honeydew made from secretions of all livings parts other than flowers. The botanical origin of nectars and secretion of plants are the chief concern of honey's composition and its properties, and carbohydrate is the main constituents (Bertelli et al. 2010).

6.1.1 Chemical Composition

The main ingredient of honey is carbohydrate; dextrose and laevulose are two important sugars, and several other sugars are there in small fraction including disaccharides and trisaccharides. Examples of disaccharides are maltose, sucrose, maltulose, turanose, isomaltose, laminaribiose, nigerose, kojibiose, gentiobiose, and trehalose, and examples of trisaccharides are maltotriose, erlose, melezitose, 1-kestose, isopanose, isomaltotriose, panose, and endorse. All different kinds of plants have these sugars in small quantities (Bogdanov et al. 2004). Apart from sugars, many kinds of organic acids exist, for example, gluconic, lactic, formic, butyric, tartaric, pyruvic, acetic, citric, oxalic, succinic, malic, maleic, α-ketoglutaric, glucose-6-phosphate, pyroglutamic, and glycolic acid. The most common acid is gluconic acid that is formed from oxidation of glucose's first carbon by the enzyme glucose oxidase. The presence of an enzyme in honey makes it unique in a contest of a medicinal point of view. Yeasts, nectar, pollen, bee, and microorganisms are sources of enzyme. Key enzymes are glucose oxidase, catalase, acid phosphatase, invertase, and diastase. Enzyme activities are destroyed on heating (Hebbar et al. 2003).

The physical and biological properties depend upon the quality and quantity of organic acids; role of enzymes are cardinal. Mineral contents are the influencing factor for storage; rich contents are less suitable at low temperature. Honey of floral origin has mineral content varying from 0.02 to 0.1% (Olga et al. 2012). Quality of honey concerning nutritional, granulation, flavor, texture, and its medicinal value are controlled by the presence of ingredient such as moisture content, reducing sugars, electrical conductivity, free acids, sucrose content, and hydroxymethylfurfural (HMF). For the production of honey in industry, physicochemical properties are very important. The International Honey Commission (IHC) imposed fixed composition to maintain the quality of honey. The chief ingredient is sugar, 95% weight. It is a complex mixture of concentrated sugar solution and has main ingredients fructose and glucose (Aljadi and Kamaruddin 2004). Ratio of glucose to fructose in any kind of honey depends upon the nectar's source. Other bioactive substances like organic acids, proteins, amino acids, minerals, polyphenols, vitamins, and aroma also have impact on the quality of honey (Ferreira et al. 2009; Ramanauskiene et al. 2012). Taste and color qualities of honey come up with the presence of sugars, amino acids, minerals, and phenolic compounds, but aroma is because of volatile substance presence. The percentage of protein reported in different kinds of honey with a small portion of enzymes is less than 0.5% (Yao et al. 2005).

To maintain the quality, a directive requirement is necessary for the standard composition by the regulatory bodies. At the international level, the Codex Alimentary Standard commission (FAO 1981) imposed the compositional criteria, i.e., fixed the acidity, apparent reducing sugar, 5-hydroxymethylfurfural (HMF), mineral content, moisture, and water-insoluble solids (Belay et al. 2013). In an acidic environment, a chemical reaction takes place reducing sugars and giving HMF. The level of HMF deciding the age and overheating of the honey, its concentration fixed by regulatory bodies with a highest limit of 40 mg/kg exception, 80 mg/kg for tropical honey.

6.1.2 Biological Activities

Honey has several biological properties such as antimicrobial, antiviral, anti-inflammatory, wound and sunburn healing, antioxidant, antiparasitic, antidiabetic, antimutagenic, and anticancer activities (Gomes et al. 2010). Pharmacological study research by a team of scientist reported that natural honey has the potential to cure gastric and cardiovascular disease without an increase in body weight, apart from these observed advantageous effects on fertility by enhancing the effects of hormones related to fertility (Alvarez-Suarez et al. 2010a; Mosavat et al. 2014). Patients of type I and II diabetes are advised to take honey because of its lower glycemic index value. Uses of honey by diabetic patients find pharmacological change that helps to cure the disease, raise the hemoglobin level, restore secretion of insulin, reduce blood glucose level, and refine lipid profile. The phenolic content of honey establishes antioxidant properties and intensity of color reported by the researcher (Piljac-Žegarac et al. 2009). Other therapeutic effects exhibited by honey are anticarcinogenic, anti-inflammatory, antiatherogenic, antithrombotic, immune-modulating, and analgesic activities because of phenolic contents (Yaghoobi et al. 2008). Athletes generally take honey as a source of energy. Infection by bacteria either gram-positive or gram-negative including aerobes and anaerobes is studied in around 60 types. Treatment of such bacterial infection by honey has been reported (Molan 2006). In Egypt, back to 1553–1550 BC, medical practitioners uses honey for the treatment of wound, urination, and obesity. Similarly, Galen, a renowned physician prescribed honey to cure the disease of poisoning and intestinal disturbance. Honey was prescribed by the greatest medical authority of medieval times Ibn-Sina (Ave-Sina) to cure the diseases like runny nose, digestion of food, improve appetite, boost up the memory power, increase the blood circulation, and enhance the intelligence. Advanced research published that honey can cure several diseases and boost up the immune system of the body. A balanced composition of various content improves the resistance against the pathogenic organism. Intestinal infection caused by nematodes, including ascariasis and hookworm is also cured by honey (Sajid and Azim 2012). The presence of glycoproteins and peptides are responsible for immunomodulatory properties which exhibit as these molecules are interfering with the innate immune system in humans (Mesaik et al. 2014). The nutritive and medicinal values of natural honey with unique flavor make it very expensive and demanding. To fulfil the requirements of the consumer with low cost leads to adulteration. Used adulterants are very difficult to detect; they replace the natural properties and finally lead to decreasing both nutritive and medicinal values. Thus, global authenticity is a very important concern for the consumer as well as the manufacturer.

6.2 Adulteration

Adulteration makes the quality poorer, as well as the safety of the product is questionable. The adulterants are chemical substances, which lower the medicinal and nutritive values which harm human beings; almost 128 chemicals are reported as adulterants. In the market, adulterants available are generally starch syrup, inverted syrup, starch or inverted syrup fed to bees, and in some places low-grade honey is mixed with standard quality of honey. The process of adulteration may be a direct or indirect method. If a substance is directly added to honey, it is called direct method, and when honey bee is fed with chemicals and industrial sugars, it is called indirect method. So the detection of adulterants added through indirect method is very difficult as compared to adulterants added through direct method (Fig. 6.1). Examples of industrial sugar as adulterants that are frequently used are high fructose corn syrups (HFCS), high fructose insulin syrups (HFIS), invert syrups (IS), and corn syrups (CS). Syrup and invert sugar adulterants have been used which are chemically similar to a pure substance in which the concentration of honey is increased and is very difficult to detect. In quality control by the analyst, it is very difficult to determine the differences between pure and adulterated honey by analytical methods (Mehryar and Esmaiili 2011). Rice syrup as adulterants is also available in the market.

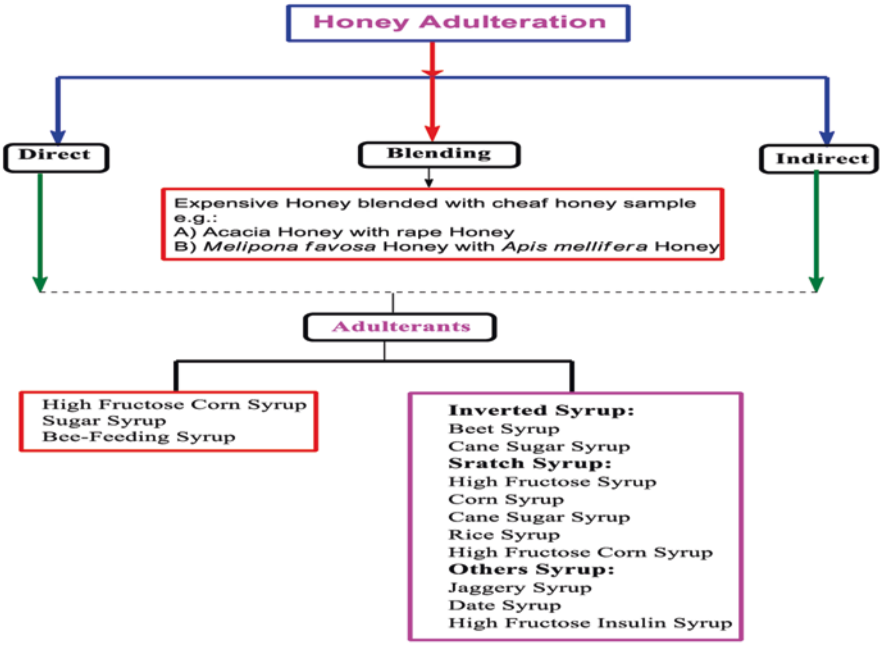

Fig. 6.1 Different types of honey adulterations

6.3 Authenticity

Presently, few authorities like Codex Alimentarius standard, the EU Honey Directive, and other legislations work at the national level to regulate the food authenticity. But, growing business globally and its medicinal uses require making an international standard regulation for authenticity. For the perfect authenticity of honey, we need to focus on two important points, first is its origin and second is the mode of production. Origin of honey may be either geographical or botanical while its production is defined by the way of harvesting and processing. The regulatory bodies International Honey Commission lately examined the Codex Alimentarius (CA) standards and European Community standards. In several countries, few adulterants are very easily available for adulteration in the market such as cheaper sweeteners from beet or canes like corn syrups (glucose), high fructose corn syrup (HFCS), saccharose syrups, and invert sugar syrups (Tosun 2013). Artificial honey is also manufactured in several places in the world adulterated with bee feeding of sugars or syrups (Bogdanov and Gallmann 2008). Monofloral honey has high market value as it is recognized by the consumer, but it is adulterated with cheaper multifloral honey (Soares et al. 2015). Authenticity of honey, and to find out its purity, is a very important task. The purity of honey must be checked by the processors, retailers, consumers, and regulatory authorities in every step by analytical methods followed by the regulation of regulatory bodies at national and international levels.

6.4 Detection Methods and Techniques

Authentication of honey of botanical origin by classical approach is common practice and monofloral origin by sensory and physicochemical analyses; melissopalynological analysis examines floral pollen grains in honey by microscopic origin (Bogdanov et al. 2004). Many factors are responsible for fraction of pollen content in honey such as species of plant, collection time, and nectar yield from a male or female flower. Pollen is sometimes collected from the bee's honey sac and illegally added to honey (Donarski et al. 2008). This method is not sufficient for the identification, but it has to be analyzed by the sensory method and physicochemical characteristics as pollen contents have some natural variation. Authentication with this method is a very tedious job, and numbers of physicochemical parameters are obligatory for the characterization. Classical authentication techniques have a limitation; modern analytical techniques are used to find out the origins of honey which is more reliable. Modern instruments like liquid chromatography and mass spectrometer (LCMS), infrared spectrometer, Raman spectrometer, nuclear magnetic resonance (NMR), and flame ionization detectors (FID) or sensor arrays are used. Research has been reported that adulterants like exogenous sugars or the addition of sugar syrups have been detected by different analytical techniques (Baroni et al. 2006). Sugar composition of honey is studied by high-performance liquid chromatography (HPLC) and chemometric method. Volatile composition and floral origin are determined by solid-phase microextraction (SPME) and gas chromatography coupled with mass spectrometry (GC–MS) (Baroni et al. 2006). By chemometric analysis, principal component analysis (PCA) and linear discriminate analysis (LDA) have been employed to estimate the controlling variables and, a likeness of honey samples. The molecular genetics approach is used to find out the composition, and geographical and entomological origins of honey (Chin and Sowndhararajan 2020) (Fig. 6.2).

6.4.1 Qualitative Physicochemical Analysis for Honey Identification

Honey quality is based on physicochemical parameters; it could be useful for the assessment of its origin. Routine determination of physicochemical parameters, water content, electrical conductivity, sugar content, fructose/glucose ratio, enzyme activity, color, ash value, optical rotation, pH value, acidity, and hydroxymethylfurfural (HMF) content is commonly used for both QC and processing control of honey. Several factors influence the final values of these parameters in honey. For water content, the most important factor is air humidity. The self-life of honey degrades when the content of water is high, low density, and high electrical conductivity. The density of honey is influenced by water content, lesser density in high water content and vice versa, less than 5% sucrose in honey indicates good quality of honey, and if the percentage is more than 5%, it indicates sucrose may be unripe and it is not completely converted by enzyme invertase into glucose and fructose (Ouchemoukh et al. 2007).

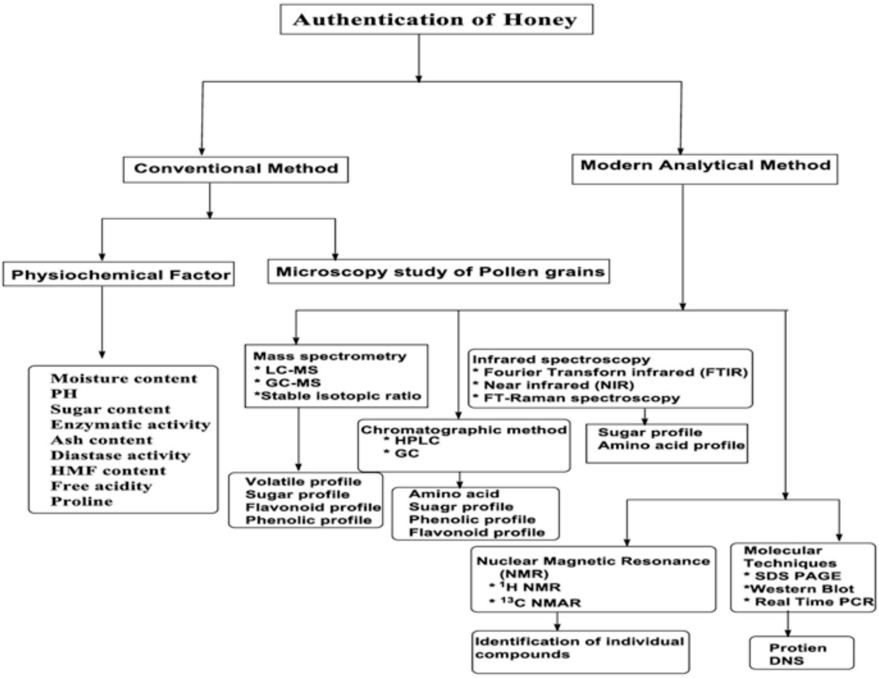

Fig. 6.2 Classical and modern analytical methods used for honey authentication

Quality indicators of honey, i.e., invertase activity, diastase, and HMF indicate freshness and overheating of honey. The presence of low quantity diastase indicates low natural amylase in honey (Pasias et al. 2017). The purity of honey is checked by the presence of HMF, higher content indicates overheated, aged, and the duration of storage is long and in poor condition. Recommended standard is (80 mg/kg). The self-life of honey is 1 year and should be consumed (Khalil et al. 2010). Mineral content determined the electrical conductivity (EC), its value varies from region to region, for example, the values of electrical conductivity are 4.18 and 1.98 ms/cm for geographical regions Yemen and Egypt, respectively, and 0.53 and 0.67 ms/cm for Saudi and Kashmir, respectively (El Sohaimy et al. 2015). Acidity is because of the presence of gluconic acid, esters, lactones, and inorganic ions of chloride and phosphate, and the pH value of honey varies as the season of extraction varies. The pH values below 3.5 spoilage the honey. The pH value of fresh honey is 4.1–4.6, and the standard range is 3.4–6.1 (Codex Alimentarius 2001). Conversion of sugar into organic acid is fermentation; in such condition, the acidic value of honey is high, and acidity of honey influences the microbial spoilage and sustains honey flavor.

6.4.2 Microscopic Analysis of Pollen Grains

The subject, learning for pollen grains and spores is called palynology (early branch is called Melissopalynology). Resin, nectar, water, and pollen are a source of energy for the honeybee. In pollens, the amounts of protein, minerals, vitamins, and fats are abundant. To know about the origin of honey, either it is geographical or botanical, the fingerprint of pollen and honeydew elements are very helpful. With microscope pollens, it can be identified, and traditionally it is used for quality analysis (Hermosín et al. 2003). The procedure adopted for microscopic analysis is weigh accurately 10 g of honey and dissolve in a volume of 20 mL warm water (40 °C) after that at 2000 rpm centrifuge for two times for a duration of 10 min. Dregs are taken out, dried, put on a slide having chemical glycerin, gelatin, and stained with fuschin alcohol solution. Observe this slide under a microscope for the examination of pollen. This analysis indicates only morphological characteristics, not genus or species. Results of the study are analyzed and categorized into four types: very frequent, frequent, rare, and sporadic percentage of pollen in each category is 45%, 16–45% 3–15%, and less than 3%, respectively. Microscopic analysis along with physicochemical analysis is very helpful to recognize the standard of honey for the business purpose (Louveaux et al. 1970).

6.4.3 Detection of Adulterants by Microscope

A routine practice to detect the adulterants by a microscope is a classical method. Adulterants like cane sugar and acid hydrolyzed cane sugar syrup,in cane sugar, epidermis cells and single rings of ring vessel particles are present along with sugar cane starch and sclereids. With microscopic analysis, other instrumental techniques like HPLC are used for the estimation of glucose, sucrose, fructose, and HMF along with the determined the value of pH, water content, and electrical conductivity that is very helpful to find out the adulterants (Kerkvliet et al. 1995). Chemical analysis data reveal whether the honey is adulterated or heated. Qualitative physiochemical analysis for honey identification is presented in Table 6.1 (Sadasivam and Manickam 1996; Patil 2016).

6.4.4 Analytical Technique

Spectroscopic techniques are quick and noninvasive and do not require tedious sample preparation, which often involves laborious work and consumption of large amounts of organic solvents, reagents, and time. Therefore, they can be considered a green analytical alternative. The noninvasive nature of spectroscopic techniques allows investigation of intact food samples, which is particularly appropriate for high-throughput screening, especially in commercial production plants quality control.

Classical techniques have limitations for authentication; advanced analytical techniques have adopted for the examination of botanical and geographical origins of honey. Also, carbohydrate profile, mineral content, aroma profiles, and phenolic and flavonoid composition were studied (Ouchemoukh et al. 2010; Jerkovic et al. 2009). Chromatographic techniques like thin-layer chromatography (TLC)

Table 6.1 Qualitative physiochemical analysis for honey identification

Test	Procedure
Molisch's test	Detection of carbohydrate: take accurate volume of 2 mL sample in the test tube then add few drops of Molisch reagent +1 mL of concentrated H_2SO_4. If a red-violet color ring develops at the junction of the two liquids, it specifies the presence of carbohydrates in the honey sample
Fehling's test	Detection of reducing sugar: transfer a specific volume of sample in a test tube then add equal volume of Fehling's solution A and B in the test tube. After shaking, keep it in a boiling water bath for few minutes. Development of a brownish-red precipitate indicates the presence of reducing sugar
Benedict's test	Detection of reducing sugar: 2 mL of Benedict's reagent is mixed with a small volume of samples; heat the mixture for 5 min on a boiling water bath, then the mixture is cooled under tap water. Development of green, yellow, or red color indicates the presence of reducing sugar in honey samples
Seliwanoff's test	Detection of ketose sugar like fructose: two drops of each sample solution is heated with 2 mL Seliwanoff's reagent in boiling water bath. Development of a deep red color specifies the presence of ketose sugar. Colored is formed with in 30 s
Adulteration confirmation test	The collected honey samples were analyzed for adulterants. Following physical tests are carried out to identify the purity and adulterants added to the sample
Flame test	The presence of added water in each honey sample is determined by putting a drop of honey on a laboratory Bunsen burner by using cotton wick. The presence of added water is confirmed by the observation of cracking sound without flame. Pure honey gives smokeless flame
Fiehe's test	Detection of added sugar: weigh accurately 2 g of honey sample dissolved in 10 mL of water and mix properly. Extract the sample with solvent diethyl ether ($C_2H_5OC_2H_5$) of volume 30 mL in a separating funnel. Prepare resorcinol solution (1 g of resublimed resorcinol in 100 mL of hydrochloric acid), take fresh 2 mL of this solution and add to extract. Shake the solution properly. Development of cherry red color within a minute indicates the presence of added sugar. No significance of other color

employed for amino acid determination. Gas chromatography (GC) and high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), and high-performance anion-exchange chromatography-pulsed amperometric detection (HPAEC-PAD) were used for the estimation of adulterants like high fructose corn syrups (HFCS) and corn syrups (CS) in the sample (Verzera et al. 2014). The analytical methods also used for the determination of adulterant are differential scanning calorimetry (DSC), electrochemical analysis, enzymatic methods, vibrational spectroscopy like mid-infrared (MIR), near-infrared (NIR) spectroscopy, Raman techniques, isotope ratio mass spectrometry coupled with an elemental analyzer, low-field nuclear magnetic resonance, stable isotope analysis, and others such as flame ionization detectors (FID) or sensor arrays (Wang et al. 2010; Kropf et al. 2010). In developing countries, microscopic analysis is a method of choice, and modern analytical techniques are bearable. Apart from the discussed technique, several special methods are used to find out the adulterants like three-dimensional fluorescence spectroscopy (3DFS) coupled with multivariate calibration, electronic honey quality analyzer, fiber-optic displacement sensor (FODS), and an electronic tongue. Detection and estimation of adulterants in the sample are very easily performed with adulterant kits, development for an enzyme label, which can make a difference in the color of the sample matrix (Table 6.2: important merits and demerits of analytical techniques).

Table 6.2 Important merits and demerits of the discussed techniques

Detection technique	Merits	Demerits	References
Melisso palynological analysis and other physicochemical parameters detection	Simple or no sample preparation; best for unifloral honeys of same geographical origin	Wide range of thresholds; could not work for honey from close geographical zones	Castro-Vazquez et al. (2014)
Chromatographic analysis	Complex, volatile and nonvolatile, wide variety of analytes are readily analyzed	Honey origin is difficult to be identified	Kamboj et al. (2013)
High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD)	Did not require derivatization; shorter total analysis time	Need specialized equipment to handle to high-pH mobile phases; no method flexibility to resolve an interfering peak	Xue et al. (2013)
Front phase fluorimetric spectroscopy	Botanical origin of polyfloral honeys can be identified easily; highly sensitive in comparison to other spectroscopic technique	Geographical origin estimation could not be done accurately	Ruoff et al. (2006)
Fourier transform infrared spectroscopy	Botanical origin of polyfloral honeys can be identified easily; short analysis time	Geographical origin estimation could not be done	Wang et al. (2010)
Fourier transform Raman spectroscopy	No water interference and minimal fluorescence interference; detect adulteration from the same plant source	Aqueous, dark colored samples at high temperatures increase interferences	Pierna et al. (2011)
Stable isotope ratio mass spectrometry (SIRMS)	Wide applicability and versatility to be coupled with several different interfaces	Lack of availability of SIRMS standards and standardized methods; not suitable for routine analysis	Cengiz et al. (2014)
Ultra-performance liquid chromatography-quadrupole/time of flight-mass spectrometry (UPLC-Q/TOF-MS)	It was possible to identify several components which cannot be detected by diode array using combination of detection with retention time for accurate molecular mass to obtain phenolic acids and flavonoids from ethyl acetate extracts of different honeys (sunflower, lime, clover)	Deficiency of this high sensitive's technique and not suitable for analysis	Trautvetter et al. (2009)

(continued)

Table 6.2 (continued)

Detection technique	Merits	Demerits	References
Nuclear magnetic resonance	Fingerprint technique so easy to identify a specific biomarker for a class of sample; minimal sample processing; non-destructive nature	Extensive chemometric analysis is required which makes it complicated for routine analysis	Consonni and Cagliani (2008)
Western blot	Development of a novel method based on honey proteins to determine floral origin of honey samples using SDS-PAGE immune blot or Western blot techniques	To sort the proteins by size, charge, or other differences in individual protein bands	Baroni et al. (2002)
Atomic absorption spectrophotometer	Characterized different types of honey produced in the Canary Islands according to their mineral contents using atomic absorption spectrophotometer	Only solutions can be analyzed, relatively large sample quantities are required	de Alda-Garcilope et al. (2012)

6.4.4.1 Infrared Spectroscopy

Infrared spectrometer is a very useful technique for honey sample analysis. Some research has been reported. Infrared different vibration range was employed for the estimation of honey in botanical origin, eight monofloral and polyfloral honey sample authenticated by near-infrared spectrometer also, performed a quantitative examination of various type of sample. FTIR and chemometrics were used for botanical origin studies (Ruoff et al. 2005; Kelly et al. 2004). Fourier transform infrared spectrometer (FTIR) with attenuated total reflectance (ATR) is used for the determination of various food parameters. Organic compounds present in honey give signals in the range MIR (4000–400 cm^{-1}) and NIR (10,000–4000 cm^{-1}) originate from the vibrational and rotational modes (stretching, bending, and rotating). The signals that originate due to NIR are complex overtones and high-frequency combinations of fundamental vibrations at shorter wavelengths. The wavelength range of MIR gives sharper, resolved, and informative peaks which indicate the botanical and geographical origins reported by Ruoff et al. FT-MIR gives more efficient information of 11 types of unifloral (acacia, alpine rose, chestnut, dandelion, heather, lime, grape, fir honeydew, metcalfa honeydew, and oak honeydew) and polyfloral kinds of honey ($n = 411$ samples; 15). The characteristic spectral line between 800 and 1500 cm^{-1} is observed. Spectra of various samples of honey correspond to the C–O and C–C stretching regions of the saccharides between 950 and 1050 cm^{-1} (Wu et al. 2017). IR spectroscopy and Raman spectroscopy have a disadvantage: during the analysis of the sample, the duration exposed to heat may lead to sample destruction. Overcome such problem by adopting a procedure to expose less irradiation duration and increasing the number of the scan experimented attenuated total reflection (ATR). Prominent absorption lines in the mid-IR region indicate the presence of water in the samples. The botanical origin of honey is indicated by

FTIR spectroscopy and attenuated total reflection (ATR) sampling technique (de la Mata et al. 2012). Differentiate HFCS-adulterated and unadulterated honey by spectra obtained through fiber optic diffuse reflectance NIR spectrometer acquired within the 10,000–4000 cm^{-1} range. A perfect PLS model used to differentiate pure and adulterated honey samples is within the range of 6000–10,000 cm^{-1} (Chen et al. 2011).

6.4.4.2 Nuclear Magnetic Resonance (NMR)

For structure determination, this instrument is best for different organic compounds present in honey sample and gives a better understanding of the complex structure (Cazor et al. 2006). Compare to the other analytical technique, NMR provides better information about the sample composition and metabolites also, have property non-invasive nature, the relative ease and rapidity of data execution in a single run (Ribeiro et al. 2014). Data produced by NMR extract useful information with multivariate analysis, several published paper indicate methods which are useful to categorize honey samples according to their botanical origin. NMR analysis performed as per the demand several choices are available such as principal component analysis (PCA), hierarchical cluster analysis (HCA), K-nearest neighbor (KNN), soft independent modeling of class analogies (SIMCA), and orthogonal PLS (OPLS)-DA. In addition to the one-dimensional (1D) technique, two-dimensional (2D) NMR experiments were also employed for the analysis (Lolli et al. 2008). Five types of botanical origin (robinia, chestnut, citrus, eucalyptus, and polyfloral) honey have been differentiated by ^{1}H-NMR and heteronuclear multiple-bond correlation (HMBC) experimented by taking 72 honey samples. Developed general DA models that had cross-validation accuracy rates of 92% in the case of D2O ^{1}H–^{13}C HMBC spectra and 97% in the case of DMSO-d6 ^{1}H–^{13}C HMBC spectra (Simova et al. 2012). ^{1}H- and ^{13}C-NMR analyzed, confirmed the protons and methylene group carbon in quercitol on TOCSY spectroscopy for the given sample. Honey sample of oak honeydew is differentiated from the other honey sample by the presence or absence of quercitol. ^{1}H-NMR is more useful as compare to ^{13}C-NMR because of sensitivity is high. ^{13}C-NMR technique is employed for the estimation of saccharides in authentic Greek honey samples, a method which was developed for the estimation has been validated as according to ICH guideline, performed experiment for validation on accuracy, linearity, range, limit of detection, etc. The samples taken have either single sugar molecules or artificial mixtures of isoglucose (glucopyranose and fructose) (Kazalaki et al. 2015). Monofloral honey ingredients like carboxylic acids, amino acids, ethanol, and hydroxymethylfurfural estimated by ^{1}H-NMR (Beretta et al. 2009). Various samples from different botanical origin were analyzed by HPLC-DAD-ESI-MS and multidimensional diffusion-ordered (DOSY) NMR, and ingredients like quinoline alkaloids and the biosynthetic precursor, i.e. kynurenic acid (KA), have been estimated. Concentration level of quinoline alkaloids distinguishes chestnut honey from others (Cho et al. 2015).

6.4.4.3 Hyphenated Technique (Mass Spectrometer Coupled with Chromatography Techniques)

The semi-volatile and volatile substances in honey separated and identified by LC–MS and GC–MS techniques. Flavor of honey-based on the concentration of volatile substance and its variation in concentration are related to floral origin. Estimation of these substances by technique headspace (HS) solid-phase micro-extraction (SPME) (Escriche et al. 2011), with this technique followed by GC–MS of various volatile substances, is identified and quantified in a different honey sample. Various types of samples were analyzed: 35 volatile components (Spanish honey), 62 compounds (Greek honey), 31 compounds (16 samples from European countries), and 26 compounds (70 authentic Turkish honey) (Alissandrakis et al. 2007; Senyuva et al. 2009). Italian thistle honey was analyzed by the HS-SPME method, and 40 volatile compounds were characterized and reported by Bianchi et al. (2011). HS-SPME/GC-MS extended with chemometric studies to estimate the organic volatile compounds, and 42 unifloral samples of five floral origins were studied (Spanik et al. 2014). SPME-GC-MS instrument used for the investigation of volatile consituents have chiral carbon. Flavonoid component of honey was studied using TLC–MS diode array detection system and electrospray ionization mass spectrometry (LC–DAD–ESI/MS). Seven types of Slovenian honey samples were extracted by solid-phase method followed by liquid chromatography, and their botanical origin was reported. Different types of honey-like strawberry tree honey, chaste honey, and rape honey studied floral origin by the high-performance liquid chromatography–diode array detection–tandem mass spectrometry (HPLC–DAD–MS/MS) method (Zhou et al. 2014). Floral markers such as kaempferol, morin, and ferulic acid are used to distinguish chaste honey from rape honey (Oelschlaegel et al. 2012). Ultra-performance liquid chromatography–photodiode array detection–mass spectrometer (UPLC–PDA–MS/MS) had been used to examine the volatile composition of Manuka honey sample; solid-phase extraction was first performed. Constituents like kojic acid, unedone, 5-methyl-3-furan carboxylic acid, 3-hydroxy-1-(2-methoxyphenyl) penta-1,4-dione, and lumichrome were identified in Manuka honey sample. Advanced technique ultra-performance liquid chromatography–quadrupole/time of flight mass spectrometry (UPLC–Q/TOF-MS) was used to detect phenolic acids and flavonoids based on the retention time of individual compound of a sample; sunflower, lime, clover, rape, and honeydew were extracted with solvent ethyl acetate (Trautvetter et al. 2009).

6.4.4.4 Inductively Coupled Plasma–Mass Spectrometry (ICP–MS)

Atomic absorption spectrometric technique is one of the key instruments used for food analysis. This technique is very popular in the food industry. This instrument is used for the estimation of multiple elements in the sample, and the advantage is that very high sensitivity detects low-level concentration elements. Brazilian kinds of honey of the geographical origin have been analyzed by ICP-MS with combination data mining approaches (Batista et al. 2012).

6.4.4.5 Stable Isotopic Ratio Mass Spectrometry (SIRMS)

This analytical technique used for the detection of more sophisticated adulterations. The method is based on the differences in the metabolic enrichment of the ^{13}C isotope due to the different photosynthetic pathways of the C3 and the C4 plants. The slower-reacting $^{13}CO_2$ is depleted to a larger extent in C3 plants than in C4 plants during CO_2 fixation, making it possible to detect the addition of cheap C4 sugar because of its different $\delta^{13}C$ value (i.e., the $^{13}C/^{12}C$ isotope ratio related to Vienna Pee Dee Belemnite as a standard reference material, expressed as a percentage; 100). The method was later improved by using the isolated honey protein as an internal standard, which enhanced sensitivity and lowered the LOD for C4 sugars from around 20 to 7%. However, the main drawbacks of this technique are the impossibility to detect the addition of C3 syrups, which is why their fraudulent use is on the rise and the working hypothesis that assumed a correlation between the floral origins of honey and its proteins (Cotte et al. 2007). The $\delta^{13}C$ value indicates the adulteration, the addition of C4 sugar in pure honey changes the value of $\delta^{13}C$, if it is less negative than 23.5, it indicates to be adulterated (Padovan et al. 2003).

6.4.4.6 Chromatographic Methods

Chromatographic techniques provide reliable separation and quantification of macro and micro components of highly similar chemical structures in complex matrixes such as food products. The chromatographic fingerprint profile is a well-established assay of authenticity concerning botanical and geographical origins of honey. Fingerprint analysis can be defined as a set of characteristic chromatographic signals leading to sample pattern recognition (classification). However, authentication of adulteration is usually done by matching measured compound profiles with predetermined target values. In these studies, LC was applied for the identification of proteins, amino acids, carbohydrates, vitamins, phenolic compounds, triglycerides, chiral compounds, and pigments, whereas GC was used for the analysis of naturally volatile or semi-volatile molecules (Doner et al. 1979). Maltose/isomaltose ratios in different kinds of honey and high fructose corn syrup were determined using gas chromatographic (GC) method.

6.4.4.7 High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC gained very high popularity for the authentication of honey and has an advantage over the other chromatography like GC and HPLC because it is easy to handle and low-cost and has the ability to simultaneously analyze multiple samples on the same plate. Automation increases precision and accuracy of the developed method. By this technique, authenticity of honey production is confirmed, but it is not possible to authenticate the botanical or geographical origin (Morlock and Schwack 2008). Puscas et al. reported adulterants in several samples of Romanian kinds of honey by high-performance thin-layer chromatography (HPTLC) combined with image analysis. In several honey, estimated proline, leucine and phenylalanine and their enantiomeric ratios by HPTLC (Rizelio et al. 2012).

6.4.4.8 High-Performance Liquid Chromatography (HPLC)

Estimation of phenolic and amino acid component experimented by this analytical technique which is used for the assessment of its authenticity (floral and geographical origins). Campone et al. (2014) analyzed the honey sample reported 5 phenolic acids and 10 flavonoids used dispersive liquid-liquid microextraction followed by HPLC analysis. This chromatographic technique developed chromatogram providing very complex information which is helpful to differentiate whether the honey is of botanical, geographical, or entomological origin. The performance of this analysis is increased when this instrument is coupled with MS (Zhou et al. 2014). Determination of phenolics compounds in honey needs to be performed with several steps like isolation from a sample matrix, analytical separation, identification, and quantification.

6.4.4.9 High-Performance Anion Exchange Chromatography (HPAEC)

Carbohydrate is one of the main constituents present in honey, and this analytical tool is very powerful because of its ability to separate all classes of aldols, amino sugar, and mono-, oligo-, and polysaccharides based on their structural features such as size, composition, atomicity, and linkage isomerism. Adulterants, structurally based on carbohydrate, are detected by HPAEC–PAD instruments. HPAEC profiles of polysaccharides are also used for the detection of adulteration with CS after pretreatment of the sample with reversed-phase SPE to remove monosaccharides and small oligosaccharides, and to concentrate traces of polysaccharides.

6.4.4.10 Gas Chromatography (GC)

It is a technique used to analyze volatile organic components (VOCs); mono-, di-, and trisaccharides; and pesticide residues in honey. In the majority of studies evaluating the authenticity of honey, GC was combined with MS to identify different substances within a test sample. GC is also a suitable technique for the detection of honey adulteration due to its relatively high resolution and sensitivity for the determination of mono-, di-, and trisaccharides. A literature survey revealed that GC was used mainly for the detection of honey adulterations carried out by the addition of sugar syrups such as HFCS, CS, and IS (Zhou et al. 2014).

6.4.4.11 Electrochemical Methods

These techniques provide a high level of sensitivity and selectivity, although some of them require cumbersome sample preparation steps. Electrochemical techniques are as fast, simple, and cheap as some of the already-mentioned techniques; however, they also provide valuable information about the redox properties of honey constituents. Therefore, they have been extensively applied in the honey analysis.

6.4.4.12 Voltammetry and Electronic Tongue

Several advantages, such as high sensitivity, versatility, simplicity, and robustness, make voltammetry a powerful electroanalytical technique. So far, cyclic, stripping, pulse, and alternating current voltammetry methods have been developed by a significant number of researchers for the analysis of different organic and inorganic compounds, as well as antioxidative activity. The electronic tongue (e-tongue) is a novel device consisting of arrays of nonselective gas or liquid sensors coupled with pattern recognition software. The e-tongue analyzes the complex natural sample as a whole without the need for separating it into simpler components. So far, there are just a few papers related to the determination of the botanical origin of honey using an e-tongue. Eight different botanical types of honey and five geographically different acacia kinds of honey were classified using an e-tongue in combination with PCA, HCA, and artificial NNs (105). Voltammetric e-tongue (VE-tongue) has been composed of six working electrodes (gold, silver, platinum, palladium, tungsten, and titanium) in a standard three-electrode configuration and is used for the classification of various kinds of monofloral kinds of honey-based on multifrequency large amplitude pulse.

6.4.4.13 Electrophoresis

Proteins are found as minor components in honey and originate from honey bees, plants, pollen, and nectar. Electrophoresis, although rarely applied as an electrochemical technique in honey analysis, provides the simultaneous analysis of a large number of samples under the same conditions. In 1987, the first silver-staining sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) method was described by Marshall and Williams (109) for the detection of 19 protein bands in Australian kinds of honey of different plant origins. Baroni et al. (2002) reported a novel analytical method for the assessment of floral origin in kinds of honey-based on the study of proteins using SDS-PAGE. The authors used honey proteins as chemical markers for the floral origin of eucalyptus honey. They also found that pollen from different plants could be significantly differentiated through SDS-PAGE coupled with DA (110).

6.4.4.14 Capillary Electrophoresis (CE)

This is becoming a popular electroanalytical technique for the separation and identification of phenolic compounds, carbohydrates, amino acids, organic acids, and cations in honey. High speed, resolution, simplicity, low operating costs, and short analysis times make CE an alternative technique to HPLC for the analysis of different target compounds in honey. Also, MS in combination with CE provides a high level of sensitivity and selectivity.

Acknowledgment The authors are very thankful to the Faculty of Pharmacy, Maulna Azad University, Jodhpur, Rajashthan, INDIA for valuable support.

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7 Honey and Its Derivatives: A New Perspective on Its Antimicrobial Activities

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Abstract

Honey is a well-known and historically important sweet food which possesses immense antimicrobial properties. Numerous varieties of honey are present in nature, and all of these honey varieties contain certain key ingredients, which confer upon them various antimicrobial properties. These antimicrobial key ingredients include polyphenolic compounds, hydrogen peroxide, methylglyoxal, and bee-defensin among several others. Honey is nowadays used extensively in modern medicine as potent antibiotic for the treatment of surface wounds and burns. It is also used in combination with other antibiotics to treat antibiotic resistance. As an antifungal agent, honey is used to treat the athlete's foot (tinea pedis), jock itch (tinea cruris), and ringworm of face, scalp, nail, and hand (tinea corporus). In this chapter, we aim to provide a brief overview of various types of honey and their composition and describe extensively its various antimicrobial properties and how these properties are exploited in modern medicine as an alternative to popular therapeutics or in conjunction with it.

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Keywords

Honey · Honeybee · Antioxidant · Antimicrobial · Antifungal · Antiviral

Abbreviations

AT	Agastache honey
CAT	Catalase
G6PD	Glucose 6 phosphate dehydrogenase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
H_2O_2	Hydrogen peroxide
LOOH	Lipid hydroperoxide
MAPKs	Mitogen-activated protein kinases
MK	Manuka honey
NO	Nitric oxide
O_2^-	Superoxide
OH^-	Hydroxyl
$ONOO^-$	Peroxynitrite
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PKB	Protein kinase B
PKC	Protein kinase C
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RO	Alkoxy
ROO	Peroxyl
SOD	Superoxide dismutase

7.1 Introduction

7.1.1 Honey

Honey is perhaps the oldest consumed food in human history and is still used as natural sweetener and health supplement. The consumption of honey dates back to 5500 BC and is duly mentioned in the manuscripts of various civilizations of Egypt, China, and India. Most of the ancient civilizations, including the Greeks, Romans, Egyptians, Babylonians, Persians, Mayans, Indians, and Chinese utilized honey and its derivatives for nutritional and medicinal reasons (Israili, 2014; Samarghandian et al. 2017; Ahmed et al. 2018). Interestingly, honey is the sole biological commodity of insect origin that is consumed so widely by humans for its nutritional, cosmetic, and therapeutic benefits (Simon et al. 2009; Ahmed and Othman 2013a; Othman 2012a, b).

The syrupy product which humans consume is derived by various species of bees (Apis mellifera; Family: Apidae) from flower nectars. During collection, this nectar gets mixed with the enzymes of saliva present in the honey sack, gets digested/processed and is then finally regurgitated back into the cells of the hive to store it for future use (Michener 2013; Abd Jalil et al. 2017). The most common source of honey consumed by humans is produced by Apis mellifera (and numerous other subspecies like *A. m. anatolica*, *A. m. carnica*, *A. m. caucasica*, and *A. m. ssp. sicula*); however, there are other species like A. andreniformis, A. caucasica, A. cerana, A. dorsata, A. florea, A. indica, and A. ligustica; Plebeia wittmanni, Tetragonisca angustula fiebrigi, and Trigona carbonaria which are known to produce quality honey also (Israili 2014). Honey is one of the most common foods used widely across all ages and gender, all over the world. Honey does not need any special methods of preservation and can be transported and stored at around 25 °C–37 °C in a dark and dry place (Israili 2014; Samarghandian et al. 2017; Othman 2012b; Bell 2007).

The nutritional and therapeutic properties of honey and its concomitant uses have been well described in almost all religious scriptures encompassing all faiths and cultures. Among the three most followed religions of world—Christianity, Islam, and Judaism—honey is mentioned in all the Holy books—Bible, Quran, and Talmud, respectively. In all, honey is regarded as the important food having both nutritional and healing properties (Israili 2014; Rosner 2000; Purbafrani et al. 2014).

7.1.2 Composition

The composition, physical and chemical properties, flavor, color, and consistency of honey varies with floral source, geographical areas, climate, storage conditions, and the type of bees (Samarghandian et al. 2017; Castro-Vázquez et al. 2009; Manyi-Loh et al. 2011; Chang et al. 2011; Brudzynski and Kim 2011). Usually honey is named based either upon various geographic locations where it is produced/harvested or the floral sources or trees on which the hives are found. There are around 300 or more unique types of honey available, of which some 35 types are most used (Lusby et al. 2002). A few different types of honey available around the world are presented in Table 7.1.

It is reported that honey usually contains about 600 different compounds of which carbohydrates contribute about 95–97% of its dry weight and primarily consist of two main sugars—glucose (31%) and fructose (38%). They are derived from the digestion of floral nectar disaccharides by bee salivary enzymes. Sugar content in honey in turn is responsible for crystallization, viscosity, thermal, and rheological properties (Nguyen et al. 2018). Sugars are also responsible for provision of energy equivalent to 300 kcal/100 g of honey, which constitutes about 15% of the recommended daily allowance (Samarghandian et al. 2017). Honey has a moisture content of 15.6% and total solid content of >82.0% (Israili 2014; Samarghandian et al. 2017; Lusby et al. 2002; Rahman 2013; Masalha et al. 2018). Honey is one of the few energy-dense foods in nature with a low glycemic index (40; range, 31–78), pH

Table 7.1 Various honeys and their floral and chemical markers

Honey type	Floral marker	Chemical markers
Acacia honey	Cis-linalool oxide and heptanal	Kaempferol–rhamnosides and rhamnosyl–glucosides
Agastashe honey	Bicyclo undec-4-ene, 4,11,11-trimethyl-8-methylene	Phenol, 2,4-bis(1,1-dimethylethyl) and Estragole
Chestnut honey	2-Aminoacetophenone, 1-phenylethanol	p-Coumaric and ferulic acids
Manuka and tea tree honey	Estragole, Apigenin	Acetanisole and methyl 3,5-dimethoxybenzoate
Tualang honey	5-Methyl furfural, 2-furylmethylketone	Catechin, 2-hydroxycinnamic acid
Eucalyptus honey	2-Hydroxy-5-methyl-3-hexanone, 3-hydroxy-5-methyl-2-hexanone	Myricetin, tricetin, and luteolin
Lime tree honey	Carvacrol and *p*-cymene	
Citrus honey	Limonyl alcohol, sinensal isomers, and α-4-dimethyl-3-cyclohexene-1-acetaldehyde	
Ulmo honey	4-Vinylanisole, benzylaldehyde, ethyl benzoate, ethyl anisate, lyrame, linalool, and damascenone	
Heather honey		Myricetin, myricetin-3-methyl ether, tricetin
Jelly bush		Linalool and nonanal
Turkish honey		3-Carene
Sage honeys		p-Coumaric, p-hydroxybenzoic, and ferulic acid

Nolan et al. (2019), Cianciosi et al. (2018), Ahmed et al. (2018), Samarghandian et al. (2017), Kaškonienė and Venskutonis (2010) and Ahmed and Othman (2013a)

(3.9; range, 3.2–4.5), and total acidity (29.12 meq/kg; range, 8.68–59.49 meq/kg) (Masalha et al. 2018; Feas et al. 2010; Islam et al. 2012; Escuredo et al. 2013). Table 7.2 enlists the average content of constituents generally present in honey.

Apart from carbohydrates, which constitute 95–97% of the solid fraction, honey contains almost all amino acids (except asparagine and glutamine) (Iglesias et al. 2004), various proteinaceous enzymes (like acid-phosphatase, catalase, diastase, glucose oxidase, and invertases) (Wilkins and Lu 1995), minerals (31 of them including phosphorus, sodium, calcium, potassium, sulfur, magnesium, chlorine) (Zhou et al. 2013), vitamins, vitamin C being the most abundant; however, it also contains small amounts of thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), and pyridoxine (B6) (Ajibola et al. 2012) and other organic acids (Daniele et al. 2012), such as flavonoids, polyphenols, alkaloids, glycosides, anthraquinone, and volatile compounds (Jerkovic et al. 2010a, b; Zhou et al. 2002). There are around 26 amino acids present in honey, of them proline constitutes about 50–85% of the total amino acid content, which is primarily produced by the bees' salivary

Table 7.2 Chemical composition of the most consumed types of honey

Amount in 100 g of honey					
Component	g	Vitamins	mg	Minerals	mg
Water	16.9–18	Ascorbic acid	2.2–2.5	Calcium	3–31
Carbohydrates (total)	64.9–73.1	Thamin	0.0–0.01	Potassium	40–3500
Fructose	35.6–41.8	Riboflavin	0.01–0.02	Copper	0.02–0.6
Glucose	25.4–28.1	Niacin	0.1–0.2	Iron	0.03–4.0
Maltose	1.8–2.7	Pantothenic acid	0.02–0.11	Magnesium	0.7–13.0
Sucrose	0.23–1.21	Pyridoxine	0.01–0.32	Manganese	0.02–2.0
Organic acids	0.5–0.7			Phosphorus	2.0–15.0
Proteins and amino acid	0.50–1			Sodium	1.6–17.0
				Zinc	0.05–2.0
				Selenium	0.001–0.003

Nolan et al. (2019), Nguyen et al. (2019), Cianciosi et al. (2018), Ahmed et al. (2018), Samarghandian et al. (2017) and Escuredo et al. (2013)

secretions. Proline content is therefore often used as a parameter to evaluate the maturation degree of honey. Other amino acids include alanine, glutamic acid, isoleucine, leucine, phenylalanine, and tyrosine (Masalha et al. 2018; Hermosín et al. 2003; Perez et al. 2007). Gluconic acid, an oxidative product of glucose is the main organic acid constituent of honey. In addition, small amounts of acetic acid, citric acid, and formic acid are also present; all of which provide the acidic (pH) property to the honey (Mato et al. 2003).

Numerous studies have demonstrated that there are approximately 600 important volatile compounds present in honey which are responsible for most of its potential therapeutic effects. These include acid esters, alcohols, aldehydes, hydrocarbons, ketones, benzene and its derivatives, pyran, terpene and its derivatives, isoprenoids, and lesser amounts of sulfur, furan, and other cyclic compounds (Ajibola et al. 2012). Among them the two main bioactive volatile molecules present in honey are flavonoids and phenolic acids (and its derivatives) (Ahmed et al. 2018; Ahmed and Othman 2013a, b; Manyi-Loh et al. 2011; Cook and Samman 1996; Erejuwa et al. 2012).

The main flavonoids present in honey are apigenin, chrysin, galangin, hesperetin, kaempferol, pinocembrin, and quercetin, and the most important phenolic acids are ascorbic, benzoic, caffeic, chlorogenic, p-coumaric, ellagic, ferulic, gallic, 3-hydroxybenzoic, rosmarinic, and vanillic acids (Masalha et al. 2018; Erejuwa et al. 2012; Kenjerić et al. 2008; Kassim et al. 2010; Khalil et al. 2011; Petrus et al. 2011). Most of these two classes of chemicals perform their activities by having synergistic interaction with each other to yield a variety of antioxidant, antifungal, anti-inflammatory, antimicrobial, antiviral, antiproliferative, antimetastatic, hypotensive, hypocholesterolemic, immune-modulating, vasodilative, anti-mutagenic, and antitumor activities (Israili 2014; Samarghandian et al. 2017; Lusby et al. 2002; Masalha

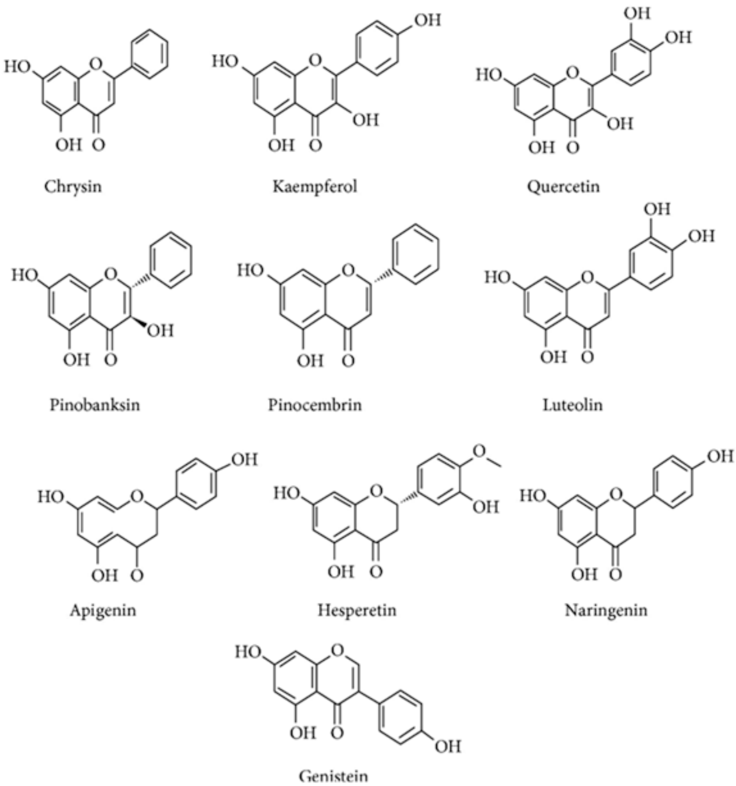

Fig. 7.1 Chemical structures of flavonoids in honey

et al. 2018; Ahmed and Othman 2013b; Cook and Samman 1996; Erejuwa et al. 2012; Kenjerić et al. 2008; Kassim et al. 2009, 2010; Al-Mamary et al. 2002; Gomes et al. 2010; Irish et al. 2006; Rakha et al. 2008; Ferreira et al. 2009; Gannabathula et al. 2012; Frankel et al. 1998; Mckibben and Engeseth 2002; Wang et al. 2002; Gribel and Pashinskii 1990; Al-Waili 2004a; Miguel et al. 2017; Cianciosi et al. 2018). The various flavonoids and phenolic acids present in honey are depicted in the Figs. 7.1 and 7.2 (Table 7.3).

7.2 Biological and Medicinal Effects of Honey

The biological and medicinal effects of honey depend heavily upon the bioavailability of its various constituents especially the phytochemical compounds, as well as their mode of absorption and metabolism (Israili 2014; Samarghandian et al. 2017; Ajibola et al. 2012). Since there is a huge diversity of secondary metabolites in plants which are usually used as food by bees, this variance concomitantly affects the phytochemical profiles in honey as well (Nicolson et al. 2007). Because of its varied constituents being actually accumulated by honeybees from various plant

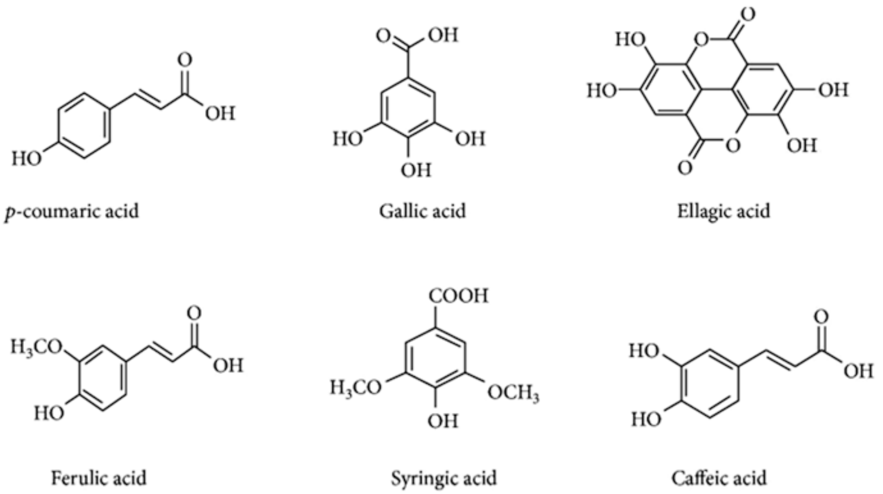

Fig. 7.2 Chemical structures of phenolic acids in honey

Table 7.3 Common phenolic acids and flavonoids in different honeys

Phenolic acids	Formula	Flavonoids	Formula
2-*cis*,4-*trans* Abscisic acid	$C_{15}H_{20}O_4$	Apigenin	$C_{15}H_{10}O_5$
2-Hydroxycinnamic acid	$C_9H_8O_3$	Catechin	$C_{15}H_{14}O_6$
Caffeic acid	$C_9H_8O_4$	Chrysin	$C_{15}H_{10}O_4$
Chlorogenic acid	$C_{16}H_{18}O_9$	Galangin	$C_{15}H_{10}O_5$
Cinnamic acid	$C_9H_8O_2$	Genistein	$C_{15}H_{10}O_5$
Ellagic acid	$C_{14}H_6O_8$	Isorhamnetin	$C_{16}H_{12}O_7$
Ferulic acid	$C_{10}H_{10}O_4$	Kaempferol	$C_{15}H_{10}O_6$
Gallic acid	$C_7H_6O_5$	Luteolin	$C_{15}H_{10}O_6$
p-Coumaric acid	$C_9H_8O_3$	Myricetin	$C_{15}H_{10}O_8$
p-Hydroxybenzoic acid	$C_7H_6O_3$	Pinobanksin	$C_{15}H_{12}O_5$
Protocatechuic acid	$C_7H_6O_4$	Pinocembrin	$C_{15}H_{12}O_4$
Sinapic acid	$C_{11}H_{12}O_5$	Quercetin	$C_{15}H_{10}O_7$
Syringic acid	$C_9H_{10}O_5$	Rutin	$C_{27}H_{30}O_{16}$
Vanillic acid	$C_8H_8O_4$		

Cianciosi et al. (2018), Samarghandian et al. (2017), Escuredo et al. (2013) and Erejuwa et al. (2012)

sources, honey has been referred to as the rediscovered remedy and identified by many researchers as one of the best source of dietary antioxidants (Ahmed et al. 2018; Erejuwa et al. 2012, Khalil et al. 2011; Nicolson et al. 2007).

7.2.1 Antioxidant Effects

Antioxidants are identified as the agents which neutralize the deleterious effects of the oxidative substances/chemicals. Free radical species are derived either directly from oxygen called as reactive oxygen species (ROS) like superoxide (O_2^-), hydroxyl (OH^-), hydrogen peroxide (H_2O_2), or nitrogen called as reactive nitrogen species (RNS) like nitric oxide (NO), peroxynitrite ($ONOO^-$) or from lipids like alkoxy (RO), peroxyl (ROO), lipid hydroperoxide (LOOH) radical. Usually these substances cause oxidative stress due to excessive generation of free radical species beyond the capacity of the antioxidant defense system to sequester them and hence result in the oxidative damage (Lobo et al. 2010).

Generally, the generated free radicals are highly reactive and unstable. They contain an unpaired electron, therefore behave as oxidants or reductants (Pisoschi and Pop 2015). Free radicals can damage almost all biological molecules like carbohydrates, lipids, proteins, and nucleic acids (DNA) (Lobo et al. 2010). Oxidative stress has been reported to make a significant impact on the etiology of all inflammatory diseases (arthritis, adult respiratory diseases syndrome, glomerulonephritis, lupus erythematous, vasculitis), atherosclerosis, alcoholism, aging, asthma, acquired immunodeficiency syndrome, cancers, diabetes, emphysema, gastric ulcers, hemochromatosis, hypertension and preeclampsia, ischemic diseases (heart diseases, intestinal ischemia, stroke), organ transplantation, neurological disorder (Alzheimer's disease, Parkinson's disease, muscular dystrophy), nephritis, smoking-related diseases, rheumatoid arthritis and osteoarthritis, and many other diseases (Finkel and Holbrook 2000; Lobo et al. 2010; Rahal et al. 2014; Nguyen et al. 2019).

Nature has bestowed every organism with some well-developed self-defense mechanisms to counteract the deleterious impact of free radicals constituting a direct repairing, physical defense, and antioxidant systems (Rahal et al. 2014; Nguyen et al. 2019). An antioxidant system within an individual is of two categories: enzymatic and non-enzymatic. Enzymatic antioxidants are represented by an interacting network of three main enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). This detoxification system operates primarily with SOD catalyzing the initial step and then various peroxidases removing hydrogen peroxide in conjunction with catalases (Sies 1997). Nonenzymatic antioxidants include all chemicals having capacity to quench the radicals by directly interacting with them by donating an electron or hydrogen and include ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, ubiquinol, flavonoids, and other antioxidants (Lobo et al. 2010). (Table 7.4 shows various free radicals and the antioxidants which act upon them and Fig. 7.3 shows various antioxidant preset in nature.)

Table 7.4 Various radical species and the antioxidants that act upon them for quenching

Radical species		Antioxidants
$^1O_2$	Singlet oxygen	Vitamin A, β-carotene, vitamin E
$O_2^{\cdot-}$	Superoxide	Superoxide dismutase, β-carotene, vitamin E
$OH^{\cdot}$	Hydroxyl	
$RO^{\cdot}$	Alkoxyl	
$ROO^{\cdot}$	Peroxyl	Vitamin C, vitamin E
H_2O_2	Hydrogen peroxide	Catalase, glutathione peroxidase
LOOH	Lipid peroxides	Glutathione peroxidase
NO	Nitric oxide	Glutathione peroxidase

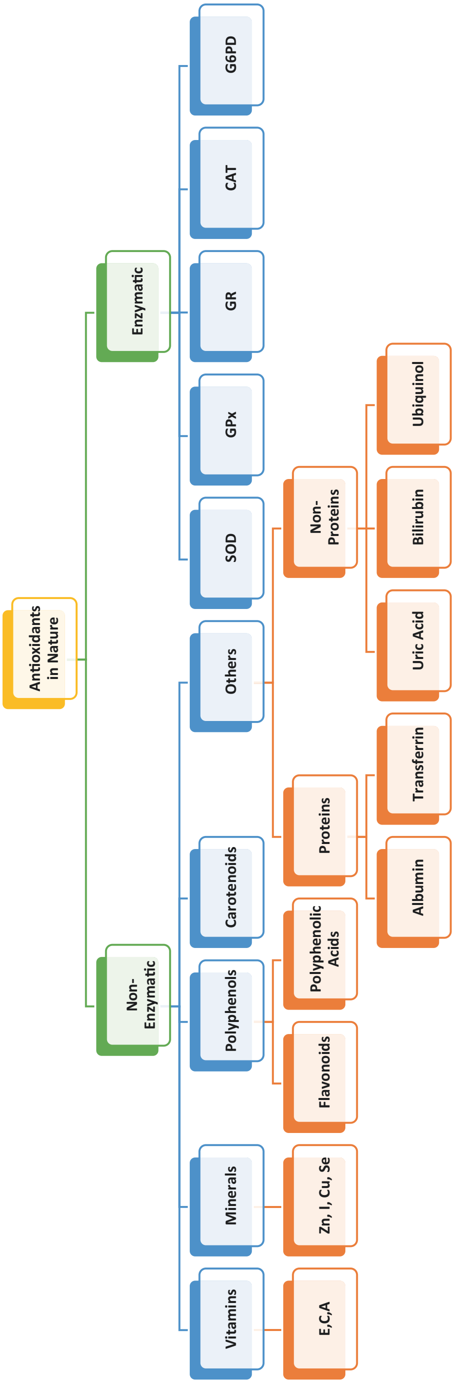

Fig. 7.3 Various antioxidants found in nature

As already mentioned, honey is a balanced concoction of a wide range of active organic molecules—vitamins and phytochemicals, and these have been recognized to be mainly responsible for its antioxidant capacity (AOC) (Ahmed and Othman 2013a; Rice-Evans and Miller 1996). Usually, these molecules act in a synergistic manner to scavenge the free radicals by forming more stable and less toxic molecules (Gheldof and Engeseth 2002; Musa Özcan and Al Juhaimi 2015). Furthermore, phenolic compounds within honey are responsible for the radiation absorbance and hence its color and brightness and in this regard it has been reported that darker honey has higher antioxidant value/concentration (Gheldof and Engeseth 2002; Musa Özcan and Al Juhaimi 2015; Samarghandian et al. 2017).

Numerous studies have been reported on the protective effects honey plays during oxidative stress. The two important mechanisms, reported by researchers, through which honey exerts its protective effect against free radical damage is via (1) antioxidant enzymes (such as SOD, catalase) and (2) numerous phenolic compounds which scavenge or trap free radical species and thereby induce cellular antioxidant systems, both enzymatic and nonenzymatic (Ahmed et al. 2018; Alvarez-Suarez et al. 2014; Erejuwa et al. 2014; Musa Özcan and Al Juhaimi 2015).

Considerable literature is available on the AOC for a wide variety of honeys available from different geographical and botanical origins (Afroz et al. 2016; Escuredo et al. 2013; Estevinho et al. 2012; Pontis et al. 2014; Alvarez-Suarez et al. 2018; Bertoncelj et al. 2007; Socha et al. 2009; Ulloa et al. 2015).

Phenolic acids (carboxylic acid derivatives of phenol) consist of two main parts—a phenolic ring and one functional group, at least, of organic carboxylic acid. They are further categorized depending upon the structure as: C6-C1 structure (e.g., syringic, vanillic, and gallic acids), C6-C2 (e.g., acetophenones and phenylacetic acids), and C6-C3 (e.g., p-coumaric, ferulic, and caffeic acids). Mostly, phenolic compounds are attached to the structural constituents/molecules of the plant (cellulose, lignin) and to other forms of organic molecules like glucose, other sugars, and/or flavonoids (Padayachee et al. 2012) (Fig. 7.1).

The second class of active antioxidant molecules in honey are flavonoids, which are natural low molecular weight and water-soluble chemical compounds. They contain two benzene rings, alternated by a three-carbon linear chain (C6-C3-C6). This structure is often arranged in the form of three rings with 15 carbon atoms called A, B, and C (Fig. 7.2). Generally, flavonoids have at least two phenolic groups (OH) and are often linked with sugars to exist as glycosides, which make flavonoids water soluble. The sugars involved in glycoside formation include mainly glucose and also arabinose, galactose, glucorhamnose, rhamnose, rutinose and xylose. If they are not associated with sugars, they are referred to as aglycones and are therefore further classified as per the degree of oxidation of the C ring as anthocyanins, anthocyanidins, flavones, flavanols, flavonols, flavanonols, flavanones, and isoflavones. The amplest flavonoids found in honey are flavones, flavanols, and flavonols (Moniruzzaman et al. 2014) (Fig. 7.2).

Although the exact antioxidant mechanism possessed by honey is not fully known, researchers have demonstrated that consumption of honey (1.2 g/kg) elevated both amount and activity of the constituting antioxidant agents like β-carotene, vitamin C, vitamin E, and glutathione reductase in healthy individuals (Al-Waili 2003). A number of different possibilities for the antioxidant effects of honey have been proposed which include sequestration of free radicals, donation of hydrogen, metallic ion chelation, hydroxyl ion capture by flavonoids, and superoxide dismutase activity (Al-Mamary et al. 2002; van Acker et al. 1996; Ahmed et al. 2018). The AOC of honey can be measured as antiradical activity using number of standardized assays like oxygen radical absorbance capacity (ORAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging, and ferric reducing antioxidant power (FRAP) (Erejuwa et al. 2012).

Additionally, both enzymatic and nonenzymatic antioxidant constituents of the honey have been reported to act at distinct cellular levels to prevent oxidation of the essential macromolecules and/or activating gene expressions, which is otherwise known to provoke an antioxidant response (Ahmed et al. 2018; Musa Özcan and Al Juhaimi 2015). Also, phytochemicals present in honey, especially polyphenols, can activate intracellular signaling producing a wide range of second messengers and activated enzymes like mitogen-activated protein kinases (MAPKs), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), tyrosine kinases, protein kinase B (PKB)/Akt, and protein kinase C (PKC) which have a protective effect in the cells (Torre 2017). Figure 7.4 shows various identified mechanisms for the antioxidant effects of honey.

Fig. 7.4 Identified mechanisms for the antioxidant effects of honey

7.2.2 Antibacterial Effects

Antibacterial effect of honey is accredited to the existence of numerous inert antibiotic factors in it. These include both physical aspects and chemical constituents (Cushnie and Lamb 2005). The physical factors are its low water activity (Aw), acidic/low pH, high osmotic pressure (because of sugars), low protein content, high carbon to nitrogen ratio, low redox potential due to high content of reducing sugars, and viscosity that limit dissolved oxygen and all of these prevent bacterial growth (Tan et al. 2009;). In addition to these physical properties, the glucose oxidase enzyme system, presence of flavonoids, phenolic acids like pinocembrin, syringic acid, terpenes, and lysozyme (Agbaje et al. 2006; Cianciosi et al. 2018) also contribute towards antibacterial properties of honey. A substantial antibiotic role in honey is because of bee defensin-1 (antimicrobial peptide), peroxidases which produce hydrogen peroxide (H_2O_2), methylglyoxal (phytochemical), and glucose oxidase and catalase enzymes (Mandal and Mandal 2011). Defensin-1 is one of the antimicrobial peptides (AMP) among others like apidaecin, abaecin, and hymenoptaecin, all of which are present in bee hemolymph and hypopharyngeal glands (6). Its bactericidal properties are due to its ability to disrupt bacterial cell membrane by creating holes in it and via stimulation of MMP-9 secretions from keratinocytes (Ganz 2003; Bucekova et al. 2017). Table 7.5 lists some of the common polyphenolic compounds present in honey together with their mechanism of action.

Interestingly, it has been observed that the dilution of honey increases its antibiotic effect by H_2O_2 because of direct effect on the ability of glucose oxidase enzyme to effectively bind to the glucose and produce a steady source of H_2O_2 (Brudzynski 2006). The levels of H_2O_2 in any form of honey is dependent upon the source of the nectar on which bees feed to produce honey which in turn affects the two critical factors—the content of catalase and the extent of glucose oxidase (Brudzynski et al. 2011, 2017). There are numerous other mechanisms which have been identified for the role played by H_2O_2 as antimicrobial agent. It has been shown that H_2O_2 also mediates its effect via stimulating insulin receptor (IR) which is essential for the uptake of glucose and amino acids required for the cellular growth and proliferation especially of monocytes and lymphocytes. As honey can serve as the best source of essential biomolecules, it provides critical energy for phagocytes to engulf the bacteria (Abuharfeil et al. 1999). Figure 7.5 shows the various identified mechanisms for the antibacterial effects of honey.

Furthermore, honey which is devoid of H_2O_2 (after its removal) still possesses the significant antimicrobial activity referred to as the nonperoxide activity, which is attributed to the presence of numerous other active substances. One of these nonperoxide and highly reactive class of compounds is 1,2-dicarbonyls, which are generated through caramelization or Maillard reactions in the carbohydrate-rich foods (Arena et al. 2011; Degen et al. 2012). These compounds are produced as intermediates of nonenzymatic reaction between glucose and free amino groups which form advanced glycation end products (AGEs). Those produced from hexoses are 3-deoxyglucosone (3-DG) and glucoson while those derived from disaccharides and oligosaccharides are 3-deoxypentosone (3-DP) (Schalkwijk et al. 1999; Arena et al. 2011; Degen et al. 2012).

Table 7.5 Common polyphenolic compounds found within honey and their antimicrobial mechanism of action

Phenolic acids	Mechanism	Flavonoids	Mechanism
Caffeic acid	Oxidative stress	Apigenin	Inhibits DNA gyrase
Chlorogenic acid	Increase in membrane permeability resulting in cytoplasmic and nucleotide leakage	Catechin	Hydrogen peroxide generation
Ferulic acid	Cell membrane dysfunction and changes in cell morphology	Chrysin	Inhibits DNA gyrase
Gallic acid	Cell membrane disruption resulting in pore formation and intracellular leakage	Galangin	Inhibition of peptidoglycan and ribosome synthesis
p-Coumaric acid	Cell membrane disruption and binding to bacterial DNA	Genistein	Disruption to topoisomerase-II DNA cleavage complex
Syringic acid	Cell membrane dysfunction	Isorhamnetin	Unknown
2-*cis*, A-*trans* Abscisic acid	Unknown	Kaempferol	Inhibits DNA gyrase
2-Hydroxycinnamic acid	Unknown	Luteolin	Inhibits FAS-I in mycobacteria and inhibits DNA helicase DnaB and RecBCD
Cinnamic acid	Unknown	Myricetin	Inhibits DNA B helicase
Ellagic acid	Unknown	Pinocembrin	Induces cell lysis
p-Hydroxybenzoic acid	Unknown	Quercetin	Disrupts membranes, transport, and motility
Protocatechuic acid	Unknown	Rutin	Induces topoisomerase IV–mediated DNA cleavage
Sinapic acid	Unknown	Naringenin	Unknown
Vannilic acid	Unknown	Pinobanksin	Unknown

Nolan et al. (2019), Cianciosi et al. (2018), Samarghandian et al. (2017), Erejuwa et al. (2012) and Escuredo et al. (2013)

Methylglyoxal (MGO) and its synthesis precursor dihydroxyacetone (DHA) are both active bacterial growth inhibitors working via urease inhibition, which otherwise facilitates bacteria to acclimatize and grow swiftly by ammonia production in low pH environment (Rückriemen et al. 2017). MGO is the chief antimicrobial constituent in manuka honey and is clinically used to rate honey as “Unique Manuka Factor” (UMF) directly related to its percentage in honey (Roberts et al. 2015). MGO is produced from dihydroxyacetone by either nonenzymatic or enzymatic (by methylglyoxal synthase) conversion (Adams et al. 2009). It has been demonstrated that the amino acid additions (of arginine and lysine) can enhance the production of MGO within honey, so does heating it to 37 °C (Adams et al. 2009; Johnston et al. 2018). MGO works by altering the structure of bacterial motility proteins in fimbriae and flagella, thereby limiting them (Rabie et al. 2016).

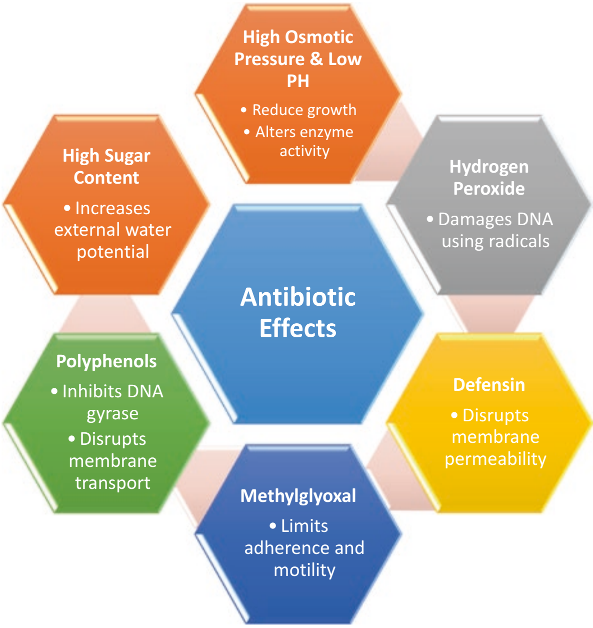

Fig. 7.5 Identified mechanisms for the antibiotic effects of honey

Furthermore, a recent report revealed that honey utilizes two main mechanisms to fight bacterial infections: first by inhibiting the bacterial quorum sensing (QS) system which hinders the expression of various gene regulons like las, MvfR, and rhl, and its associated virulence factors and second by its bactericidal constituents which aggressively kill bacterial cells (Wang et al. 2012). Since honey also contains some quantities of propolis and pollen, a part of its antibacterial activity can also be attributed to these antimicrobial constituents (Viuda-Martos et al. 2008; Redzic et al. 2011).

For any drug to be antibiotic, a minimum inhibitory concentration (MIC) is necessary to be possessed which is tolerant to the cells as well. It is defined as the lowest concentration of an antimicrobial (like an antifungal, antibiotic, or bacteriostatic) drug that inhibits the observable growth of any microorganism after an overnight incubation (Israili 2014). Numerous researchers have reported that the antibacterial activity of honey is equivalent to the minimum inhibitory concentration (MIC), and hence, for the complete growth inhibition of microorganisms, only minimum concentration is required (Samarghandian et al. 2017).

Honey exhibits both bacteriostatic and bactericidal capacities against a wide range of Gram-positive and Gram-negative bacteria (Tan et al. 2009; Alvarez-Suarez et al. 2010; Chang et al. 2011; Israili 2014). Furthermore, honey having a monofloral origin is found to possess potent antibacterial activity than others, and some pathogens are more susceptible than others to a certain type of monofloral honey (Al-Waili 2004b; Lee et al. 2008; Tan et al. 2009; Kumar et al. 2010; Sherlock et al.

Table 7.6 List of microorganisms that have been found to be sensitive to honey

Gram-positive strains	Gram-negative strains
Streptococcus pyogenes	*Stenotrophomonas maltophilia*
Coagulase negative staphylococci	*Acinetobacter baumannii*
Methicillin-resistant Staphylococcus aureus (MRSA)	*Salmonella enterica Serovar typhi*
Streptococcus agalactiae	*Pseudomonas aeruginosa*
Staphylococcus aureus	*Proteus mirabilis*
Coagulase-negative Staphylococcus aureus (CONS)	*Shigella flexneri*
Hemolytic streptococci	*Escherichia coli*
Enterococcus	*Enterobacter cloacae*
Streptococcus mutans	*Shigella sonnei*
Streptococcus sobrinus	*Salmonella typhi*
Actinomyces viscosus	*Klebsiella pneumonia*
	Stenotrophomonas maltophilia
	Burkholderia cepacia
	Helicobacter pylori
	Campylobacter spp.
	Porphyromonas gingivalis

Alvarez-Suarez et al. (2010), Chang et al. (2011) and Israili (2014)

2010; Voidarou et al. 2011; Kwakman et al. 2011; Mandal and Mandal 2011; Cooper and Jenkins 2012). Table 7.6 lists various bacterial strains which are limited by honey.

Bactericidal activities of monofloral origin honeys against many pathogens like S. aureus, P. aeruginosa, *Streptococcus mutans*, and MRSA are demonstrated to be because of numerous mechanisms, a few of which are extensive cellular disruption affecting structural integrity, prevention of cell separation, producing cells with cross-walls, thereby preventing the normal growth and progression, enhanced lysis of cells, affecting normal cell shapes, blocking the attachment of bacteria to tissues, inhibiting formation of biofilms, downregulation of stress protein A of MRSA, and reducing expression of fibronectin-binding proteins (Alnaqdy et al. 2005; Henriques et al. 2010, 2011; Kwakman et al. 2011; Jenkins et al. 2010; Jenkins and Cooper 2012; Maddocks et al. 2012; Nassar et al. 2012).

In addition to using honey alone, it is also used synergistically with numerous antibiotics like gentamicin, amikacin, ceftazidime, methylglyoxal pipercillin, carbenicillin, or amikacin (Karayil et al. 1998; Al-Jabri et al. 2005; Mukherjee et al. 2011), especially to reverse the bacterial resistance, e.g., oxacillin-resistant Gram-negative MRSA, vancomycin-resistant Enterococcus (Jenkins and Cooper 2012; Boukraâ and Sulaiman 2009; Mandal and Mandal 2011; Israili 2014). Also, it has been reported that honey's antibiotic spectrum against various resistant isolates of Burkholderia cepacia, E. coli, Enterococcus faecium, P. aeruginosa, S. epidermidis, S. aureus, MRSA, and β-lactamase-producing E. coli gets enhanced and broadened by the addition of some compounds like synthetic peptide "Bactericidal Peptide 2" (Kwakman et al. 2011), starch (Boukraâ and Sulaiman 2009), royal jelly (Boukraâ 2008), or thyme (*Thymus ciliatus*) powder (Abdellah et al. 2012).

One of the important medical areas where honey's antibacterial activity is extensively utilized is in the management of wounds. Honey is among one of the ancient medicines used in treating infected wounds, and nowadays, it is used in medical field especially in conditions where conventional therapeutic medicine fails (Minden-Birkenmaier and Bowlin 2018). Historically, honey's use find mention in a Sumerian tablet of 2100–2000 BC where it is referred to as an ointment. Aristotle (384–322 BC) reported honey as "*good as a salve for sore eyes and wounds*" (Mandal and Mandal 2011). Topical application of honey has been demonstrated to rapidly clear and heal deep surgical wound infections to facilitate the healing process. For highly infected wounds that are resistant to the conventional therapy of antibiotics and antiseptics, honey has been shown to promote quick healing (Ahmed et al. 2003).

Normal wound healing is a multistep process which includes several events taking place concomitantly with each other like coagulation, cell proliferation, inflammation, tissue remodeling, and replacement of injured tissue (Falanga 2005). Honey has been extensively and effectively used in clinical practice to manage simple wounds, burns, various ulcers, necrotic tissues, diabetic foot, and postoperative split skin wounds (Visavadia et al. 2008; Cianciosi et al. 2018; Ahmed et al. 2018). Honey can sterilize the wounds, stimulates tissue re-growth, rapidly clears infection, enhances debridement, suppresses inflammation, stimulates angiogenesis, tissue granulation, and epithelial growth while reducing edema and scar formation (Falanga 2005; Visavadia et al. 2008; Lee et al. 2011a; Lund-Nielsen et al. 2011a; Orey 2011; Efem et al. 1992; Vardi et al. 1998; Molan 1999, 2001, 2006; Moore et al. 2001; Lusby et al. 2002; Ingle et al. 2006; Boukraâ and Sulaiman 2010; Al-Waili et al. 2011a; Kegels 2011; Sioma-Markowska 2011; Smaropoulos et al. 2011; Jull et al. 2013; Biglari et al. 2013; Mohamed et al. 2015).

Honey helps in eliminating necrotic tissues of the wound, improves its remodeling, and furthermore prevents bacterial growth within it which is critical for healing process (Koenig and Roh 2016). Honey-coated dressing has been reported to be effective in reducing morbidity linked with first- and second-degree burns as well as in aiding to reduce the rehabilitation time (Baghel et al. 2009; Wijesinghe et al. 2009). Also, bandages coated with manuka honey were reported to be as effective as the silver-coated bandages in decreasing and limiting the size of malignant wounds (Lund-Nielsen et al. 2011b).

Recently, honey has been found to cause enhanced activation and production of monocytes, lymphocytes, phagocytes, and/or macrophages which affect the secretion of numerous cytokines like IL-1β, IL-6, and TNF-α, thereby expediting the healing process (Lin et al. 2003;). It has also been found to activate expression and secretion of IL-6 and TNF-α in IL-6-deficient mice at the injury site which enhances the healing process (Tonks et al. 2007; Molan and Rhodes 2015). Honey's high sugar composition and osmolarity play a pivotal role in healing process, as osmotic effect pulls out the water from the wound bed through the outflow of lymph, enhanced by the effective blood circulation at the wound site. Honey is also directly involved in the ameliorative effects during the oxidative stress by activating 5′- adenosine monophosphate-activated protein kinase (AMPK) and antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). These enzymatic antioxidants are known to initiate proliferation and exodus of dermal fibroblasts as well as to enhance the mitochondrial function to assist in wound healing (Molan and Rhodes 2015; Alvarez-Suarez et al. 2018).

One of the pivotal factors in antibiotic resistance is the ability of microbes to make biofilms (a layer of extracellular matrix after adhesion to surface), which protect them from killing and static effects of antibiotics (Stewart and Costerton 2001). The antibiofilm capability is because of honey's ability to disrupt the quorum sensing in the biofilm itself (Minden-Birkenmaier and Bowlin 2018). Honey has been demonstrated to be able to penetrate the biofilms and in turn recover the aggressive infection by eradicating bacterial colonies. Numerous biofilms of pathogenic strains like extended-spectrum beta-lactamases (ESBL), Clostridium difficile, *Klebsiella pneumonia*, methicillin-resistant Staphylococcus epidermidis (MRSE), Pseudomonas aeruginosa, Proteus mirabilis, Pseudomonas aeruginosa (PA), Staphylococcus aureus (SA), and enterohemorrhagic E. coli have been demonstrated to be limited or eradicated by the application of honey (Merckoll et al. 2009; Halstead et al. 2017). Honey acts to prevent the attachment of bacterial strains with the fibronectin of the tissue in the wound, thereby halting biofilm growth and in addition limiting the expression of fibronectin binding surface proteins like Sfb1 and Sof, both of which are key requirements for binding of bacteria to the fibronectin (Maddocks et al. 2012). It has also been shown to suppress the expression of three critical proteins: curli genes (csgBAC), quorum sensing genes (AI-2 importer and indole biosynthesis), and virulence genes (LEE genes) in E. coli to limit its virulence. High sugar content of honey has also been shown to play critical role in repressing biofilm formation (Lee et al. 2011b).

7.2.3 Antifungal Properties

Honey has been reported to exhibit wide spectrum of antifungal activity which is equivalent to numerous pharmaceutical antifungal preparations (Israili 2014). Maria et al. reported in vitro antifungal activity of honey after observing the limitation of growth of *Candida albicans*, *Candida krusei*, and *Cryptococcus neoformans* by the application of honey (Maria et al. 2011). It was also reported that honey distillate inhibited the resistant strains of *C. albicans* (Obaseiki-Ebor and Afonya 1984). Honey has antifungal activity against *Aspergillus flavus, Aspergillus niger, Candida albicans, Microsporum gypseum, Malassezia species*, *Penicillium chrysogenum*, and *Saccharomyces* (Anyanwu 2012). The potential antifungal effect of honey is due to the presence of three active systems: glucose oxidase and H_2O_2 production, high sugar contents and osmotic pressure, and methylglyoxal (Cushnie and Lamb 2005; Kwakman et al. 2010; Al-Waili et al. 2011b). Al-Waili et al. (2011b) have reported that honey in concentrations of 30%–50% is inhibiting the growth of C. albicans. Similarly, Irish et al. (2006) found that various honeys had antifungal activity against *C. albicans*, *Candida glabrata*, and *Candida dubliniensis*. Also, Khosravi et al. (2008) found that honey has antifungal activity

Fig. 7.6 Identified mechanisms for the antifungal effects of honey

against C. albicans, C. dubliniensis. C. glabrata, C. kefyr, C. parapsilosis, and C. tropicalis. Figure 7.6 lists various identified mechanisms for the antifungal effects of honey.

Although the actual mechanism of how honey limits the fungal growth is not well known, several theories have been proposed. Some of the important theories of which include as, by preventing formation of biofilm, disrupting the established biofilms, changing exopolysaccharide structure, distorting integrity of cell membranes, shrinking cellular surface, retarding growth, and enhancing apoptotic pathways (Ahmed et al. 2018; Ahmed and Othman 2013a; Moussa et al. 2012; Cancliracci et al. 2012; Khosravi et al. 2008). Many reports have demonstrated that flavonoid constituents of honey negatively affect the fungal growth, by inhibiting critical cellular processes essential for the growth of germ tube as well as affecting external cellular membrane morphology and integrity. Additionally, flavonoid extract affects the hyphal transition by arresting the viable cells in the G_o/G_1 phase and/or G_2/M phase (Canonico et al. 2014).

Even though numerous honeys derived from various resources have demonstrated potent antibacterial activities, this however does not necessarily mean that they do possess antifungal activity as well. Manuka honey although possessing a potent antibacterial activity has a weak activity against fungus like *C. albicans* and *dermatophytes* (Brady et al. 1996; Anand et al. 2019a). *C. albicans* is known to cause candidiasis while most fungal skin infection in humans are caused by *dermatophytes* like T. mentagrophytes and T. rubrum. Also, athletes' foot (*tinea pedis*), jock itch (*tinea cruris*), ringworm of scalp, nail, face, and hand (*tinea corporis*) are also results of dermatophyte infections (Havlickova et al. 2008; Anand et al. 2019b). Recently, various honey varieties including Agastache, tea tree honey, and manuka honey were demonstrated to be effective in countering dermatophytes (*T. mentagrophytes* and *T. rubrum*) and C. albicans.

Agastache honey was most effective against *dermatophytes* (zone diameter, 19.5–20 mm) and *C. albicans* at 40% concentration while tea tree and manuka honey were effective at 80% (Anand et al. 2019b). Furthermore, Moussa et al. (2012) reported the antifungal action of various honey's against *C. albicans* and Rhodototorula sp. Generally, *C. albicans* was more susceptible to get inhibited by all varieties of honeys than the dermatophytes (Anand et al. 2019b). However, it was reported that fluconazole-resistant *C. albicans* was inhibited by Turkish honey (Rhododendron, Orange and Eucalyptus) in a concentration range of about 40–80% (MIC values) (Koc et al. 2009). Also, jujube honey (*Zizyphus spina-christi*) has potent antifungal properties against *C. albicans* at 40% (w/v) (MIC) and could effectively impede the formation of *C. albicans* biofilms and lead to disruption of established biofilms (Ansari et al. 2013).

The antifungal activity of Agstache honey is attributed to numerous volatile organic compounds in it. Some of the major compounds reported are as: benzaldehyde, estragole (12.31%), ethyl ester (5.68%), hexadecanoic acid, phenol, 2,4-bis(1,1-dimethylethyl) (12.77%), nonanoic acid, ethyl ester (7.22%), 2-propenoic acid, 3-phenyl-, ethyl ester (6.32%), 4 methoxy (5.17%), β-Caryophyllene (4.67%), nonanal (3.19%), and 2H-benzimidazol-2-one, 1,3-dihydro-5-methyl- (2.34%). In addition, Agstache honey was reported to contain limonene at a concertation of 0.11% and trace amounts of menthone, pulegone, methyl eugenol (Yamani et al. 2014).

7.2.4 Anti-Viral Properties

In literature, a limited number of studies are present who have reported the antiviral activity of honey. The earliest and important study on the antiviral effects of honey were on varicella zoster virus (HZV) infected human malignant melanoma (MeWo) cells, which reported the reduction of viral plaques by treatment with manuka and clover honey (Shahzad and Cohrs 2012). Similarly, experiments on influenza virus (H1N1)–infected Madin–Darby canine kidney (MDCK) cells also demonstrated the inhibitory effects of various types of honey especially for manuka honey which exhibited higher antiviral activity synergistically in combination with numerous antiviral compounds (Watanabe et al. 2014). Furthermore, manuka honeys have been found to be successful against rubella virus and herpes simplex virus (HSV-1) in vitro (Zeina et al. 1996; Ghapanchi et al. 2011; Hashemipour et al. 2014). Additionally, honey has been reported to heal herpetic lesions effectively especially occurring in labial and genital sites owing to its ability of inhibiting prostaglandins at the affected site (Al-Waili 2004c). A recent research report has extensively described honey's antiviral activity against respiratory syncytial virus (Feás and Estevinho 2011). Figure 7.7 lists various identified mechanisms for the antiviral effects of honey.

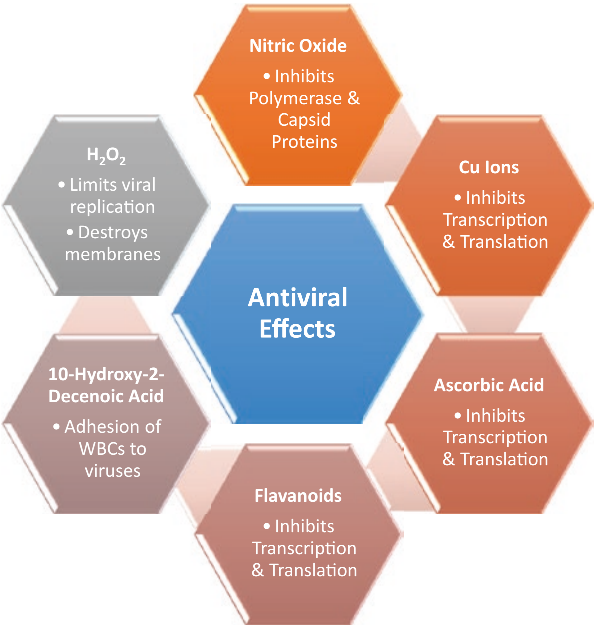

Fig. 7.7 Identified mechanisms for the antiviral effects of honey

Since honey does contain various secretions from honeybee's salivary and pharyngeal glands, it has been found to contain high concentrations of nitric oxide (NO) metabolites, nitrite, and nitrate (Al-Waili 2003). NO is reported to be responsible for the provision of host defense against both DNA- and RNA-based viruses, by preventing their replication. Thus, NO can slow down the development of viral lesions especially in the genital regions (Al-Waili 2003; Al-Waili and Boni 2003). In its identified mechanism of action, NO not only represses replication by interfering with viral polymerase but also inhibits the translation and assembly of viral capsid proteins. The flavonoid, copper, H_2O_2, and ascorbic acid present in honey have also been reported to prevent viral transcription and replication, thereby inhibiting their life cycle (Miguel et al. 2017; Ahmed et al. 2018; Khan et al. 2018). For royal jelly honey, antiviral activity has been credited to the activity of 10-hydroxy-2-decenoic acid (10-HAD), which is known to stimulate white blood cells (WBCs), resulting in their adhesion to viruses culminating in their destruction (Shahzad and Cohrs 2012).

7.3 Conclusion

As discussed extensively, honey can be described as the miracle food which besides being a power-packed diet also has an extensive medicinal property. It is extensively used as a kind of panacea since ages for a wide variety of diseases, and its antioxidant, antibacterial, antifungal, and antiviral properties have been well established in literature. However, there are some adverse effects of using honey also, which are overshadowed in literature, which may be specifically associated with the contaminants present in it (Israili 2014). These contaminants are usually from floral source, environment, or microbes. This is beyond the scope of this chapter to discuss them in detail. Also, as there is no prescribed or effective therapeutic dose of honey for adults, due care and diligence are required when honey is consumed for treating chronic ailments especially in diabetes, gastrointestinal problems, or treating wounds. This is one of the challenging aspects in using honey. The quality, efficacy, dosage, and formulations are major challenges in standardizing its medical and clinical usage.

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8 Traditional and Modern Applications of Honey: An Insight

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Abstract

Honey has been used for its nutritional and medicinal values since the Stone Age. Being one of the oldest foods known to humans, honey as a natural product has become an important part of food, economy and health care for most of the population. Honey stands as the most vastly discussed natural product across religions and civilizations. Traditional knowledge of these natural products has served as the base for many breakthrough discoveries, especially in the medicinal field. Today honey holds a strong position among its natural counterparts in terms of global market. This chapter provides an in-depth review of historical evidences of honey in different civilizations, religions and cultures, its use as an ethnomedicine, its application in different traditional system of medicine like Unani and Ayurveda, its physico-chemical properties, its modern application as antioxidant, antimicrobial, wound healing and antiviral agent, its application in ophthalmology, cough, diabetes and inflammation, intellectual properties and patent insights on honey, and industry and marketing insights of honey.

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_8

Keywords

Honey · Ethnomedicine · Natural products · Unani medicine · Ayurvedic medicine

8.1 Introduction

Since ancient times, humans have been using many natural products for their nutritional and medicinal values. These natural products have been an important part of food, economy and health care for most of the population. Traditional knowledge of these natural products has served as the base for many breakthrough discoveries especially in the medicinal field. One of the most vastly discussed natural product in many civilizations and religions around the globe is honey. Since Stone age, honey is being used by humans (Ajibola et al. 2012). Many cave paintings depicting humans and honey have been traced to be about 8000 years old. Different religious books like the Holy Quran, New and Old Testaments of the Bible, the Vedas, etc. have discussed the importance and use of honey for humans (Mcintosh 1995; Beck and Smedley 1997). These discussions in different scriptures undoubtedly prove that honey is not a new discovery and that its application as nutritive food and ethnomedicine goes way back to Before Christ times. However, still researchers are continuously studying honey for its modern medicinal application, and thus, it is still one of the most frequently used natural product of medicinal value in the world.

8.2 Honey in the Prehistory, Civilizations and Religion

Honey has been an important part of many ancient civilizations. Thus, it would not be wrong to state that honey has been used by humans since the earliest habitable days on earth. It has been indicated that honey has been considered highly nutritious in many primitive tribal diets (Zucoloto 2011). Honey with meat makes up 20% of the food for the Hadza of Tanzania (Woodburn 1963). People of Mbuti pygmies from Congo gets 80% of their total energy from honey during its season. Similarly, honey has been an important ingredient for Veddas of Sri Lanka (Crane 1983). As per the reports by Meehan, every Anbarraian of Australia consumes about 2 kg of honey annually (Meehan 1982). Honey has been an important part of diet and culture for Guayaki Indians of Paraguay. In Australia, both men and women take active part in the hunt for honey; however, in most of the countries, men are responsible for the same.

Many cultural evidences have been reported which suggests that honey was an important ingredient of food, therapeutic and religious source (Cartland 1970). In Egyptian civilization, honey was used as offerings to the gods and was also used for purposes like boat building, mummifying, etc. Honey was considered as a valuable gift to gods and was considered of highest devotion and worship. Honey filled in jars was buried with the coffins as part of sustenance for the life after death. In the tomb of pharaoh in Thebes, archaeologists have found pots made up of clay in which honey was kept. Similar honey pots were also reported by archaeologists from Tutankhamen's tomb. In Egypt, Mesopotamia and other regions, burying dead with honey was a common practice. Whether it is a rumour or fact, it is difficult to conclude about Alexander the Great being buried in honey. Honey was also used in the pharaoh's weddings (Hajar 2002). Greeks and Romans took this culture from the Egyptians which was passed to the Europe of the medieval period. In their culture, honey was taken for about a month after weeding for euphoria and weeding. Thus, came the word "honeymoon" from the culture, a custom of after-marriage ceremony still practised today. In the Roman culture, honey was used in wide range of liquors and dishes (Hajar 2002).

Both Islam and Christianity religions have endorsed honey (Zumla and Lulat 1989). Honey had been mentioned 54 times in the Holy Old Testament. In the Holy Quran, the Prophet pronounced that "Honey is a remedy for all disorder/diseases.". The Prophet stated that honey is not only an exquisite food but also an extraordinary healing molecule. Honey is considered as a talisman by the followers of Islam (Bogdanov 2009). Because of such outstanding characteristic, Imam Bukhari entitled chapter four of his Kitab al-Tibb (book of medicine) as "al-Dawa'bi al-Asal wa Qawlihi Ta'ala 'Fihi Shifa li al-Nas which means that treatment with honey and the statement of Allah: where is healing for men."

8.3 Honey as an Ethnomedicine

There are many records in ancient written books which confirms the application of honey as medicine for a wide variety of diseases (Ransome 1937). Many ancient books, tablets and scrolls excavated from different regions of the world especially from the Mesopotamia indicate that honey was prescribed and was a very common ingredient for a variety of illness (Hajar 2002). Honey was the most frequently used medicine in the Egyptian medicine and was reported to be used for both external and internal applications as listed by Edwin Smith Papyri and Ebers. As per Ebers Papyrus (1550 BC), honey was mentioned in 147 prescriptions for external applications. Also, as per Smith Papyrus (1700 BC), honey was also used in wound treatment. As described by Ebers Papyrus, honey was used to make ointments and had application in the surgical wounds of circumcision. He further added that ointment for ears can be made with two-thirds of honey and one-third of oil. Honey was also used to provide protection against bacteria and protect from different infections. Honey not only was used as medicine in Egypt but were also a common ingredient in Greek, Indian, Roman and Arabic medicine systems. *De Materia Medica* compiled by Dioscorides (AD 40–90) is one of most extensively compiled pharmacological text until fifteenth century. It has description of almost 1000 simple drugs and 600 plants with details about using milk and honey as medicinal and dietic value. As per Dioscorides, honey has the potential to treat stomach disease, haemorrhoids and cough. It is also helpful in treating swelling and pain of the ear. Gargling with honey reduces swelling of tonsil.

Germans were reported to use cod liver oils and honey for fistulas, boils, burns, ulcerations, etc. (Newman 1983). Ali Bin Hamzah AlBasri, a philosopher from Arab, recommended cooked honey as a good antidote for poisonous therapeutic molecules and induces vomiting, whereas raw honey for the treatment of swollen intestine. Al Razi famously known as Rhazes AD 864–932, a renowned physician, has written that honey ointment with vinegar and flour made with honey are good for nerve injuries, skin diseases and bladder wounds. Al Razi wrote the Encyclopaedia of Medicine known as Al Hawi which is one of the most comprehensive medicinal text which was translated to Latin from Arabic language in the early thirteenth century and was used as a standard medical text until the 1700s. Honey has been discussed as one of the best treatment for gum problems, is an extraordinary mouth wash and whitens teeth if it is rubbed on teeth. According to Ibn Sina (famously known as Avicenna), whose medical treatise is known as the Canon, was treated as a standard medical text by Arabs and Europeans until 1800s, describes honey as an active ingredient which prolongs life and has antiageing potential. He also stated that honey is beneficial for lung diseases like early stages of tuberculosis and was also used for insomnia. In *Veda*, a Hindu scripture, there are description of lotus honey for the treatment of eye diseases, as topical eye ointment for measles. *Shi Jing* is an ancient Chinese medical text dated sixth century BC, according to which honey is very beneficial for stomach and spleen (Bogdanov 2009). Honey has also been used in the African traditional medical system. It has been discussed to be used in leg ulcers, earaches, gastric ulcers and constipation (Molan 1999). Table 8.1 enlists the traditional applications of honey.

8.4 Honey in the Unani System of Medicine

In Unani system of medicine, honey is known as Asl Khalis. It is used in the treatment of many internal and external ailments. According to Unani system of medicine, the use of honey for treating diseases is as old as the history of mankind itself. Honey is the base for many Unani medicines acting as nutritive and preservative agent (Vohra and Khan 1979). Prophet Mohammed SAW (PBUH) considered honey for wound healing and treating diarrhoea (Banerjee et al. 2003). Honey does not support the growth of microorganisms due to its low water activity (Prescott et al. 1999). In ancient Unani medicines, honey was effectively used for the treatment of wounds. The modern medicinal studies have verified the superiority of honey on standard medical treatments for burns, skin ulcers and wounds.

The importance of honey can be understood by the fact that there is an entire chapter in the Holy Qur'an called al-Nahl (the Bee). According to one of the Hadith (teachings of Prophet Muhammad (PBUH) and recorded in Sahi Bukhari, the Prophet used to strongly recommend honey for healing purposes. The Prophet used to drink honey, i.e., a cup of water mixed well with a teaspoon of honey in the early morning before taking anything. Recent researches conclude that such drink activates the digestive system to work in an efficient way. Furthermore, honey has been reported to be far more superior to conventional antibiotics in treating infections and

Table 8.1 Traditional application of honey

Source	Use	Reference
Egyptian Civilization	Dressing for wounds, embalming fluid	Crane (1977)
Babylonian era	Tablet recipes based on honey, curative ointment, healing plasters	Davidson (2006)
Ebers papyrus (1550 BC)	Honey was mentioned in more than 140 external preparations for baldness, burns, sores, skin diseases like scurvy; also prescribed for circumcision surgery, suppository, inflammation and stiff joints	Jones (2001, Jones 2017)
Ancient Greeks	Regular intake for vigour and longer life	Lahanas (2016)
Aristotle (384–322 BC)	Sore eyes and wounds	Chepulis (2008)
Hippocrates of Kos (c. 460 to c. 370 BC)	Honey was used to clean sores, ulcers, sunning sores and heal carbuncles	Chepulis (2008)
Pedanius Dioscorides (c. 40–90 AD)	Treatment of wound	Dioscorides (2000)
Ali Ibn Al-Husain-al-Sina/Avicenna (980–1037)	Honey and myrrh were used to reduce the amount of exudates coming out of wounds	Kanal (1975)
Teodrico Borgognoni (1205–1296)	Cleaning of wound	Popp (1995)
Pliny the elder (Roman naturalist)	Detergent for cleaning ear	Pliny the Elder (1855)
Traditional Chinese medication	Infectious diseases, gastrointestinal diseases, allergic and immunologic disorders	Siu-Wan (2007)
Ayurvedic medical system	Sore throat, cold and cough, bronchial asthma, eye diseases, sleep disturbance, eczema, stomach ulcers, dermatitis, arthritis, diabetes mellitus, vomiting, hypertension, stress and fatigue, dehydration, hiccups, diarrhoea, polyuria, obesity, leprosy, weakness, bad breath, morning sickness, bed wetting, hemiplegia, burns, wounds and minor cuts, allergies and tooth pain	Ediriweera and Premarathna (2012)

without imparting any major side effect. In another study, it was concluded that bacteria-killing properties of honey increases by twofold when diluted with water (Ghaleb 2008). Table 8.2 summarizes the pharmacological actions (Af'aal) of honey as per Unani system of medicine (Vohra and Khan 1979; Kabeeruddin 1933; Ibn-ul-Qaaf 1233–1286; Ibn Sina n.d. 980–1037; Ghani 1926).

In the Unani system of medicine, no side effects of honey have been reported. However, in one of the studies, spores of Clostridia have been reported, which may possess some risk of wound botulism (Banerjee et al. 2003). In a published report, 66 patients were hospitalized with symptoms of nausea, vomiting, dizziness, weakness, salivation, hypotension, syncope and bradycardia for several hours after

Table 8.2 Pharmacological actions of honey as per Unani system of medicine

Terminology in Unani medicinal system	Terminology in modern medicinal system
Muhallil-e-Waram	Anti-inflammatory
Mughazzi	Nutrient
Musakkin-e-Auja'a	Pain killer
Jali	Detergent
Mufattit e Hisa't	Lithotryptic
Mufatteh Sudad	Deobstruent
Muqawwi e Bah	Aphrodisiac
Muqawwi e Mida	Stomach tonic
Daf e Taffun	Antiseptic
Mushtahi	Appetizer
Munaffith e Balgham	Expectorant
Hadim	Digestive
Musaffi e Dam	Blood purifier
Mundamil-e-Qurooh	Wound healing

ingesting honey. The patients were treated with i.v. fluids and atropine. No patient was reported dead (Yilmaz et al. 2006). The modern application of honey is limited by the lack of clinical trials which need special consideration of researchers.

8.5 Honey in the Ayurvedic System of Medicine

Honey in ayurvedic medicine is also known by the name: Madhu, Madhvika, Kshaudra, Mahika, Varti, Vanta, Pushparasodbhava, Bhrungavanta, Saragha and Makshikavanta (Shastri Brahmashankar 1984). Honey is considered as one of the most common medicines in ayurvedic medicine and is considered to be used for both internal and external purposes. Honey is an excellent preservative and sweetening agent as is widely used as the same in Ayurveda. Honey is also exploited in the treatment of obesity, diabetes, leprosy, diarrhoea, wound healing, hiccups, blood filled vomits, asthma, cough, eye disease, phlegm, etc. Honey provides the base as vehicle alongside other medicaments in order to improve the safety and efficacy profile of the medicine. As per Ayurveda, honey is classified into eight different types depending on the type of bee which collects the honey and the qualities which the honey possesses (Table 8.3) (Shastri Brahmashankar 1984).

8.6 Physico-Chemical Properties of Honey

Apart from taste and composition, honey has numerous other properties. Honey is a viscid fluid with glue-type characteristic which is dependent on a number of substances and composition (Mandal and Mandal 2011). Honey has a natural

Table 8.3 Classification of honey according to ayurvedic system of medicine

Type of honey	Characteristic
Makshikam	Used to treat hepatitis, cough, piles, asthma, eye disease, tuberculosis
Kshoudram	Used to treat diabetes
Bhraamaram	Used to treat blood filled vomiting
Aardhyam	Used in effectively treating anaemia, cough and eye diseases
Pauthikam	Used for treating urinary infections and diabetes
Chathram	Used in treating infection by worm with bloody vomits and diabetes
Daalam	It is dry and used to improve digestion and conditions of diabetes, cough and vomiting
Ouddalakam	Used in skin diseases, and helps in bringing about clarity to the voice

ability to hold water and thus acts as a super humectant and is an excellent moisturizer. The viscosity of honey gives it foaming property (Olaitan et al. 2007). Honey shows variation in colour and can show vast difference from being colourless to black or sharp amber colour. The change in colour depends upon the botanical source, age and storage condition while the transparency of honey depends upon the number of suspended pollens in it (Ramirez-Arriaga et al. 2011). The colour of honey changes upon crystallization, it usually becomes lighter in colour due to the crystal of glucose which are white in colour. Water content in honey is inversely proportion to the crystallization process. The percentage of glucose will be higher if the water content is less and thus more frequent crystallization process will be seen and vice versa (Al-Habsi et al. 2013). Pure honey contains vitamins, minerals, enzymes and amino acids, but the major constituents are water and sugar. Fructose is the major form of sugar found in honey. However, honey also contains maltose and sucrose also. Few oligosaccharides are also present in honey. The fructo-oligosaccharides in honey serves as probiotic agents (Ezz El-Arab et al. 2006). Water is the second major component of honey. Organic acids like gluconic acid are also found in honey. These organic acids provide an acidic taste to honey (Rahman et al. 2014). Potassium, sulphur, sodium phosphorous, magnesium and calcium are some major minerals while iron, zinc and copper make up the minor mineral component of honey (Sampath et al. 2010; Rashed and Soltan 2004; Lachman et al. 2007).

Proteins in concentration range 0.1–0.5% are also in honey (Da Silva et al. 2016). The percentage of proteins in honey varies depending upon the origin of honeybee (Jagdish and Joseph 2004; Won et al. 2009). Honey contains B-complex vitamins like vitamin B1 (thiamine), B2 (riboflavin), B3 (niacin), vitamin C, nicotinic acid, vitamin B6, pantothenic acid and nitrogenous compounds (Moundoi et al. 2001). Enzymes like glucose oxidase, invertase, catalase, amylase, etc. also make up the chemical composition of honey (Olaitan et al. 2007; Mafra et al. 2015).

Phenolic compounds also constitute chemical makeup of honey (Estevinho et al. 2008; Bravo 1998). Due to the presence of phenolic components imparts anti-inflammatory, analgesic, anti-carcinogenic, immune modulating, anti-atherogenic and anti-thrombotic activity in honey (Vinson et al. 1998). Flavonoids and phenolic acids act as an indicator in determining honey's botanical origin (Yao et al. 2003).

The antioxidant activity of phenolics are related to the quenching, metal ion chelation, free radical scavenging and hydrogen donation properties (Kucuk et al. 2007; Pandey and Rizvi 2009).

8.7 Honey and Its Modern Applications

From ancient time, honey has been used for treating microbial infections and wound although medical experts have rediscovered honey, particularly where conventional therapeutic molecules do not show significant effect. At a recent time, manuka honey has been revealed to exhibit antimicrobial activity against bacteria, e.g. *Helicobacter pylori* and *Staphylococcus aureus* making it a potential therapeutic agent for the treatment of wound healing and gastrointestinal ulcers (Alvarez-Suarez et al. 2010). Due to their highly thick nature, which assist to provide a protective layer and maintain a moist wound surrounding that relives healing (Molan et al. 1992, 2002, 2004). Furthermore, honey has shown its effect against inflammation, cough, diabetics and burns. The topical formulation of honey can also assist healing of wounds with methicillin-resistant S. aureus compared to conventional therapy. Recent study found that manuka, jelly and pasture honey are effective in promoting the monocytes (the precursors of macrophages), which secrete a cytokine like tumour necrosis factor-α (TNF-α), and this cell signalling protein is known to promote the wound repair. In addition, honey has a potential to prevent the reactive intermediate release which limits tissue degeneration during wound healing by macrophages. Thus, the immunomodulating attributes of honey plays an important role in wound repair (Mandal and Mandal 2011; Hananeh et al. 2015). Honey as a therapeutic regimen for gastrointestinal disorders such as gastritis and peptic ulcer is supported by ancient medical treatment as well as from results in modern times. Recent studies have also proven that honey may initiate the repair of intestinal mucosa and the growth of new tissues and functions as an anti-inflammatory agent. Honey exhibits antioxidant activity due to the presence of compounds such as polyphenols and flavonoids (Bogdanov et al. 2008).

8.7.1 Antioxidant Activity

The term "oxidative stress" explains the insufficient stability between protection from oxidation and free radicals (Bogdanov et al. 2008). Antioxidants are substances that can prevent cell damages caused by free radicals. Antioxidants are sometimes called free radical scavengers. Oxidation is a process that produces free radicals leading to chain reactions that could harm the body function of organisms. Free radical is also called as reactive oxygen species (ROS).

The antioxidant can be obtained from natural or artificial source. Mainly plant-based foods are rich in antioxidants. The body also generates some antioxidants, known as endogenous antioxidants and those which comes from outside are called exogenous antioxidants.

Honey possess antioxidant activity primarily due to the presence of flavonoid compounds like ellagic acid, gallic acid, ferulic acid, caffeic acid, etc. and also flavonoids such as kaemferol, quercetin, apigenin, and naringenin these chemical constituents may work simultaneously to provide a synergistic effect (Johnston et al. 2005; Turkmen et al. 2006; Rakha et al. 2008). Therefore, it has been suggested that honey could serve as an alternative to sodium tripolyphosphate preservative in food to preserve the food and to interrupt lipid oxidation (Johnston et al. 2005). The geographical source of honey has the highest effect on its antioxidant property (Eteraf-Oskouei and Najafi 2013). Total phenolics contents and the colour of honey are strongly associated with its antioxidant activity. Several reports find that the dark colour of honey has higher phenolic contents and subsequently higher antioxidant activity (Beretta et al. 2005; Al-Mamary et al. 2002). Another study optimized a combination of Trigona sp. Honey (TH) and Musa paradisiaca (MP), and via the response, surface methodology in formulating high antioxidant jelly was investigated for total carbohydrate content and antioxidant capacity. The optimized formulation comprising 20% of Musa paradisiaca and 20% Trigona species honey showed significant antioxidant activity (Nasyriq et al. 2019).

Due to antioxidant potential, honey possesses several preventive properties against cancer, cardiovascular disease, inflammatory and neurological disorders, also ageing (Kishore et al. 2011). The chemical constituents, i.e. polyphenols and phenolic acids found in honey, may vary based on their geographical origin; for instance, rosemary honey have flavanol kaempferol, whereas sunflower honey have quercetin (Akan and Garip 2011). Another researcher reported that phenolics obtained from monofloral honeys prevent oxidative damage of human red blood cell (RBC) membranes. Study showed that honey restrains RBC oxidative destruction due to its incorporation into cell membrane and ability to introduce into the cytosol. It comprises suitable antioxidants that are responsible for biological action, protection and rise RBC functions (Alvarez-Suarez et al. 2012). Hazirah et al. investigated the antioxidant properties of stingless bee honey (kelulut honey) and its reaction on lymphoblastoid cell line. Viability of cell was estimated using 3-(4,5-dimethylthiazol2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, and it was demonstrated by MTS assay that kelulut honey was increased in cell viability which may be modulated via their antioxidant attributes of honey (Hazirah et al. 2019).

8.7.2 Antimicrobial Activity

Natural honey has been used as traditional medicine since long times. Dustmann reported that honey possess antibacterial activity which was first recognized by van et al. in 1892 (Dustmann 1979). Several variables have been revealed to contribute to the antibacterial action of honey, such as mostly due to a high sugar concentration, low water content and its high viscosity, which assists to provide a protective barrier to inhibit infection. Furthermore, presence of hydrogen peroxide content has showed obvious antimicrobial effects (Najla 2019). Honey has broad spectrum antibiotic activity, and it is found to be sensitive to most multidrug-resistant bacteria

Table 8.4 Bacteria and other microbials detected to be sensitive to honey[a]

Actinomyces pyogenes	Rubella virus
Shigella species	*Serrata marcescens*
Pseudomonas aeruginosa	*Campylobacter coli*
Bacillus anthracis	*Salmonella typhimurium*
Salmonella choleraesuis	*Corynebacterium diphtheria*
Campylobacter jejuni	*Staphylococcus aureus*
Echinococcus parasite	*Salmonella typhi*
Enterococcus avium	*Candida albicans*
Leishmania parasite	*Enterococcus faecalis*
Shigella species	*Streptococcus agalactiae*
Trichophyton mentagrophytes	*Streptococcus dysgalactiae*
Vibrio cholerae	*Enterococcus faecium*
Streptococcus faecalis uberis	*Nocardia asteroids*
Enterococcus raffinosus	*Mycobacterium tuberculosis*
Trichophyton rubrum	*Epidermophyton floccosum*
Trichophyton tonsurans	*Streptococcus uberis*
Streptococcus mutans	*Microsporum canis*
Helicobacter pylori	*Streptococcus pneumonia*
Serrata marcescens	*Escherichia coli*
Streptococcus pyogenes	*Proteus species*
Klebsiella pneumonia	*Haemophilus influenza*

[a]Adapted from Molan (1992, 2001) and Cooper et al. (2002)

mentioned in the given table. Alvarez and his coworkers reported that all types of honey possess antimicrobial activity and suggested that formation of hydrogen peroxide as an antibacterial product for diminishing the bacterial infections by the analysed native Cuban honeys. Even though, the Cuban honeys can vary in their chemical constituent's composition percentage, and still they displayed antimicrobial potential against both Gram-negative and Gram-positive bacteria (Alvarez-Suarez et al. 2010). Manuka honey contains unusually abundant amount of the antibacterial compound methylglyoxal, which makes it as a potential antibacterial agent. The Unique Manuka Factor (UMF) grading system infers the methylglyoxal concentration in commercially available manuka honey. An experiment on manuka honey was reported with antimicrobial activity against the multidrug resistance, with their potential activity against Gram-positive and Gram-negative bacteria (Girma et al. 2019).

Natural honey action against the bacteria and other microbials infections are listed in Table 8.4. A study investigated the treatment of methicillin-resistant Staphylococcus aureus against each category of clinical isolates retrieved from wound infection. Several concentrations of honey (25–100%) were tested for bacteriostatic and bactericidal activities. Among the different concentration of honey, honey-2 at 75% v/v concentration had high antibacterial potency than other concentrations of honey (Mama et al. 2019).

Molan and his coworkers in several studies suggested that dissimilarities occur in the antifungal and antibacterial activities of about 200 New Zealand honeys, whereas all exhibit potency (Molan et al. 1992, 2002, 2004).

8.7.3 Wound Healing Activity

Natural honey used for wound healing activity is reported in scientific literatures. Russians used honey during First World War for the prevention of wound infection and speedy wound healing activity. Besides, Germans used honey in amalgamation with cod liver oil for the management of ulcers, burns, fistulas and boils (Bansal et al. 2005; Eteraf-Oskouei and Najafi 2013). Honey is explored to be reactive in approximately all types of wounds such as abrasion, amputations, septic wounds, cracked nipples, varicose and sickle cell ulcers, leprosy, diabetic and fistula. Honey is used as wound dressing to promote healing action and eliminate wound infection swiftly. Wound healing activity of honey is primarily due to its antimicrobial property, keeping wound moistly and greater viscosity to protect from the infection (Manisha et al. 2011; Hananeh et al. 2015). Application of honey on open wounds has been reported, whereas on burns it has both soothing and healing effects. Honey is therapeutically effective on numerous types of wounds, where other treatments are not successful and also minimize the probability of wound infection. Honey has a curative role in gingivitis and periodontal disease (Khan et al. 2007).

Sterilized manuka honey used as dressing pads was very effective for the complete healing of knee amputation case in a young boy in 10 weeks (Dunford et al. 2000). Honey dressing fastens healing process, sterilizes wound and minimizes pain and hospital stay (Subrahmanyam 1991).

Clinical trials have recommended that honey bandages exhibited well recovery in patient with burns associated with amniotic membrane dressing, using boiled potato and silver sulfadiazine dressing (Eteraf-Oskouei and Najafi 2013). Febriyenti et al. examined the potentiality of honey film and gel to boost the incision wounds and healing of burns on the outer skin of white female Sprague-Dawley female rats. The experimental result concluded that honey film has a remarkable effect on the wound associated with burn in comparison to the positive and negative control (Febriyenti et al. 2019).

8.7.4 Antiviral Activity

Besides antimicrobial effects, natural honey also exhibited antiviral effect (Jibril et al. 2019). Topical use of honey was therapeutically effective in the management of frequent wounds from genital and labial herpes in comparison to acyclovir (antiviral) cream. Al-Waili et al. reported that rubella virus activity inhibited by honey (Al-Waili 2004a). Semprini et al. reported a randomized controlled trial on kanuka honey versus acyclovir for the topical therapy of herpes simplex labialis. The results showed that there were no serious adverse effects with the kanuka honey (Semprini et al. 2019).

8.7.5 Honey Used in Ophthalmology

Globally, honey is used as traditional medicine for the management of several ophthalmological conditions, thermal and chemical burns to eyes, corneal injuries and conjunctivitis, etc. (Shenoy et al. 2009). Obaseiki and his coworkers reported that recovery was observed in 85% of patients using honey as ointments by 102 patients of nonresponsive eye disorders. Honey used in infective conjunctivitis minimizes redness and swelling and quickens bacterial elimination (Al-Waili 2004a).

8.7.6 Honey Used in Cough

Cough is the utmost common problem for all people reporting to general physicians. It is very general problem especially in infant and children associated with etio-pathological causes.

Cohon and his coworkers investigated that a single nightmare dose of three different types of honey (eucalyptus, citrus, and labiatae honey) has better recovery in contrast to placebo effect on nocturnal cough and upper respiratory tract infection in 300 children's (Cohen et al. 2012).

8.7.7 Antidiabetic Activity

Glycaemic index of honey is significantly lower compared with glucose in normal type I and type II diabetes. Honey assists to decrease the absorption of digested food due to its lower glycaemic index. It considerably rises low levels of blood glucose in diabetic patients compared with dextrose and decreases the levels of homocysteine, C-reactive protein and lipid profile in normal and hyperlipidaemic patients (Bansal et al. 2005). Al-Waili and coworkers reported that honey accelerates the production of insulin and lipid profile and reduces the level of blood glucose (Al-Waili 2004b).

8.7.8 Anti-Inflammatory Activity

Many studies revealed that honey retards the actions of cyclooxygenase-1 and cyclooxygenase-2, hence exhibits immunomodulatory and anti-inflammatory actions (Markelov and Trushin 2006; Al-Waili 2003). Besides, intake of diluted honey exhibited reduced levels of prostaglandins and thromboxane in plasma of common peoples. Al-waili and coworkers reported that honey has also been beneficial in the treatment of eczema, psoriasis and dandruff. Bilsel and coworkers have verified that efficacy of honey is equally effective as compared to prednisolone (steroidal drug) for its anti-inflammatory potential (Bilsel et al. 2002). Modern drugs used for the treatment of inflammation have severe adverse effects, whereas honey

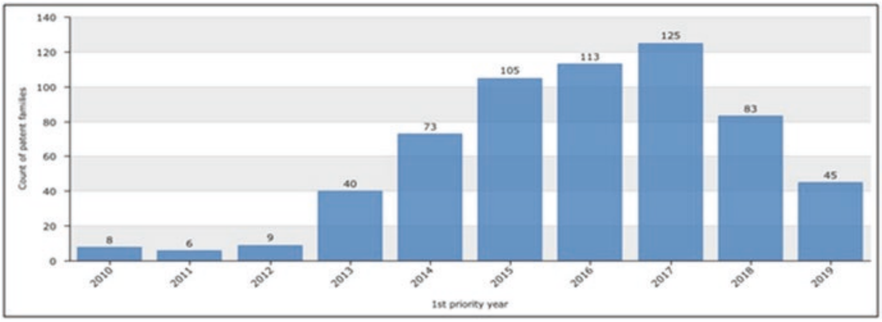

Fig. 8.1 The graph representing the number of patent application filed on honey verses priority years

has no adverse effects when used in the anti-inflammatory activity (Al-Waili and Boni 2003; Molan 2001).

Biluca et al. investigated the free radical scavenging activity and anti-inflammatory effect of phenolic compounds against lipopolysaccharide stimulated RAW264.7 macrophages via stimulation of inflammatory cytokines, in stingless bee honey. All these findings showed that stingless bee honey could be a promising source of antioxidant and anti-inflammatory effect which may promote health benefits when included in the food (Biluca et al. 2020).

8.8 Intellectual Property/Patent Insights on Honey

According to the focus of this book chapter, search strategies were built in paid patent database such as orbit intelligence to identify potential patents of interest regarding the scope of the chapter. The retrieved patent documents were manually screened, in order to prepare a relevant set of patents on the technological space. This study was limited to the last 5 years and found 619 relevant patents focused on technology trends such as various types of honey and its application in various fields such as pharmaceuticals and fast moving consumer goods.

Figure 8.1 illustrates the evolution of patent filing overtime, making it possible to understand the dynamics of the industrial and market value of honey. It is also possible to distinguish peaks or troughs in the number of application files, depending on research and development (R&D) budgets or broader economic or even strategic changes. After 2017, there was a drastic decline in the number of patents filed which indicates that symptomatic of a substantial decline in R&D or intellectual property budgets.

Figure 8.2 depicts the number of the priority patent application filed in the various national offices. This graph provides information on the patent strategy in the sector studied and is an excellent indicator of the main research and development locations, as generally, players file priority patent applications locally. Origin of patent application corresponds to the first country where the patent application was filed, usually determining the place of R&D. This indicator reveals the most

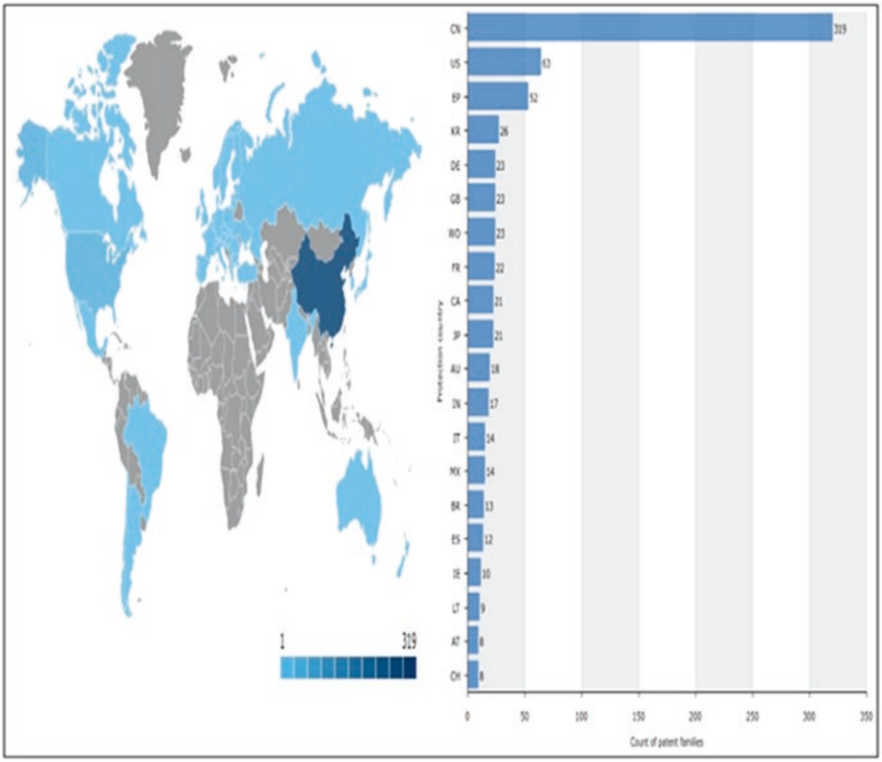

Fig. 8.2 Figure representing the patent application filing on honey in various national patent offices

innovating countries in this subject matter. One patent family can have several patent family members, so it can span on several different countries. This indicator also shows the most important countries to be protected in this domain, where consumer market needs to be protected.

8.9 Industry Insights

Due to increased awareness about the benefit of honey, the global honey market size is forecasted to a significant gain in the forthcoming years. The health-conscious world is actively focusing on sugar substitutes, and honey is a promising natural sugar that is extensively consumed throughout the world. In addition, honey is supposed to raise traction as it shows potential anti-inflammatory, antioxidant, antibacterial and antimicrobial properties. Honey is a rich source of nutrients which makes it as a key ingredient in several applications such as energy drinks, alcoholic drinks like mead, bakery products, confectionery and bars.

The global honey market share was estimated at USD 8.4 billion in 2018 and is expected to generate approximately USD 10.3 billion by 2025 (Globenewswire

2019). The global honey market is divided into various regions including North America, Asia Pacific, Europe and the rest of the world. In which, the Asia Pacific segment is predicted to hold its dominance all over the projected period due to an increase in the production and consumption volume of honey. The estimated amount in the Asia Pacific region is projected to reach around 1162.8 tons in terms of volume by 2023. Some of the potential players in the global honey market are (1) Capilano Honey (Australia), (2) Dabur (India), (3) Comvita Ltd. (New Zealand), (4) Bee Maid Honey (Canada), (5) Billy Bee Honey Products (Canada), (6) Lamex Food Group (USA) and (7) Beeyond The Hive (USA) (Market Research Future 2019).

8.10 Conclusion

Until now, inventors pay more attention to investigation of therapeutic molecules from natural source and assume that products from natural sources may provide a potential remedy and an alternate to synthetic molecules, medicine products from natural source cannot bloom. Among the natural products, honey is one of the most promising natural products in the traditional therapy, and researchers also believe honey as potential medicine for various types of diseases or disorders. The most popular effect of honey is antibacterial, antioxidant, skin burns and postoperative wound healing and anti-inflammatory. Furthermore, honey has already shown potential in treating cardiovascular diseases, diabetics and gastrointestinal issues. This chapter may help medical practitioner with significant evidence supporting the use of honey in the various field such as medical and diet. Further investigations are needed to establish all aspects of honey. More experimental and clinical trials are intended to validate the authenticity of various types of honey either alone or as an additive in therapy.

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9 Recent Advances in the Discovery of Bioactive Components from Natural Honey

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Abstract

Honey is one of the most valued natural products introduced to mankind since antiquity. Traditionally, honey is not only used as a food product but also as an alternative remedy for clinical conditions ranging from wound healing to cancer treatment. Honey contains about 200 beneficial bioactive constituents primarily comprising glucose and fructose and it also encompasses some vitamins, amino acids, minerals, and enzymes from fructo-oligosaccharides. Honey is an essential source of phenolic compounds and it is of great interest to see the amount and type of phenolic acids and flavonoids as they are responsible for nutraceutical properties as well as promising pharmacological functions such as antimicrobial, antidiabetic, anticancer, neuroprotective, cardioprotective, and wound healing properties. Additionally, several recent reports have also verified that the phenolic compound profile in honey is closely linked to the botanical and, often, the geographic origin of this food product. In this book chapter, therapeutic effects associated with the bioactive compounds in natural honey have been thoroughly discussed.

Keywords

Honey · Therapeutic effects · Phenolic acids · Flavonoids

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_9

9.1 Introduction

It is generally accepted that a natural product imparts better health benefits than that of synthetic source. However, this frontier is still open for the debate, and many investigations are going-on on this subject (Topliss et al. 2002). In the recent past, plant-derived natural products have been used at larger scale due to the occurrence of vital components such as vitamins, enzymes, phytochemicals hormones, antioxidants, minerals, and other nutritional components. These substances provide vital nutrients for human use and avert the nutrition-associated diseases and thus help in improving the health of the human beings (Atanasov et al. 2015).

Honey is the most appreciated and valued product of natural origin produced by honeybees (*Apis mellifera* L.). It is an essential food product that has been excruciatingly used for its ethnopharmacological applications. Honey contains nearly 200 beneficial bioactive components majorly comprising fructose and glucose as well as fructo-oligosaccharides (Chow 2002), vitamins, minerals, amino acids, and enzymes (Da Silva et al. 2016). Its constitution varies and depends on the plants on which the bee nourishes. Any natural honey type contains flavonoids (e.g., quercetin, kaempferol, chrysin, galangin, pinocembrin, apigenin, and hesperidin), phenolic acids (such as p-coumaric, caffeic, ferulic acids, and ellagic), antioxidants (SOD: superoxide dismutase, ascorbic acid, GSH: reduced glutathione peptides, tocopherols, CAT: catalase, and Maillard reaction products). These chemotypes induce synergistic antioxidant effect and mostly act in mishmashes (Alvarez-Suarez et al. 2010; Johnston et al. 2005; Turkmen et al. 2006; Rakha et al. 2008; Al-Mamary et al. 2002). Evidence suggests that honey possesses several health-associated effects such as antioxidant (Ahmed and Othman 2013), anti-inflammatory, antimicrobial activity (antibacterial, antifungal, and antiviral) against diverse human pathogens (Khalil et al. 2012) and anticancer activity against different kinds of tumors by targeting diverse molecular pathways that play key role in cell division and antidiabetic activity with the reduction of fructosamine, glucose, and glycosylated hemoglobin concentrations in serum (Estevinho et al. 2008). Honey also exerts protective effects in the lungs against asthma and respiratory infections, in the gastrointestinal tract (Abdulrhman et al. 2008) in the cardiovascular system as well as in the nervous system by preventing the low-density lipoproteins (LDL) oxidation (Ghosh and Playford 2003). Although numerous studies were done on nectar honey types, only a few are accounted for.

This book chapter is a comprehensive update which highlights the recent advances in the discovery of bioactive components from natural honey. Moreover, therapeutic role (Fig. 9.1) of honey in antimicrobial, antidiabetic, anticancer, wound healing, apoptotic, and ophthalmological conditions has been thoroughly discussed.

9.2 Composition of Honey

The composition of all natural honey types relies upon the plant species on which the honeybee feeds. Major components of all natural honey types remain same. The average composition of natural honey is summarized in Table 9.1.

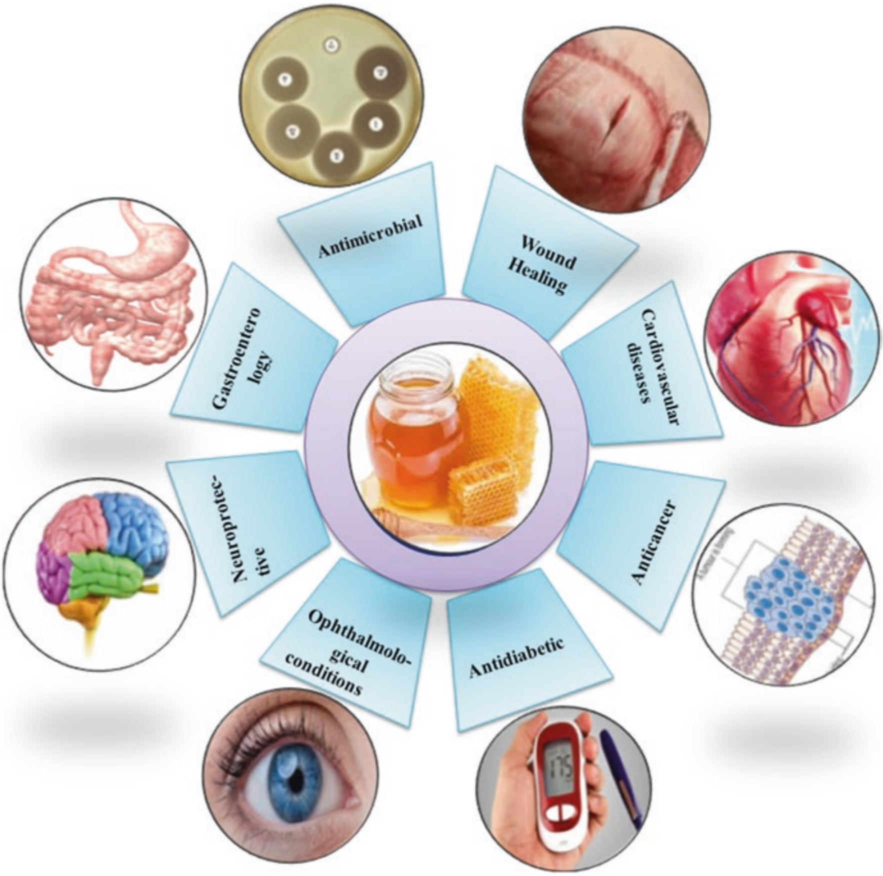


Fig. 9.1 Therapeutic properties of honey

Table 9.1 Average composition of honey (Adapted and modified from the references (White et al. 1962; Amy and Carlos 1996))

Constituents	Average (%)
Moisture	17.2
Glucose	31.28
Fructose	38.19
Sucrose	1.31
Disaccharides, calculated as maltose	7.31
Higher sugars	1.5
Lactone as gluconolactone	0.14
Total acid as gluconic	0.57
Free acid as gluconic	0.43
Nitrogen	0.041
Ash	0.169

9.3 Bioactive Compounds in Honey

Honey contains several essential bioactive components such as vitamins (retinol, thiamine, riboflavin, pyridoxal phosphate, ascorbic acid, tocopherol, menadiol, niacin, pantothenic acid), enzymes, fatty acids, and phenolic compounds (octadecanoic acid, hydroxybenzoic acid, cinnamic acid, flavonoids, and ethyl ester) (Bogdanov et al. 2008; Muhammad et al. 2015). Chemoprofile of honey also encompasses pinocembrin, acacetin, apigenin, and acids like ferulic acid and abscisic acid (Marghitas et al. 2010). The amino acid composition of physiological significance is arginine, cysteine, proline, aspartic acid, and glutamic acid (Qamer et al. 2007). The presence of this dynamic compound profile indicates better insightful of the potent biological role of honey in the management of human diseases. The bioactive compounds identified in honey are summarized in Table 9.2.

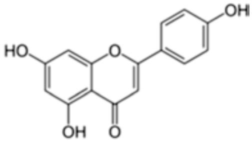
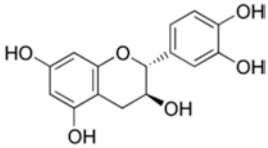
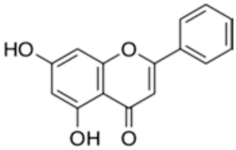
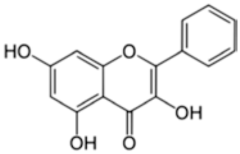
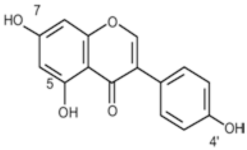
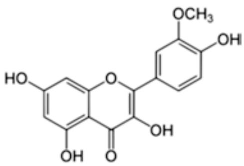
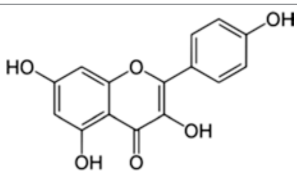
9.4 Antibacterial Activity

Antimicrobial activities of honey are majorly credited to the phenolics present in honey, including benzoic acid derivatives, flavonoids, and other volatile compounds (Pita-Calvo and Vázquez 2017). The other main factors that append to antimicrobial activity of honey include the enzymatic oxidation of glucose as well as some of its physical aspects (Beretta et al. 2007; Cushnie and Lamb 2005). Moreover, acidic (low pH) environment, high carbon (C)/nitrogen (N) ratio, high osmotic pressure/low water activity (WA), low protein content, and high level of reducing sugars lead to low redox potential; a viscosity that is limiting the dissolved oxygen content as well as other chemotypes/phytochemicals can contribute to the antimicrobial activity of honey.

Honey has long been exploited as a remedy for the control of microbial infections. It exerts an inhibitory effect against nearly 60 bacterial species that comprise aerobic and anaerobic, Gram-negatives and Gram-positive bacteria (Olaitan et al. 2007). Honey inhibits the growth by manifold (Al-Waili 2004). Previous investigations on antimicrobial activity of honey (Visavadia et al. 2006) indicated its antimicrobial activity against several pathogenic bacteria, including *Salmonella typhimurium*, *Escherichia coli*, *S. aureus, Enterobacter aerogenes* (Lusby et al. 2005; Visavadia et al. 2006). The spectrum of antibacterial effect of honey also encompasses different types of *methicillin-resistant S. aureus* (MRSA), β-*hemolytic streptococci* and vancomycin resistant *Enterococci* (VRE) (Allen et al. 2000; Kingsley 2001). The coagulase negative staphylococci are very akin to *S. aureus* (Cooper et al. 2002; Abhishek et al. 2010) in their sensitivity to honey and more sensitive than Enterococcus species and *Pseudomonas aeruginosa* (Cooper et al. 2002). Recent investigations reported antibacterial activity of against *Aeromonas hydrophilia*, *Salmonella enteric*, and *Klebsiella pneumoniae* (Table 9.3).

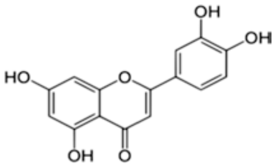
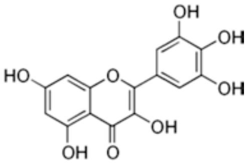
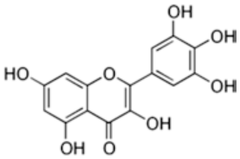
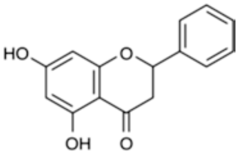
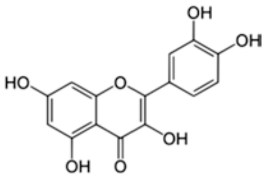
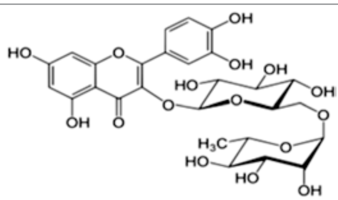
Neat honey exhibits inhibitory effects against fungi, and diluted honey inhibits the production of toxin by these microorganisms (Al-Waili and Haq 2004). Inhibitory activity of honey has also been reported against some yeast. Growth

Table 9.2 Most common phenolic compounds identified in honey

	Structure	Significance	Reference
(a) Flavonoids			
Apigenin		Inhibits the proinflammatory mediators release, induces anticancer and immunomodulatory effects, protects endothelium-dependent vasorelaxation of the aorta	Jin et al. (2009)
Catechin		Protects against ischemia-reperfusion-induced nerve cell death	Inanami et al. (1998)
Chrysin		Controls proliferation of cell by activating p38-MAPK via accumulation of p21Wafi/Cip1	Weng et al. (2005)
Galangin		Antitumor activity apoptosis induction, elevates the cytotoxic activity, anticlastogenic effects, inhibits osteoclastic bone destruction as well as osteoclastogenesis	Hossen et al. (2017)
Genistein		Anti-inflammatory effects via STAT-1 and NF-κB activations	Hämäläinen et al. (2007)
Isorhamnetin		Inhibits NF-κB activation by inhibiting the ions expression and no production in stimulated macrophages	Hämäläinen et al. (2007)
Kaempferol		Downregulates the lipid peroxidation and cell division and enhances the susceptibility to apoptosis	Almasaudi et al. (2016)

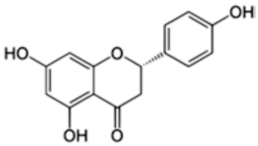
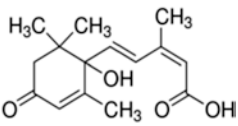
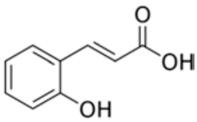
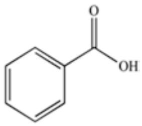
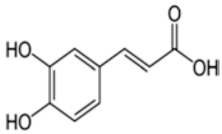
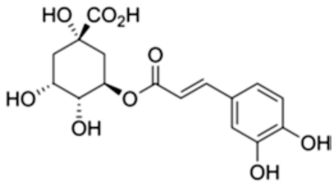
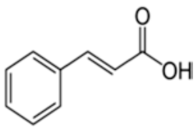
(continued)

Table 9.2 (continued)

	Structure	Significance	Reference
Luteolin		Antidiabetic effect through several mechanisms to reduce blood sugar levels	Jung et al. (2004), Mcdougall and Stewart (2005) and Rouse et al. (2014)
Myricetin		Reduces ROS and free radical generation after ischemic injury cell swelling	Gordon and Roedig-Penman (1998)
Pinobanksin		Antiproliferative effect, inhibits peroxidation of LDL, reduces oxidative stress and antimutagenic effect, improves cognition	Hossen et al. (2017)
Pinocembrin		Neuroprotective, effects, ameliorates effect against blood–brain barrier injury, prevents atherosclerosis, improvement in memory impairment, induces apoptosis, reduces cardiac arrhythmia infarct size, inhibits inflammatory mediators and ameliorates nephrotoxicity	Hossen et al. (2017)
Quercetin		Antidiabetic effect via several mechanisms to reduce blood sugar levels	Jung et al. (2004), Mcdougall and Stewart (2005) and Rouse et al. (2014)
Rutin		Inhibits in vitro platelet aggregation by binding to the A2 receptor of thromboxane	O'malley et al. (1995)

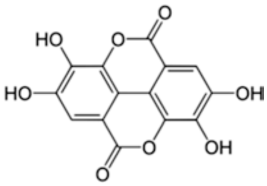
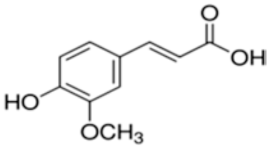
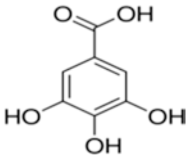
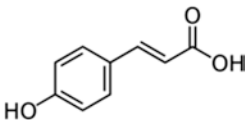
(continued)

Table 9.2 (continued)

	Structure	Significance	Reference
Narigenin		Alters the fluidity in both outer and inner membrane of hydrophilic and hydrophobic regions	Tsuchiya and Iinuma (2000)
(b) Phenolic acids			
2-*cis*, 4-*trans* Abscisic acid		Antidiabetic activity	Uzor et al. (2017)
2-Hydroxycinnamic acid		Antibacterial activity against *Mycobacterium tuberculosis* and *M. bovis* with an MIC ranging between 122 and 244 μM	Guzman et al. (2014)
Benzoic acid		Inhibits the active uptake of some amino and oxo acids in *Escherichia coli and Bacillus subtilis*	Russell and Chopra 1996; Park et al. 2001
Caffeic acid		Inhibits the oxidative stress in the rats that were overloaded by iron, reduces the lipid peroxidation, and increases the tocopherol (vitamin E levels) in the plasma	Lafay et al. (2005)
Chlorogenic acid		Neuroprotective effects by preventing methylmercury-induced apoptosis of PC12 cells	Li et al. (2008)
Cinnamic acid		Improves the insulin resistance and glucose homeostasis by increasing the glucose uptake, pancreatic β-cell functionality, and reducing the dipeptidyl peptidase-4 and protein glycation	Adisakwattana (2017)

(continued)

Table 9.2 (continued)

	Structure	Significance	Reference
Ellagic acid		Anti-inflammatory activity, prevents high fat/ carbohydrate diet-induced metabolic syndrome, induces anticancer effect, prevents kidney toxicity, inhibits protein kinase CK2, ameliorates cisplatin induced injuries to sperm quality, redox system, and the histologic structure of the rat testicles, hepatoprotective, cardioprotective, gastroprotective effects, inhibits the cell proliferation	
Ferulic acid		Induces glucose uptake by increasing the expression of P13K and GLUT4 transcripts via P13K dependent signaling pathways	Prabhakar and Doble (2009)
Gallic acid		Protects against the bacterial cytotoxicity, exhibits antimicrobial activity, prevents oxidative stress, induces apoptosis, exhibits cardioprotective, hepatoprotective, and gastroprotective effect, induces antihyperglycemic, anti-lipid peroxidative effects, induces anti-melanogenic, pro-inflammatory activities	Hossen et al. (2017)
p-Coumaric acid		Cardioprotective role, antioxidant effects on LDL cholesterol oxidation, pesticide detoxification	Hossen et al. (2017)

(continued)

Table 9.2 (continued)

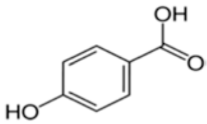
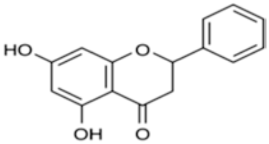
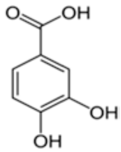
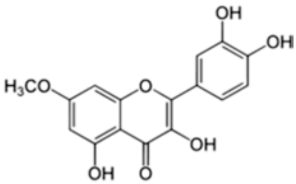
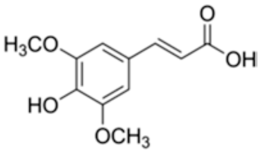
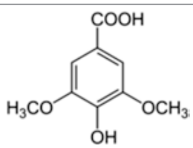
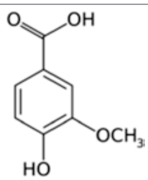
	Structure	Significance	Reference
p-Hydroxybenzoic acid		Antifungal and antimicrobial effects, antiproliferative effects against PC-3 and MCF-7 cells, lowers the expression of adhesion molecules in HAEC	Spilioti et al. (2014)
Pinocembrin		Antibacterial, antifungal, anticancer, and neuroprotective activities	Rasul et al. (2013)
Protocatechuic acid		Antioxidant and anti-inflammatory roles, antioxidant and hepatoprotective effects	Rasul et al. (2013)
Rhamnetin		Anti-inflammatory effect, reduces pro-inflammatory cytokines levels by regulating the c-Jun NH2-terminal kinase 1 and p38 MAPK signaling pathway	Jnawali et al. (2014)
Sinapic acid		Acetylcholinesterase inhibitor, potential antioxidative agent, and antimutagenic by inhibiting the carcinogenesis and the induction of inflammatory cytokines	Nićiforović and Abramovič (2014)
Syringic acid		Nephroprotective, hepatoprotective antidiabetes, cardioprotective, anticancer, antimicrobial, antioxidant, anti-inflammatory, and antiendotoxic activities	Srinivasulu et al. (2018)
Vanillic acid		Exhibits estrogen-like effects in osteoblast-like UMR 106 cells by MAP kinase (MEK/ERK)-mediated ER signaling pathway	Xiao et al. (2014)

Table 9.3 Bacteria that are sensitive to honey (Molan 1992, 1997)

Bacterial strain	Clinical importance	Reference
Actinomyces pyogenes	Endometritis	
Corynebacterium diphtheriae	Diphtheria	Molan (1992)
Escherichia coli	Urinary tract infection, diarrhea, septicemia, wound infections	Chauhan et al. (2010)
Bacillus anthracis	Anthrax	Molan (1997)
Haemophilus influenzae	meningitis, ear infections, sinusitis, respiratory infections	Molan (1997)
K. pneumoniae	Pneumonia	Molan (1997)
Helicobacter pylori	Chronic gastritis, peptic ulcer, gastric malignancies	Molan (1997)
Mycobacterium tuberculosis	Tuberculosis	Molan (1992)
Proteus sp.	Septicemia, urinary infections, wound infection	Molan (1997)
Salmonella sp: Salmonella typhi, Salmonella typhimurium Salmonella cholerae-suis	Typhoid, enteric fever	Mulu et al. (2004), Chauhan et al. (2010), and Molan (1992)
Nocardia asteroids, Microsp. Canis, M.. gypseum	Mastitis	Molan (1992)
Shigella sp.	Dysentery	Molan (1997)
Serratia marcescens	Wound infections, septicemia	Molan (1997)
Pseudomonas aeruginosa	Wound infection, urinary tract infections, diabetic foot ulcer	Chauhan et al. (2010)
Streptococcus faecalis	Urinary tract infections	Molan (1992)
Streptococcus mutans	Dental carries	Molan (1992)
Staphylococcus aureus	Community acquired and nosocomial infection	Molan (1992)
Streptococcus pneumoniae	Meningitis, sinusitis, ear infections, pneumonia	Molan (1997)
Streptococcus pyogenes	Ear infections, impetigo, rheumatic fever, puerperal fever, scarlet fever, wound infections, sore throat	Molan (1997)
Trichophyton rubrum, T. tonsurans, T. mentagrophytes var., *Epiderm floccosum*	Tinea	Molan (1997)
Vibrio choleriae	Cholera	Molan (1992)
Aeromonas schubertii	Burn, wound infection	Hassanein et al. (2010)
Stenotrophomonas maltophilia	Pneumonia, urinary tract infection, blood stream infection, nosocomial infection	Tan et al. (2009)

inhibitory effects of honey has also been against other species of Aspergillus, Penicillium, and against all the common dermatophytes (Brady et al. 1997; Sampath Kumar et al. 2010). *Candida albicans* (causative agent of Candidiasis) also exhibits some sensitivity to honey (Obaseiki-Ebor and Afonya 1984; Bansal et al. 2005).

Surface mycoses such as ringworm and athletes foot cutaneous have also been reported to exhibit sensitivity to honey. This sensitivity is attributed to the inhibition of fungal and bacterial growth (Bansal et al. 2005). Additionally, topical application of honey has been shown to be effective in treating the seborrheic dermatitis and dandruff (Al-Waili 2005; Bansal et al. 2005)

9.5 Wound Healing

The use of honey in wound dressing dates back to ancient times. Its effectiveness in wound healing in the modern science has become available only recently. The treatment effects of honey for both acute wounds and superficial partial thickness burns are almost equal or a little better than conventional treatments (Yaghoobi and Kazerouni 2013). The wound dressing capacity of honey is due to the combinatorial effects that act in synergism to accelerate the process of wound healing. Wound healing capacity of honey is the widely studied and most effective application of honey (Medhi et al. 2008). In World War I, the Russians used honey to stop wound infection and to expedite wound healing. Honey combined with cod liver oil was used by Germans to treat burns, boils, fistulas, and ulcers (Bansal et al. 2005). All wound types including skin abrasion, bed sores/decubitus ulcers, septic wounds, abscess, burns, amputation, chill blains, surgical wound, abdominal wound (burst), nipples cracking, fistulas, diabetic, cervical, leprosy, traumatic, malignant, varicose, sickle cell ulcers, wounds of abdominal wall, and perineum have been indicated to be responding to honey treatment. Honey therapy as wound dressing leads to the initiation of healing process and removal of the infection. Honey has sanitization action on wounds, stimulates tissue regeneration, and reduces inflammation.

Treatment of cutaneous wounds in rabbits with honey was found to reduce edema (swelling), lower the inflammation, lessen the necrosis, attenuate the epithelialization, and improve wound contraction. On histological examination, honey has also been demonstrated to accelerate wound healing on cutaneous wounds in murine model (Bashkaran et al. 2011).

The application of honey (dressings soaked with natural honey) in diabetic wounds as topical wound dressings resulted in excellent treatment effects. Application of honey improved the diabetic wound and the rate of leg or foot amputations which in turn enhanced the life quality and productivity (Makhdoom et al. 2009).

In a double-blind randomized controlled clinical trial, healing time with honey dressing was found to be equivalent to hydrogel dressings in the abrasions or minor lacerations patients (Ingle et al. 2006). Similar effects in average healing times were observed with honey, paraffin gauze, or iodoform gauze in the studies of randomized, double-blind controlled clinical trial (McIntosh and Thomson 2006) and a randomized single-blind controlled clinical trial, respectively. A meta-analysis of these minor acute wounds indicated no statistically significant difference in mean time to healing between honey and conventional dressing (Marshall et al. 2005).

9.6 Cardiovascular Disease

The promising role of honey in the treatment of cardiovascular diseases is attributed to the presence of polyphenols (Habauzit and Morand 2012) such as quercetin, kaempferol, and caffeic acid phenethyl ester (CAPE). Polyphenols are the valuable natural products in honey for managing the blood pressure (Sánchez-Moreno et al. 2006). Quercetin lowers the risk of stroke and coronary heart disease (Zahedi et al. 2013). Kaempferol prevents the accumulation of the low-density lipoprotein (LDL) cholesterol that poses the great risk for cardiac diseases. The role of polyphenols in the prevention of the cardiovascular diseases is mainly due to oxidization of LDL cholesterol, scheming the vasodilatation of heart vessels and reversing platelet clotting in the blood circulation. Honey repressed blood coagulation through each of the three coagulation cascades including extrinsic, intrinsic, and the common cascade and thus reducing the fibrinogen levels. Owing to these excellent features, honey is believed to counteract the process of formation of atherosclerotic plaques that are associated with the development of cardiac disorders. Thus, the atherosclerosis that contributes to arterial hardening and narrow down of the lumen of the vessel are effectively neutralized (Kas'ianenko et al. 2010).

9.7 Anticancer Activity

Recent studies provide the strong evidences that honey induces anticancer effects through several mechanisms such as modification of the immune responses, apoptosis, anti-mutagenic, anti-proliferative, and anti-inflammatory pathways (Eddy et al. 2008). Honey has also been reported to inhibit the cell division, induce the apoptosis, modulate the cell cycle progression, and induce the mitochondrial membrane depolarization in several types of cancer cells including cervical cancer cells, adenocarcinoma epithelial cells (Pichichero et al. 2010), skin cancer cells (melanoma), (Erejuwa et al. 2014), and endometrial cancer cells (Yaacob et al. 2013; Tsiapara et al. 2009).

The potential of honey as an ameliorating agent has been indicated in all stages including prevention, progression, and treatment of the disease. Most of the investigations have been documented in in vitro, and they have been performed out on several types of cell lines and numerous types of honey. Several studies have also been performed out in animal models (mice/rats) with induced or transplanted tumor (Miguel et al. 2017). Honey operates at different stages of cancer including the initiation, cell multiplication, and disease progression. The mechanism of anticancer effects of honey includes induction of apoptosis (physiological form of cell death), arrest of cell cycle, oxidative stress reduction, the lowering of inflammation, the induction of mitochondrial outer membrane permeabilization (MOMP), and angiogenesis inhibition (Orsolic et al. 2003).

Honey has been found to induce apoptosis in cancer cells through mitochondrial membrane depolarization (Fauzi et al. 2011). Honey has been reported to elevate poly-ADP-ribose polymerase (PARP) cleavage and caspase 3 activation in colon cancer cell lines of humans owing to its high content of amino acid (tryptophan) and phenolic compounds (Jaganathan and Mandal 2009). Additionally, honey induces cell death in colon cancer cell lines by modulating the expression levels of pro- and anti-apoptotic proteins (Jaganathan and Mandal 2010). Honey elevates the expression of p53, proapoptotic protein Bax, and caspase and decreases the expression of anti-apoptotic protein Bcl-2 (Jaganathan and Mandal 2010). Honey attenuates the generation of ROS leading to p53 activation which in turn fine tune the expression of pro- and anti-apoptotic proteins like Bax and Bcl-2 (Jaganathan and Mandal 2010).

9.8 Honey and Diabetes

Diabetes is a metabolic disease with multifactorial and diverse causes. Diabetes mellitus, a chronic disorder, is one of the leading diseases in the modern world, and >285 million people were estimated to have the disorder in 2010. It is estimated that 438 million people will develop diabetes mellitus by the year 2030 globally (Shaw et al. 2010). Diabetes prevalence is either hereditary or can develop any time during life.

It has been indicated in numerous studies that use of honey results in decrease in the blood sugar levels in partial insulin deficiency diabetic rats in which diabetes was induced by simultaneous administration of streptozocin (STZ)-nicotinamide. Rats treated with honey for about 1 month showed a significant reduction of fetal bovine serum (FBS) level compared to the control (untreated) diabetic rats that is credited to a remarkable improvement in serum insulin level. Additionally, treatment with honey considerably increased catalase (antioxidative enzyme) expression as indicated in the immunohistochemical analysis, which lowered the oxidative stress in the pancreas and promoted the healing of the pancreatic tissue (Aziz et al. 2017). L-Phenylalanine amino acid present in honey have been indicated for stimulating the insulin release from pancreas which improves the glucose tolerance in diabetic rats (Aziz et al. 2017).

It has been investigated that a 3-month ingestion of honey in type 1 diabetic patients induced a significant reduction in fasting blood glucose, serum triglycerides (TGs), total cholesterol (TC), LDL, and a significant rise in fasting C-peptide and 2-h postprandial C-peptide. Additionally, a prolonged ingestion of honey triggered considerable reductions in fasting serum glucose, 2-h postprandial serum glucose, serum TGs, and HbA1C (Abdulrhman et al. 2013). These findings indicated that long-term ingestion of honey has improved the metabolic imbalances of type 1 diabetes mellitus.

9.9 Nervous System

Honey plays a key role in the neuroprotection owing to the presence of polyphenols. Honey prevents the generation of ROS, which are toxic to the central nervous system. Polyphenols in honey neutralize various neurological pathologies involved in the process of aging. Additionally, polyphenols in honey prevent the accumulation of misfolded proteins, such as β-amyloid plaques, that have central role in some age-related neurological pathologies (Syarifah-Noratiqah et al. 2018).

It has been investigated that administration of honey to kainic acid (KA)-induced neurodegeneration in the cortex of male Sprague–Dawley rats resulted in the decrease in the neurodegeneration in the rat cerebral cortex, and this property is attributed to its antioxidant property of honey (Sairazi et al. 2017). Additionally, The neuroprotective effects of honey owing to its antioxidant rich potential were also examined in cultured astrocytes. These cells were exposed to honey at the different doses (0.1%, 0.3%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 3%, and 5% [*v*/*v*]) for 24 h followed by hydrogen peroxide (H_2O_2) at the concentration of 100 μmol/L for 3 h. Cell viability was analyzed with MTT assay. Honey treatment prevented the cell death in a dose–response manner compared with H_2O_2-treated cells. Honey at the dose of 1% had the most significant effect (Ali and Kunugi 2019). The neuroprotective effects of honey flavonoid extract (HFE) on the production of pro-inflammatory mediators in lipopolysaccharide-activated N13 microglia cells were examined. The findings from this study indicated that the HFE considerably inhibited the release of TNF-α and IL-1β (pro-inflammatory cytokines). The expression of iNOS and the production of ROS were also considerably inhibited. In this study, it was indicated that HFE is a potent inhibitor of microglial cell activation and thus a potential neuropreventive–therapeutic substance involving neuroinflammation (Candiracci et al. 2012).

Earlier study indicated that pretreatment with honey to Sprague–Dawley rats that were exposed to hypoxia-induced memory deficits reduced the neuronal damage in hippocampus of rats and improved the memory of the rats (Abdulmajeed et al. 2016).

On in vivo use, administration of different honey samples to mice induced the behavioral effects including central inhibitory effects, antinociceptive, anxiolytic, as well as antidepressant effects. Additionally, significant hypnotic and partial protection of picrotoxin-induced convulsions was also observed. These findings provide crystal clear indications that honey can be used as nutraceutical agents (Akanmu et al. 2011). In another study, preemptive administration of honey (Tualang honey) at the dose of 1.2 and 2.4 g/kg body weight reduced the pain responses in male Sprague–Dawley rats (Aziz et al. 2014).

9.10 Ophthalmological Conditions

Ophthalmology is one of the most promising areas of application of honey. Ample source of investigation provides the strong evidences that honey can be successfully used in the management of different ophthalmological conditions. In vitro corneal fibroblast cell lines isolated from New Zealand white rabbits have indicated that honey promoted wound healing by improving the healing process, maintaining corneal crystallin and retaining the production of type I collagen as well as by decreasing the scar development risks through reduction of myofibroblasts transformation which may be a potent natural adjunct for corneal wound treatment (Yusof et al.

2019). In another study of contact lens-induced corneal ulcer, complementary treatment with honey was explored, and it was indicated that honey is an effective antimicrobial agent for corneal ulcers treatment. Additionally, honey exerts promising antibiofilm and anti-inflammatory effects and thus becomes an attractive ophthalmologic agent (Majtanova et al. 2015). Other studies in which ophthalmological use has been explored include dry eye syndrome (Albietz and Lenton 2006; Jankauskiene et al. 2007), bullous keratopathy (Sethi and Rai 2005), and opacities of the cornea after herpetic keratitis (Mozherenkov and Prokofjeva 1991).

Honey has been found to exhibit antiangiogenic and anti-inflammatory properties on corneal abrasions and endotoxin-induced keratitis in Lewis rats in which keratitis was induced by topical application of *P. aeruginosa* endotoxin to scarified corneas (Uwaydat et al. 2011).

The effectiveness and safety of topical honey eye drops was evaluated in the clinical trial in the patients with diagnosed vernal keratoconjunctivitis (VKC). Honey drop in VKC patients resulted in the significant increase in eye pressure and decrease in redness as well as limbal papillae (Salehi et al. 2014).

9.11 Gastroenterology

Protective effects of honey on the gastrointestinal tract have been established in several studies. Rats fed with honey demonstrated a modulation in the lactic acid bacteria in the intestines possibly indicating the role of honey in modulating the gut microbiota (Shamala et al. 2000). The antimicrobial activity of different types of honey against *H. pylori* isolated from patient stomach with gastric diseases has been determined. The antimicrobial potential of honey against *H. pylori* was evaluated by minimum inhibitory/minimum bactericidal concentration. *H. Pylori* has indicated to be susceptible to honey with a median level of antimicrobial activity due to the presence of H_2O_2 (20%) concentration (McGovern et al. 1999).

All the honey samples tested in the study indicated a high antibacterial activity with obvious therapeutic potential (Manisha and Shyamapada 2011). Furthermore, honey also acted against gastric ulcers in indomethacin and alcohol-induced rat models (Ali 1995; Gharzouli et al. 2002). Honey inhibits the production of prostaglandin and stimulates the sensory nerves in the stomach that respond to capsaicin (Ali 1995). This accounts for the antioxidant properties of honey. The effects of natural honey on absolute ethanol-induced gastric lesions were also studied in rats. Honey demonstrated the healing properties in acetylsalicylic acid–induced gastric ulcer in rats. The healing properties demonstrated by the honey were equivalent to the cimetidine (used for the treatment and prevention of certain types of stomach ulcer) (Bukhari et al. 2011).

Honey ingestion has been found to resolve the gastroenteritis and diarrhea quickly (Haffejee and Moosa 1985; Bansal et al. 2005). Ingestion of honey at the dosage of 5.0% (v/v) decreased the length of diarrhea associated with bacterial gastroenteritis when compared to sugar solution in replacement fluid concentration. However, No change was observed in viral gastroenteritis. The addition of honey to rehyderation fluids resulted in increase in K and H_2O uptake with no increasing in sodium uptake (Bansal et al. 2005). Pretreatment with honey at the dose of 2 g/kg body weight ameliorated indomethacin-induced gastric lesions, myeloperoxidase activity and microvascular permeability of the stomach in the rats that were administered (orally) honey (Nasutia et al. 2006).

9.12 Concluding Remarks

This chapter summarizes the recent update on the identification of bioactive components from natural. Use of honey as a valued natural product as well as traditional medicine has been appreciated from the time immoral. Its effectiveness in the modern medicine for the treatment of human diseases has become available only recently. The major effects of honey include its antibacterial activity against a wide spectrum of bacteria, fungi, and yeast. Additionally, the role of honey in the treatment of diabetes, wound healing, eye care, neuroprotection, and gastroenterology has been well established in several studies and has been thoroughly discussed. The diverse pharmacological property of honey is due to its constituents such as phenolics, peptides, vitamins, enzymes, organic acids, and Maillard reaction products which plays an vital role in its useful effects for the management of human diseases.

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Honey: Types, Composition and Antimicrobial Mechanisms

10

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Abstract

Honey has been broadly recognized as a source of nourishment and medication by both old and new generations. It has been utilized by people to treat numerous illnesses through topical application for at least 2700 years, but recent researches have revealed the antiseptic and antimicrobial activities of honey. It has been

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_10

seen as a powerful agent that can be used in various human pathologies. Different clinical examinations have affirmed that the use of honey to cutaneous injuries which are severely infected removes contamination from the injury and enhances tissue healing. Honey has been perceived as a successful antioxidant and antimicrobial agent for centuries. Utilized mainly for treating burns and surface wounds, it has thus been developed into clinical medicine, as medical grade honey. In spite of this, the underlying interest in the utilization of honey for antimicrobial treatment was significantly reduced, as antibiotics were discovered and used. Nonetheless, due to the alarming increment in the occurrence of organisms with antimicrobial resistance, specifically the expansion in multidrug resistance (MDR), the quantity of efficient antibiotics is decreasing at a larger rate than new medications are created. This serious situation has made numerous scientists to think back to the pre-antibiotic period for creating solutions, directing their consideration towards the mechanisms of action of antimicrobial activity of honey.

Keywords

Honey · Polyphenols · Constituents · Antimicrobial · Nectar · Apis mellifera

10.1 Introduction

Honey is having an extensive history of human utilization as an oldest sugar and nourishment source. Honey was referenced in the manuscripts of Egypt, China and India since 5500 BC. While the medical utilization of honey has been documented, at least since 2000 BC, it is recently that the utilization of honey in wound administration has become extensively available (Cooper et al. 2002). In both clinical medications and the safeguarding of nourishments, the utilization of natural items is turning into a perpetually well-known approach. Increase in their ubiquity is because of their powerful activities and normally extremely low lethality. As per the World Health Organization insights, in some developed countries, up to 80% of the population had utilized natural items in their primary health services (WHO 2014). Furthermore, 80% of individuals rely upon these sorts of treatment in Asian nations, for example, India and China. Products of natural origin can be utilized in the revelation of new antimicrobial medications as well as in treating many infectious diseases. Researchers have established that natural items are commonly more acknowledged by people; if these substitute methodologies are efficient, this may decrease the dependence on manufactured substances (Slover et al. 2009). In addition, the investigation of such natural products may prompt the detection of an active compound that might be utilized to evade some environmental risks or potentially, in mammalian cells have an ameliorative effect on certain diseases (Mahady et al. 2008). For these new active components, herbs, plant separates, honey and fundamental oils are mainly the well-known sources (Slover et al. 2009), and these items have been seen to be very efficient against a variety of inflammatory cases and bacterial contaminations (Molan 2009). Honey is one of the best examples of a naturally accessible product and is the main concentrated sugar found in nature. In many nations, it has been utilized for centuries as a cure for numerous diseases, long before the information existed on the reasons of contamination. Honey has been perceived to be powerful in practically all instances of disease and for the advancement of healing particularly in wounds and burns (Mandal and Mandal 2011). Throughout the past, honey has been utilized in various societies, with contrasting applications. The ancient Egyptians utilized honey for wound dressing as well as a topical ointment and for preserving their dead, while the old Greeks utilized it to treat fever, gout, pain and wound healing (Eteraf-Oskouei and Najafi 2013). The principal observations about the antimicrobial action of honey were made in the year 1892, and since then, honey was shown to have a wide range of activity, hindering both Gram-positive and Gram-negative microbes, including Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Listeria monocytogenes and their multidrug-resistant counterparts (Dustmann 1979; Laallam et al. 2015). The effectiveness of honey against these living beings is reliant on the kind of honey utilized, because of variations in geographical area, bee health, botanical origin and the processing of honey (Cokcetin et al. 2016; El Sohaimy et al. 2015; Sherlock et al. 2010). It is apparent that numerous diverse sorts of honey can be found throughout the globe as various regions will have diverse flora, which will have an effect on the preparation and activity of various kinds of honey. Moreover, it is feasible to recognize honey into two fundamental types: first, floral honey that is set up from the nectar of blossoms (blossom honey) and second, honeydew honey that is made from the plant secretions (living part) or the discharges of plant sucking insects (Sanz et al. 2005a; Bentabol et al. 2011).

Honey has been chiefly utilized as a source of nourishment as well as therapeutic agent all the way through the history, across a wide and diverse variety of communities (White 1966). The antimicrobial properties of honey along with healing process and immune system activation are the major reasons for its universal recognition (de Abreu Franchini et al. 2007; Tonks et al. 2007). Furthermore, in spite of its extensive history as medication, honey was not perceived as a therapeutic agent by present day medication until recent past, perhaps because of limited understanding of its range and mechanism of antibacterial property (Blair et al. 2009). Numerous studies in the last two decades have looked into identification, mechanism of action and synergistic nature of its bioactive compounds (Blair et al. 2009; Tan et al. 2009; Brudzynski and Lannigan 2012; Majtan et al. 2014). In this manner, honey has recently become a part of conventional medicine for wound healing (Vandamme et al. 2013; Molan 2006; Bonn 2003). However, presently only a limited number of honey brands (Manuka, Medihoney) are accessible for the treatment of wound infections (Langemo et al. 2009; Simon et al. 2009). This identification is because of the high non-peroxide antibacterial activity of honey, identified specifically in Manuka honey (Allen et al. 1991). Manuka honey has been standardized according to phenol equivalence and labelled as Unique Manuka Factor (UMF) (Allen et al. 1991). A number of studies have found that honey also contains prebiotics, probiotics and zinc along with multiple antibacterial substances (Hernandez et al. 2005; Sanz et al. 2005b; Olofsson and Va'squez 2008; Robert and Ismail 2009; Vásquez et al. 2012). The existence of such valuable substances in honey has considerable clinical implications as far as treatment of diarrhoea is considered, since the existing treatment protocols for diarrhoea prohibits the usage of antibiotics and instead recommend the use of prebiotics, probiotics and zinc along with rehydration therapy (Guarino et al. 2008; Dinleyici et al. 2015; Dickinson and Surawicz 2014; Aachary and Prapulla 2011). The enhanced consumer utilization of complementary medicines has encouraged an expanding enthusiasm for nonconventional as well as traditional clinical medicines. One of the treatments that have gotten a lot of consideration is honey, and it has an extensive custom of utilization in different clinical frameworks (Majno 1975; Zumla and Lulat 1989), and over the previous decade, many researchers have centred their interest towards this natural product (Postmes et al. 1993; Greenwood 1993; Molan 1998; Moore et al. 2001). While honey has various therapeutic uses and has been utilized as a preservative agent for food, it is typically well identified for its benefits in wound treatment. Honey helps in maintaining moisture within wound environment that advances healing, and for the prevention of infection a protective barrier is provided by its high viscosity. Also, the mild acidic property of honey and release of lower levels of hydrogen peroxide help in wound healing and add to its antibacterial activity. It is this antibacterial property of honey which plays a vital role, in advancing healing of wound which is infected (Dunford et al. 2000; Lusby et al. 2002). There are two honeys in Australia, Manuka honey and Medihoney that are available as therapeutic honeys appropriate for treating ulcers, burns and infected wounds. Certainly, most of the available research right now in this field has been done utilizing either Manuka honey or Medihoney (Honey Scientific Report 1998). Both Manuka honey and Medihoney are obtained from *Leptospermum* spp., and it is not surprising that because of this reason, there is similarity in their activity. Earlier researches have revealed that both the honeys have a particular antibacterial property because of a non-hydrogen peroxide mechanism (Molan and Russell 1988; Weston 2000), that is, the Unique Manuka Factor (UMF). On the other hand, the distinction in Minimum Inhibitory Concentration (MIC) for antibacterial property among Unique Manuka Factor honeys and other honeys are often very little, usually <5% (Willix et al. 1992; Molan and Brett 1998), and the implication of this in medical domain is not clear. Yet, honey does have important prospective to help in healing of the wound, and this has been confirmed time and again (Molan and Brett 1998); Dunford et al. 2000; Natarajan et al. 2001). Manuka honey that is obtained from Leptospermum species inhibits the growth of Gram-positive microorganism, *Enterococcus faecalis,* while *E. coli* (Gram-negative microorganism) was seen to show more resistance towards honey treatment (Kumar et al. 2014). Several researches that were conducted on Chinese Buckwheat (Fagopyrum esculentum) and Manuka honey revealed a minimum inhibitory concentration of 60% (w/v) against *P. aeruginosa* and 5% (w/v) against *S. aureus* (Deng et al. 2018). Comparable outcome of linen vine honey revealed that *S. aureus* was more vulnerable than *P. aeruginosa* (Alvarez-suarez et al. 2010a). One more research assessing the efficiency of honey throughout a range of botanical origins recognized more vulnerability generally with respect to Gram-positive organisms, *Staphylococcus epidermidis* and *S. aureus*, and also reduced susceptibility or no impact towards the Gram-negative organisms, *P. aeruginosa* and *E. coli* (Matzen et al. 2018). One more investigation observing the antimicrobial action in case of Polish honey against *S. aureus* established a minimum inhibitory concentration of only 1.56% (w/v) (Grecka et al. 2018). However, many different investigations carried out on Gram-positive bacteria have revealed that they are more resistant to honey (Isla et al. 2011; Escuredo et al. 2012; Fyfe et al. 2017). Mohapatra et al. (2011) recognized that the Gram-positive microbes were less susceptible to honey than Gram-negative microbes, proposing the reason might be the high hydrogen peroxide concentration and osmolality of the samples. With respect to Rubus honey, the most susceptible organism was *Proteus mirabilis*, displaying an MIC of 7.8–31.3 mg/mL, while *S. aureus* showed an MIC range of up to 125 mg/mL (Escuredo et al. 2012). Besides, honeys of monofloral and multifloral origin were found to be more efficient against Gram-negative microbes compared to Gram-positive microbes, with *P. aeruginosa* establishing an MIC of 100 mg/mL, while as *S. aureus* showed an MIC of 250 mg/mL and *E. feacalis* extending from 200–250 mg/mL, also few honey samples do not show any effect on Gram-positive microbes (Isla et al. 2011). Besides, an investigation carried out on Egyptian honey recognized Sidr honey was the only efficient honey against *S. aureus*, having an MIC of 100% and just four honey samples out of six were efficient against *Streptococcus mutans*. All tested samples were found to be efficient against *K. pneumoniae and P. mirabilis*, having an MIC of 50% or less. Just one sample was not found to be efficient against *E. coli*, and three out of six samples were not efficient against *P. aeruginosa*; however, the MIC values of inhibitory samples were 50% or less (El-Borai et al. 2018). Also, it has been revealed that when given a treatment of a range of Scottish honey samples, *Acinetobacter calcoaceticus* was found to be the most affected, in comparison to *P. aeruginosa, E. coli*, and *S. aureus* (Fyfe et al. 2017). This diversity of results proposes that all honeys are not equivalent and their efficiency varies largely, delineating the importance of geographical area and botanical origin on the antimicrobial action displayed by a particular honey. Interestingly, through researches, it has been seen that no organism has acquired resistance against honey (Maddocks and Jenkins 2013). Furthermore, in methicillin-resistant Staphylococcus aureus (MRSA), sub-inhibitory dosages of honey have been found to re-establish oxacillin susceptibility (Jenkins and Cooper 2012). Initial investigations regarding honey have revealed some important aspects that result to its antimicrobial activity, these include low pH, polyphenolic compounds, hydrogen peroxide, high sugar content, and the detection of an "Inhibine" (Albaridi 2019; Dustmann 1979; Molan 1992). In addition, investigations exploring the reason for honey being a potent antimicrobial agent revealed that inhibine was a 1,2-dicarbonyl compound in the form of methylglyoxal which is an effective antimicrobial found primarily in Manuka honey (Mavric et al. 2008). Bee defensin-1, a bee-derived protein, a potential antimicrobial agent within honey was also identified through recent studies (Bucekova et al. 2019). This furthers the argument that honey contains a number of antimicrobial components, and their activity is not governed by a single antimicrobial agent. Furthermore, the effectiveness of honey as an antimicrobial agent is enhanced by synergistic operation of its multiple components.

10.2 Nomenclature and Classification of Honey

Honey is basically a sugar solution (saturated or supersaturated) which is prepared by honeybees and some other insects. Honeybees and the insects collect nectar from the flower and transform the nectar by adding up enzymes to it and then store it as a source of food, so that it can be used in dearth periods (Crane and Visscher 2009). Honey is predominantly prepared by honeybees (social insects) which have a perennial life cycle, although few other insects also contribute towards honey production. The bees are mostly categorized into various groups which include all honeybees (Apis spp.), Nectarina wasps in South America, as well as stingless bees (*Melipona and Trigona spp*.) and a number of honey ant species, particularly *Melophorus inflatus* that are found in Australia. In addition, other social wasps and bumblebees (*Bombus spp.*) are also present, but they generate a little amount of honey (Crane 1999). Honey is mainly classified into two categories (Fig. 10.1). These are nectar honey and honeydew honey.

10.2.1 Nectar Honey

The European Commission Council Directive (EU 1102001) characterizes nectar honey as a naturally occurring sweet compound that is prepared by *Apis mellifera*. Bees collect the plant nectar, convert it by combining with particular substances produced by them, deposit the nectar, dry out, store and leave in honeycombs, so that it can get ripened and mature (EU 1102001).

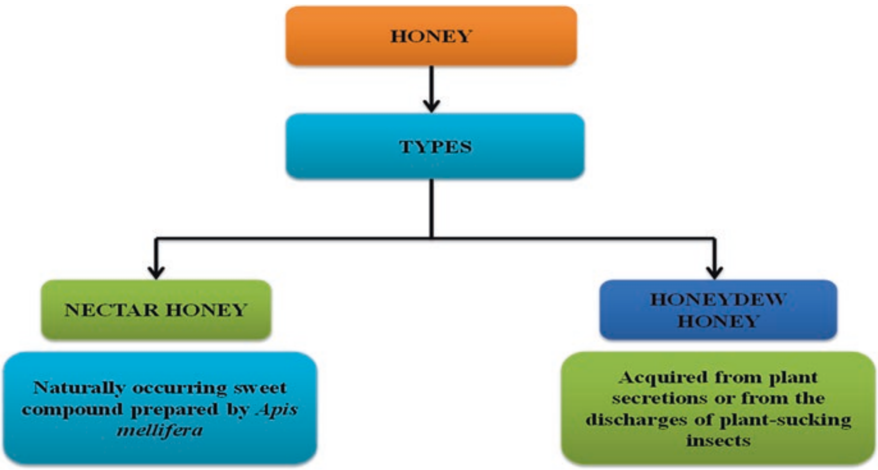

Fig. 10.1 Classification of honey

10.2.2 Honeydew Honey

The European Commission Council Directive (EU 1102001) characterizes honeydew honey as a source of nourishment that is acquired from plant secretions or from the discharges of plant sucking insects. These insects perforate the leaves or other plant parts and feed on the sap of the plants, discharge the excess in the form of droplets, which are later accumulated by the bees as honeydew (EU 1102001). The differentiation between nectar and honeydew honeys can be done with the help of pollen analysis, but through their physicochemical analysis, these can be better differentiated. The honeydew honeys have a high electrical conductivity, pH, ash, acidity and darker colour, as well as a high di- and trisaccharide concentration and a low monosaccharide concentration (Mateo and Reig 1998). Furthermore, honeydew honey contains algae and fungi cells, but their presence in not related to its origin (Bogdanov et al. 1997).

10.3 Honey Production

Apis mellifera (honeybee) is of enormous significance for people as it acts as a pollinator of both domestic and commercial crops and also provides honey which is of high nutritional value (Ratnieks and Carreck 2010; Potts et al. 2010). Honeybee loss because of the interfacing divers of diseases and pests, absence of genetic diversity, introduction of agrochemicals and apicultural mismanagement leads to extensive concern for the future potential of honeybees with respect to providing the services (Ratnieks and Carreck 2010; Potts et al. 2010). The composition and the quality of honey produced depend on numerous elements which includes bee health, geographical location of bee hive, flower composition, flowering phenology and yearly changes in local flora (Galimberti et al. 2014). Commercially, there are various kinds of honey that are available (creamed, chunk, comb, granulated or crystallized) with numerous diverse degrees of processing (heat processed, centrifuged, pressed, drained) (Anklam 1998). There are three castes within a honeybee hive: first is queen (alpha), second is worker (beta) and the third one is drone (gamma) bees (Havenhand 2010), and it is their aggregate effort which results in honey production. Honeybees prepare honey by utilizing nectar that is collected from flowering plants; nectar is a sugar containing liquid that is secreted by glands known as nectaries.

The worker bees travel up to 9 km in a single trip for collecting nectar (Havenhand 2010). The carbohydrate and sucrose that is present in nectar is hydrolysed to generate fructose and glucose (Kubota et al. 2004). The nectar is then ingested and regurgitated upon their arrival to the hive, by large number of worker bees inside the honey comb. The regurgitation and wing fanning process lead to evaporation which results in decreased water content, then the ripening of honey takes place with time (Fig. 10.2). Honeybees store honey as food for the winter season when there is non-availability of nectar or pollen. For human consumption,

Fig. 10.2 Process of production of honey

the excess honey can be extracted from the honey comb (Havenhand 2010). Kubota et al. (2004) depicted the way by which hypopharyngeal gland secretes glucosidase III in European bees. This glucosidase III is released into the nectar and is accountable for the release of hydrogen peroxide (Bucekova et al. 2014). In order to feed honeybee larvae, honeybees collect pollen grains as they visit flowering plants (Galimberti et al. 2014). Using nectar–saliva mixture, thick pellets of pollen are prepared from these pollen grains. Honeybees collect the exudates as an alternative to nectar, from sap-sucking insects. Honeydew is collected more often from insects that feed on sap of conifers and other anemophilous species (Oddo et al. 2004). For making propolis, the tree resin which is vigorously obtained from different plants is mixed with wax and later on stored in bee hive because it has antimicrobial properties (Wilson et al. 2013).

10.4 Honeybee: Life Cycle

Apis mellifera (honeybee), which is responsible for producing honey by collecting nectar from the flowers of different plants, is one of the eminent manifestations of God. In a bee hive, there are three kinds of bees: first, the only reproducing female that is the queen bee; second, the male bees known as drone bees; and third, the non-reproducing female bees called as worker bees. In order to produce eggs, the queen bee mates with the drones, fertilizes and resides the eggs in bee hive (comb) cells. Within 3–4 days, the eggs hatch to produce larvae. These larvae then grow to form pupae, and these pupae transform into new worker bees (female bees); the already existing worker bees then feed the new ones (Bishop 2005). A huge number of individual bees may be present in a characteristic bee colony which primarily comprise of the sterile worker bees (sterile female bees). Once the queen mates with the drones, the drones die, and the queen bee may have a life expectancy of almost 3–4 years (Bishop 2005; Rueppell et al. 2007). Honey is formed by the honeybees in their "honey stomach." The honeybees have two types of stomachs: normal stomach, which carries out the normal functions in honeybee, and the other one is particularly useful for preparing honey. Apis mellifera generally collects the nectar which is typically a sugar-rich transparent fluid comprising of mainly water (80%) and sugars (20%), from different flowers using their tongue (Zhu et al. 2016). In order to fill the "honey stomachs" with nectar, one worker bee usually visits approximately dozens of flowers. After that the processing of nectar is immediately done with the help of digestive enzymes which include catalase, amylase, acid phosphorylase and glucose oxidase, which converts sucrose into glucose and fructose (Zhu et al. 2016).

Once the worker bee arrives at the bee hive, the nectar is spitted into the mouth of different bees, thus starting the process of regurgitation. This process vitally helps in the production of honey that is the final product from raw nectar, with the help of the impact imparted by the digestive enzymes secreted by bees. The process of regurgitation proceeds for around 20 min, and then the final product (raw honey) is spitted into the honey comb cells. This final product is quite vulnerable for the attack by different microbes, as it has a high moisture content of about 80%. Then the honeybees flutter the wings to decrease excessive moisture, which creates a strong draft resulting in evaporation, thus reducing the moisture content to <20%. When the honey is dried, the honeybees seal the cells of the comb with wax in order to store it for future consumption. It is by the activity of honeybees that the wax is also produced from honey (Nicolson and Human 2008). There are usually two techniques that can be utilized for the extraction of honey from the hive. The first method is a conventional technique in which bees are calmed down or moved away from the bee hive by applying smoke in the hive. The moment bees are calmed down or moved away from the bee hive, honey is extracted by squeezing the hive. In the second method, the combs are placed in a metallic bowl soon after the bees are moved away, and then the burning coal is placed on the combs which results in melting down of honey and bee wax. These are then drained out from a hole, where honey is collected. However, the above-mentioned conventional techniques are not so effective and are being taken over by the modern techniques (Ediriweera and Premarathna 2012). Mechanical extractor which works on the concept of centrifugal force is a contemporary honey extraction method. It comprises a container that has a frame basket, this basket spins and tosses out the honey from the comb without damaging it and, hence, can be reutilized again by the honeybees.

10.5 Honey and Its Composition

Honey is a complex mixture consisting of water, carbohydrates and other minor compounds (Garcia et al. 1986; Cortes et al. 2011). The composition of honey is affected by the flower type as unifloral and polyfloral, as well as climatic and regional parameters (Cortes et al. 2011). Honey majorly comprises carbohydrates (82.4%), water (17.1%), amino acids (0.5%), minerals, vitamins and various minor compounds (Table 10.1) (Garcia et al. 1986; Montenegro and Fredes 2008; Cortes et al. 2011). In association with the geographical and botanical origin, there are various heavy metals that have been extracted from honey (Cortes et al. 2011). Alvarez-Suarez et al. (2010a, b) revealed that honey approximately contains 181 substances and, therefore, is a supersaturated solution. Honey was known as a significant carbohydrate sweetener before producing industrial sugar (Bogdanov et al. 2008; Alvarez-suarez et al. 2010b). Honey is being used as a nutrient as well as a medicine in many human societies (Bogdanov et al. 2008). The particular colour, flavour, texture and aroma of honey depends on a variety of factors; these include the flower type, honeydew and plants, their foraging habits, the physiological behaviour of the bee, post-collection processing and climatic conditions. Honey is prepared throughout the world, and it estimates approximately to 1.2 million tons per year, but it still constitutes only about less than 1% of the total sugar production worldwide (Bogdanov et al. 2008). Honey consumption varies worldwide; in European Union, it is 0.3–1.8 kg per capita, and in Argentina and China, it is 0.1–0.2 kg per capita annually. Researchers have so far been successful in isolating about 600 compounds from honey. Typically, the compounds that are present in honey can perhaps be obtained straight away from the plant source, by converting them with the help of metabolic activities of the bee, or from handling, heating, storage and microbial and environmental contamination (Manyi-Loh et al. 2001). The isolated chemicals belong to different chemical families, which include aldehyde, alcohol, hydrocarbon, ketone, norisoprenoids, furan and pyran, acid, cyclic compounds, benzene and its derivatives, ester, sulphur and terpenes. Due to the temperature at which honey has been stored and other storage conditions, the volatile compounds of honey may vary. In addition, the geographical location and the floral composition may greatly influence the composition of honey. During the storage process of honey, the volatile substances are produced by nonenzymatic activity, and the heat labile compounds may get destroyed, which results in change in the organic components of

Table 10.1 Different components of honey and their concentration

Component	Concentration
Water	17.1%
Carbohydrates	82.4%
Fructose	38.5%
Glucose	31%
Amino acids	0.5%
Protein	0.25%
Gluconic acid	0.23–0.98%

honey (Manyi-Loh et al. 2001). Honey is a nutritive compound as it is composed of numerous contents. Despite health benefits of honey, it can also get contaminated with pesticides, heavy metals and antibiotics that are present in the environment (Bogdanov 2006). There are some plants which contain poisonous compounds such as pyrazolidine and diterpenoids; bees can also use these for collecting nectar and may result in honey contamination (Edgar et al. 2002; Bogdanov et al. 2008). A brief description of some important components of honey is given below:

10.5.1 Carbohydrate

Honey being a highly saturated sugar solution generally comprises around 17.1% water. The major sugar is fructose which constitutes about 38.5%, followed by another sugar glucose which is about 31%, and disaccharides, trisaccharides and oligosaccharides in small quantities are also present (Crane 1976). Honey is the most suitable sweetener that is being utilized by the consumers, as an alternate to other sweeteners because of its unique flavour and higher nutritional value (Cortes et al. 2011). Besides 25 different forms of oligosaccharides, the most common sugars that are found in honey are monosaccharide, glucose and fructose (Siddiqui 1970). The major oligosaccharides include turnose, panose, sucrose, maltose, palatinose, 6-kestose, 1-ketose and trehalose (Bogdanov et al. 2008). The fructose and glucose present in honey soon after digestion provide instant energy to the body. However, as per the human standards, honey should be considered as a food supplement and not as a complete food. A dosage of 20 g of honey can provide only about 3% of daily energy requirement.

10.5.2 Protein, Enzymes and Amino Acids

Honey comprises about 0.25% protein content which majorly consists of amino acids and enzymes. Invertase, glucose oxidase and diastase are the key enzymes that are present in honey. As per the recommended daily requirement for human consumption, the consumption of honey as a protein source is not adequate (Bogdanov et al. 2008). Although the amino acid content in honey is often small, the extensive range of almost 18 amino acids (essential as well as nonessential) that are found in honey is exclusive and varies by floral origin. The main amino acid found in honey is proline, and lysine is the second most common amino acid. Tyrosine, glutamic acid, phenylalanine and aspartic acids are the various other amino acids that are found in honey. The glucose oxidase reaction yields glutamic acid as a product. While as the proline and other amino acids are contributed by the nectar, pollens or by the bees themselves (Crane 1976). Numerous enzymes which include glucose oxidase, acid phosphatase, invertase, diastase (amylase) and catalase are also found in honey (Crane 1976). Glutamic acid and hydrogen peroxide are prepared from glucose by glucose oxidase reaction, and it also results in the production of glucolactone that occurs in equilibrium with gluconic acid. The hydrogen peroxide found in honey thus acts as a contributing factor towards the antimicrobial activity of honey. The sucrose is converted to glucose and fructose with the help of invertase enzyme. The bees add invertase to the nectar in the form of fructo-invertase or gluco-invertase (Ensminger et al. 1983). After the extraction of honey, it contains a little quantity of invertase enzyme, and this enzyme may continue its activity there. However, high temperature results in inactivation of invertase enzyme.

10.5.3 Vitamins, Minerals and Other Compounds

Besides carbohydrates, proteins and enzymes, honey additionally contains different quantities of trace elements and minerals which include calcium, sodium, magnesium, zinc, potassium, phosphorous, copper, manganese, iron, selenium and chromium (Bogdanov et al. 2008). There are certain essential vitamins that are found in trace amounts; these include niacin, riboflavin, thiamine, phyllochinon, ascorbic acid, pyridoxine and pantothenic acid.

10.5.4 Polyphenols

Polyphenols are the finest vital groups that are found in plants, incorporating almost 8000 varied recognized structures (Bravo 1998; Estevinho et al. 2008). Polyphenols are affirmed to exhibit anti-inflammatory, antiatherogenic, analgesic activities, immune modulating, anti-carcinogenic and anti-thrombotic activities (Vinson et al. 1998). The possible indicators of the botanical source of honey are phenolic acids and flavonoids which are basically the phenolic compounds of honey (Yao et al. 2003). There are numerous diverse mechanisms which include singlet oxygen quenching, metal ion chelation, hydrogen donation, free radical scavenging and substrate carrying out for radicals such as hydroxyl and superoxide, to which the antioxidant activity of phenolic compounds is related to (Kucuk et al. 2007; Pandey and Rizvi 2009). The phenolic compounds that are isolated from honey can be categorized as flavonoles (quercetin, fisetin, kaempferol, myricetin, galangin), flavones (luteolin genkwanin, wogonin, apigenin, acacetin, tricetin), flavanones (hesperidin pinobanksin, pinocembrin naringenin, naringin), phenolic acid (vanillic acid, p-hydroxybenzoic acid, caffeic acid, gallic acid, syringic acid, p-coumaric acid, cinnamic acid, chlorogenic acid, ferulic acid, rosmarinic acid and derivative forms), tannins (ellagic acid) and coumarins (coumarin), as recognized by Abubakar et al. (2012).

10.5.5 Flavouring Agents

The aroma profile is the most significant attribute for the assessment of genuineness and organoleptic nature of any food product. As far as the consumer point of view is concerned, the flavour of honey is the most essential criteria. It is the volatile and semi-volatile organic components on which the aroma of the honey mostly depends (Jerkovic et al. 2006). The colour, taste and flavour of the honey may perhaps differ depending upon the botanical origin. Sugar is one of the most essential flavouring agents. The aroma of honey is dependent on factors like the type and quantity of amino acids and has also found to be connected with various types of phenolic compounds that are separated from various kinds of honey (Bogdanov et al. 2007). There are about 56–500 mg/kg polyphenols present in honey, and these depend usually on the type of honey (Al-mamary et al. 2002). Chrysin, luteolin, quercetin, galangin and apigenin are some of the important phenols that are found in honey (Tomas-Barberan et al. 2001). In general, the dark colour honey will have a prominent flavour compared to mild coloured honey which will have a mild flavour (Castro-Varquez et al. 2003; Kaskoniene et al. 2008).

10.5.6 Water Content

The determination of water content of honey is a significant quality parameter to avoid the spoilage of honey which can occur because of fermentation process. The moisture content affects the shelf life and the quality of honey; therefore, it is not like several other parameters which are alternatively accepted (Bogdanov et al. 2004). The International Honey Commission (IHC) has set a highest concentration of 20 g of water per 100 g of honey, for any honey sample to be acknowledged for honey trade. The other parameters of honey like glucose crystallization and viscosity are directly affected by its moisture content (Bogdanov et al. 2004). The moisture content of honey can be assessed with the help of many techniques which include Karl Fischer titration, gravimetric technique or refractive index (Sanchez et al. 2010).

10.5.7 Organic Acids

Honey contains mostly 30 organic acids (Mato et al. 2003), although the main organic acid that is found in honey is gluconic acid. It is found in the range of 0.23–0.98%, which is produced with the activity of the enzyme glucose oxidase (White 1975).

10.6 Honey: Antimicrobial Activity

Honey obtained from several plant source shows intense antimicrobial action (Szweda 2017). Manuka honey exhibits efficient action against *Salmonella aureus, Escherichia coli, Enterobacter aerogenes* and *S. typhimurium* (Lusby et al. 2005; Visavadia et al. 2006). Hovenia monofloral honey has been reported capable of showing antibacterial action Gram-negative and -positive bacteria that are found in various foodstuffs (Park et al. 2020). Researches on buckwheat honey also revealed that it showed strong antibacterial activity (Dżugan et al. 2020). Antibacterial property is an essential trait of honey for its selection in medical purpose and also serves an important criterion to assess honey in terms of quality (Godocikova et al. 2020). The osmotic pressure of honey is usually high because of its high sugar concentration, resulting in low water activity (Aw), of range 0.562–0.62 (Bogdanov et al. 1997), which makes the osmolarity to play a fundamental function in determining the antimicrobial action in case of undiluted honeys. When the water activity is between 0.94 and 0.99, it completely inhibits the growth of numerous bacterial species (Molan 1992). Acidity is another factor that plays a major role in determining the antimicrobial property of honey. Though it was considered to have a major role in antibacterial activity, current investigations have confirmed the acidity as a minor role player in determining the antibacterial activity of honey (Molan 1992).

10.6.1 Antimicrobial Activity: Mechanisms

Although a number of factors are responsible for the antimicrobial action of honey, the enzymatic glucose-oxidation reaction and few physical properties of honey contribute majorly towards its antimicrobial activity. Other factors comprise low pH/acidic environment, low protein content, low water content/high osmotic pressure, high carbon to nitrogen ratio, viscosity, and low redox potential that limits dissolved oxygen and other phytochemicals/chemical agents (Snowden and Cliver 1996). Dr. Peter Molan, the most well-known honey researcher, has carried out investigation on honey-related antimicrobial components and came out with major findings that are available on the website of University of Waikato, Hamilton New Zealand. The major findings include:

- The water activity (Aw) of honey is low, resulting in small amount of water availability that limits the bacterial and yeast growth. If the Aw of honey is in the range of 0.94–0.99, it will encourage the growth of many bacterial species. The Aw of ripened honey is in the range of 0.56–0.62, which prevents yeast growth. The bacterial species that grow rapidly at an Aw of 0.99 will not be affected with diluted honeys which have a higher value of Aw.
- The acidic property of honey inhibits the growth of several pathogens. For certain pathogens usually causing wound infections, the minimum pH value ranges from 4 to 4.5. This antibacterial efficiency of honey resulting from its acidity gets reduced whenever the honey is diluted particularly with body fluids which raise its pH.
- Honeybees secrete glucose oxidase, an enzyme that helps in the preparation of honey from nectar. It transforms glucose into gluconic acid and hydrogen peroxide in the presence of water and oxygen. During the ripening of honey, sterilization and preservation are carried out by gluconic acid and hydrogen peroxide. The pure honey has an insignificant content of hydrogen peroxide and active glucose oxidase. The hydrogen peroxide is quickly decomposed into oxygen and water by ascorbic acids and transition ions, while in case of low pH, the enzyme

is inactivated. On the other hand, there occurs a 2500- to 50,000-fold increase in enzyme activity on dilution of honey and formation of a slow release antiseptic which do not damage the tissue.

However, the generation of peroxide is not responsible for the entire observed antibacterial action. A number of other constituents found in honey with antibacterial property are found in small amounts and do not contribute significantly towards its antibacterial activity. These include benzyl alcohol, 3,4,5-trimethoxybenzoic acid, terpenes, 1,4-dihydroxybenzene, pinocembrin, 3,5-dimethoxy-4-hydroxybenzoic acid, 2-hydroxybenzoic acid, methyl-3,5-dimethoxy-4-hydroxybenzoate, and 2-hydroxy-3-phenylpropionic acid. Different researches provided the evidence for the presence of non-peroxide antimicrobial factors. One such research is treating the honey with heat resulting in inactivation of glucose oxidase, and another is treating the honey with catalase resulting in the elimination of peroxide activity. All kinds of honey does not have the same antimicrobial potential because of the differences in concentration of non-peroxide factors as well as peroxide production owing to difference in floral source and honey processing. Certain factors like metal ions, catalase and ascorbic acid can destroy hydrogen peroxide while as glucose oxidase enzyme may get wiped out by light and heat. The antibacterial potential can be assessed by determining the Inhibine Number, and it is the degree of dilution up to which the antibacterial activity of honey can be retained.

The majority of the researchers nowadays represented the antimicrobial activity of honey in terms of minimum inhibitory concentration (MIC). It is defined as the minimum concentration of honey that is essential for absolute inhibition of microbial growth (Saranraj and Sivasakthivelan 2012). A variety of studies carried out on a large number of honey samples showed a broad range of antimicrobial activity and several with low level of activity (Allen et al. 1991). While there is still lot more work to be done to clearly recognize the antimicrobial action of all honeys, there are few researches which revealed high levels of antimicrobial activity in honeydew honey from coniferous forests of Central Europe and Manuka honey obtained from *Leptospermum* species of New Zealand, as it had the maximum levels of non-peroxide activity among 26 different samples of honey with diverse floral origin. It strongly inhibited the growth of *Escherichia coli and Staphylococcus aureus* (Willix et al. 1992). An in vitro study conducted for comparing the antibacterial potential of pasture honey and Manuka honey on coagulase positive strains (*Staphylococcus aureus*) collected from contaminated wounds reported slight difference in their sensitivity towards both the honeys. However, Manuka honey which has non-peroxidal antibacterial action and pasture honey which has a high peroxide generation were both efficient at low concentrations of 2–3% v/v and 3–4% v/v, respectively (Cooper et al. 1999).

Today most of the researches have revealed the antimicrobial activity of honey in many microbial strains including clinical isolates, with the help of in vitro antimicrobial assays. There are only few studies that have revealed the antimicrobial activity of honey in vivo, with relation to wound infections. Cooper et al. (2001) reported, in a 38-year-old female who was suffering from a *S. aureus* infected recalcitrant surgical wound, when treated with Manuka honey impregnated dressings and oral coamoxiclav resulted in wound healing and bacterial clearance after 7 days of commencement of the treatment. For 3 years, the wound had failed to respond to any conventional treatment before the commencement of honey/antibiotic therapy. More researches have reported many controversial findings. Gethin and Cowman (2008) studied the treatment of Manuka honey or hydrogel in 108 patients suffering from venous leg ulcers. They found out that the Manuka honey efficiently eradicated methicillin-resistant *Staphylococcus aureus* (MRSA) from 70% of MRSA-infected wounds, and the hydrogel eliminated MRSA only from 16% of wounds. Jull et al. (2008) during a clinical trial of 368 patients treated with Manuka honey impregnated dressings or usual care revealed no major difference in incidence of venous leg ulcer infections. One more study reported that when patients undergoing peritoneal dialysis were treated with Medihoney antibacterial gel or the topical antibiotic mupirocin, no major difference was reported with respect to development of peritoneal dialysis-related infections (Johnson et al. 2014).

10.7 Conclusion

Today, researchers give more consideration to medications with natural origin and consider that the products with natural origin may prove to be efficient therapeutics compared to synthetic ones. Honey is the most significant natural product, being utilized for diverse medicinal purposes since long back. Although, honey has a significant role in conventional medication, scientists also believe that honey can also be used as an efficient medicine in treating various kinds of diseases. Researchers have revealed that honey has a significant antimicrobial activity. The antimicrobial and wound healing effectiveness of honey is specific to season, flower and region. All honeys do not necessarily show the same antimicrobial potential as it can vary due to the difference in pH, quantity of active principles, sugar content, different vulnerability of a variety of bacterial strains and storage conditions, though some honey samples do not show any significant antimicrobial activity. The use of honey does not result in developing antibiotic resistance in microorganisms, unlike some other traditional local chemotherapeutics, and hence can be used continuously. In case of highly drug-resistant bacterial infections, honey has the capability to immensely reduce the requirement of last resort drugs, since currently the antimicrobial resistance in case of honey is not seen. The utilization of honey in future will be prolonged to a large extent. This is because of the increase in multidrug-resistant organisms (MDR) which causes infections that cannot be treated even with multiple classes of antibiotics, predominantly because honey has been found to have the capability of reversing certain antibiotic resistance mechanisms. Hence, the renewal of this substitute antimicrobial agent represents potential therapeutic avenue in controlling the rising frequency of antibiotic-resistant bacterial infections.

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Honey as Component of Diet: Importance and Scope

11

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Abstract

Natural honey (NH) is a highly nutritious substance and is considered as one of nature's wonders which has been used by all cultures, traditions and civilizations as a food and medicine. Natural honey (NH) is a by-product made by honeybees by using nectar of flowers and sugary non-floral deposits obtained from plants that is then converted into honey by a process of regurgitation and evaporation. Later the honeybees store honey as a primary source of food in wax honeycombs inside the beehive. Honey is classified on the basis of processing, physical, chemical, and nutritional properties. Honey also plays a part in symbolism and religion. The appearance, quality, sensory perception, and composition of NH vary greatly depending on the nectar source, environmental and climatic conditions. Honey's main constituents include carbohydrates, primarily fructose and glucose although it also contains various oligosaccharide sugars. Besides these NH, also contains minute quantities of amino acids, proteins, enzymes, trace elements, minerals, vitamins, aroma substances, and polyphenols. NH shows a vast range of health and nutritional properties. NH imparts antimicrobial, anti-inflammatory, antioxidant, immune boosting property, antiviral, antiparasitory,

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_11

antimutagenic, and antitumor effects. Honey has also been well known for treatment of gastrointestinal disorders like peptic ulcers, gastroenteritis, and gastritis. Thus NH has proved a valuable nutritional food and medicinal entity.

Keywords

Natural honey (NH) · Composition · Scope · Nutritional and health effects

11.1 Introduction

The use of NH as a rich nutritive food and medicine has been done by mankind from the immemorial times. Honey is a sweet, viscous complex substance synthesized by honeybees (*Apis mellifera*) from nectar, a sugary liquid of flowers, or from exudation of plants or secretions of plant sucking insects living on plants, respectively. The honey bees gather, convert, and mix these plant parts in the comb with their own enzymes (diastase, invertase, and glucose oxidase), thereby storing and leaving this for ripening and developing (Can et al. 2015). In ancient times, the only natural food sweetener that was available to *Homo sapiens* was honey. Infact the relation between bees and man is very old and has started since the Stone Age (Crane 1983). The earlier people of ancient civilizations were prepared to sacrifice his life to get sweet honey. First instance of NH being used as medicine and emollient reflects back to a Sumerian tablet dating 2100–2000 BC (Crane 1975). For rich nutritious and medicinal value, NH has been used by most of the ancient civilizations (Crane 1975, 1999; Jones 2001; Allsop and Miller 1996). The utilization of NH by humans is believed to be 8000 years old as portrayed by the works of art of Stone Age (Bansal et al. 2005). The ancient Egyptians, Chinese, Assyrians, Greeks, and Romans used NH to treat wounds and gut diseases (Al-Jabri 2005). Natural honey is used by people of different ages as it overcomes all the barriers of culture and ethnicity. All religions all cultures advocate and embrace the use of NH. In our religion (Islam), an entire chapter is depicted in the Holy Qur'an, entitled "Surah al-Nahl," which means "Honey Bee" which advocates use of NH as food and medicine (The Holy Quran). Our Prophet Muhammad SAW has firmly mentioned the use of NH for healing and curative purposes as mentioned in the book of hadith (Al-Bukhari 1976). It is also mentioned in the Chap. 16 of the Holy Qur'an that NH is an essential nutritious and health boosting food) (An-Nahl 1990a, b). The well-known insight about honey being good for health has its origin from folklore. An important role in folklore has been played by myth. There are numerous written and oral records about the vitalizing honey's life-giving qualities. Avicenna, a great Iranian scientist and physician, has strongly recommended NH as best available remedy for tuberculosis treatment (Asadi-Pooya et al. 2003). To the mankind, NH has been considered as one of the most unique gifts of nature as described by Indian Ayurveda medicine. Ayurveda texts consider NH as a great medicine to treat disorders of skin like burns and wounds, insomnia, cardiac pain, palpitation, anemia, teeth and gums, lung imbalances, weak digestion, irritating cough and eye ailments (Honey in History 2008). Honey is considered as highly valuable food by Veddas, Wild Men of Sri Lanka and Australian aboriginal tribes as they risk their lives to obtain it. The people of ancient Egypt considered NH as the most renowned medicine that was used in 900 remedies (Al-Jabri 2005). Most of the medicines used by Egyptians contained honey in combination with milk and wine. NH was used by ancient Egyptians and presented to their deities as a sacrifice (Zumla and Lulat 1989). Honey was also used as a topical ointment and antibacterial agent for healing wounds by ancient people of Egypt (Honey 2012). In ancient Greece, NH was used to treat several diseases. A beverage of ancient Greece was Oenomel that consists of unfermented juice of grapes and NH that was utilized to treat gout and various nervous disorders (Honey 2012). Hippocrates, prescribed a simple diet, which consisted of honey and other substances with honey being the main ingredient. He gave a mixture of honey and vinegar (oxymel) for pain, honey, and water (hydromel) for thirst and a combination of NH, water and other substituents for treating acute fevers (Zumla and Lulat 1989). Further NH was also used for treating baldness, cough, sore throat, wound healing, contraception, topical antisepsis, laxative action, eye diseases, prevention, and treatment of scars (Bansal et al. 2005). Thus NH was used by the folklore people as a great nutritious food and medicine for treating numerous diseases.

11.2 Classification of Honey

The honey is classified into various classes and the major forms that are available after the process of packaging are raw, pasteurized, strained, granulated, ultrafiltered, chunk, ultrasonicated, comb, whipped, and dried as enlisted in Table 11.1 (Crane 1975; Ustunol and Gandhi 2001; Decaix 1976).

11.3 Chemical Composition of Natural Honey

NH possesses all the essential components that are required for a healthy balanced diet. NH is an energy rich source of food and is readily digestible. More than 200 compounds are present in NH, a major portion of which are carbohydrates and water accounting for about 95% of honey's dry weight and the remaining part includes amino acids, proteins, enzymes, vitamins, organic acids, polyphenols, and aroma substances (White 1979). NH's nutritional and chemical constituents are strongly affected by the plants or botanical sources on which the honey bees feed (Persano and Piro 2004). The importance of honey with regard to its nutritional aspect lies in its manifold physiological effects irrespective of the recommended daily intake being required is small.

Table 11.1 Natural honey and its types

Class	Description
Raw honey	Raw honey exists in the beehive in its original form and is obtained by various processes such as extraction, settling, straining, etc. The production of raw honey requires minimal processing and no heat treatment
Pasteurized honey	Pasteurized honey is obtained during the process of pasteurization in which the honey is heated to a high temperature of about 161 °F (71.7 °C). The pasteurization cycle kills the cells of yeast, and also causes micro-crystal liquefaction in NH, thus preventing start of visual crystallization
Strained honey	Strained honey is obtained after the honey passes over a mesh medium to extract suspended matter like propolis, wax, and dirt without the removal of minerals, enzymes, and pollens
Granulated honey	Granulated honey or crystallized honey is typically made when glucose levels in NH has slowly crystallized in monohydrate form from a solution (Bogdanov 2015). This form of honey returns to liquid form when it is placed in a vessel with lukewarm water which is at a temperature of 120 °F (49 °C)
Ultrafiltered honey	Ultrafiltered honey is obtained when the honey is heated at a temperature of 150–170 °F (65–77 °C) under high pressure. The honey is then filtered through fine filters in order to extract all pollen grains and extraneous solids (Bogdanov 2015)
Chunk honey	In chunk honey at least one or more portions of comb honey are soaked in collected liquid honey and placed in big mouth containers
Ultrasonicated honey	This type of honey is retrieved through a nonthermal process which, along with inhibition of crystal formation, destroys the yeast cells
Comb honey	Comb honey is what usually exists in the honeybees' wax comb, which is typically collected from honey supers by using standard wooden frames where the comb is cut in chunks before being packed
Whipped or creamed honey	Whipped honey contains small crystals that are present in large numbers and the honey produced in this process is smooth with a ubiquitous constancy
Dried honey	In dried honey, moisture content is removed from the liquified form honey in order to make complete solid, nonsticky granules. Drying and anticaking agents are usually used during this process

11.4 Carbohydrates

The key constituents in NH are the carbohydrates that includes fructose which is a monosaccharide accounting for 32.56–38.2% and glucose, also a monosaccharide that accounts about 28.54–31.3%, thereby constituting about 85–95% of total sugars (Moundoi et al. 2001; Ezz El-Arab et al. 2006). In NH the only monosaccharides present are glucose and fructose. There are about 25 different complex oligosaccharide sugars found in natural honey (Doner 1977; Siddiqui 1970). In blossom honey, the principal oligosaccharides are primarily the disaccharides such as sucrose, maltose, isomaltose, melibiose, turanose, panose, nigerose, palatinose, maltotriose, 1-kestose, 6-kestose, and (Bogdanov 2015; Ezz El-Arab et al. 2006; Yun 1996; Chow 2002). Honeydew honey, on the other hand, contains higher levels

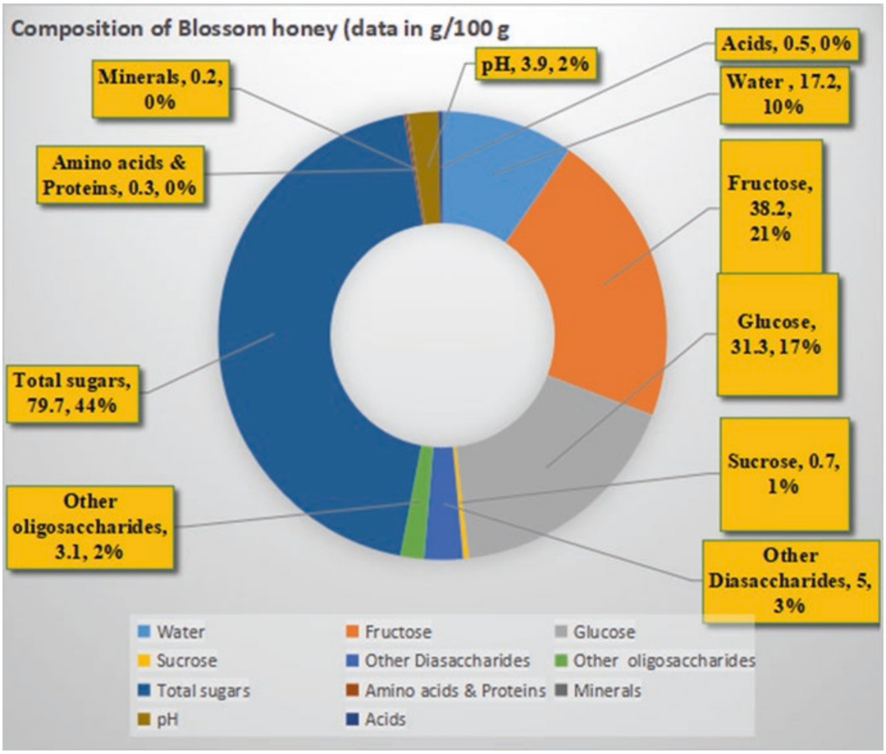

Fig. 11.1 Pie chart depicting composition of blossom honey (data in g/100 g)

of oligosaccharides such as melezitose and raffinose relative to blossom honey. It was also found that 4–5% fructo-oligosaccharides present in natural honey serve act as probiotics (Ezz El-Arab et al. 2006; Chow 2002). The second most essential constituent of NH is water, averaging for about 17.2%. After the intake of honey the principal carbohydrates that are present in human body during the process of digestion are fructose and glucose which can readily serve as energy sources to meet the requisite requirements. The average amount carbohydrates present in blossom honey and honeydew honey is shown in Figs. 11.1 and 11.2 as represented separately by pie charts (1 and 2).

11.5 Proteins, Enzymes, and Amino Acids

Proteins are present in NH only in minute amounts that make up about 0.5% and mostly exist as free amino acids and enzymes. A recent report demonstrated that according to the origin of honeybee the quantities of specific protein were different (Jenkins and Cooper 2012). Natural honey contains a variety of enzymes, however invertase (saccharase), diastase (amylase), and glucose oxidase are key enzyme

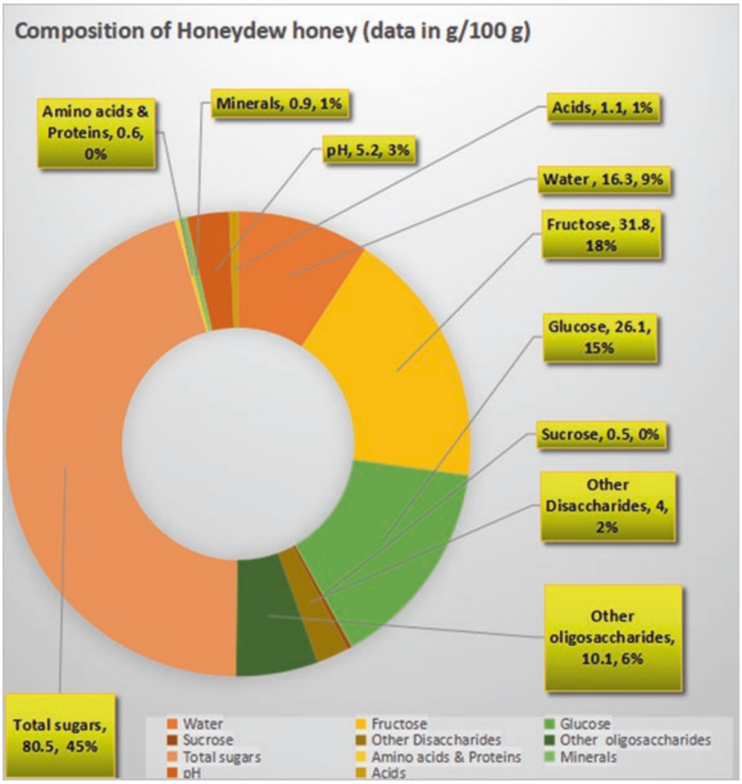

Fig. 11.2 Pie chart depicting composition of honeydew honey (data in g/100 g)

molecules involved in the production of NH (Olaitan et al. 2007). The enzyme invertase found in honey converts sucrose into invert sugars (dextrose and levulose). The enzyme glucose oxidase acts on the glucose molecule to produce hydrogen peroxide (H_2O_2) and gluconic acid, the former provides antimicrobial property and the later helps in calcium absorption. The enzyme amylase acts on the long chains of starch to produce dextrin and maltose. The average amount of amino acids and proteins that are found in blossom and honeydew honey are shown in Figs. 11.1 and 11.2 as represented separately by pie charts (1 and 2).

11.6 Organic Acids

Natural honey contains various organic acids which constitute 0.57% of dry weight and largely contribute primarily to its complex flavor. An essential organic acid found in NH is gluconic acid, which is the by-product of glucose digestion produced by the glucose oxidase enzyme (White 1979). Other acids that are present in NH are acetic acid, malic acid, butyric acid, citric acid, lactic acid, formic acid, pyroglutamic acid, succinic acid, and inorganic acids like hydrochloric acid and phosphoric. The acidic nature of NH is due to the organic acids which largely render to its great sweetness. Average amount of organic acids present in blossom honey and honeydew honey is shown in Figs. 11.1 and 11.2 as represented separately by pie charts (1 and 2).

11.7 Minerals

Natural honey also contains minerals with composition that ranges from 0.1 to 1.0%. The major mineral found in honey are potassium (K), followed by calcium (Ca), magnesium (Mg), sodium (Na), sulphur (S), and phosphorus (P). In NH, the trace elements found are iron (Fe), copper (Cu), fluorine (F), zinc (Zn), iodine (I), manganese (Mn), and selenium (Se) (Yun 1996; Chow 2002; White 1975). In human nutrition, other elements that be can be important are boron, sulphur, fluorine, cobalt, silicon, iodine and molybdenum. The amount of minerals varies in different unifloral honeys (Bengsch 1992a, b). The daily requirement of minerals is too low which makes its requirement marginal in daily diet. The average amount of minerals present in blossom honey and honeydew honey are shown in Figs. 11.1 and 11.2 as represented separately by pie charts (1 and 2).

The amount of choline present in NH is 0.3–25 mg/kg whereas acetylcholine accounts for 0.06–5 mg/kg (Heitkamp 1984). Choline plays a vital role in cellular membrane composition, repair, brain function and cardiovascular activities, while as acetylcholine mainly functions as a neurotransmitter.

According to the studies carried out by White (1975), Bogdanov et al. (2003) the composition of constituents in blossom honey (pie chart 1) and honeydew honey (pie chart 2) varies and is depicted in Figs. 11.1 and 11.2, respectively:

11.8 Vitamins

Natural honey also contains vitamins which includes vitamin C, & B (thiamine (B_1), riboflavin (B_2), niacin (B_3), pyridoxine ($B_{6)}$, pantothenic acid (B5), and folic acid (B_9) (Olaitan et al. 2007). The amount of vitamins required is so small and that their recommended daily intake (RDI) is marginal. NH contains vitamins in minute amounts as depicted in Tables 11.2 and 11.3.

Table 11.2 Amount of vitamins in 100 g honey

Vitamins	mg/Kg
Thiamine (B_1)	0.00–0.01
Riboflavin (B_2)	0.01–0.02
Pyridoxine (B_6)	0.01–0.32
Niacin (B_3)	0.10–0.20
Pantothenic acid (B_5)	0.02–0.11
Folic acid (B_9)	0.002
Ascorbic acid (C)	2.2–2.5
Phyllochinon (K)	0.025

Table 11.3 Honey nutrients values in 100 g (White 1975; Conti 2000; Terrab et al. 2004; Iskander 1995; Rodriguez-Otero et al. 1994; Golob et al. 2005; Yilmaz and Yavuz 1999; Bengsch 1992a, b; Bogdanov and Matzke 2003)

Ingredient	Amount in 100 g
Energy (kcal)	
Carbohydrates (kcal)	300
Fats (g)	0
Proteins (g)	0.5
Minerals (mg)	
Sodium (Na)	1.6–17
Calcium (Ca)	3–31
Potassium (K)	40–3500
Magnesium (Mg)	0.7–13
Phosphorus (P)	2–15
Zinc (Zn)	0.05–2
Copper (Cu)	0.02–0.6
Iron (Fe)	0.03–4
Manganese (Mn)	0.02–2
Chromium (Cr)	0.01–0.3
Selenium (Se)	0.002–0.01

11.9 Aroma Compounds and Polyphenols

Depending on the origin, a wide range of honey exists which have different tastes and colors (Crane et al. 1984). However sugars are the main compounds that make up the taste. Natural honey that has higher amount of fructose (e.g., acacia) is sweeter than those which have a high glucose concentration (e.g., rape).The quantitative and qualitative proportion of amino acids and organic acids within NH determines its aroma. Numerous studies for detection of aroma substances in different types of natural honey have been put forth and they have identified about 500 different volatile compounds. Different forms of NH have varied aroma composition which ultimately depends on their botanical origin (Bogdanov et al. 2007). The flavor of honey is an important factor that determines its quality for being used in food industry and other areas.

Based on appearance and functional characteristics of NH, an important group of compounds that have been discovered are the polyphenols. In different types of honey total polyphenols that have been found account for 56–500 mg/kg, respectively (Al-Mamary et al. 2002; Gheldof and Engeseth 2002). The main polyphenols present in NH are flavonoids such as keampferol, quecertin, apigenin, luteolin, galangin, chrysin), phenolic acids and derivatives of phenolic acid (Tomas-Barberan et al. 2001). An important characteristic feature of these compounds is their antioxidant properties. As flavonoids, are the main polyphenols, their concentration ranges from 60 to 460 μg/100 g of honey and various studies have reported that samples produced higher amounts of flavonoids during a dry season with high temperatures (Kenjeric et al. 2007).

11.10 Physical Properties of Natural Honey

In addition to taste and composition, NH is a viscous liquid with various essential properties. The water content and composition of NH varies because viscosity of honey is dependent on a number of factors. Another property of NH is hygroscopicity that describes its capacity to absorb and retain moisture from the surrounding environment. Natural honey has a water content of 18.8% or less, which additionally has the property of extracting moisture from air at a relative humidity greater than 60%. NH shows variation in surface tension which is totally dependent on the biological origin and the nature of different colloidal substances present. The foaming characteristics of NH are due to high viscosity along with presence of these substances (Olaitan et al. 2007). NH has a pH of 3.2–4.5 and this relative acidic pH levels prevents many bacteria from growing. Further there also exists variation in color of liquid honey which usually is transparent and colorless (as water) to dark amber or black. Also the color of NH varies on the basis of botanical origin, storage conditions, age, etc., however the amount of particulate matter such as pollens also determines the transparency or clarity of natural honey (Olaitan et al. 2007). The least known colors of NH that are available are bright yellow (sunflower), reddish undertones (chest nut), grayish (eucalyptus), and greenish (honeydew). The light color of honey after crystallization is due to the white glucose crystals. The production of monohydrate crystals of glucose causes crystallization of honey which varies with number, shape, dimension, quality, composition, and storage conditions (Olaitan et al. 2007). Further the process of crystallization is faster if the water content is lower and the glucose content is higher in honey (Olaitan et al. 2007).

11.11 Beneficial Effects of Honey in Physiological Processes

The constituents of NH include bioactive compounds that are necessary for metabolism and physiological processes. Regular use of natural honey encourages physiological processes such as development and strengthens physical activities such as exercise and other sporting events (Kreider et al. 2002). Honey is considered a complete meal, as it includes essential constituents of a balanced diet, particularly micronutrients which support digestion and its main dietary components promote healthy growth (Kreider et al. 2002). Studies have reported increased gain in body weight in rats fed that were with fed with honey blossom honey. Calcium as a constituent element of honey is believed to contribute to enhanced bone growth and mineralization in rodents from this study. Some of the important beneficial properties that are provided by natural honey are shown graphically in Fig. 11.3, however the general description is given below:

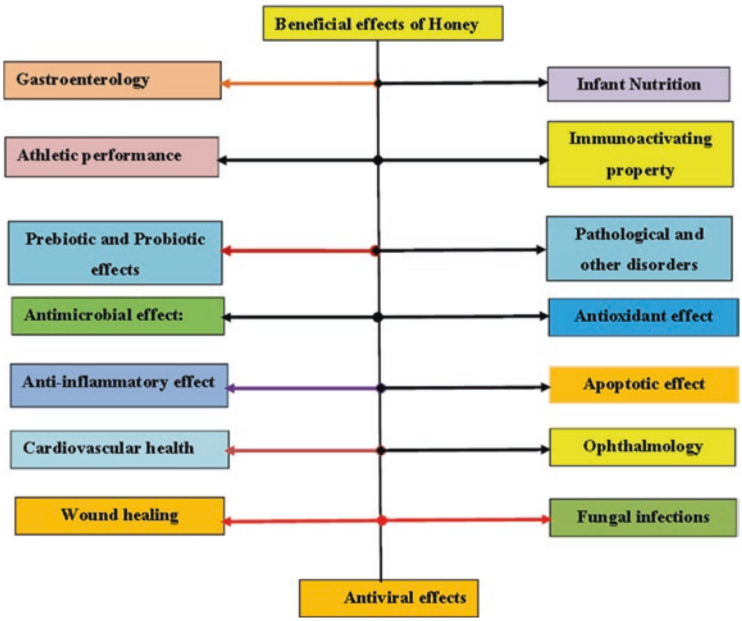

Fig. 11.3 A general overview of beneficial properties of natural honey

11.12 Gastroenterology

Natural honey (NH) has been used to treat various gastrointestinal disorders. Different studies have supported the growth stimulating property of honey, as it was involved in enhanced gastrointestinal function (Ajibola 2013). One of the Hadith mentioned in Sahih Al-Bukhari by our Prophet Muhammad (SAW) have recommended the use of honey against diarrhea (Potschinkova 1992; Cherbuliez and Domerego 2003; Khotkina 1955; Ludyanskii 1994; Menshikov and Feidman 1949; Mladenov 1978; Slobodianiuk and Slobodianiuk 1969) and Arab countries (Salem 1981), NH has been used to prevent and treat gastrointestinal disorders including gastritis, peptic ulcers, and gastroenteritis. It has been reported that NH inhibits Helicobacter pylori that is responsible for causing gastritis and peptic ulcers (Al Somal et al. 1994; Ali et al. 1991; Osato et al. 1999). In experimental rats, NH acted against gastric ulcers which was induced by indomethacin, alcohol, ammonia, and aspirin (Ali 1995a, b; Gharzouli et al. 2001, 2002). Two possible mechanisms have been suggested that provide NH these protective properties. The first one is the antioxidant property of honey and the second mechanism suggests that NH stimulates sensory nerves of the stomach, which then show response to capsaicin (Ali 1995a; Nasuti et al. 2006). The use of NH in indomethacin-induced gastritis rat models showed decreased stomach ulcer index, myelperoxidase and microvascular permeability activities (Ali 1995a). Further NH also maintains the amount of non-protein sulfhydryl compounds (e.g., glutathione) in gastric tissues (Al Swayeh and Ali 1998; Ali 1995b, 1997).

11.13 Infant Nutrition

In infant nutrition, a general recommendation that has been used since the last century is the use of honey in diet and there are some interesting observations that have been reported. Honey has found applications in infant nutrition as well and studies have reported that the blood formation was better along with regular weight gain in infants fed on honey-containing diet as compared to honey-free diet. It has been found that babies have a higher weight gain and also a higher hemoglobin content, better skin tone when fed with honey instead of sucrose and studies have shown less throw up than controls on sucrose. Besides better weight increase in infants exposed to honey, they were least vulnerable to diseases than infants who were usually fed with blood building agents. Some other studies have reported increased calcium intake into the blood, leading to lighter and thinner feces in infants fed with honey (Bianchi 1977; Ajibola 2015a). In infant diet, the positive results of honey are due to its direct role in the digestion process. Several studies have reported well-established oligosaccharide effect on *B. bifidus* (Rivero-Urgell and Santamaria-Orleans 2001). In one study, babies were given a mixture of milk and honey and the results of which showed a regular weight gain with rich acidophilic microorganism like *B. bifidus* (Hubner 1958). Other study in infants that were fed with honey and milk were found to have improved hematological profile, less frequent diarrhea, increased uptake of calcium in blood leading to lighter and thinner feces (Hubner 1958).

11.14 Athletic Performance

Besides enhancing growth, the results provided by Sports Nutrition and Exercise Laboratory has shown that natural honey can provide constant energy as compared to commercially available glucose during vigorous physical exercises. Consumption of natural honey during some form of physical activity raises heart rate and maintains a relatively steady level of glucose in blood, thus making it a better substitute for glucose and an efficient source of carbohydrates (Ajibola 2013, 2015b). The fasted athletes did not show any physical or psychological signs of hypoglycemia (Leutholz and Kreider 2001), as they had consumed NH before during training (Earnest et al. 2000).

11.15 Immuno-activating Property

Honey also exhibits immuno-activating properties in humans as daily use of honey for 2 weeks of 1.2 g/kg body weight, observable were found: iron in serum increased by 20 and ferritin in plasma decreased by 11%, monocyte increased by 50%, lymphocyte and eosinophilic percentages rise marginally (Al-Waili 2003; Bogdanov et al. 2008). Further in serum reduction of immunoglobulin E (34%), lactate dehydrogenase (41%), AST (aspartate transaminase) (22%), ALT (alanine transaminase) (18%), CK (creatine kinase), and fasting sugar (5%) (Bogdanov et al. 2008; Al-Waili

2003). Lastly, an increase in levels of blood copper (33%) and a minor increase in magnesium, zinc, hemoglobin and packed cell volume was also observed (Bogdanov et al. 2008; Al-Waili 2003).

11.16 Prebiotic and Probiotic Effects

Oligosaccharides in honey, mostly panose, are believed to contribute to its prebiotic effect thereby causing an increase of lactobacilli and bifidobacteria. Increase in the apex of lactobacillus plantarum and lactobacillus acidophilus has been observed in intestines (small and large) of honey fed rats where as in vitro sucrose failed to produce any such effect (Yun 1996; Ajibola 2015b). Other studies have shown probiotic effect only of fresh honey for about 2–3 months only that contained *Bifidus* and *Lactobacilus* bacteria. In addition, NH shows laxative effect on individual's digestive system. However, in some cases fructose malabsorption or insufficient absorption, ingestion of fairly large quantities of natural honey (70–95 g) can only show a mild laxative effect (Ladas et al. 1995). The supply of calcium is other nutraceutical property of NH. Honey consumption provides calcium which is an essential mineral and is easily absorbed in the body which further enhances bone mass growth. As a consequence of this, there occurs a reduction in chances of developing osteoporosis or low bone mass in the elderly population. Studies in animal models has shown that calcium absorption was increased with regular intake of honey (Bogdanov et al. 2008; Olofsson and Vasquez 2008; Chepulis and Starkey 2008).

11.17 Pathological and Other Disorders

The importance of natural products for medicine and well-being has been enormous throughout our evolution and has often been regarded as the primary means of treating diseases and injuries. In recent years, an alternate branch of medicine, known as apitherapy or bee products therapy, has been developed that offers treatment mainly focused on using natural honey and various bee products (Bansal et al. 2005). Honey plays a vital part as antioxidant, antibacterial agent, anti-inflammatory molecule, increases skin graft adherence and healing of wounds. Scientific literature has recognized the importance of honey and a strong evidence has evolved supporting its antibacterial and antioxidant existence, that helps in treatment of cough, fertility problems and healing of wounds (Alvarez-Suarez et al. 2010a, b). NH has found its uses in a wide array of pathological conditions (acute and chronic infections) in humans which particularly includes gastrointestinal ailments, dental infections, neonatal conditions ophthalmological diseases, metabolic diseases, urinary tract infections and neoplastic diseases (Bogdanov et al. 2008; English et al. 2004). Among the various health claims, the most widely investigated property of NH is its potent healing ability that has been utilized in the therapeutic and surgical management of wounds (Havsteen 2002; Ames et al. 1993; Abubakar et al. 2012).

11.18 Antimicrobial Effect

Honey's healing ability is because of its strong antibacterial properties, high viscosity and moisture content that helps to protect against infections by providing a protection barrier (Bogdanov et al. 2008). The unique antibacterial property of honey is due to large amounts of sugar molecules, strong acids, low water activity, hydrogen peroxide (White et al. 1963), flavonoids (Cushnie and Lamb 2005), and phenolic acids (Weston et al. 1999), bee defensin-1 and methylglyoxal. However, manuka honey still shows significant antibacterial non-peroxide activity even though when hydrogen peroxide activity is blocked. Some studies have reported that non-peroxide parameters like lysozyme, flavonoids and phenolic acids may be involved, however it is believed that lower honey pH and high levels of sugar (high osmolarity) may also hinder the microbial growth (Bogdanov et al. 2008; Yatsunami and Echigo 1984). Both Gram positive bacteria and Gram negative bacteria show response to honey therapy, for instance, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *E. coli*. Other microbial pathogens that have found to respond to honey treatment include Rubella virus, *Candida albicans*, *Trichophyton mentagrophytes*, and *Leishmania* parasites (Molan 1992a, b, 1997; Bogdanov 1997).

11.19 Antioxidant Effect

NH also possesses strong antioxidant properties in addition to the antibacterial activity. It boosts natural mechanism against diseases by serving as natural dietary antioxidant. Antioxidants scavenge free radicals to protect the body from oxidative stress responsible for cellular dysfunction, metabolic and cardiovascular diseases (CVD's) pathogenesis as well as ageing (Ames et al. 1993). The production of free radicals by oxidative reaction may destroy cells, tissues and finally the physiological functions (Al-Mamary et al. 2002). The redox properties of NH are due to its chemical constituents like phenolic compounds, flavonoids, vitamins, proteins, amino acids, and organic acids. There occurs a tremendous variation in the antioxidant potential of NH which largely is dependent on floral source. These characteristic properties may be due to the variations in the constituents of secondary metabolites of plants and enzyme activity. Some studies have shown that the antioxidant property positively correlates with the dark color and water content of the natural honey (Beretta et al. 2005; D'Arcy 2005; Gheldof et al. 2002; Aljadi and Kamaruddin 2004; Inoue et al. 2005; Fahey and Stephenson 2002; Blasa et al. 2006; Nagai et al. 2006; Perez et al. 2007; Frankel et al. 1998).

11.20 Anti-inflammatory Effect

In animal models, cell cultures, and human clinical trials, the anti-inflammatory potential of NH has been studied by various studies (Al-Waili and Boni 2003; Candiracci et al. 2012). In humans after intake of 70 g of NH, the mean plasma levels of thromboxane B(2) decreased by 7%, 34%, and 35% whereas levels of PGE(2) were reduced to 14%, 10%, 19% at 1, 2 and 3 h respectively. After further intake of NH, the concentration of PGF (2α) was reduced to 31% after 2 h and by 14% at 3 h. The concentration of thromboxane B(2), PGE(2), PGF (2α) in plasma after 15 days of NH intake were decreased to 48%, 63%, and 50%, respectively (Bilsel et al. 2002). In NH, the flavonoid and phenolic compounds possess significant properties that suppress the pro-inflammatory activities of cyclooxygenase-2 (COX-2) and/or inducible nitric oxidase synthase (iNOS) (Al-Waili and Boni 2003; Viuda-Martos et al. 2008).

11.21 Apoptotic Effect

Honey acts as potent anticancer agent by exerting apoptotic effect either by upregulating and modulating proapoptotic proteins including p53, caspase 3, caspase 9, Bax or by downregulating antiapoptotic protein Bcl-2. Earlier research has related honey's chemopreventive action to its hydrogen peroxide-releasing property, but recent studies have implicated the role of phytochemical antioxidants in induction of cell apoptosis. Since honey contains phenolic derivatives, which exhibit antitumor, anti-inflammatory effects (Bang et al. 2003; Lopez-Lazaro 2006). It has been observed that either oral administration of honey or intravenous injection mediates its apoptotic effect by activating various proapoptotic proteins and by downregulating antiapoptotic proteins. Honey's apoptotic properties thus render it a potential natural product as an anticancer agent (Facino 2001; Bogdanov et al. 2008).

11.22 Cardiovascular Health

In the developed countries of the world, more deaths and disability are caused by ischemic heart disease (IHD) (Selwyn and Braunwald 2004). Among them the serious manifestations of IHD are arrhythmias and myocardial infarctions. Anti-arrhythmic drugs are commonly used for the management of such conditions. However, in some patients the use of anti-arrhythmic medications poses serious health hazards like lethal arrhythmias, it is therefore important to use such drugs that will produce fewer side effects and will have more efficacy (Hume and Grant 2007). From ancient times, NH was used due to its medicinal aspect but most of the previous studies that have been reported in animals have particularly focused on various risk factors of cardiovascular disease like hyperlipidemia and generation of free radical species (Schramm et al. 2003; Chepulis 2007; Bahrami et al. 2008; Yaghoobi et al. 2008). The flavonoids and phenolic compounds that are present in NH have proved effective for treating cardiovascular diseases. These phenolic compounds possess protective effects which primarily include antithrombotic, anti-ischemic, antioxidant, and vasorelaxant. A study has reported that flavonoids perform three functions i.e., improves coronary vasodilation, decrease ability of platelets to form clot, and prevents oxidizing of LDL's in order to decrease the risk of CHD (Khalil and Sulaiman 2010). It has also been found that NH decreases venous blood pressure which might be important for diminishing the congestion in the venous system (Rakha et al. 2008).

11.23 Ophthalmology

Natural honey has been used around the globe to cure several ophthalmological diseases like conjunctivitis, keratitis, blepharitis, corneal injury, chemical and thermal burns to eyes (Meda et al. 2004; Shenoy et al. 2009). A study in which NH was used as an ointment was conducted in 102 persons with nonresponsive disorders of eye, the results of which demonstrated that the condition improved in 85% patients while as in remaining 15% cases the disease did not progress at all. The use of NH in infectious conjunctivitis decreased redness, pus discharge, swelling, and eradicated bacteria (Bansal et al. 2005; Obaseiki and Afonya 1984; Al-Waili 2004).

11.24 Wound Healing

The use of NH that has been mostly studied and found to be most effective is in healing of wounds (Medhi et al. 2008). During the World War I, NH was used by the Russians to treat wound infection and to accelerate healing of wounds. The ancient Germans used a mixture of NH and cod liver oil for curing boils, burns, ulcers, and fistulas (Bansal et al. 2005). Natural therapy using honey has found to be most effective against all kinds of wounds such as abrasion, abscess, bed sores, amputation, burns, ulcers, burst abdominal wound, varicose & sickle cell ulcers, diabetic wound, septic wounds, leprosy, surgical wounds or wounds of abdominal wall and perineum, etc. In wound dressing the healing process is quickly stimulated by the use of NH besides clearing the infection. NH has been found to reduce inflammation, cleaning wounds and stimulate regenerate tissue. During tissue dressing, the pads impregnated with honey acts as nonadhesive (Bansal et al. 2005; Efem 1988; Al-Waili 2005). However, the elucidation of exact process of wound healing using honey is still not known.

11.25 Fungal Infections

Natural honey is capable of inhibiting the growth of fungi whereas diluted honey functions to inhibit the production of toxins (Al-Waili and Haq 2004). It has been found that NH possesses antifungal properties against yeast and species of Penicillium and Aspergillus (Sampath Kumar et al. 2010; Brady et al. 1997), *Candida albicans*, which causes Candidiasis also responds to honey therapy (Obaseiki and Afonya 1984; Bansal et al. 2005). It has also been reported that mycoses such as the ringworm and athletes foot respond to honey therapy (Bansal et al. 2005). Some research data studies have suggested that topical honey was quite successful to treat seborrheic dermatitis and dandruff (Al-Waili 2001, 2005).

11.26 Antiviral Effects

Natural honey has been found to have antiviral property in addition to the antibacterial and antifungal qualities. In one study conducted by Al-Waili (2004) in which he compared the effects of topical NH and acyclovir cream in treating genital herpes and recurrent labial lesions and found that NH was quite effective and safe in managing the signs and symptoms (Al-Waili 2004). It has also been found that NH inhibits the activity of rubella virus (Al-Waili and Haq 2004).

11.27 Scope of Use of Honey

Honey produced all over the world is well known for its nutritional and therapeutic properties. NH has been used in various ways as an artificial sweetening and flavoring agent. NH has found application as food additive based on its antibacterial and antioxidant activity (Nagai et al. 2006). In probiotic products of milk, NH can be used as a prebiotic additive based on its oligosaccharide content which acts as growth enhancer. There are sufficient evidences in support of use of honey for beneficial physiological effects in adults as well as infants older than 1 year (Ajibola 2015a; Yun 1996; Ladas et al. 1995). The health claims for honey include physiological processes like growth, physical performance and fitness, bone health, mental state and regulation of body weight. However, there is enough evidence that supports the use of NH in managing various disease conditions like diet-related cardiovascular disease, cancer, osteoporosis, etc. (Kreider et al. 2002; Ajibola 2013; Al-Bukhaari 1994). Research studies have found that natural honey may have a medicinal effect because of its antibacterial, anti-inflammatory, apoptotic, and antioxidant qualities. Antioxidant property of natural honey confers gastroprotective role such that it can help to prevent, cure, and treat some GIT disorders that includes gastritis, ulcers, and gastroenteritis (Ajibola 2015b). Moreover, NH is a folklore topical medicine to treat various ailments including eye diseases, wounds, dental plaque, gingivitis, periodontics. The only possible explanation for this behavior is its antibacterial properties, which prevent the bacteria from growing. Overall, the use of NH in diet reduces the pathogenesis as compared to other conventional antibacterial agents (Bansal et al. 2005; Ajibola et al. 2012). However, there are some research studies which have tested the effectiveness of honey for medicinal applications, however further research studies are required to validate all these aspects of natural honey. The primary issue about the therapeutic use of honey in present medicinal field is its variability in its constitution and inadequate clinical trials. Apicultural activities should be promoted worldwide in order to increase the production and availability of natural honey as it has the potential to replace refined sugars and traditional medicines. Moreover, it would also promote the use of NH as a reasonably inexpensive source of energy and an alternate cost-effective medication for most disorders.

11.28 Conclusion

Most countries of the world produce NH and it has been used in Ayurvedic as well as Yunani medicines for centuries. Generally NH is regarded as a rich nutritional and medicinal diet mainly due to the presence of various sugar molecules, water and also various vitamins, particularly B complex and vitamin C. There occurs a tremendous variation in composition as well as their medicinal uses among honeys of different floral origin. Natural honey contains various bioactive compounds and possesses various nutritional and biological effects. The presence of numerous substances present in natural honey has rendered it of utmost importance as a nutritional food and as a promising therapeutic agent.

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Positive Influence of Honey on Human Health

12

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Abstract

Use of honey is advocated by the people of all religions, traditions, and cultural beliefs, and it is one of the most valued natural products owing to its nutritional and medicinal properties. Honey is known to be the rich in sugars, phenolic compounds, free organic acids, and enzymes. It also contains lipids, amino acids, trace elements, vitamins, and few toxic compounds. It has been known to exert neuroprotective, cardioprotective, gastroprotective, antidiabetic, antioxidant, antimicrobial, anticancer, and anti-inflammatory activities. This chapter focusses on the positive influence of honey on human health and the mechanisms involved in the same. It also sheds light on the chemical composition and the ongoing clinical trials on honey.

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_12

Keywords

Honey · Chemical composition · Health benefits · Clinical trials on honey

12.1 Introduction

Honey is a natural product formed by honeybees (*Apis mellifera*; Family: Apidae) from nectars of the flowers and offer huge benefits to human beings because of nutritional and medicinal values (Samarghandian et al. 2017; Subramanian et al. 2007). Approximately 1.20 million tons per annum of honey is produced globally and its major producers are Argentina, China, Mexico, Ukraine and the United States (Meo et al. 2017).

Use of honey has been advocated by all religious and cultural beliefs and all traditions due to its nutritional and medicinal values and has been accepted by all civilization and generations whether ancient and modern. A whole chapter named Surah al-Nahl which means a chapter of the honey bee is mentioned in Holy Qur'an, which contains a verse And your Lord inspired the bee(s), saying: "Take your habitations in the mountains and in the trees and in what they erect. Then, eat of all fruits, and follow the ways of your Lord made easy (for you)". There comes forth from their bellies, a drink of varying colour wherein is healing for men. Verily, in this is indeed a sign for people who think". The final Prophet Muhammad (S.A.W) emphasized the utilization of honey because of its restorative, therapeutic and healing property. Use of honey has also been advocated in books of Exodus, Judges, Mathew and Proverbs in the Bible (Ajibola et al. 2012).

Consumption of honey is also popular with ancient civilization such as Greeks, Chinese, Egyptians, Romans, Mayans and Babylonians (Samarghandian et al. 2017). Honey bee colony exhibits a perennial life cycle. Honey bees are divided into three categories viz., Queens, Drones (mates the new queens) and workers. The queens are responsible for producing egg and lay it in the comb's cells. Within 3–4 days, the eggs give off larvae. Worker bees fed these larvae after which the larva pupates by crossing several stages of development in the cells (Ediriweera and Premarathna 2012). The Worker bees collect nectar from the flower by inserting their long hollow tube (formed from labium and maxillae) to into the flowers. After extracting the nectar they pass it into the honey sac or crop through their oesophagus. The nectar which mainly contains sucrose is converted into invert sugar by the enzyme invertase present in the saliva of the worker bee (Khemchand et al. 2015). In the hive, the bee ingests the nectar and regurgitate it several rounds until the nectar is partly digested. The bees continue to carry this performance until the product of the desired quality is obtained. The honeycomb is remained unsealed until 80% of water present in honey is evaporated by a strong draft created by the wings of the bees inside the hive. The dried form of honey thus obtained is sealed inside the cells of the honeycomb (Ediriweera and Premarathna 2012).

Honey is plentiful in carbohydrate and contains other components such as polyphenols, organic acids, proteins, amino acids, minerals, vitamins and over 500 enzymes.

These components impart medicinal property to honey such as antioxidant, anti-inflammatory and antimicrobial potential. There are approximately 320 different range of honey, the composition of which depends upon plant variety from which the nectar is obtained and the environment in which the plants mature (Hills et al. 2019).

This chapter gives insights into the health benefits associated with the consumption of honey and brief about the composition and clinical trials of the honey.

12.2 Composition of Honey

Honey has been identified to possess 181 different substances and some of these are exclusive to honey only (Przybylowski and Wilczynska 2001). Honey contains approximately 8% of sugar which is dissolved in 17–20% of water. The composition of honey is dependent upon factors such as air, water and soil, the geographical region from which it is collected, type of flowers visited by bees and environmental condition where the plants grow and mature (Hack-Gil et al. 1988). The nutritional and medicinal value of honey is attributed to both major and minor components present in it**.** The ideal density of honey is 1.52 g/mL at 15 °C, Optically, it is laevorotatory due to the presence of high fructose content. The caloric value of honey on an average is 3500 calories/kg (Czipa et al. 2019). The common range of specific gravity is 1.3648–1.4101 g/mL (Gairola et al. 2013). The viscosity of honey ranges from 10 to 30 poises (James et al. 2009).

There are various factors which regulate the colour, taste, aroma and chemical composition of honey. The colour of the honey varies based on its nectar source, season, duration between the collection of nectar and harvesting of honey, production and storage. Dark honey is known to be rich in minerals. Reactions such as Maillard reaction, caramelization or tannates and polyphenols make the honey to appear darker. Darker honey tends to have strong flavour compared to pale honey. The physiochemical properties of honey are related to the quality of storage, texture, granulation, flavour and its medicinal and nutritional properties and are important for the industry (Przybylowski and Wilczynska 2001). Different chemical constituents present in honey are mentioned in Table 12.1.

12.3 Health Benefits of Honey

Apitherapy is defined as the use of honey derived products and other bee products for medicinal purposes (Ab Wahab et al. 2018).

12.3.1 Neuroprotective Effect

Honey has also been reported to prevent cognitive impairment and dementia due to its antioxidant and brain's cholinergic system enhancing properties. It has been hypothesized that Tualang honey could remove ROS and regress oxidative stress that was caused during noise-induced stress. In ovariectomized stressed rat, Tualang

Table 12.1 Chemical constituents of honey

S. No	Constituents		Name	References
1	Sugars	Monosaccharides	Glucose, fructose	Da Silva et al. (2016)
		Disaccharides	Sucrose, maltose, turanose, isomaltose, maltulose, trehalose, nigerose, kojibiose, maltotriose, melezitose	
		Oligosaccharides	Maltotriose, melezitoze, erlose, theanderose, panose, isomaltosyltetraose, 1-ketose, centose, isopanose, isomaltosyl glucose, isomaltosyltriose, isomaltosyltaose	Afroz et al. (2016)
2	Phenolic compounds	Flavonoids	Hesperetin, pinocembrin, naringenin, chrysin, apigenin, luteolin, tricetin, galangin, kaempferol, quercitin, isorhamnetin, myricetin, pinobanksin	Santos-Buelga and González-Paramás (2017)
		Phenolic acids	4-hydroxybenzoic acid, protocatechuic acid, gallic acid, vanillic acid, syringic acid, cinnamic acid, p-coumaric acid, caffeic acid, ferulic acid, phenylacetic acid, mendelic acid, homogentisic acid, phenylpropanoic acid, rosmarinic acid	
3	Free organic acids		Gluconic acid, acetic acid, butyric acid, citric acid, formic acid, malic acid,, oxalic acid, succinic acid, lactic acid fumaric acid, pyroglutamic acid, maleic acid, α- ketoglutaric acid	Afroz et al. (2016)
4	Enzymes		Diastase, amylase, invertase, sucrase, sucrose hydrolase, saccharase, glucose oxidase, catalase, acid phosphatase, protease, esterase, β-glucosidase	White Jr. (1978)
5	Lipids		Glycerides, sterols, phospholipids, palmitic acid, oleic acid, lauric acid, miristic acid, stearic acid, linoleic acid	Machado De-Melo et al. (2017)
6	Amino acids		Proline, phenylalanine, tyrosine, lysine, arginine, glutamic acid, histidine, valine , methionine, cysteine, valine, a-alanine, tyrosine	Hermosín et al. (2003)
7	Trace elements		Aluminium, arsen, barium, boron, bromine cadmium, chlorine, cobalt, flouride, iodide lead, lithium, molybdenum, nickel, rubidium silicium, strontium, sulfur, vanadium, zirkonium	Bogdanov et al. (2016)
8	Amino acids		Proline, phenylalanine, tyrosine, lysine, arginine, glutamic acid, histidine, valine methionine, cysteine, valine, a-alanine, tyrosine	Hermosín et al. (2003)
9	Vitamins		Phyllochinon, thiamin, riboflavin, pyridoxin, niacin, panthothenic acid, ascorbic acid	Bogdanov et al. (2008)
10	Toxic compounds		Grayanotoxins, polycyclic aromatic hydrocarbons (PAHs), polyhydroxylated cyclic hydrocarbons (diterpenoids)	Machado De-Melo et al. (2017)

honey improved memory performance. In a study, stress was induced in the rats using noise. Following the exposure of noise, it was demonstrated that the stressed rats contained a less number of neurons in hippocampus and medial prefrontal cortex region compared to a non-stressed rat which resulted in short- and long-term decrease in memory in stressed rats compared to nonstressed rat. Administration of Tualang honey imparted neuroprotection; the histopathological study revealed an enhanced number of neuron in hippocampal and medial prefrontal cortex region and short- and long-term memory was also improved in the stressed rats (Azman et al. 2018).

Astrocytes, the neuroglia which forms a part of the blood–brain barrier (BBB) are one of the main locations that express genes responsible for neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. Neuroinflammation and oxidative stress lead to the dramatic transformation of astrocytes called "reactive astrocytes" which is characterized by alterations in morphological and functional aspects that degrade biomolecules such as DNA leading to the occurrence of neuropsychiatric disorders. In a study, the antioxidant potential of honey was examined in astrocytes cell culture. Astrocytes from the cortex of pups of Wistar rats were reseeded in 96-well microplate at a density of 1.8 × 104 cells/well with complete Dulbecco's Modified Eagle Medium (DMEM) containing 10% foetal bovine serum (FBS) as a medium. The cell was treated with various concentrations of honey (from 0.1 to 5% v/v) for 24 h which was followed by H_2O_2 (100 μmol/L) treatment for 3 h followed by MTT assay. The result of the study demonstrated that H_2O_2 treatment resulted in substantial cell death due to oxidative stress. Whereas with honey, the cell viability was preserved compared to H_2O_2-treated cells, and the most significant effect of honey was observed at 1% concentration. The investigator concluded that honey exerts a protective effect on Astrocytes (Ali and Kunugi 2019).

Neuroprotective effect of Tualang honey was investigated against kainic acid (KA)-induced neurodegeneration and oxidative stress in rats. KA administration is known to induce excitotoxicity and develop well-characterized seizures due to its ability to cause severe oxidative stress. Pretreatment with Tualang honey was able to reduce neuronal degeneration in the piriform cortex following KA administration, attenuated decreased of total antioxidant level, and increased thiobarbituric acid reactive substances level in cerebral cortex however it was not able to prevent KA-induced seizures (Mohd Sairazi et al. 2017).

All these above studies demonstrate the neuroprotective effect of honey which is attributed to the mechanism mentioned below.

12.3.1.1 Mechanism

1. Honey is reported to contain choline and acetylcholine which enhances the brain cholinergic system enhancing property.
2. It imparts neuroprotection due to its antioxidant potential.
3. The neuroprotective potential of Tualang honey was attributed to its high flavonoids content, enzymes such as peroxidase, catalase and glucose oxidase and non-enzymatic antioxidants such as ascorbic acid, α-tocopherol, carotenoids (Azman et al. 2018).

4. Gallic acid, caffeic acid present in Tualang honey has been demonstrated to lessen the seizure behaviour, decreased oxidative stress and apoptosis and prevent memory impairment (Sairazi et al. 2017).
5. Tualang honey has also been shown to enhance brain-derived neurotrophic factor (BDNF) and *acetylcholine* (ACh) levels, and decrease *acetylcholinesterase* (AChE) in the brain homogenates (Othman et al. 2015).

12.3.2 Respiratory Infections

Respiratory tract infection has a high prevalence, especially in children. Various microorganisms such as *H. influenza*, *S. aureus*, *P. aeruginosa*, *K. pneumonia*, and *S. pyogenes* are responsible for respiratory infections such as influenza, pneumonia, nosocomial infection, pneumonia in debilitated individual and pharyngitis (El-Kased 2016). *P. aeruginosa* is known to disturb the homeostasis of the upper and lower respiratory tract. *P. aeruginosa* utilizes its flagella, pili, lipopolysaccharides, lipoproteins and type III secretion system to interact with host cell resulting in epithelial damage by recruiting immune cells subsets (Curran et al. 2018). In a study, Macuna honey lowered *P. aeruginosa* viability in an ex vivo infection model and was believed to serve as potential therapeutics for upper respiratory tract infections especially in resistance is observed against other antimicrobials (Roberts et al. 2019). Theoretically, drugs such as diphenhydramine and histamine or other cold medicines are effective in relieving runny nose and cough. However, the evidence has proved it another way, that is, only to relieve rhinorrhoea and not cough and if taken in large doses these could pose a potential threat to child's health. Honey on contrary serves a safer alternative for treating distressing cough in children however it is restricted for infants less than 1 year of age (Chan 2014).

On oral administration, honey first acts on the upper respiratory tract topically followed by its action on lower respiratory tract followed by its absorption in the systemic circulation. In a study, a simulated sugar solution mimicking the property of honey (the high sugar content and low water content) was prepared and it showed an amazing antimicrobial effect. The antimicrobial potential of honey and simulated sugar were checked. Honey at 75% of its concentration and simulated sugar at 100% of its concentration inhibited all the tested bacterial isolates (El-Kased 2016).

A darker variety of honey named buckwheat honey which is higher in phenolic content is demonstrated to relieve nocturnal cough and improve sleep quality in children and parents. The relief by honey was found to be superior then dextromethorphan (Paul et al. 2007). Effects of aerosolised honey were studied against OVA-induced injury in the rabbit airway to find whether honey alleviates asthma-related histopathological changes. The results revealed that honey 25 and 50% significantly decreased the epithelial and mucosal thickening in Asthma which is one of asthma's characterized changes. Aerolised honey also inhibited hyperplasia of goblet cells and eliminated the mucus overproduction and hence, it proved to be useful in decreasing histopathological changes in asthma and prevention of allergen-induced asthma. It can also provide symptomatic relief in asthma (Kamaruzaman et al. 2014).

12.3.2.1 Mechanism

1. The antimicrobial action of honey against pathogen causing respiratory tract infection was attributed to its high sugar content which exerts the high osmotic pressure in both crude honey and simulated sugar solution. The study also revealed that apart from high osmotic pressure other components may also be responsible for antimicrobial effects because honey at limited dilution showed better antimicrobial activity. Presence of enzyme glucose oxidase was counted as one of the reasons for honey's antimicrobial effects as it releases hydrogen peroxide on dilution which is a potent antimicrobial compound (El-Kased 2016).
2. World Health Organization has postulated that the benefit of honey in cough is attributed to its demulcent effect. There is also a hypothesis that sweet substances such as honey relief dry/unproductive cough by its demulcent effect on pharynx and larynx which is attributed to reflex salivation and enhancing the secretion of airway mucus. And, their beneficial effect in productive cough is attributed to the improvement of mucociliary clearance in airways. It has been also suggested that the interaction between opioid responsive sensory fibre which are stimulated following the consumption of sweet substances and gustatory nerves via a central nervous system may be responsible for antitussive action of honey (Paul et al. 2007).

12.3.3 Cardioprotective Effect

Intake of honey has been shown to improve the cardiovascular diseases in the patients as well as healthy people at risk. Several studies affirmed that consumption of honey is associated with the positive impact on metabolic and cardiovascular health as revealed by recording some health profiles. The cardioprotective effect of honey was confirmed by another study; in which experimental rats were fed with a diet supplemented with natural honey or sugar (golden syrup, GS) for 84 days and compared their metabolic response in metabolic syndrome (MetS). The rat with GS administration had a significantly increased level of glucose and triglycerides and caused hyperinsulinemia, hepatomegaly, hypercholesterolemia, fatty liver and visceral adiposity and the rat with NH administration did not display the above-mentioned risk factors (Ajibola et al. 2012).

In a study, a reduction in systolic blood pressure was noted after 3 weeks of consumption of Malaysian Tualang honey in streptozotocin-induced diabetic spontaneously hypertensive rats. This effect was attributed to the reduction of oxidative stress in the kidney. Similarly, in another study, after 60 and 120 min of honey inhalation, a significant reduction in systolic and diastolic blood pressure was noted in the hypertensive patient. Honey also increases the NO level, which is also responsible for mitigating hypertension. C-reactive protein (CRP) is an important biomarker of cardiovascular disease risk and its levels increase in various deteriorating cardiovascular conditions. Honey, on oral administration, can reduce CRP (Al-Waili et al. 2013).

The postmenopausal state is highly correlated with the prevalence of cardiovascular disease and hence in a pilot study systolic blood pressure (SBP) was measured in postmenopausal women in two groups, one receiving Tualang honey and other receiving HRT and the result demonstrated that group receiving Tualang honey reduced systolic blood pressure significantly compared to the one receiving HRT (Ab Wahab et al. 2018). However, obtained from the Black Sea coast of Turkey contained acetylandromedol, occasionally was reported to cause atrioventricular block (AVB), arterial hypotension and bradycardia but none of the fatalities was reported.

In a report, "Mad" honey which is utilized to treat gastric pains, hypertension and bowel disorders (as alternative medicine), may increase the change of atrioventricular block (AVB) when consumed in about "one teaspoonful". The toxicity is attributed to grayanotoxin present in it (Ozhan et al. 2004).

In isoproterenol-induced myocardial infarction in Wistar rats, pretreatment with honey restored the reduced levels of enzymes such as glutathione peroxidase, superoxide dismutase, and glutathione reductase including creatine kinase-MB, aspartate transaminase, lactate dehydrogenase and alanine transaminase. In another model of myocardial infarction, administration of honey improved cardiac troponin I, total cholesterol, triglyceride and lipid peroxidation (LPO) products (Ahmed et al. 2018).

In a study, the effects of natural honey were evaluated in ischemia/ reperfusion (I/R) induced wounds in isolated rat heart. The result of the study confirmed the consumption of honey for 45 days produce significant anti-infarction and anti-arrhythmic activity and serve preconditioning agent in such condition. In another study, pretreatment of stressed rat for 1 h with natural honey (5 g/kg), before adrenaline (100 μg/kg) administration preserve them from epinephrine-induced vasomotor dysfunction and cardiac disorder. The researchers clinched that this protective effect of honey was due to nitric oxide release from endothelium (Ahmad et al. 2017).

12.3.3.1 Mechanism

1. Honey has been shown to benefit cardiovascular disease via several mechanisms such as ameliorating endothelial function and coronary dilatation, platelet aggregation inhibition, reduction of inflammatory responses, reduction of oxidative stress (Ab Wahab et al. 2018).
2. Some flavonoids present in honey are known to increase the bioavailability of nitric oxide, a very well-known mediator for vasodilatation. For instance, rutin enhances eNOS gene expression and its activity and promotes the production of NO. Similarly, catechin and quercetin in honey exert a negative effect on aortic atherosclerotic lesion development (Ahmed et al. 2018).
3. Lowering of total cholesterol, low-density lipoprotein (LDL)-cholesterol, TGs, glucose level, C-reactive protein and increase of high-density lipoprotein (HDL) cholesterol in blood was noted (Ajibola et al. 2012).
4. Free radicles and reactive oxygen species (ROS) remains the key reason in the pathogenesis of the cardiovascular disease (Vallianou et al. 2014).
5. Honey is enriched with various natural antioxidants such as flavonoids, polyphenols (like caffeic acid, acacetin, quercetin, phenethyl ester (CAPE), galangin, and kaempferol), Vitamin C, and monophenolics and is reported to exert cardioprotective effect (Khalil and Sulaiman 2010; Samarghandian et al. 2017). And hence, can lower the levels of free radicals and reactive oxygen species (ROS) to exert cardioprotective effects.

12.3.4 Antidiabetic Effect

Diabetes mellitus is a complicated metabolic condition which is characterized by elevated glucose levels which occur due to deficiency of insulin or nonfunctional insulin. It is also characterized by abnormal lipoprotein and carbohydrate metabolism. Honey has known to display antidiabetic effects from preclinical to clinical studies and it has been referred to as a potential antidiabetic agent by researchers.

Inhalation of honey (60% w/v) was found to reduce glucose levels in type 2 diabetes mellitus. The most important component of honey which is known to exert antidiabetic effect is fructose. Fructose controls blood glucose level by regulating the insulin response system. It follows protein- and energy-mediated diffusion and taken up by the receptor GLUT5 and/or GLUT2. Both glucose and fructose increases the expression of GLUT2 mRNA. However, fructose increases the expression of GLUT5 mRNA specifically facilitating its fast absorption (Ahmed et al. 2018).

Natural honey has demonstrated to reduce the fasting blood glucose in obese by 4.2%. It is also reported to significantly improve the metabolism of glucose in type 2 diabetes. The glucose-lowering effect of the honey is contributed to fructose, antioxidant, oligosaccharides and trace elements present in it. However, there are some studies which demonstrate that consumption of Tualang honey for 4 months significantly increased fasting blood glucose levels compared to their baseline levels. Increase in the level of HbA1c after 8 weeks consumption of honey supplement was also noted. Therefore, cautious usage of honey is recommended in diabetic individuals (Ab Wahab et al. 2018).

It was found in a study that oral consumption of honey at various doses for 4 weeks increased body weight, total antioxidant status, activities of catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, and superoxide dismutase activity in diabetic rats, significantly. Tualang honey reduced elevated malondialdehyde level and re-establish superoxide dismutase and catalase activities in streptozotocin-induced diabetic rats. The hypoglycaemic effect of honey was attributed to its antioxidant property on the pancreas. Combination of Glibenclamide or Metformin with honey improved glycaemic control in the streptozotocin-induced diabetic rat (Ahmed et al. 2018).

Natural honey has been considered as non-injurious and curative agent in diabetes in the folklore. Presently, it is used as a sweetening agent in eastern/Unani herbal antidiabetic preparations (Akhtar and Khan 1989).

12.3.4.1 Mechanism

1. The most important component of honey which is known to exert antidiabetic effect is fructose (Ahmed et al. 2018). Honey contains 21–43% of fructose and the ratio of fructose to glucose is from 0.4 to 1.6 or higher. Compared to glucose and sucrose which have glycaemic index of 100 and 60, respectively, the glycaemic index of fructose is 19. Fructose display blood-glucose-lowering effect via several mechanisms such as reduction of intestinal absorption rate, gastric emptying time prolongation, and reduction of food intake (Bobiş et al. 2018).

Fructose plays a pivotal role in glucose uptake by the liver and storage of glucose as glycogen by stimulating glucokinase in hepatocytes. Fructose control blood glucose level by regulating the insulin response system. It interacts with GLUT5 and/or GLUT2 receptor via protein and energy mediated diffusion. Both glucose and fructose increases the expression of GLUT2 mRNA. However, fructose specifically increases the expression of GLUT5 mRNA, facilitating its fast absorption.

2. Honey also modulates the insulin signalling pathway. PI3K/Akt is a key component in insulin signalling which is known for cell growth and survival. In a study, the effect of the honey extract on Akt-activated insulin signalling pathway in pancreatic cells under hyperglycaemic condition was explored. Insulin resistance progression was described by enhanced levels of MAPK, NF-κB, and insulin receptor substrate 1 (IRS-1) serine phosphorylation and reduced Akt expression and insulin content. The results of the study revealed that pretreatment of honey along with quercetin improved insulin content and resistance. Alternatively, expression of Akt was increased and that of IRS-1 serine phosphorylation, NF-κB, and MAPK was decreased following honey treatment (Ahmed et al. 2018).
3. Glucose present in honey along with fructose promotes the absorption of fructose and enhances its delivery to liver thereby promoting its hepatic action. Honey also protects pancreas, an important organ in diabetes against oxidative stress.
4. Administration of fructose alone or with sucrose is demonstrated to ameliorate homeostasis of glucose and response of insulin when compared to the rats receiving glucose. Enzymatic cleavage of proinsulin to insulin is accompanied by co-secretion of C-peptide by the pancreatic cell and, is considered as a good insulin secretion marker. Honey raises the preprandial C-peptide levels compared to sucrose and glucose in non-diabetic individuals. This suggests the direct stimulatory effect of honey on the healthy pancreas beta cells.
5. As it is well known that diabetic wounds are slower to heal compared to typical wounds. Honey was used since folklore for healing various kinds of wounds. Recently, honey is reported to be used in diabetic wound management. The mechanism involved is: when honey is diluted with water or body fluid it forms hypochlorite anions and hydroxyl radical at the wound site. Moreover, the antioxidants of honey reduce the ROS and inflammation, fight against microorganism around the wound enhancing the healing process. Honey also contains nitric oxide metabolite which again ameliorates the healing process. In a study effect of manuka honey was studied in wound healing and result demonstrated that manuka honey has a healing effect on wound due to its antioxidant capacity and cell proliferation promoting property (Bobiş et al. 2018).
6. After several up and down in one of the clinical trial conducted, "honey as a sole treatment to type 2 diabetes" the investigator concluded that honey benefits the macrovascular complications of DM in a patient under the trail. The mechanism for the same includes a reduction in weight, reduction in blood pressure in hypertensive patients, preservation of Apo A-1 and attenuation of postprandial hyperglycaemia. The investigator also revealed that honey might heal the pancreatic

insult and ameliorate insulin resistance. However, two patient in the same study developed microvascular complication like enhanced peripheral neuropathy, development of the diabetic foot, non-proliferative retinopathy and cataract. Contrary to this, a patient who already had peripheral neuropathy did not show progress in this condition (Abdulrhman 2016).

12.3.5 Antioxidant Effect

Free radicals and reactive oxygen species generated during various metabolic process exhibits the tendency to interact with the lipid and protein composition of the cell membranes, DNA and enzymes. Thanks to antioxidants which seize free radicals before any damage caused. Honey is a rich source of antioxidant. Darker the honey more is its antioxidant value (Samarghandian et al. 2017). Oxidative stress contributes to the pathogenesis of various diseases and disorders such as Cancer, mutagenesis, ageing, atherosclerosis and many degenerative lingering diseases. Natural defence system present in the cells consists of catalase, superoxide dismutase, peroxidase, vitamin C, tocopherol and polyphenols. The antioxidant property of Honey is attributed to its flavonoids and phenolic acids, carotenes, organic acids, sugars, amino acids, protein, Maillard reaction products. Honey (1.2 g/kg) has demonstrated to increase the activity of other antioxidants such as ascorbic acid, glutathione reductase, beta carotene in healthy individuals (Ahmed et al. 2018).

Honey is rich in several phenolics (viz, ferulic acids, p-coumarin, caffeic acids, ellagic acids,) flavonoids (viz., quercetin, kaempferol, apigenin, pinocembrin, hesperetin, chrysin and galangin), vitamin C & E, superoxide dismutase and catalase. These antioxidants synergize with each other to exert its antioxidant effect. The antioxidant activity of honey is strongly correlated with its total phenolics content and its colour. Darker is the colour of honey, more is its phenolic content, consequently more phenolic content means more antioxidant potential (Vallianou et al. 2014).

In vitro and in vivo studies have demonstrated the antioxidant potential of honey. For instance, in an in vitro study free radical scavenging property of honey was demonstrated against (1,1-diphenyl-2-picrylhydrazyl, peroxyl radicals, 2,2′-azinobis [3-ethylbenzothiazoline-6-sulphonic acid] and nitric oxide. Honey also reduced ferric cations, form metal ion chelates, inhibit lipid peroxidation and β-carotene bleaching (Miguel et al. 2017).

12.3.5.1 Mechanism

1. The proposed mechanism of honey's antioxidant activity include sequestration of free radical, chelation of metallic ion, donation of hydrogen, superoxide radical action and flavonoids substrate action for hydroxyl group (Ahmed et al. 2018).
2. The in vivo antioxidant study of honey revealed that it stimulates antioxidant defence system such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione *S*-transferase, tissues of rat and mice.

3. Honey also contains gluconic, malic, and citric acids which exhibit antioxidant property, as these chelates metal ions and enhancing the antioxidant potential of flavonoids (Miguel et al. 2017).

12.3.6 Antimicrobial Effects

Recently investigators have reported antimicrobial property of the honey. Many researchers have found that natural honey possesses broad-spectrum antibacterial potential against oral and food spoilage bacteria. Plenty of honey is sold nowadays with systemized antibacterial potential. For instance, honey obtained from *Leptospermum scoparium* has reported possessing an inhibitory effect on approximately 60 species of aerobic, anaerobic, gram-positive and gram-negative bacteria. Tualang honey is effective against the various wound and enteric bacteria.

Back to 2100–2000 BC, honey was mentioned as a drug and ointment. Aristotle (384–322 BC) described pale honey as being "good as a salve for sore eyes and wounds". Manuka honey exhibit inhibitory potential against pathogenic bacteria such as *Staphylococcus aureus* (*S. aureus*) and *Helicobacter pylori* (*H. pylori*) *Escherichia coli* (*E. coli*), *Enterobacter aerogenes*, *Salmonella typhimurium. Breakthrough in this regard is the potential of honey against* methicillin-resistant *S. aureus* (MRSA), β-*haemolytic streptococci* and vancomycin-resistant *Enterococci* (VRE), coagulase-negative *staphylococci* (Mandal and Mandal 2011).

In a study antibacterial activity of honey was checked against MRSA isolates from wound infection using disk diffusion technique. The result of the study demonstrated that honey in the range of 18.75–37.5% v/v completely inhibited the growth of MRSA. Although, not all honey displays the same degree of antibacterial potential (Mama et al. 2019). Honey has also been demonstrated to have antiviral activity. In a study antiviral activity of honey, royal jelly and acyclovir were checked against herpes simplex virus-1 via Vero cells cultured in the Dulbecco's Modified Eagle's Medium (DMEM) along with 10% foetal bovine serum (FBS). The result of the study revealed that honey at 500 μg/mL concentration displayed an inhibitory effect against HSV-1(Hashemipour et al. 2014). Honey has also been demonstrated to be active against the rubella virus and at 5% of honey concentration complete inhibition of rubella virus was achieved (Ghapanchi et al. 2011). Manuka honey and clover honey demonstrated to be active against varicella-zoster virus (Shahzad and Cohrs 2012).

Honey is also known for its antifungal activity. It is active against *Rhodotorula* sp. *C. albicans, Candida glabrata*, and *Candida dubliniensis*, *Candida parapsilosis, Candida tropicalis, Candida kefyr, Candida glabrata*, and *Candida dubliniensis* (Moussa et al. 2012). In a study, hydrogen peroxide type honey was demonstrated to exhibit greater antifungal effects against dermatophyte fungi. Prophylactic use of honey is recommended to prevent some serious infection. For instance, the effectiveness of honey when placed around the catheters was same as mupiron or povidone-iodine in checking exit site infection. Honey could also be used for treating vaginal candidiasis when incorporated into pessary (Irish & Dee 2006). *Agastache* honey was found to be the most effective honey against *T. mentagrophytes* and *T. rubrum*, closely followed by Tea tree honey, with Manuka honey showing some activity. The antifungal activity of honey was due to production of H_2O_2, the presence of volatile and phenolic compound (Anand et al. 2019).

12.3.6.1 Mechanism

1. The antibacterial property of honey is due to its capacity to liberate hydrogen peroxide, moisture drawing capacity from the environment, hence causing dehydration of bacteria, high osmolarity due to the high content of sugar, acidity (pH 3.2 and 4.5) and presence of non-peroxide components such as methylglyoxal (MGO).
2. The most important mechanism of antibacterial activity is the liberation of hydrogen peroxide due to dilution of honey hence activating enzymes such as glucose oxidase which carry oxidation of glucose to gluconic acid and H_2O_2.
3. On the other hand "non-peroxide honey" is honey which preserves its antimicrobial potential even in the presence of catalase (which normally decreases the antibacterial activity of honey). The constituents responsible for the non-peroxide honey's antibacterial are methyl syringate and methylglyoxal especially in manuka honey derived from the manuka tree (*L. scoparium*) (Mandal and Mandal 2011).
4. The antiviral effect of honey might be due to the existence of glucose oxidase which generates gluconic acid and hydrogen peroxide (Ghapanchi et al. 2011).
5. The antifungal potential of honey is attributed to the production of H_2O_2, the presence of volatile and phenolic compound (Anand et al. 2019).

12.4 Anticancer Activity

Studies demonstrating the anticancer potential of honey cover cell and tissue culture, preclinical to clinical trials. Honey contains flavonoids, phenolic acids, sugars, enzymes, amino acids, protein and miscellaneous compounds. The polyphenols and phenolic compounds present in honey have been demonstrated to possess anti-leukemic potential against various leukemic cell lines. The anticancer potential of honey has been studied extensively and has been reported against various cancers cell lines such as breast, colorectal, endometrial, prostate, renal and oral cancers. Additional benefits of honey are that it supplements the antitumour activity of drugs such as cyclophosphamide and fluorouracil.

In a study, the treatment with Tualang honey reduced the tumour incidence and delayed the tumour initiation (Ahmed and Othman 2017). Malaysian jungle Tualang honey was proved to be potential anticancer agents against human breast, cervical, oral and osteosarcoma cancer cell lines (Othman 2012a, b).

Patients with breast cancer display abnormal and poor blood parameters. It was demonstrated that values of blood parameter of the rats treated with TH were nearer to that of normal rats. TH has been proved to potentiate the haematological parameters such as RBCs, Hb, PCV, eosinophils, lymphocytes and platelet compared to negative control rats which had reduced value of RBC, Hb, PCV, lymphocytes and platelets (Ahmed and Othman 2017).

Manuka honey was demonstrated to possess an inhibitory effect on cellular proliferation in human breast cancer MCF-7, murine melanoma B16.F1, and mouse colon carcinoma CT26 cell lines in time and dose-dependent manner. Similarly, thyme honey has been proved to have an inhibitory effect on proliferation in breast, endometrial and prostate cancer cell lines. Gelam and nenas honey also displayed antiproliferative effect against colon cancer cell lines (Porcza et al. 2016).

The cytotoxic effect of Tualang honey against breast cancer cell lines was proved by enhanced leakage of lactate dehydrogenase (LDH) from the cell membranes. The investigator found that the cytotoxic effect of TH was limited to breast cancer cell lines and not to the normal breast cell line, which is a pivotal characteristic of the good chemotherapeutic agent.

Honey having greater phenolic content employed a higher antitumor effect (Erejuwa et al. 2014). However, the beneficial effect of honey against cancer draw sceptics as it also contains sugars such as glucose, fructose, sucrose and maltose which itself are carcinogenic (Othman 2012a, b).

12.4.1 Mechanism Involved

1. *Induction of apoptosis*: Cancer cells are characterized by uncontrolled proliferation of cells and inadequate apoptotic mechanism. Two apoptotic mechanisms are followed by cell: (1) the caspase 8 or death-receptor pathway, (2) caspase 9 or mitochondrial pathway. Manuka honey induces caspase 9 which in succession cause activation of executor protein caspase 3. It also induces DNA fragmentation, PARP activation and decreased Bcl-2 expression. All these effects ultimately induce apoptosis on the cancer cells.

   In human colon cancer cell lines, honey enhances the level of caspase 3 activation and *poly (ADP-ribose) polymerase (PARP)* cleavage, upregulates and modulates pro and antiapoptotic protein expression, this effect of honey was due to its high phenolic and tryptophan content. Honey upregulates caspase 3, p53, and proapoptotic protein Bax expression and downregulates antiapoptotic protein Bcl2 expression (Ahmed and Othman 2013).

   Rats in TH treated group also displayed to normalize E2 (increased level of which is associated with risk of breast cancer in postmenopausal women) and Apaf-1 and caspase-9 (decreased level of which is associated with higher chances of cancer) compared with the rats in negative control group indicating TH acts as a natural estrogen-lowering agent (Ahmed and Othman 2017). The inhibitory effect of manuka honey on cellular proliferation was attributed to caspase-9-dependant apoptotic pathway activation (Porcza et al. 2016).
2. *Mitochondrial membrane depolarization*: Honey depolarizes mitochondrial membrane which leads to induction of apoptosis (Ahmed and Othman 2013). TH treated tumour hindered expression of Bcl-xL (overexpression of which is associated with metastasis in a breast cancer patient). The mechanism involves

blockade of mitochondrial swelling and membrane hyperpolarization hence inducing cellular proliferation and apoptosis (Ahmed and Othman 2017). Other mechanisms such as activation of the mitochondrial intrinsic pathway during which several proteins such as cytochrome c are released leading to cell death. Honey is also known to reduce the mitochondrial membrane potential which leads to mitochondrial membrane hyperpolarization ultimately causing leakage of intermembrane space proteins into the cytosol and ultimately cell death (Erejuwa et al. 2014).

3. *Cell cycle arrest*: Tumour cells are characterized by aberrant proliferation and hence, are a pivotal target for conventional and novel chemotherapeutics. DNA alterations initiate cell growth arrest at G0/G1 and G2/M phases or apoptosis. Inhibition of cell cycle in S and M phases are the target of many chemotherapeutic drugs. Honey treatment on the bladder (T24, 253 J, RT4, and MBT-2), colon and human melanoma cancerous (A375) cell lines arrested cell growth in the G0/G1 phase (Porcza et al. 2016). The honey treatment causes substantial arrest of the cell cycle in the sub-G1 phase in bladder cancer cell lines. The ability of honey to arrest is attributed to the presence of the several flavonoids and phenolic compounds present in it. Chrysin, quercetin and kaempferol are some of the flavonoids and phenolic compound present in honey in large quantities which arrest the cell cycle in G0/G1, G1 and G2/M in various cancer cell lines (Erejuwa et al. 2014).

   Tumorigenesis has also been linked to dysregulation and/or overexpression of cell cycle growth factors such as cyclin-dependent kinases (CDK) and cyclin D1. Ki-67, the nuclear protein which is expressed during G1, S, G2, and mitosis of the cell cycle, is a novel marker to investigate the "growth fraction" of cell proliferation. Honey has been proved to arrest the cell cycle. Honey when administered with *Aloe vera* solution markedly decreased Ki67-LI expression in tumour cells of rats. Honey has been reported to block the cell cycle in many cancerous cell lines via downregulation of various cellular pathway through kinases, ornithine decarboxylase and tyrosine cyclooxygenase (Ahmed and Othman 2013).

4. *Miscellaneous*: Antitumour activity of jungle honey is attributed to the chemotactic induction for neutrophils and generation of reactive oxygen species. Honey acts via several mechanisms such as stimulating the release of TNF-alpha (tumour necrosis factor-alpha), inducing apoptosis, inhibiting lipoprotein oxidation and causing cell cycle arrest (Othman 2012a, b).

   The proliferative effect of honey may also be attributed to its capacity to generate hydrogen peroxide, hence liberate radicals. Apoptosis induction by honey is also caused by depletion of intracellular non-protein thiols (Erejuwa et al. 2014).

12.4.2 Gastroprotective Effect

In the Greek, Chinese, Romans and Egyptian honey is used for treating stomach-related diseases and wounds. It has the efficacy to impart cytoprotection of the stomach. There are dozens of studies which highlight the gastroprotective effects of honey against ulcer. In a study, the antiulcer effect of honey, turmeric and honey turmeric combination were seen. The honey treated group at 2125 mg/kg dose showed the healing percentage of 49.10% which was as significant as the group treated with Omeprazole. However, the same effect was not observed with the high dose of 4250 mg/kg BW. Honey also reduced the loss of body weight compared to nontreated rats.

In a study, the effect of honey (monofloral and polyfloral) and honey-like solution (a mixture of glucose-fructose-sucrose-maltose) on gastroprotection against ethanol, indomethacin, or ASA-HCL gastric ulcers were evaluated. Both honey and honey-like solution prevented lesions formation of the gastric mucosa. In another study, it was found that after treatment with honey for 2 weeks, 66% of the animals showed recovery from gastric ulcers and extension of treatment for 6 weeks showed that 83.4% of animals had no lesions on gastric mucosa against NSAIDS-induced gastric ulcers (Fazalda et al. 2018).

Similarly, in one more study, the antiulcer effect of honey was evaluated against aspirin (200 mg/kg BW). On the histopathological study of gastric mucosa in the aspirin-treated group presented necrosis and desquamation of lamina epithelium of gastric mucosa and necrosis of lamina propria whereas in honey-treated group apparent normal gastric mucosa reduction in gastric juice volume and increase in pH was noted (Header et al. 2016).

In vitro growth of Helicobacter pylori (H. pylori) which is one of the main etiology of ulcers is inhibited by honey (Bukhari et al. 2011). Unifloral manuka honey has been demonstrated to have significant gastroprotective activity against ethanol-induced stomach ulcer (Almasaudi et al. 2016).

12.4.2.1 Mechanism

1. Honey contains high levels of flavonoids which are believed to prevent the gastric ulcers formation through its antioxidant and anti-secretary mechanisms.
2. The gastroprotective effect of honey may also be attributed to the fact that the pH of honey is 3.88 which plays a role in increasing the pH of the gastric juice (Fazalda et al. 2018).
3. The osmotic effect of honey cause dilution of the necrotizing agent in lumen, delay the gastric emptying, release nitric oxide and non-protein sulfhydryls. Hyperosmolarity of honey also increases prostacyclin synthesis in antral and fundic mucosa of the rat (Gharzouli et al. 2002).
4. Gastroprotective effect of manuka honey was attributed to increased glycoprotein production, preservation of gastric mucosal GSH, decreased lipid peroxidation product MDA and increased formation of nitric oxide (Almasaudi et al. 2016).
5. Gastroprotective effect of manuka honey was attributed to increased glycoprotein production, preservation of gastric mucosal GSH, decreased lipid peroxidation product MDA and increased formation of nitric oxide. It also increased the antioxidant capacity of GPx and superoxide dismutase. Manuka honey inhibits proinflammatory cytokines such as TNF-α (important modulator of apoptotic cell death in gastric mucosa), IL-1β, and IL-6 (Almasaudi et al. 2016).

12.4.3 Anti-inflammatory Property

Honey promotes wound healing process via modulating inflammatory response by the dual effect. It prevents the extended inflammatory response by suppressing the production and growth of inflammatory cells at the wound and it warrants occurrence of normal healing by stimulating the production of proinflammatory cytokines. Nuclear factor-kappa beta (NF-KB), a transcription factor is a chief inflammatory marker. It amplifies the inflammatory response by activation proinflammatory cytokines such as interleukin (IL)-6, IL-8, and tumour necrosis factor-α (TNF-α) which further activates an important mediator of inflammation, nitric oxide. Flavonoids in honey inhibit NF-KB activation and nitric oxide production.

New Zealand honey, particularly Manuka and Kanuka honey have been suggested to have significant anti-inflammatory potential. These act by reducing the production of neutrophil superoxide. Manuka honey has also been associated with the decreased inflammatory response in ulcerative colitis due to its antioxidant activity (Tomblin et al. 2014). Gelam and manuka honey has been demonstrated to have anti-inflammatory potential. Anti-inflammatory effect of Acacia honey was confirmed against LPS-stimulated RAW264.7 cells (Kim et al. 2018).

12.4.3.1 Mechanism

1. Chrysin, quercetin and galangin, flavonoids found in honey was demonstrated to suppress the activity of inducible nitric oxide synthase (iNOs) and cyclooxygenase-2 (COX-2), enzymes which are responsible for producing inflammatory mediators (Ahmed et al. 2018).
2. Manuka honey has been demonstrated to activate IL-10, IL-1, IL-6 (an anti-inflammatory cytokine), TNF-α and IL-1β (proinflammatory cytokines) via toll-like receptors (TLR) and growth factors PDGF and TGF-β. Manuka honey was also able to scavenge superoxide anion and inhibit ROS production due to its phenolic content (Tsang et al. 2015).
3. Manuka honey has also been demonstrated to reduce superoxide production, oedema and leukocyte infiltration in a mice model. Anti-inflammatory effect of Manuka honey was attributed to modulation of the TLR1/TLR2 signalling pathway (Tsang et al. 2015).
4. It has also been said that higher phenolic content display increased anti-inflammatory effect (Ruiz et al. 2017; Hadagali and Chua 2014).
5. Acacia honey works by interfering with NF-κB and MAPK/ATF2 signalling pathways resulting in inhibition of potent proinflammatory mediators such as iNOS, NO, IL-6, IL-1β TNF-α, and MCP-1 (Kim et al. 2018).
6. Inhibition of matrix metalloproteinase-9 (MMP) (Hadagali and Chua 2014).
7. Inhibition of leukocyte infiltration (Hadagali and Chua 2014).

12.5 Clinical Trials on Honey

Trails are being conducted to take honey from bench to bedside for treating some of the serious disease and disorders. Table 12.2 summarizes ongoing clinical trials on honey.

Table 12.2 Clinical trials on honey

S. No	Honey type	Condition	Title of the trial	Clinical trial number/ID	Study phase	Recruitment status
1	Manuka honey	Wound healing	Honey Dressings for Local Wound Care of Split Thickness Skin Graft and Free Tissue Transfer Donor Sites: A Prospective, Randomized, Controlled Trial	NCT02259491	Phase 4	Terminated
2	Honey and hydrogel product	Diabetic foot	The Healing Effects of Honey and Hydrogel Products on The Diabetic Foot	NCT03816618	Early phase 1	Not yet recruiting
3	Raw honey	Poor quality sleep	Honey to Improve Sleep Quality: A Feasibility Study	NCT03567395	Not applicable	Completed
4	Ziziphus honey	Idiopathic dilated cardiomyopathy	Honey Supplementation in Children With Idiopathic Dilated Cardiomyopathy: A Randomized Controlled Study	NCT02987322	Phase 2 Phase 3	Completed
5	Dietary supplement	Type 1 diabetes mellitus	Metabolic Effects of Honey in Type 1 Diabetes Mellitus: a Cross Over Randomized Controlled Pilot Study	NCT01554566	Phase 2	Completed
6	Manuka honey	Dysphagia, lung cancer, pain, eosinophilia	Manuka Honey in Preventing Esophagitis-Related Pain in Patients Receiving Chemotherapy and Radiation Therapy For Lung Cancer	NCT01262560	Phase 2	Completed
7	Manuka honey	Radiotherapy-induced mucositis, head, and neck cancer	A Randomized Placebo-Controlled Trial of Manuka Honey for Oral Mucositis Due to Radiation Therapy for Cancer	NCT00615420	Phase 3	Completed
8	Dietary supplement: honey	Hepatitis A	The Effects of Honey, as a Dietary Supplement in Children With Hepatitis A	NCT02300792	Phase 2	Completed
9	Honey (Madu Nusantara)	Laveration of face, arm, and leg, wound injuries	Comparison of Honey and Povidone-Iodine in Wound Healing on Acute Laceration Wounds: A Randomized Controlled Trial Study	NCT03641053	Phase 3	Completed

	Natural honey	Cough, acute upper respiratory tract infection	A Randomized, Double-Blind Study to Evaluate the Efficacy and Tolerability of a Cough Syrup Containing Specific Plant Extracts (Poliflav M.A.) and Honey Versus Placebo in Cough Due to Upper Respiratory Tract Infection	NCT03218696	Not applicable	Not yet recruiting
10	Natural honey	Cough	Efficacy & Tolerability of a Specific Plantain, Thyme and Honey Cough Syrup vs Placebo in Child Cough Due to Common Cold	NCT02486835	Not applicable	Completed
11	Natural honey	Inflammatory indices in sedentary subjects	Effect of High Intensity Interval Training and Honey Consumption on Some Inflammatory Indices in Sedentary Subjects	UMIN000039156		Completed
12	Manuka and leptospermum honey	Bed sores, pressure ulcer, pressure sore, critically III children	Use of Honey Versus Standard Treatment for Pressure Ulcers in Critically Ill Children- A Randomized Controlled Trial	NCT03391310	Not applicable	Completed

12.6 Conclusion

This chapter deals mainly with health benefits associated with the consumption of honey and the possible mechanism through which the benefits are exerted. The chapter also briefs about chemical constituents and ongoing clinical trials of the honey. Honey is known to influence almost all the body system positively. The common mechanism through which honey positively regulates the various systems of the body is its antioxidant potential attributed to its high phenolic content. Considering the above-mentioned review, consumption of honey thus can improve the overall health of an individual.

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Different Types of Honey and Their Properties

13

Rabia Farooq, Sabhiya Majid, Aamir Hanif, Ahila Ashraf, and Andleeb Khan

Abstract

Honey is a sweet viscous liquid produced by several species of honey bees (Genus Apis). These insects mainly feed on the floral nectar and by enzymatic activity and evaporation of water produce honey from this nectar by regurgitation. Honey has several varieties and is regarded as the superfood with several pharmaceutical properties. This chapter gives a detailed outline about composition, classification, and pharmaceutical and other applications of honey.

Keywords

Honey · Types of honey · Pharmaceutical properties of honey

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_13

13.1 Introduction

Honey a sweet, dark golden, sticky liquid formed by bees from Genera Apis. These bees collect sap from different flowers or from the secretions of plant sucking insects knows as aphids Genus *Rhynchota* (Alvarez-Suarez et al. 2014).

The nectar collected by the bees is ripened into honey by inversion of its sugar i.e., sucrose into fructose and glucose. Honey is supersaturated with sugars, mainly fructose. The sugars in honey have more calorific value than artificial sweeteners (Bogdanov et al. 2008). Honey is stored in the beehive, known as honeycomb made up of beeswax and propolis. Worker bees secrete beeswax while as propolis is a plant resin. The practice of collecting honey is known as "Apiculture." Raw honey is obtained by extraction from beehive as such. It is not processed and contains pollen, bee wax with high antioxidant properties (Crane 1975). Pasteurized honey is obtained by heating the honey at 72 °C or more to destroy yeast cells and prevent crystallization. Dried honey has water content removed from it to form solid granules, mainly used as toppings or garnish foods (Thacker and Emily 2012).

Worldwide production of honey was around 1.9 million tonnes in 2018. Main producer is China, followed by Turkey, Iran, Ukraine, the United States, and India. The color of honey contrasts from pale yellow to dark brown, depending upon the amount of phytochemicals present. Honey contains number of essential organic acids and minerals which is accountable for its high electrical conductivity but in at the same time, it is a poor conductor of heat. The density of honey varies from 1.38 to 1.45 kg/L at 20 °C and pH from 3.4 to 6.1. Depending upon the type of honey, 100 g of honey provides about 304 kcal of energy and its glycemic index ranges from 31 to 78.

13.2 Constituents of Honey

Honey is regarded as a complete food; it contains all compounds which makes it a supernatural food. All its properties, whether wound healing, antioxidant, anti-inflammatory, antitumor, and many more are due to the phytochemicals present in it. Below mentioned are the compounds present in the honey and Fig. 13.1 represent their proportion.

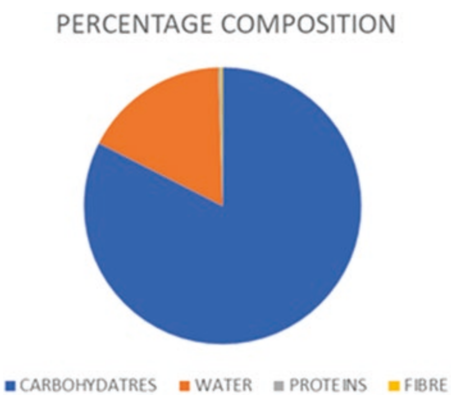

Fig. 13.1 General composition of honey

13.2.1 Carbohydrates

Main part of honey are carbohydrates (82%) which includes monosaccharides like fructose (38%) and glucose (31%); disaccharides includes sucrose, maltose, turanose, maltulose, isomaltose, and few oligosaccharides.

13.2.2 Proteins and Amino Acids

Honey contains 18 amino acids and most abundant one is proline (16 μg/g) and proteins in honey include Major royal jelly proteins (MRJP) in honey such as MRJP-1/2/5/7. MRJP-1 are abundant ones (Chua et al. 2015).

Enzymes like invertase, amylase, catalase, glucose oxidase, acid phosphorylase, glutathione reductase (G-RH) are present.

13.2.3 Vitamins and Minerals

Honey contains vitamin C, vitamin B complex and minerals like calcium, magnesium, phosphorus, selenium, potassium, manganese (Shown in Fig. 13.2).

13.2.4 Other Compounds

Organic acids like: acetic, butanoic, palmitic, citric, formic, succinic, lactic, malic, gluconic acids and some aromatic ones. Honeybees during nectar collection

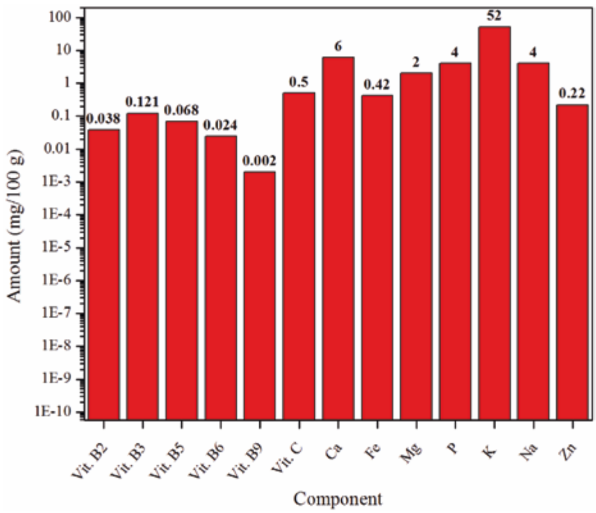

Fig. 13.2 Amounts of different vitamins and minerals in honey

transform phytochemicals of host plant into honey. So, the secondary metabolites in plants attributes to varying properties in honey composition (Nicolson et al. 2007). Mainly phenolics and flavanones constitute the phytochemicals in honey. Phenolics include acids like caffeic, p-coumaric, gallic, vanillic, ellagic, chlorogenic, phenyl-lactic, hydroxy benzoic, and hydroxy cinnamic acids. Flavonoids include (apigenin, chrysin, quercetin, galangin, myricetin, hesperidin, kaempferol, and quercetin) (Balasundram et al. 2006).

However, the floral source, season, geographical location, all influence honey's different properties and composition.

13.3 Types of Honey

Honey is categorized into different types depending upon the floral source. Pollen content in honey also depends upon the floral source. Even weather conditions, temperature also have influence on honey composition.

Monofloral honey is produced when honeybees collect nectar from one type of flower only. So, this kind of honey varies in taste and color, depending upon the type of flower available for nectar in an area for bees. Examples: Clover, Orange blossom, Blueberry, Buckwheat honey are North American Monofloral honeys.

Polyfloral also known as Wildflower honey is when nectar is collected from different types of flowers.

Honeydew honey is formed by honeybees by not from nectar but from the sweet secretions from plant sack sucking insects. It is not as sweet as honey taken from the nectar and is dark brown in color. Germany's Blackforest, Greece pine honey are some examples.

In this chapter, we will discuss some important varieties of honey and their properties.

13.3.1 Acacia Honey

Source: Monofloral honey taken from honeybees who took nectar from black locust tree (*Robinia pseudoacacia*) found in Europe and North America. It is available throughout the year and is very common.

Composition: Among the sugars, fructose is present in large concentration. So, is considered as one of the sweetest honeys (Moniruzzaman et al. 2013). Sucrose content is less, and its pH is around 3.4 (Jaafar et al. 2012). It's mild so it doesn't affect the taste of food in which it is mixed. Its moisture content varies between 12.33 and 17.46% (Terrab et al. 2003). The whole sugar content present in this type of honey is found to be between 62.33 and 70.00 g/100 g. Proline content was found to be around 517.55 mg/kg, proline content indicates sugar adulteration as well as honey ripeness and the value must be above 183 mg/kg (Bogdanov et al. 2008). Among vitamins: Vitamin A, E, and C are present (Muhammad et al. 2015).

Activities: Acacia honey contains high concentration of fructose than glucose, it can remain as liquid for long, and is slow to crystallize. Due to the presence of various phytochemicals, the properties of Acacia honey are as under:

Antioxidant: Acacia is known to contain highest percentage of phenolic compounds than flavonoids, which is the cause of its scavenging property (Moniruzzaman et al. 2013). In a study, Acacia honey is shown to increase GSH, superoxide dismutase (SOD) and catalase enzyme levels when sodium arsenite is injected to rats. So, it reduces free radicals in them and protects the cells against lipid peroxidation and other harmful effects of free radicals (Aliyu et al. 2013). Acacia suppresses generation of free radicals and reactive oxygen species (ROS) as observed in rat models and so prevents the cells against damage (Erejuwa et al. 2012; Muhammad et al. 2014). More catalase activity but less antimicrobial activity has been observed in this honey (Bucekova et al. 2019). It also shows anti-inflammatory action (Muhammad et al. 2016). However, when compared with other types of honey, Acacia honey possess less antioxidant properties.

Immunomodulatory: Acacia honey is observed to increase blood cells which protects against infection, rate of cell and nuclear division. Honey also prevents chromosomal aberrations in lymphocytes, suggesting an immune protective role of honey.

Wound Healing: In a study conducted on rats, Acacia honey is found to increase the area of wound contraction, collagen strengthening, formation of new connective tissues at wound site, increase in blood vessels at wound surface. Collagen formation also occurs due to increase in hydroxyproline content in the rats (Iftikhar et al. 2010). Even corneal wound healing is regulated by using Acacia honey.

Antiproliferative: Acacia honey shows antiproliferative activity on cancer cells, it helps the cells to halt cell division at either Go or G1 phase (Pichichero et al. 2010). It is known to decrease prostate cell in NIH/3T3 PC-3 and NCI-H460 cell lines by causing downregulation of tumor suppressor gene p53 and act bcl-2 and preventing cell division and leading to apoptosis (Muhammad et al. 2012, 2013).

Other Activities: Acacia honey is found to be more potential than antilipidemic drugs like orlistat to prevent obesity. It is taken by diabetic patients as well due to its low sucrose content. Acacia also decrease serum aminotransferases and urea, creatinine levels so hepatoprotective as well as nephroprotective (Ker-Woon et al. 2014).

13.3.2 Clover Honey

Source: Monofloral honey is taken from honeybees who took nectar from white clover (*Medicago sativa* L), found in Canada, the United States, and New Zealand. It is very common and widely available.

Composition: Its color varies from white to dark amber. Clover honey is moisture rich as it contains more glucose than fructose. It also crystallizes readily and is hygroscopic.

Activities: Clover honey exhibited the strongest antibacterial activity when compared with sixteen different types of honey. It shows antibacterial action against both Gram positive as well as Gram-negative bacteria *Staphylococcus aureus* and *Escherichia coli* (Chang et al. 2011). The antibacterial property of clover honey is attributed to its pH, water content, and hydroxymethyl furfurals. But it has been observed its antibacterial activity diminishes with time so does water content and pH (Badawy et al. 2004).

In a study, clover honey is used as topical dressing over diabetic foot ulcers where 43% of complete healing was observed besides lower bacterial load on the wounds (Moghazy et al. 2010). Even it is used as bandage on teeth sutures, scratches, etc.

Clover honey has antiviral properties. In a study varicella zoster human melanoma cell line was supplemented with clover honey and antiviral activity has been observed (Shahzad and Cohrs 2012). Antiviral activity also depends upon the concentration of honey taken. Like other types, clover honey is having antioxidant, anti-inflammatory properties as well. It also has lipid lowering effect. Consumption of honey reduces cholesterol, low density lipoproteins (LDL), triglycerides while cause increase in high density lipoprotein levels (HDL) (Rasad et al. 2018).

13.3.3 Buckwheat Honey

Sources: Monofloral honey, taken from honeybees who took nectar from buckwheat flowers (*Fagopyrum esculentum Moehch*) found in America, Canada, Japan, China, Russia, and the Holland. China is the main producer. However, this plant has less flowering season, so buckwheat honey is scarce. *Fagopyrum esculentum Moehch* has about 15 species all over the world.

Composition: As buckwheat flowers are darker in color so the color of this type of honey is darker amber in color with some reddish tint. Darker honeys are rich in phenols so possess more properties. Its taste is somewhat different than other types. It has more content of fructose (31–39%) than glucose (27–35%) (Pasini et al. 2013) and it contains more iron, magnesium, copper, and zinc. So, buckwheat is healthier than other types. Small amounts of boron, chromium, nickel, cobalt, vanadium, and copper were present in buckwheat honey. It contains about three times more proteins than even Manuka honey (Alvarez-Suarez et al. 2010; Habib et al. 2014). But it's not consumed due to its pungent taste. Lysine and arginine contents are more in this honey.

Activities

Antioxidant and Antibacterial: Buckwheat honey is highly nutritious, and it possesses many medicinal properties. It is used to treat respiratory illnesses (sore throat, cough, nasal congestion) especially among children of age less than 18 years. It contains numerous phenolic compounds and the dominant ones include p-hydroxybenzoic acid, p-coumaric acid, and chlorogenic acid. The presence of these compounds makes this type of honey a good agent to treat cholesterol, high blood pressure, and heart diseases (Zhou et al. 2012). Buckwheat honey exhibits antibacterial activity against *Clostridium difficile, Staphylococcus aureus,* *Pseudomonas aeruginosa*. It possesses antibacterial and antioxidant activity even more than Manuka honey, which makes it a good antioxidant agent (Deng et al. 2018). It shows stronger cellular antioxidant activity (CAA) than other types and this may be due to its high mineral content (Deng et al. 2018). It acts as superoxide ions scavenger, reduces ROS levels by inhibiting complement factors, low pH, high phytochemicals—all these factors over wound contribute to its antibacterial activity and wound healing processes (van den Berg et al. 2008). Ghelof et al. studied effects of both buckwheat honey in water and buckwheat honey in tea and observed an increase in serum antioxidants after only one and a half hour. However, geographic conditions, environmental factors, altitudinal differences—have important effect on the phenolic content of honey, so difference in antioxidant activities (Oomah and Mazza 1996; Oomah et al. 1996; Kishore et al. 2010).

Anticarcinogenesis: Buckwheat honey showed strong anticancer activity. It shows higher inhibition effects by reducing cell proliferation in AGS human gastric carcinoma cell line, colon carcinogenesis and dimethylbenz (α) anthracene (DMBA)-induced mammary carcinogenesis (Kayashita et al. 1999; Liu et al. 2001; Kim et al. 2007). TBWSP31 (Tartary Buckwheat Protein Fraction) is a protein extracted from buckwheat honey and it shows a promising effect on human breast cancer cell line (Bcap37) by reducing cell proliferation and causing apoptosis (Xiaona et al. 2010). Xiao-Li et al. observed the presence of flavonoids (quercetin and rutin) in honey and which are known to possess anticancer properties. They cause apoptosis by regulating the expression of apoptotic proteins Bax/Bcl-2 and caspase-8 in MGC80-3 gastric cancer cell line (Xiao-Li et al. 2019). Trypsin inhibitor extracted from buckwheat shows the inhibitory effect on cell proliferation on hepatic carcinoma cell line H22,IM-9, and K562 cell lines by causing activation of caspases and translocation of cytochrome c to cytoplasm from mitochondria, which sequentially leads to formation of apoptosome so cell death. But trypsin inhibitor does not affect normal cells (Wang et al. 2007; Chong-Zhi et al. 2015). Other inhibitors extracted from the buckwheat are BWI-1 and BWI-2a which are also known for their anticancer effect as elucidated on acute lymphoblastic leukemia cell line (Park and Ohba 2004). Chrysin present in this honey induces apoptosis in hepatocellular cancer (HepG2), colon cancer (HCT116, DLD1), and rectal cancer (SW387) cell lines via caspase activation, AKT activation, and apoptosis (Ronnekleiv-Kelly et al. 2016; Zhang et al. 2016). In prostate (PC3) and lung cancer (A549) cell lines, concentrations as low as 10 μM were found to be effective (Samarghandian et al. 2011; Shao et al. 2012). Buckwheat honey also shows anti-invasive action on U87MG cancer cell one by repressing matrix metallopeptidases (MMP2 and 9) (Moskwa et al. 2014).

Effect on Cardiovascular Diseases and Diabetes: Buckwheat honey also showed effects on cardiovascular studies, in both animal and human studies it shows significant reduction in triglycerides, cholesterol, LDL levels. Besides decrease in blood pressure, body weight, blood glucose as well (Zhang et al. 2007a, b). Rutin, about 1.14% present in buckwheat honey is considered responsible for reduction on cholesterol concentrations. Buckwheat contains rutin at (1.14%) and dietary fibers. So, it also has profound effect on both types of diabetes mellitus, it causes reduction of fasting blood glucose (FBG), glycosylated hemoglobin (GHb) levels as observed in both animal models and humans. Quercetin, rutin, and d-chiro-inositol present in buckwheat honey possess antidiabetic properties, as they are known to decrease oxidative stress associated with diabetes (Cao et al. 2016; Han et al. 2008; Liu et al. 2009; Hęś et al. 2012; Rui et al. 2016; Bao et al. 2016). Absence of gluten in buckwheat makes it good for celiac disease patients.

Anti-fatigue Activity: Presence of protein globulin in Tartary buckwheat honey (*F. tataricum*) possesses significant anti-fatigue ability. It increases the workout time like cycling time, swimming time however it also decreases urea and blood lactic acid levels in the body. Buckwheat honey is also rich in branched chain amino acid content, which diminishes formation of 5 hydroxytryptamine (5-HT), which otherwise suppresses the ability of movement (Chaouloff et al. 1985; Zhang et al. 2005).

13.3.4 Alfalfa Honey

Sources: Monofloral, produced from the nectar of purple or blue blossoms (*Medicago sativa*), white or light amber in color and with mild fragrance. This type of honey is not as sweet as other types. Originated from North America and Canada. Alfalfa honey is less common as it's hard for bees to pollinate.

Composition: It contains more glucose than fructose content, so crystallizes quickly due to the hygroscopic nature of glucose. It contains various flavonoids which are responsible for its important properties. It contains various vitamins and minerals.

Activities: Overall phenolic content in Alfalfa honey was found to be around 0.72, which is measured as mg of Gallic acid/g of honey (Dimitrios et al. 2018). It possesses antibacterial, antioxidant properties required for wound healing. It acts as a probiotic agent, as it contains beneficial molecules which are needed for the growth of beneficial bacteria in the gastrointestinal tract, thus promotes good gut health. Alfalfa honey is known to reduce appetite, acts as lipid lowering agent. It is rich in energy so it can be used for anemia. It is also used to lower body temperature in fevers. Because of its mild flavor, it's used with other beverages, baking, toppings for salads, etc.

13.3.5 Eucalyptus Honey

Sources: Produced from the nectar of *Eucalyptus globulus*, found in Australia, California, Brazil, and South Africa. It is widely available. Jarrah, Yellow Box, Grey box, Blue Gum, River Red Gum, Ironbark, Stringybark, and Messmate are types of monofloral eucalyptus honey. Its color ranges from light amber to medium dark.

Composition: It crystallizes less rapidly, as fructose concentration is slightly more than glucose. It possesses characteristic herbal flavor and a slight after taste of menthol and this flavor may be due to hydroxyacetones, aliphatic compounds, sulfur compounds, norisoprenoids, ketones, alkanes, and monoterpenes. It possesses high vitamin C content and vitamin B9 than other types. However mineral content is less. It contains diastase enzyme but less than buckwheat honey. It has high protein content (0.91–1.24 mg/g of honey) and high levels of gentisic acid and protocatechuic acid (Serra-Bonvehí and Ventura-Coll 2003; Rossano et al. 2012).

Activities: It contains various flavonoids which make this honey as antibacterial, antioxidant, anti-inflammatory agent like luteolin, myricetin, tricetin, quercetin, kaempferol, ellagic acid, and the other nonfloral phenolics which come from propolis like pinobanksin, pinocembrin, and chrysin.

Antibacterial and Antifungal: Eucalyptus honey showed hydrogen peroxide dependent antibacterial action (Irish et al. 2011). Few types of eucalyptus didn't show any antibacterial properties (Campos 1997). Gram-positive bacteria and *E.coli* were highly vulnerable to oils and extracts of eucalyptus. Oil extract from some species (*Eucalyptus sideroxylon and Eucalyptus torquata*) were susceptible to some fungal strains like *Candida albicans, Aspergillus flavus and Aspergillus niger* (Ashour 2008).

Anti-carcinogenesis: Eucalyptus torquata oil extracts are observed to exhibit toxic effects on human breast adenocarcinoma cell line (MCF-7) (Ashour 2008). Faraul et al led a study where he observed antimetastatic effects in Ehrlich ascites carcinoma (EAC) in mice by eucalyptus extracts. It diminishes the size of the tumor and improved the life span of mice (Farhadul et al. 2012). Tricetin is a flavonoid present in the eucalyptus honey which is used in the treatment of non-small cell lung cancer patients with bone metastasis (Hung et al. 2018).

Other Activities: Eucalyptus honey possesses anti-flu and cold properties, helps to relieve nasal congestion, bronchitis, asthma. It is used to treat urinary tract infections. Eucalyptus honey used to treat and heal wounds, including cuts, burns, ulcers, due to its antiseptic and antimicrobial activities. It's used to treat joint pain, stiffness, and muscle pain as it possesses high anti-inflammatory activity. Eucalyptus oil is used treat skin diseases and is used in soaps, deodorants, mouthwashes, toothpastes, etc. It helps to relax nerves and helps in treatment of depression and migraines. Eucalyptus bee pollen showed antidiarrheal activity in rats (Campos 1997).

13.3.6 Manuka Honey

Sources: Monofloral honey, produced by the honeybees from the sap of mānuka tree (*Leptospermum scoparium*), commonly recognized as Manuka bush found in Australia and New Zealand. It is widely available. Color varies from dark cream to dark brown.

Composition: Manuka honey is highly viscous due to the presence of protein, which defines its unique presence. It possesses aromatic fragrance but slightly bitter flavor due to the presence of minerals in high quantity (Morgan 2009). It is rich in vitamins (B1, B2, B3, B5, and B6) and amino acids lysine, proline, arginine, and tyrosine. It also contains minerals like calcium, magnesium, copper, potassium, zinc, and sodium.

Activities: Besides sweetener, Manuka honey is recognized for its medicinal properties. Some important properties are as under:

Antibacterial: Manuka honey displays strong antimicrobial properties, and this is due to the presence of methylglyoxal (MGO), which is formed from dihydroxyacetone present in nectar of flowers. It shows microcidal effect on against *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) (Patton et al. 2006; Sherlock et al. 2010; Israili 2014).

Wound Healing: In 2007 Food and Drug Administration (FDA) approved Manuka honey as an agent to treat wounds. This action might be attributed to its antimicrobial as well as antioxidant properties besides it creates clean acidic microenvironment around the wound to fasten healing. Glyoxal (GO) and MGO enhances wound healing, collagen formation, and tissue regeneration by their immunomodulatory property. Manuka honey also shows advanced wound healing among burn patients (Niaz et al. 2018). This is even used to treat diabetic foot and wounds of diabetic patients as it controls inflammation as well as shows antioxidant properties (Alam et al. 2014). Even it helps to treat scares of the skin an eye lids, as observed in a randomized study (Malhotra et al. 2017).

Anticarcinogenesis: Manuka honey is known for its antitumor properties. Its antiproliferative activity was studied on three different cancer cell lines which includes murine melanoma (B16.F1) and colorectal carcinoma (CT26) as well as human breast cancer (MCF-7) cells where it decreases tumor formation via the activation of caspases, decrease in Bcl-2 expression which sequentially leads to the formation of apoptosome (Fernandez-Cabezudo et al. 2013). Manuka honey even at very low concentration (1% w/v) activate monocytes to release tumor necrosis factor-alpha (TNF-α) and interleukins- (IL-) 1β and IL-6 (Tonks et al. 2003). TNF-α is a key adipocytokine and regulator of various cell processes like apoptosis, inflammation, etc. A 5.8 KD protein in Manuka honey causes the production of TNF-α and Interleukins in macrophages via toll-like receptors (Tonks et al. 2007; Simon et al. 2009). This honey shows anti-inflammatory activity by causing increase in nitric oxide levels and decrease in prostaglandin levels (Al-Waili et al. 2011).

Hepatocellular cell line (HepG2) and HCT-116 colon cancer cells is supplemented with Manuka honey, where cell proliferation is inhibited by inducing caspase 3, Bax activity, and downregulating oncogenic β-catenin and cyclin D1. It arrests the cell cycle at Go/G1 phase and inhibits abnormal cell formation. So, Manuka honey can be considered as a promising effective adjuvant therapeutic for treatment of hepatocellular carcinoma (Al Refaey and Sultan 2018; Cianciosi et al. 2020). Some in vitro studies also observed the antitumor effect of Manuka honey on breast cancer cell lines. It induces intrinsic or caspase-9 apoptotic pathway in breast cancer. Manuka honey increases serum Apaf-1 levels among breast cancer rats, which upregulates expression of proteins, caspase-9 and p53. Manuka honey treatment also modulates immune response by ameliorating hematological and serological parameters in breast cancer (Ahmed et al. 2017).

Manuka honey even at lowest concentrations shows protective action in dermal fibroblasts, by phosphorylating AMP-activated protein kinase (AMPK), or NrF2/ARE anti-inflammatory signaling pathway (Alvarez-Suarez et al. 2016). Manuka honey has been shown to reduce the chemotherapy side effects and toxicity of the anticancer drug Paclitaxel in mice (Fernandez-Cabezudo et al. 2013).

Other Activities: Manuka honey is also used to treat cough, sore throat since ages as it fights bacteria that causes upper respiratory tract infection. It is preventive among cystic fibrosis patients. It is good for maintaining oral health, it reduces oral bacteria *like P. gingivalis and A. actinomycetemcomitans* which form plaques in the teeth, causing gingivitis and tooth decay (Schmidlin et al. 2014; Eick et al. 2014). It is used to treat acne and other skin diseases. It is also used to treat *Helicobacter pylori* caused ulcers.

13.3.7 Sage Honey

Sources: Monofloral honey, produced by the honeybees from the nectar of *Salvia officinalis* L, found in North America.

Composition: It is rich in fructose content, meaning it may be several years before it crystallizes on the shelf when kept at room temperature. Extremely sweet honey flavor makes it one of the best honeys to match with savory dishes and strong cheeses. Color differs from light amber to dark, even greenish-yellow and purple color is also present in some types. It contains high levels of lumichrome (a vitamin B2 metabolite), chrysin, quercetin, luteolin, kaempferol, apigenin, galangin, p-coumaric, and caffeic acid (Kenjerić et al. 2008).

Activities: Due to the presence of high content of flavonoids and phenolics in Sage honey, is a good source of the antioxidants that fight free radicals in the body, so is preventive against chronic diseases. It is also used as expectorant to control cough and respiratory problems. It is good for digestive problems as it contains high number of prebiotics. It shows antibacterial properties so is used to treat wounds and used for dressing.

13.3.8 Rosemary Honey

Sources: Monofloral and polyfloral honey, produced by the honeybees from the nectar of *Rosmarinus officinalis*, found in European countries (France, Italy, Portugal, and Spain).

Composition: It contains more fructose than glucose. It contains high levels of luteolin, kaempferol, chrysin, pinobanskin, pinocembrin, p-coumaric, and caffeic acid. Its color is light amber. It's rich in lithium.

Activities: Rosemary and artificial honeys induced 46–36% of apoptotic cells in human leukemia cell line (HL-60) (Morales and Haza 2013). It's used in digestive problems like indigestion, acidity. Its antiseptic and balsamic properties make it a good tonic for respiratory diseases like cold, bronchitis. It's used to elevate mood, for depression, bipolar disorder as it is rich in lithium. It's used to treat wounds, scares because of its antiseptic properties. Moreover, its anti-inflammatory properties make it good agent to treat joint pain, gout, arthritis. Besides, Rosemary honey is used as a moisturizing agent in cosmetics and creams (Jiménez Soriano et al. 1999).

13.3.9 Avocado Honey

Sources: Monofloral, taken from the nectar of avocado trees, found in Western United States, Australia, and Mexico.

Composition: Avocado honey is high in sucrose content and contains very little fructose. It does not crystallize fast. It is rich in Phosphorus and Magnesium. It has a strong flavor, but less bitter than buckwheat honey but pleasant aromatic aroma. There is a presence of one more sugar, persitol which is not found in any other type of honey (Serra Bonvehi et al. 2019). Avocado honey has a subtle and pleasant, aromatic aroma. It is richer in mineral content, diastase enzyme, phenols, flavonoids, and carotenoids. pH is around 5 (Terrab and Heredia 2004).

Activities: Avocado honey possesses strong antioxidant, free radical scavenging activity, chelating metal ions capacity. It prevents lipid membranes peroxidation caused by free radicals (Henriques et al. 2006). It shows strong antimicrobial action against *Staphylococcus aureus* and *Klebsiella pneumoniae* as it is rich in hydrogen peroxide and has capacity to inhibit bacterial biofilms (García-Tenesaca et al. 2018; Kwakman and Zaat 2012). It is immune booster with excess minerals and amino acids. Among amino acids, glycine and histidine are the dominant ones. It contains more sugars so provides more calories. Avocado extracts impedes esophageal squamous cell carcinoma and colon adenocarcinoma cell lines (Vahedi Larijani et al. 2014). It also exhibits antiproliferative effects by causing halt in cell cycle in many human cancer cell lines (Ding et al. 2007). Avocado extract was supplemented to breast cancer cell lines MDA-MB-231 an T47D and increase in caspases and poly (ADP-ribose) polymerase (PARP) cleavage was observed, which causes apoptosis (Kristanty et al. 2014; Deepti et al. 2019). In yet another study, methanolic and ethanolic avocado extracts exerted cytotoxic effects in breast cancer (MCF-7 and MDA-MB-231) cell lines (Lee et al. 2008; Abubakar et al. 2017). Carotenoids present in avocado extract causes increase in the of tumor suppressor gene p27 expression and blocks cell division at G2/M phases as observed in prostate cancer cell lines (Lu et al. 2005). D003: an acetogenin extracted from avocado is selectively shown to suppress the growth of human oral cancer cell lines via targeting the EGFR/RAS/ERK1/2 pathways or through modulation of mitochondrial ROS production (Ding et al. 2007; Steven et al. 2011). Avocado honey is also used in the treatment of neurological disorders and skin repair treatments.

13.3.10 Dandelion Honey

Source: Monofloral, produced from Dandelion (*Taraxacum officinale*), produced in Asia, Europe, North America, and New Zealand. Real Dandelion honey is rare.

Composition: Dandelion honey is rich in vitamins (A, B, C, and D) and among minerals such as iron, potassium, and zinc. It contains higher concentration of glucose than fructose (Persano and Piro 2004). It also contains flavonoids (apigenin, isoquercitrin, caffeic acid, chlorogenic acid, luteolin), terpenoids, triterpenes, and sesquiterpenes. It is a relatively strong honey blended with mild tangy notes and is dark amber in color.

Activities: Dandelion honey has strong medicinal properties. It's used to treat liver, kidney, stomach, anorexia, and digestive problems (Schütz et al. 2006; Yarnell and Abascal 2009). It's used to treat fever, warts, ophthalmic diseases, and diabetes. It also possesses anti-inflammatory, antiangiogenic, and prebiotic activities (Yarnell and Abascal 2009). It exhibits anticancer properties by targeting death-receptor mediated extrinsic pathway of apoptosis via activation of caspase-8 (Chatterjee et al. 2011; Ovadje et al. 2012). Dandelion is known to contain triterpene and lupeol which are important compounds to inhibit FLICE-like inhibitory protein (cFLIP): highly expressed in pancreatic carcinoma and other cancer cells (Hata et al. 2000; Chatterjee et al. 2011). Dandelion also showed antiproliferative activity in cancer cells as it be due to the presence of sesquiterpenes, phenolics, and triterpenes in dandelions (Hu and Kitts 2003; Jeon et al. 2008).

Dandelion honey is used to treat infections, as laxative, as diuretic, to cure breast inflammation, diarrhea, skin diseases, oral hygiene, etc.

13.4 Conclusion

This chapter summarizes the different varieties of honey and their specific properties which include Acacia, Buckwheat, Rosemary, Dandelion, etc. All these types of honey possess antibacterial, anti-inflammatory, antioxidant, and anticancer properties. Multiple mechanisms are exerted by which honey exerted these properties. But they vary with respect to the composition of phenols and flavonoids which gave them specific characteristics. Honey in future can be used as a therapeutic drug in future but more research needs to be carried out particularly human trials are needed. Above and beyond daily consumption of honey must be taken to remain healthy and wise.

Acknowledgment We would like to put on record our appreciation for the Dean and Vice Deans of College of Medicine and Faculty of Sciences, University of Bisha for encouragement and provision of a conducive ambience for contributing this chapter for the advancement of knowledge.

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Pharmaceutical Applications of Honey

14

Rehab Mohammed Elbargisy

Abstract

Currently, researchers are oriented to the use of several natural products as alternatives in curing various ailments. Among natural products, honey occupies a great position as a sweetening agent as well as a magic remedy for a large list of diseases. Several studies had been conducted on different types of honeys. At first, most of the studies were focused on the use of honey as a natural antimicrobial. Afterwards, many pharmaceutical applications have been knocked. The well-known anti-inflammatory, antioxidant, and antimicrobial characteristics of honey suggest its use to promote wound healing, relief oxidative stress in case of cardiovascular diseases and cure several infectious and inflammatory diseases. Honey has proved its effectiveness in eradication of multidrug resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), controlling blood sugar in diabetic patients, accelerating healing of wounds and chronic ulcers, improving cough and asthma, treatment of different types of cancers, and reducing symptoms associated with periodontal diseases.

Keywords

Honey · Antimicrobial · Antioxidant · Anti-inflammatory · Wound · Cough · Diabetes

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_14

14.1 Introduction

Honey is considered as a treasure trove of nature. Since antiquity, honey had been used as a cure for several diseases as well as a dietary supplement. Benefits of Honey were mentioned in the holy books, Papyrus and even engraved in stones (Pathare et al. 2015). Honey originating from a single flower is termed as monofloral honey and that produced from more than one flower is termed as polyfloral honey. There are numerous types of honey related to its botanical origin. The composition of each honey type varies with its floral source, geographical origin and season of collection as well. That's why some honeys can exert certain therapeutic activities while others cannot. About 80% of honey is fructose and glucose. Other components of honey include proteins, vitamins, minerals, flavonoids, and phenolic acids (Sakač et al. 2019). This chapter will shed light to the possible biological activities of honey and its major pharmaceutical applications in reducing symptoms of some diseases such as wounds and chronic ulcers, cough, asthma, dental problems, diabetes, and cancer. Table 14.1 shows some currently available pharmaceutical products of honey.

14.2 Honey as Antimicrobial Agent

Development of microbial resistance is the major health problem that threatens human being and livestock as well (Levy and Marshall 2004). The rapid emergence of multidrug resistance gave a warning alarm for scientists to look for new antimicrobial agents or seek in the past for ancient remedies thought to have antimicrobial activity and reevaluate their efficacy as antimicrobials. Among these remedies is the bee's honey that has been used for long time for its nutritional and therapeutic values (Mandal and Mandal 2011). Table 14.2 shows some examples of microbial pathogens that proved sensitivity to honey.

14.2.1 Honey as Antibacterial

Bee's honey has proven to have antibacterial activity against various array of bacterial pathogens either gram positive e.g., *Staphylococcus aureus*, *Bacillus anthracis*, *Streptococcus pneumonia*, *Streptococcus mutans,* and *Enterococcus faecalis* or gram negative e.g., *Escherichia coli*, *Salmonella typhi, Shigella species, Proteus species, Pseudomonas aeruginosa, Klebsiella pneumoniae, Helicobacter pylori, Haemophilus infleunza* and *Vibrio cholerae* (Abeshu and Geleta 2016).

14.2.1.1 Mechanisms Exerted by Honey as Antibacterial

The mechanism by which honey exerts its antibacterial activity is multifactorial, that's why resistance to honey is not detected in tested bacterial isolates (Cooper et al. 2010). Also, resistance to honey is minimized by its antimutagenic and

Table 14.1 A list of some available pharmaceutical products of honey

Product	Category	Manufacturer
Manuka honey	Nutritional and health benefits	Honeybee Centre
Magnificent Manuka honey soap bar	Cleansing and antibacterial for skin and hair	
Clover honey and lavender soap bar		
Wedder spoon Manuka lozenges (with eucalyptus and menthol or with ginger and Echinacea	Soothing the irritated tissues of the throat	
Day time soothing syrup with UMF™ 10+ Manuka honey	Soothing irritated throats and cough	Comvita
Night time soothing syrup with UMF™ 10+ Manuka honey		
Chewing vitamin-fortified candy from honey and wax	Substitute for chewing gums	
Skin therapy cream	Skin care products	Natural beauty
Manuka honey hydrating cream		
Clarify and illuminate cleanser		
Orange honey blossom extraordinary beauty oil		
Simple Manuka hand cream		
Honey infused hair mask	Hair care products	Gisou
Honey infused hair conditioner		
Honey infused hair wash		
Honey infused hair perfume		
Honey infused hair oil		
Revamil wound dressing	Wound and burn care products	Oswell Penda Pharmaceuticals
Revamil melginate wound dressing		
Revamil collagen wound dressing		
Revamil wound gel		
Revamil balm wound ointment		
Medihoney antibacterial wound gel		Comvita

antiproliferative activity. Previous studies reported that bacterial cells exposed to Manuka honey do not proceed in the normal cell division cycle but slowed down its division rate. This may be due to affection of honey to a genomic site in the bacteria that is important in cell division (Abdel-Azim et al. 2019).

Honey has numerous components that act individually to inhibit bacterial growth or coincide together to strengthen its power against bacteria. Mechanisms for antibacterial activity of honey can be summarized as follows:

– Honey has high osmolarity that originates from the high sugar content of honey. Most of bacteria cannot survive the high osmolarity.
– The pH of honey is low (in the acidic range), so it doesn't suit the growth of most bacterial pathogens which favors neutral pH (Mandal and Mandal 2011).

Table 14.2 Examples of some microbial pathogens that are sensitive to honey

Type of honey	Organism	Reference
Manuka honey	MRSA, *Escherichia coli, Salmonella typhi Enterobacter aerogenes*	Lusby et al. (2005), Visavadia et al. (2008)
	Helicobacter pylori	Mandal and Mandal (2011)
	Bacillus subtilis	Kwakman et al. (2011)
	Infleunza virus	Watanabe et al. (2014)
	Respiratory syncytial virus	Zareie (2011)
Heather honey "Erica species"	*Candida albicans, Candida krusei, Cryptococcus neoformans*	Feás and Estevinho (2011)
Tualang honey	*Stenotrophomonas maltophilia Acinetobacter baumannii*	Tan et al. (2009)
	Aspergillus niger, C. albicans	Hamid et al. (2018)
Ulmo honey	*Ps.aeruginosa*, MRSA, *Escherichia coli*	Sherlock et al. (2010)
Iranian honey	*C. albicans, C. krusei, C. glabrata, C. tropicalis*	Shokri and Sharifzadeh (2017)
Acacia honey, Kelulut honey	*Aspergillus niger, C.albicans*	Hamid et al. (2018)
Medihoney	*C. albicans, C. krusei, C. dubliniensis*	Irish et al. 2006)
Turkish honey	Fluconazole-resistant *C. albicans, C. krusei* and *C. glabrata*	Koc et al. (2009)
Croatian honey (Fir honeydew honey, Mint honey)	MRSA, *Acinetobacter baumannii*	Gobin et al. (2018)

– The low water content of honey along with its high osmolarity cause dehydration of the surrounding medium and bacteria as well and further bacterial cell lysis (Mandal and Mandal 2011).
– The glucose oxidase enzyme secreted by the honeybee in the nectar will be activated by body fluids to convert glucose into gluconic acid and hydrogen peroxide. The peroxide species activate cytokine production as a consequence to neutrophils activation and thus increase the inflammatory response that help bacterial killing (Vandamme et al. 2013).
– The antibacterial activity of honey may correlate to its anti-quorum sensing activity (Wang et al. 2012) which in turn affects expression of virulence genes and biofilm formation (Jenkins et al. 2014).
– Polyphenolic compounds such as flavonoids can affect bacterial growth through disruption of cell membrane function, inhibition of bacterial DNA synthesis by impairing DNA gyrase enzyme, and blocking of cell metabolism (Cushnie and Lamb 2005).
– Bee defensin-1 in the bee saliva may be transferred to honey during eructation process. This compound has strong antimicrobial activity against gram positive bacteria (Kwakman et al. 2010).
– Bee propolis may be present in raw honey and it is known for its antimicrobial activity (Campos et al. 2014).

14.2.1.2 Revamil Honey Versus Manuka Honey as Antibacterial

The antibacterial activity of honey is not a constant value against bacterial pathogens where it varies greatly with the type of honey used and the species of the bacteria. The variation in the antibacterial activity of honey is usually associated with variation in its botanical source, geographical source, weather conditions, and honey processing. All these parameters result in different chemical composition of honey even different quantities of the same components. So, some types of honey are known to have potent antibacterial activity while others have little activity. Stingless bee honey experienced inhibitory effect to the growth of gram positive and gram negative bacteria which suggests adding it as ingredient in pharmaceutical preparations (de Queiroz Pimentel et al. 2013). The antibacterial effect of this type of honey is enhanced by the diffusion of antibacterial components from propolis in the pots where it is stored (Campos et al. 2014).

There are two types of medically graded honeys that have proven their potent activity as antimicrobials, Manuka honey and Revamil honey. These two types undergo sterilization by gamma radiation to eliminate bacterial spores that may contaminate them during processing (Kwakman et al. 2011). Although effective as antimicrobial but the mechanism is limited to their chemical composition. Manuka honey originates from Manuka flower present in New Zealand. The nectar of this flower is characterized by the presence of high concentrations of dihydroxyacetone which is meanwhile converted to the effective antibacterial methylglyoxal. The mechanism suggested by this chemical is its interaction with guanine residue in RNA or DNA resulting in impairment of DNA and protein synthesis (Krymkiewicz et al. 1971). In addition to methylglyoxal Manuka honey was found to stimulate immune system to release certain mediators such as TNF-α, IL-1β, and IL-6 which help clearance of microbial infections. Manuka honey is usually assigned to a UMF value (Unique Manuka Factor) that indicates its antimicrobial potency (Tonks et al. 2007).

Revamil honey is produced under controlled process where bee colonies are kept in adjustable conditions. The controlled production process ensures that its chemical composition and further therapeutic values will be maintained. Revamil acts as antibacterial through its two major components: hydrogen peroxide and bee defensin-1. Absence of these components in manuka honey explains the difference in their antibacterial mechanism. Both honeys show potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *P. aeruginosa*, *E. coli,* and *Bacillus subtilis*. Although Revamil showed its bactericidal activity in shorter time, Manuka honey maintained its bactericidal activity even with very high dilutions against food spoiling bacteria and in this case it is more suitable to use Manuka honey in food preservation to protect against food spoiling microbes rather than Revamil honey (Kwakman et al. 2011).

14.2.2 Honey as Antifungal

Fungal infections represent a major threat to public health. *Candida albicans* comes in the first place as an opportunistic pathogen that can cause a wide range of diseases starting with oral and vaginal candidiasis to the most serious invasive condition candidemia. It is now extended to other candida species to generally affect human health (Perlin 2009). In addition to remarkable toxicity, conventional antifungal agents are now worthless against most fungal infections. This can be attributed to the improper use of antifungals which lead to emergence of resistant fungal species (Pappas et al. 2004). Researchers realized the urgent need for new antifungal agents that combine both effective therapeutic activity and minimal side effects and then natural products have shed the light to start considering as a medicament, among which is honey.

Several reports demonstrated the effective antifungal activity of honey against multiple fungal pathogens including *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, *Cryptococcus neoformans,* and *Trichosporon* species (Khosravi et al. 2008; Koc et al. 2009; Feás and Estevinho 2011). The polyphenolic compounds in honey affect its antifungal power, and these compounds vary with respect to type and concentration to the botanical origin of honey. In this regard, good antifungal activity of Rhododendron honey was observed compared to that of orange honey (Andrade et al. 1997).

Irish group limited effectiveness of honey as antifungal to topical applications which limits its value for treatment of systemic fungal infections. Honey has good role in systemic fungal infections through its clearance and protection of different body entrances such as mouth and vagina and even indwelling medical devices from contaminants. The other problem that faces honey for use in treatment of oral or vaginal candidiasis is its dilution by human body fluids. This can be overcome through its formulation as oral chewable tablets or vaginal suppositories or continuous addition of honey on infected area to maintain high concentration above the MIC value all over the therapeutic period (Irish et al. 2006).

14.2.3 Honey as Antiviral

Several problems are associated with the use of the conventional antiviral drugs in therapy. These problems can be summarized in rapid emergence of drug resistance due to high incidence of viral genetic mutations, prohibition of drug intake during pregnancy and breast feeding, toxic side effects, and even the high cost (Hashemipour et al. 2014). As a result, developing new antiviral drugs with minimum toxicity and lower drug resistance remains the priority for virologists. Scientists now focus on natural products seeking its medicinal value. The nutritional value, safety, and availability of bee's honey make it among the first products to explore its medical value. Several studies had been conducted on the antiviral potential of honey. Honey possessed antiviral activity against common viruses such as rubella virus (Zeina et al. 1996), varicella zoster virus (Shahzad and Cohrs 2012), herpes simplex-1 virus

(Al-Waili 2004), and infleunza viruses (Watanabe et al. 2014). Moreover, the duration for viral diarrhea is much decreased when honey is administered compared to the conventional antiviral drugs (Andualem 2013). The mechanism by which honey exerts its antiviral activity is not fully understood. However, it was suggested that the anti-infleunza activity of different honey samples was partly due to rutin and chrysin (Watanabe et al. 2014). On the other hand, manuka honey demonstrated the highest anti-infleunza activity that may be attributed to its content of methylglyoxal that had shown good antiviral activity in foot and mouth viral infection (Ghizatullina 1976).

14.3 Honey and Wound Healing

Wound is a breach in human body caused accidentally or through surgical operation. The wound area is characterized by the presence of damaged tissue, low oxygenation, and impaired blood circulation (Porth 2017). Based on duration for complete cure, wounds can be divided into acute wounds that are healed within 8–12 weeks and chronic wounds that do not respond to treatment within the proposed period of healing but getting worse. Rapid management of wounds is an important measure in diabetes, obesity, and elderly patients (Lu et al. 2018). Since ancient times, honey was used in wound treatment due to its physical and chemical properties. Honey has several mechanisms that are employed together to help rapid healings of wounds, described as follows and in Fig. 14.1.

1. Antimicrobial activity:

   The antibacterial and antifungal properties of honey select for its use in treatment of wounds. Wounds are at high risk for being infected. The reported

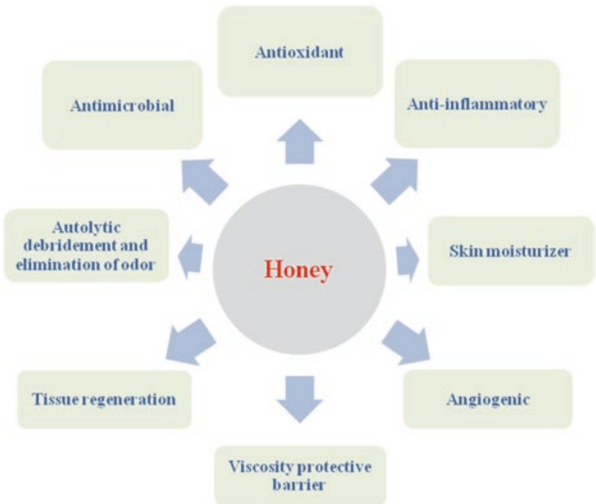

Fig. 14.1 Mechanisms employed by honey in wound healing

peroxide and non-peroxide activity of honey against common bacterial pathogens isolated from wound infections assures its importance in wound clearance from any possible microbial contaminants (Singh et al. 2012).

2. Skin moisturizing capacity:

   Maintenance of wound tissues moisturized is very essential in wound healing process. Moisture can enhance tissue oxygenation (Kurhade et al. 2013), stimulate blood circulation, stop tissue necrosis (Korting et al. 2011), decrease possibility for scar formation (Atiyeh et al. 2003), and reduce the chance for secondary microbial infections (Kurhade et al. 2013). Honey is known for its skin moisturizing capacity. The hydroxyl groups in chemical structures of different honey components such as sugars and proteins (Boateng et al. 2008), vitamins and minerals (Vanhanen et al. 2011), sorbitol, glycerin and propylene glycol (Draelos 2010), all these components contribute to honey's moisturizing effect. In addition, high osmolarity withdraws water from the surroundings to hydrate wound tissues (Alvarez-Suarez et al. 2010).

3. Honey acts as a protective barrier on wound tissues to prevent its microbial contamination (Hananeh et al. 2015).
4. Honey enhances blood circulation in wound tissues and allows for tissue regeneration through stimulating the growth of the outermost epithelial layer and the connective tissue. The increase in tissue growth that occurs upon oral administration of honey seems to be due to growth factor component rather than nutritional or environmental factors (Al-Waili et al. 2011).
5. Anti-inflammatory activity:

   Inflammation of wound tissues occurs due to invasion by a foreign matter e.g., bacteria and fungi or degeneration of tissues (Molan 1999). During inflammation, macrophages augment the production of inflammatory mediators such as nitric oxide, cytokines, and prostaglandins. These mediators cause cytotoxic effect to the target cells, but excessive production of such mediators can cause tissue damage (Kim et al. 2013). Therefore, anti-inflammatory drugs are given during therapeutic treatment of wounds. Unfortunately, the known anti-inflammatory drugs were found to decrease wound healing either by their destructive effect on the tissues (nonsteroidal anti-inflammatory drugs, e.g., aspirin) or prevention of tissue regeneration (corticosteroids e.g., dexamethasone) (Krischak et al. 2007). Honey has anti-inflammatory activity through inhibition of cyclooxygenases and prevention of overproduction of inflammatory mediators (Erguder et al. 2008). These effects are brought about by the phenolic compounds in honey. Another indirect effect of these phenolic compounds is being as antiradical compounds that protect tissues from the cytotoxic effect of inflammatory mediators. Consequently, honey can reduce swelling in wound area and in this case, better oxygenation and nutritional supplementation can reach wound tissues and encourage its growth (Molan 1999).

6. Debridement of a wound is an essential step in treatment process as necrotic tissue helps growth of infective microbes that can cause extended damage to the surrounding tissue. Honey helps removal of necrotic tissue through autolytic debridement of the wound which eliminates wound odor (Vandamme et al. 2013).

7. Antioxidant activity:

   In the first stage of wound healing, overproduction of nitric oxide leads to production of hydrogen peroxide and reactive oxygen species (ROS) (Ju et al. 2012). These species cause harm to the wound and distant body organs through activation of cellular and humoral mediated immune mechanisms (Closa 2013). Antioxidants can counteract these harmful effects by two possible mechanisms.

   (a) Enzymatic removal of free radicals through its conversion to stable harmless molecules, e.g., peroxidases, catalase, and superoxide dismutase (Ahmad et al. 2010).
   (b) Nonenzymatic removal of ROS which blocks their damaging activity and even prevents their formation by the cell, e.g., vitamin C, tocopherol, and phenolic compounds (Ahmad et al. 2010).

   Honey can improve wound healing through its antioxidant properties where it contains a wide variety of phenolic compounds that can act as antioxidant for the nonenzymatic removal of free radicals. In addition, some enzymes in honey e.g., peroxidase can also, clear the ROS and protect the cells from the destructive effect of these free radical species (Khalil et al. 2011).

Wound dressing is a crucial step that has a great effect on the healing process. Wound dressings were applied to the wound to protect the damaged tissues from being infected (Henry et al. 2019). Currently, wound dressings are loaded with active compounds that can accelerate the healing process. Several factors can control the choice of the suitable wound dressing. Patient relief, duration of application, infected wounds, sterility of dressing, ease in application or removal of dressing and the cost, all mentioned factors guide the physician to select the most suitable wound dressing (Henry et al. 2019). Honey is effectively used in wound dressing for first- and second-degree burns, diabetic ulcers, and leg ulcers. Being nontoxic, nonirritant, and easily applied and removed make honey dressing more comfortable to use than any other dressings (Bulman 1955). For pediatric patients, Revamil gel and gauze are selected as the most suitable wound dressing for the patient comfort where this type of dressing doesn't stick to wounds so it does not cause any pain in its removal (Henry et al. 2019).

14.4 Honey and Respiratory System

14.4.1 Honey and Cough

Many factors can trigger coughing reflex. The most common factors are the upper respiratory tract infections (URTIs) and inhalation of an allergen (Landau 2006). Cough is considered an annoying symptom that bothers both the sick child as well as his parents. Most symptoms of URTIs can be resolved within 1 week but cough may last for several weeks. When a family has a child with persistent cough, it means there is a lack of sleep and disturbance of life regimen to all family members where there is a high possibility for absence from school or work for the sick child and his parents, respectively (Ayazi et al. 2017).

Many drugs that are usually prescribed for controlling cough in children demonstrate undesirable side effects. Children given dextromethorphan were unable to sleep normally and those given diphenhydramine were drowsy (Paul et al. 2004). Appearance of such side effects necessitates searching for a safe alternative natural remedy. Since ancient times, honey is used to relief common cold symptoms with special regard to cough. Being cheap, available, and safe to use, honey is then recommended by the world health organization (WHO) for curing cough and other URTIs-related symptoms (World Health Organization 2001). Several studies were conducted to evaluate the effect of honey on number of coughing episodes and its severity. Honey proved to be effective in decreasing number of coughing episodes compared to over-the-counter drugs dextromethorphan and diphenhydramine (Oduwole et al. 2018). The combination of honey and coffee has proven good activity in curing post-infectious persistent cough where the damaged nerve endings by mucosal irritation and desquamation were recovered and mucosal tissue became coalescent (Raeessi et al. 2013). The mechanism by which honey causes cough sedation is proposed to be through its sweetness that induces excess saliva secretion leading to liquefaction of mucous in the affected airways and reduction of larynx irritation then relief of cough episodes (Paul 2012). Another explanation for the sedative effect of honey on cough was illustrated by Eccles. He supposed that there is possibility for interaction between sweet tasting nerve fibers and cough initiating nerve fibers. This interaction originates mainly from their anatomical relationship (Eccles 2006).

14.4.2 Honey and Asthma

Asthma is a common chronic respiratory illness that affects the lower respiratory tract (LRT) and is usually the result for exposure to allergens (Cianciosi et al. 2018). The disease manifestations include inability to breathe, chest constriction, and early morning and night coughing (Balaha et al. 2012). These manifestations result from the inflammatory cell response, the structural changes of airways, goblet cells hyperplasia, excessive mucous secretion, and blood vessels expansion. Augmentations in thickness of epithelial and subepithelial layers are the main changes in airway structures. Therefore, narrowing of airways occurs causing difficulty in breathing (Fahy et al. 2000). The long-term therapy of asthma by conventional drugs can be accompanied by several side effects such as osteopenia, ocular hypertension, marked decrease in growth rate, and oral thrush (Fanta 2009). These serious side effects encouraged seeking for more safe alternatives. The antioxidant and anti-inflammatory properties of honey reported by several studies suggest its medical value in controlling asthmatic attacks. Honey is mostly administered by oral route to produce its therapeutic effects, but the case is different in case of asthma. It was expected that inhalation of aerosolized honey will be more effective than ingested honey in relieving asthma because the amount of honey that reaches the altered airways will be much more in case of inhalation which will accelerate the curing process (Rhman 2007). Kamaruzaman et al. reported that 25 and 50% of honey aerosol inhibited goblet cells excessive proliferation. Therefore, mucous secretion was much reduced. Decrease in inflammatory cell response was observed as well. These two major effects of honey suggest its clinical importance in both improving symptoms in asthmatic patients and prevention of asthmatic attacks (Kamaruzaman et al. 2014).

14.5 Honey as Antidiabetic Agent

The most common chronic metabolic disorder that largely affects general human health and his quality in performance is diabetes mellitus (DM). This disease features uncontrolled high blood glucose level which then causes multiple symptoms starting simply with excessive urination, severe thirst, dehydration, and body weight loss. With time, these symptoms develop serious conditions such as retinopathy, kidney failure, nerve cells damage, diabetic foot infections (DFIs) that usually ends with limb amputation, dyslipidemia, and cardiovascular diseases (Bobiş et al. 2018). Diabetes mellitus is classified into two types that differ primarily in their etiology, onset of clinical manifestations and even therapeutic strategies. Type-I DM is characterized by low or non-insulin secretion by the beta pancreatic cells due to its autoimmune destruction. Inherited genes are behind this type of diabetes combined with some environmental factors that accelerate its emersion. Type II DM found to be associated with overweight and obese individuals who have improper lifestyle and diet control. In this type, the beta pancreatic cells are still able to secrete insulin to which body cells become resistant. The hyperglycemia is the characteristic sign that both types of diabetic patients share (Kokil et al. 2010).

Honey has a wide variety of nutritional and nonnutritional compounds that contribute to its different biological activities. Honey as a natural product has been used for long time as a sweetening agent. Several studies have demonstrated the hypoglycemic effect of honey upon oral administration by either laboratory animals or human which might suggest its use as an antidiabetic agent beside the conventional drug therapy (Kokil et al. 2010).

The monosaccharides, fructose and glucose, constitute about 80% of honey in total. Glucose in honey was found to promote fructose absorption (Kokil et al. 2010). Previous studies had reported the lowering effect of fructose on blood glucose level (Erejuwa et al. 2012). Slowing gastric emptying rate caused by fructose with subsequent decrease in food intake explain in part its hypoglycemic effect (Gregory et al. 1989). Besides, the prolonged contact time between fructose and intestinal receptors might adversely affect intestinal absorption of macronutrients such as carbohydrates which then improves satiety sensation (Anderson and Woodend 2003). In addition, fructose catalyzes glucokinase enzyme that help glucose uptake and its storage as glycogen in the liver (Van Schaftingen and Vandercammen 1989).

Another possible explanation for the hypoglycemic effect of honey was supposed by Abdulrahman et al. who attributed the increase in C-peptide serum level to the increased insulin secretion caused by oral administration of honey (Abdulrhman et al. 2013). The elevated insulin level results from the hydrogen peroxide stimulatory effect (evolved upon mixing of honey along with water) on beta pancreatic cells to secrete more insulin (Al-Waili 2003).

The high glucose uptake by adipose tissue causes massive production of reactive oxygen species (ROS) resulting in oxidative stress. This condition strongly stimulates development of diabetes especially type II where disruption of insulin signaling pathway makes body cells resistant to insulin. The antioxidant compounds in honey act through its free radical scavenging ability to clear the oxidative stress of the pancreas as well as the other body tissues (Kim et al. 2006). Moreover, honey acts as a protective antioxidant against lipid peroxidation and altered lipid metabolism in type II DM (Rahimi et al. 2005). Polyphenols present in honey are suggested to decrease hyperglycemia and improve lipid metabolism in diabetic patients through different pathways. The alpha amylase and alpha glucosidase enzymes are basically involved in carbohydrate hydrolysis. The inhibitory effect exerted by some polyphenolic compounds (i.e., quercetin, myricetin, and luteolin) on these enzymes beside its antioxidant activity will then reduce blood glucose level (Tadera et al. 2006; Hussain et al. 2012). The increase in peripheral glucose uptake is another way for controlling blood glucose level by the polyphenols in honey (Lee et al. 2012). Moreover, in type II DM patients, polyphenols present in honey such as luteolin proved to induce adipokines production, and thus preventing insulin resistance (Ding et al. 2010).

The level of adiponectin, a hormone found to regulate fat and glucose metabolism, is elevated upon ingestion of honey. This hormone caused marked amelioration in blood glucose level and lipid metabolism as well (Hemmati et al. 2015). In addition, Aziz et al. attributed the hypoglycemic activity of stingless bee honey to the high expression of catalase enzyme that acts as antioxidant in addition to the L-phenylalanine that stimulates insulin release (Aziz et al. 2017).

Honey is rich in highly valuable compounds that act together to exert its beneficial health effects. Reviewing different explanations for the antidiabetic effect of honey strengthens the scientists' recommendations for using honey as an adjuvant in the control of diabetes and its associated deteriorative effects.

14.6 Honey and Testosterone Hormone

Testosterone is the prime male sex hormone secreted mainly by Leydig cells in the testes and in part by the adrenal gland. This hormone controls both maturity of male sex organs and secondary sexual characters. Testosterone also, contributes in major to sperm production and sexual desire. The importance of this hormone is not limited to sexual life improvement and reproduction in male only but also it extends to normal body health e.g., body muscle mass, bone density, and generalized physical state of the body (Kloner et al. 2016). At the age of 40, testosterone level starts to decrease. Several diseases (such as cardiovascular diseases, osteoporosis, infertility, obesity, type II diabetes mellitus, and sarcopenia) were found to be associated with low testosterone level in serum (Petering and Brooks 2017).

For scientists, improving testosterone level is a major concern. Several studies targeted the effect of different food stuffs on serum testosterone level (Banihani 2018). Most of these studies were conducted on male animal populations (either associated or not with chemically induced reproduction toxicities) with different dosing range of various types of bee's honeys. The duration of the experiments done in this issue varied from 2 to 12 weeks (Banihani 2019). Studies that emphasized the role of bee's honey in elevation of testosterone level suggested different possible mechanisms. These mechanisms can be summarized as follows:

1. Honey increases the level of luteinizing hormone which in turn increases the serum testosterone level.
2. Honey participates in maintenance of healthy testicular tissues which increases their ability to produce testosterone.
3. As the oxidative stress state in the testes negatively affects testosterone production by Leydig cells, the antioxidant activity of honey promotes the following:

   (a) The antioxidant compounds in honey (either phenolic or non-phenolic) help removal of the harmful free radicals and then retrieve the Leydig cell ability to produce testosterone normally (Banihani 2019).
   (b) The antioxidant flavonoid chrysin in bee's honey increases the activity of antioxidant enzymes such as catalase and superoxide dismutase (Ciftci et al. 2012), inhibits aromatase enzyme (this enzyme stimulates testosterone conversion into estradiol (Oliveira et al. 2012), and augments StAR gene expression that encodes StAR protein, a protein involved in cholesterol transport through mitochondrial membrane for subsequent cleavage into pregnenolone in Leydig cells (Jana et al. 2008).
   (c) Quercetin, in addition to phenolic compounds present in honey (e.g., caffeic acid, rosmarinic acid, and ellagic acid) augment testosterone production in reproduction toxicity resulted from exposure to certain chemicals (Banihani 2019).

In conclusion, most researches that investigated serum testosterone level in relation to bee's honey intake revealed that there is a positive effect for oral administration of honey on elevation of serum testosterone and no harmful effects were detected in any of those studies.

14.7 Honey and Cancer

Cancer is considered one of the major causes of death all over the world (Jemal et al. 2011). Following the western life habits, ageing, awareness campaigns through different media that teach people about syptoms of cancer, amelioration in detection techniques play important role in the noticeable increment in number of recorded cancer diseased population (Kanavos 2006). It was found that types of cancers that are largely affected by prevalence of certain infectious agents predominate in developing countries, while those affected by food habits and lifestyle are more dominant in developed countries (Othman 2012). Developing cancer means genetic modifications start to occur leading to irreversible damage (Pitot 1993). Secondly, high proliferation rate of mutated cells ending with a benign mass. In the third stage, the cancerous cells extend to the distant tissues (Tubiana 1998).

Treatment of cancer undergoes several procedures according to type of cancer and its stage. Radiotherapy, surgical removal of cancerous mass, and chemotherapy are the main therapeutic strategies employed to control the disease. Besides being somewhat expensive and have limited availability in some areas, chemotherapeutic drugs used in cancer managements are known for its serious side effects that largely affect the general health of patients and its normal life practices (Chidambaram et al. 2011). These facts encourage seeking for alternative therapeutic agents that are safe, low in cost, and readily available. Honey satisfies all these criteria which favor its use as adjuvant therapy in cancer (Mendel 2004). Many studies had reported the efficacy of honey as anticancer against several types of human cancer cells. Figure 14.2 demonstrates factors stimulating cancer occurrence and possible previously explained mechanisms for the activity of honey as anticancer.

14.8 Honey as Oral Curing Agent

Oral health provides a key sign for the overall body health. Wide spectrum of oral diseases commonly occurs including dental plaque, gingivitis, malodor, alveolar osteitis, and cancer. Several studies advice the use of honey along with the basic oral medications to accelerate patient cure from different oral ailments (Seymour 2007).

Dental plaque is highly prevalent and usually associated with dental caries and other periodontal diseases. Mechanical removal is the most successful method employed to get rid of plaque and retrieve the healthy gingiva (Gupta et al. 2014). Anti-plaque agents are used along with mechanical removal techniques to support complete removal (Kayalvizhi et al. 2014). Manuka honey was reported as antibiofilm agent that decreases colonization by bacteria and dental plaque formation (Nayak et al. 2010).

In root canal inflammation, dentists find that the first measure is to debride necrotic tissue and clear microbial inhabitants. Honey as a root canal irrigant was less potent than sodium hypochlorite but more compatible with tissues (Sundaram et al. 2016).

Mucositis is an aching inflammation in mucous layer of the oral cavity. This inflammation can arise from radiotherapy or chemotherapy directed to cancerous cells. Honey was found to decrease radiation-based mucositis (Rao et al. 2017). Alveolar osteitis is an oral disease appears 2–3 days post tooth extraction. Severe pain and disintegrated blood clot are characteristic signs for alveolar osteitis. Reduction in pain, malodor, and edema was notified by Singh et al. in patients

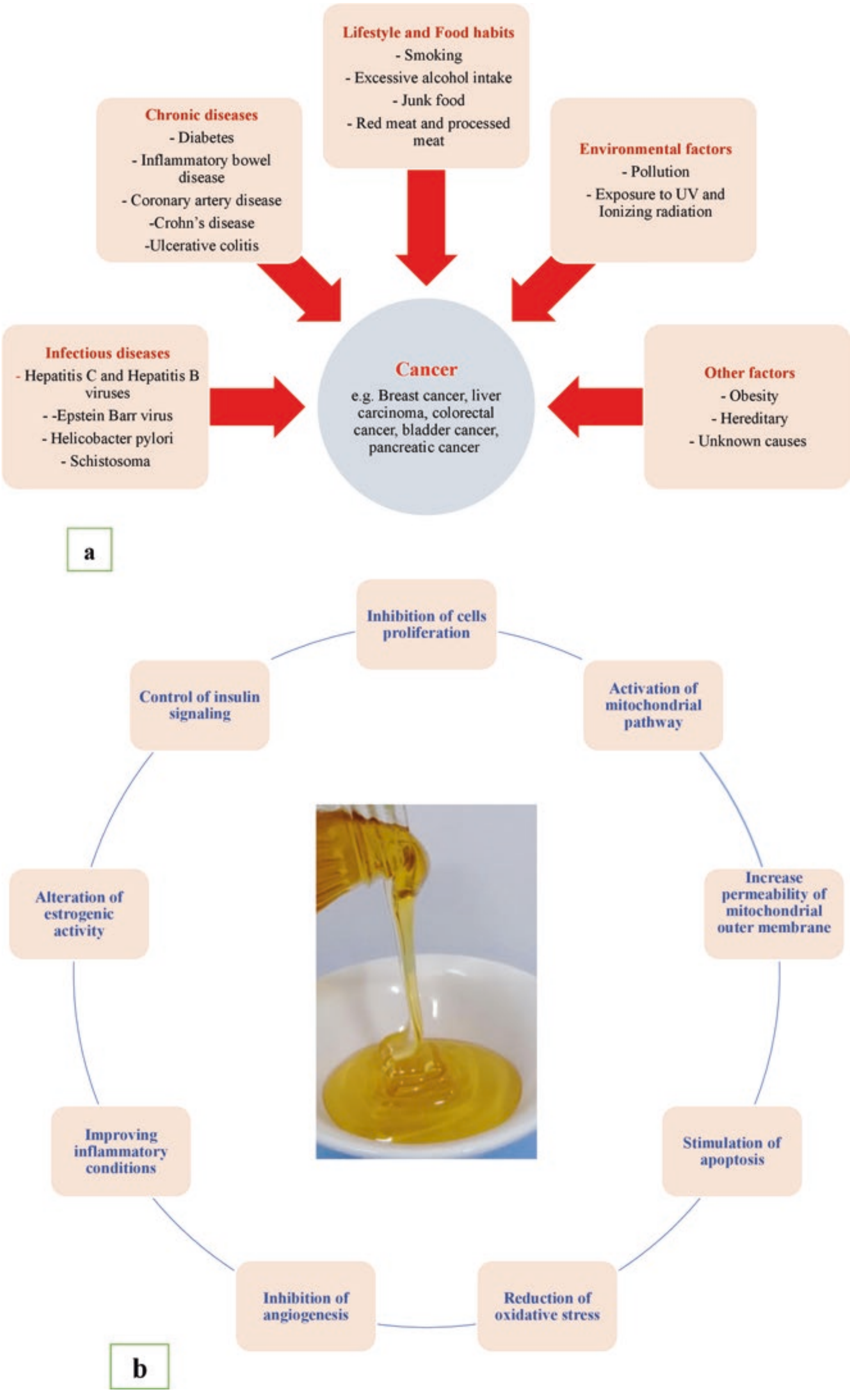

Fig. 14.2 Schematic diagram of (**a**) some causes of cancer and (**b**) mechanisms exerted by honey to combat cancer

receiving honey as adjuvant therapy for alveolar osteitis (Singh et al. 2014). Moreover, honey was beneficial in oral ulcers, stomatitis and halitosis supported by its antibacterial and anti-inflammatory properties (Pasupuleti et al. 2017).

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Properties of Honey: Its Mode of Action and Clinical Outcomes

15

Reyaz Ahmad Wani, Arif Akbar Bhat, Iyman Rasool, Syed Mubashir Yousuf, Shabhat Rasool, and Hilal Ahmad Wani

Abstract

The medicinal use of honey is well established since time immemorable. Currently, there exists a large volume of clinical research wherein the benefits of honey in health and disease have been proven beyond doubt. By the very nature of its biochemical composition, it is a nutrient par excellence comprising carbohydrates, amino acids, minerals, and electrolytes. The main focus of this chapter is to highlight the important clinical uses of honey in light of modern day medical practice. Honey has broad-spectrum antimicrobial effect, covering diverse varieties of viruses, bacteria, and fungi. Other therapeutic properties of honey include antioxidant, anti-inflammatory, immunomodulatory, cytoprotective, and antineoplastic properties. Various components of honey have been isolated, and each of its actions is attributed to one or more of its constituents. The present day use of honey ranges from simple skin conditions to difficult surgical wounds to diverse cardiovascular and gastrointestinal pathologies to anticancer remedy.

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_15

The benefits of honey as a therapeutic agent is its low cost, natural product with minimal adverse reactions, and minimal need for dose adjustments.

Keywords

Nutraceutical property · Antimicrobial agent · Wound healing · Pre- and probiotic · Honey polyphenols · Pediatric burns · Medihoney · Apitherapy

15.1 Introduction

Honey is a versatile natural product produced from plant nectars by the living machineries called honey bees. The biochemical properties of honey vary, depending mainly on the floral type. Based on the origin, honey has been classified as monofloral and polyfloral or multifloral. While monofloral honey is produced from single plant source, polyfloral honey has several botanical sources. Besides the floral honey, another variant is honeydew honey. Here honey bees feed on the plant secretions or excretion of certain plant-sucking insects belonging to genus Rhynchota. It is the source plant and the honey bee modifications thereof that imparts distinct physicochemical properties to the honey. Likewise the nutritive and medicinal properties of honey are due to the constituents present in it. At biochemical level, honey has antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, cytoprotective, antiproliferative, and many other properties that make this natural substance such a versatile agent. This chapter will focus on certain clinical attributes of honey and the active chemical ingredients responsible for such properties. Before that, let us briefly discuss some physicochemical properties of honey.

15.2 Physicochemical Properties of Honey

Honey is a sweet viscous substance ranging in color from near transparent pale yellow through ambers to dark red amber to near black depending on the source, age, storage, and climatic conditions (Bertoncelj et al. 2007; Anupama et al. 2003). The viscous nature is because of the presence of very high sugar concentration. The supersaturated nature of the solution makes it highly dense, hygroscopic with a tendency to granulate if its moisture content drops further (Olaitan et al. 2007). Honey has acidic pH, ranging from 3.2 to 4.5 (Jeffrey and Echazarreta 1996). This is due to the presence of organic acids like gluconic acid, formic acid, citric acid, and acetic acid (Majewska et al. 2019). Undue fermentation can result in increased acidity due to conversion of alcohols into organic acids (Majewska et al. 2019). The very presence of phosphates, carbonates, and other mineral salts makes honey a good buffer.

Table 15.1 Main nutrient composition of honey[a]

Component	Floral honey		Honeydew honey	
	Range	Mean	Range	Mean
Water	15–20	17.2	15–20	16.3
Sugars (Total)		79.7		80.5
Fructose	30–45	38.2	28–40	31.8
Glucose	24–40	31.3	19–32	26.1
Sucrose	0.1–4.8	0.7	0.1–4.7	0.5
Erlose	0.5–6.0	0.8	0.1–6.0	0.1
Minerals	0.1–0.5	0.2	0.6–2.0	0.9
Amino acids	0.2–0.4	0.3	0.4–0.7	0.6
Organic acids	0.2–0.8	0.5	0.8–1.5	1,1
pH value	3.2–4.5	3.9	4.5–6.5	5.2

[a]Values in g/100 g of honey (Adapted from White (1975) and Bogdanov et al. (2003))

15.3 Nutraceutical and Pharmaceutical Properties of Honey

Honey has been used as a sweetener and health food. Literature in this regard dates back to 5500 BC. It has been used by all civilizations from time to time; be it Greeks, Romans, Chinese, Egyptians, or Indians. Though honey finds thousands of uses when both traditional and modern day medicine literature is taken into account, our emphasis will only be on the important and clinically significant ones.

15.3.1 Nutrition

Natural honey is a combination of carbohydrates, peptides and amino acids, organic acids, enzymes, minerals, and vitamins in aqueous base (Table 15.1). The composition varies depending on the floral type, geographical location, season of harvest, and thermal treatment (Alvarez-Suarez et al. 2010). The predominant component of honey is the carbohydrates; nearly 83% being simple sugars like fructose and glucose while rest 17% consisting of maltose, sucrose, isomaltose, erlose, maltulose, and many others (Khan et al. 2007; Jeffrey and Echazarreta 1996). Depending on the species of the honeybees, the protein content of honey is variable. For example, while protein content in *Apis cerana* varies from 0.1 to 3.3%, it varies from 0.2 and 1.6% in *Apis mellifera* (Won et al. 2008). Proline is the main amino acid in honey, constituting about 50–85% of the amino acid pool (Hermosin et al. 2003). Enzymes in honey has two sources: invertase, glucose oxidase, and amylase originate from hypopharyngeal glands of worker honeybees. On the other hand, catalase, acid phosphatase, and a little proportion of amylase come from plants (Jeffrey and Echazarreta 1996). The minerals and vitamins constitute about 0.02% of its weight (Jeffrey and Echazarreta 1996) (Table 15.2). Potassium comprises almost 30–35% of the total mineral content of honey. Other minerals like sodium, calcium, magnesium, iron, copper, silicon, and manganese are present in small quantities (Alqarni et al. 2014). Organic acids, which impart acidity to honey, constitute 0.57% of

Table 15.2 Mineral and vitamin composition of honey

Minerals	Amount (mg/100 g)	Vitamins	Amount (mg/100 g)
Sodium (Na^+)	1.6–17	Thiamine (B1)	0.00–0.01
Calcium (Ca^{2+})	3–31	Riboflavin (B2)	0.01–0.02
Potassium (K^+)	40–3500	Niacin (B3)	0.10–0.20
Magnesium (Mg^{2+})	0.7–13	Pantothenic acid (B5)	0.02–0.11
Phosphorus (P)	2–15	Pyridoxine (B6)	0.01–0.32
Selenium (Se)	0.002–0.01	Folic acid (B9)	0.002–0.01
Copper (Cu)[a]	0.02–0.6	Ascorbic acid (C)	2.2–2.5
Iron (Fe)[a]	0.03–4	Phyllochinon (K)	0.025
Manganese (Mn)[a]	0.02–2		
Chromium (Cr)[a]	0.01–0.3		
Zinc (Zn)[a]	0.05–2		

[a]Heavy metals (Adapted from White (1975) and Bogdanov et al. (2003))

honey. The chief organic acids in honey are gluconic acid and citric acid, which also help differentiate between floral and honey drew honey (Karabagias et al. 2014).

From nutritional point of view, natural honey is an instant energy dense food with added benefits of mineral and vitamins. This makes honey a good meal for body growth and metabolism. On an average 100 g of honey provides around 304 kilocalories of energy. Since the concentration of macro and micronutrients in honey is low (far below than recommended daily allowance), other supplements are needed in an adult human being to meet requirements on day-to-day basis. Scientific research showed adequate weight gain in rats fed on natural honey (Ajibola et al. 2011). In humans, the role of honey in infant nutrition is well described and has been found to be fairly tolerated with significantly lowered crying phases of babies as compared to placebo (Ramenghi et al. 2001). Studies have shown that feeding honey in infant diet had a better weight gain, increased hemoglobin levels and were less susceptible to diseases as compared to those fed normally or with sucrose supplemented diet (Samanta et al. 1985; Liu et al. 2001). Use of honey with milk is very common practice for premature babies and children with iron-deficiency anemia or jaundice. The beneficial effects of honey in athletics are also well documented (Hills et al. 2019).

Honey has other nutritional benefits like antioxidant potential, immune booster and pre and probiotic use. Metabolic activity in human body generates a class of highly unstable reactive oxygen species (ROS) and free radicals, which inflict damage at molecular level. These harmful agents are neutralized by the scavenger system within the body. However, disequilibrium between the production and the destruction of ROS and free radicals can result in oxidative stress and the subsequent detrimental effects in the body in the form of changes at cellular and molecular level. Certain extrinsic agents when consumed as food like honey can boost the protective mechanism by its antioxidant property. Honey is rich source of antioxidants like polyphenols/phenolic acids, flavonoids, carotenoid derivatives, organic acids, vitamins (ascorbic acid), amino acids, and proteins like glucose oxidase and catalase (Khalil et al. 2010). Some of the polyphenols of honey like Caffeic acid and its phenyl ester, Chrysin, Kaempferol, Galangin, Quercetin, Acacetin, Pinocembrin, Pinobanksin, and Apigenin have shown some promise as pharmacological agent in treatment of cancer (Jaganathan and Mandal 2009a, b).

The pre- and probiotic properties of honey have been validated by various studies (Gaifullina et al. 2017). Certain oligosaccharides and some low molecular weight polysaccharides present in honey exhibit prebiotic properties. These sugars evade digestion and undergo fermentation in large intestine and provide nutrition to resident microflora. On the other hand, probiotic effect of honey is mainly due to presence of microorganisms like Bifidobacteria and Lactobacilli in freshly harvested honey.

15.3.2 Antimicrobial

The broad-spectrum antimicrobial (antibacterial, antiviral and antifungal) activity of honey has been confirmed by vast majority of clinical and in vitro studies. The mechanism of antimicrobial action of honey has been attributed to several inherent components of the product like high sugar concentration, low pH value, glucose oxidase, bee defensin-1 and others (Molan 1992a, b; Mandal and Mandal 2011; Israili 2014; Kwakman et al. 2011; Kwakman and Zaat 2012). Due to high sugar content, certain bacteria which are osmosensitive are eliminated (bacteriocidal action), while as growth of few other bacteria resistant to changes in osmotic pressure is hampered (bacteriostatic action). Likewise acidic pH due to presence of organic acids like gluconic acid can have bacteriocidal or bacteriostatic action. The production of gluconic acid from glucose is catalyzed by enzyme glucose oxidase. The byproduct of this reaction is hydrogen peroxide which is a strong antimicrobial agent. Defensin-1 is a peptide secreted by the hypopharyngeal glands of honeybee and shows activity against Gram-positive bacteria, including *Bacillus subtilis* and *Staphylococcus aureus*. Some factors are typical of the variant of the honey like high methylglyoxal (MGO) levels in Manuka honey. However, the antimicrobial role of MGO and few others is still not clear. Other factors play an indirect role by exerting counter-productive response against the ill effects of microbial agents like anti-inflammatory action, antioxidant action, immunomodulation, and lysosomal cytolysis.

In clinical practice, honey has exhibited high anti staphylococcal and anti-*Helicobacter pylori* activity. Because of its excellent anti staphylococcal activity including Methicillin-resistent *Staphylococcus aureus* (MRSA), it can be used in wounds, burns, and certain soft tissue infections. Certain animal studies have shown that honey has a potential to be used in treatment of infected surgical wounds (Al-Waili 2004a, b). Local application of honey on infected wounds was found to hamper signs of inflammation and facilitated clearance of infection. Tissue erythema and edema was lessened, time for complete resolution of lesion and time for eradication of bacterial infection due to *S. aureus* or Klebsiella sp. was reduced. Its antimicrobial activity was found to be comparable to that of local antibiotics. Using agar diffusion assay, Nzeako and Al-Namaani investigated activity of different samples of honey against *Helicobacter pylori* (*H. pylori*) (Nzeako and Al-Namaani 2006). They concluded that all of them effectively inhibited the growth of *H. pylori*. Low gastric pH affects the activity of glucose oxidase enzyme present in honey, thus hampering generation of hydrogen peroxide, crucial antimicrobial agent. Thus, to execute anti *H. pylori* action in acid peptic disorders, components other than H_2O_2 come into play. Urease and xanthine oxidase, the important virulence factors of *H. pylori*, were found to be inhibited by phenolic components in honey (Sahin 2016). Further in one of the human clinical studies involving 150 patients with dyspepsia, consumption of honey at least once weekly significantly reduced the risk of development of *H. pylori* infection (Boyanova et al. 2015). In an in vitro study, researchers found that Mycobacteria failed to grow in culture media containing 10 and 20% honey (Asadi-Pooya et al. 2003). However in vivo studies supporting its role in tuberculosis are still at large. This future endeavor could solve the current problem of multidrug resistant (MDR) tuberculosis. It has been found that gram-negative bacteria are less sensitive to antimicrobial effects of honey, such as the lesser activity of honey against *P. aeruginosa* and *E. coli* in comparison with *S. aureus*. Honey effectively eradicates biofilms formed by *P. aeruginosa* (Cooper et al. 2014). The antibacterial spectrum of honey also includes organisms like *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., and others. Due to its broad-spectrum antimicrobial action, honey is a potential wound dressing material.

Honey is effective antifungal with its antifungal action comparable to azole group of antifungals when used against Candida species (Irish et al. 2006; Majidi Poya and Khodavandi 2018). However, due to topical use, its role becomes limited in systemic fungal infections like candidemia, wherein it can act as an adjunct to antifungal medications. Virucidal action of honey is supported by its anti-influenza and anti-Varicella Zoster virus effects of honey (Watanabe et al. 2014; Shahzad and Cohrs 2012). MGO in Manuka honey has shown potent antiviral activity in in vitro studies (Watanabe et al. 2014). Other uses include labial and genital herpes.

15.3.3 Antioxidant

Honey polyphenols, catalase, peroxidase, glucose oxidase, vitamin C, and carotenoids are important dietary antioxidants present in honey. Antioxidant property of honey has important clinical implications such as use in cardiovascular diseases, diabetes, cancers, osteoporosis, neurodegenerative diseases, etc. One of the factors studied extensively in etiopathogenesis of aging and age-related disorders is oxidative stress. Free radicals and reactive oxygen species can harm cells by reacting with cell membrane components or proteins or DNA and can alter cell structure or function. Polyphenols and other antioxidants protect cells through scavenging of free radicals. Another action of polyphenols is pro-oxidant action that helps in apoptosis of cancer cells.

There seems to be a relationship between antioxidant potential and color of honey, with darker varieties of honey providing higher antioxidant levels. The antioxidant activity of honey polyphenols is measured in vitro by comparing the oxygen radical absorbance capacity (ORAC) with the total phenolic concentration.

15.3.4 Anti-inflammatory and Wound Healing

Various clinical observational and animal experimental studies have described the anti-inflammatory activity of honey (Efem 1993; Subrahmanyam 1996, 1998; Benhanifia et al. 2011; Oryan and Zaker 1998). The mechanism of action is an overlap between antimicrobial and antioxidant property and a direct anti-inflammatory role. The latter is the justification for its clinical use in nonmicrobial inflammatory conditions like arthritis, vasculitis, atherosclerosis, and senile neurodegenerative disorders like Parkinson's and Alzheimer's disease. Since the antimicrobial and antioxidant nature of honey has already been elaborated, the anti-inflammatory activity needs a word here. The anti-inflammatory effects of honey are mainly attributed to phenolic compounds and flavonoids present in the honey (Palmieri et al. 2012; Kassim et al. 2010). Chrysin, a flavonoid found in honey, has been reported to suppresses lipopolysaccharide-induced COX-2 expression (Woo et al. 2005) and thus the release of nitric oxide (NO) and pro-inflammatory cytokines such as tumor necrosis factor (TNF-α) and interleukin (IL-1β) gets inhibited (Palmieri et al. 2012). Researchers have isolated two more flavonoids from honey, namely apigenin and kaempferol. They act by suppression of TNF-α induced matrix metalloproteinase (MMP-9) expression in experimental human cell line, HaCaT (Majtan et al. 2013). In human endothelial cells, apigenin was also found to inhibit TNFα-induced MMP-9 expression by modulation of Akt signaling pathway (Palmieri et al. 2012). Further it has been found that IL-1β-induced MMP-9 mRNA expression in osteoblasts is inhibited by apigenin (Yang et al. 2012). Similarly there is evidence to support the role of kaempferol as a potent inhibitor of MMP-2 and MMP-9 (Li et al. 2009). Medicated manuka honey has been used effectively in conservative treatment of exomphalos major (Nicoara et al. 2006). Briefly the anti-inflammatory actions of honey can be summed up by its effect on various inflammatory mediatory pathways like: (a) inhibition of production and rapid scavenging of free radical and ROS (Zare et al. 2007), (b) inhibition of chemotaxis and thus decreased leukocyte infiltration (Leong et al. 2012), (c) inhibitory effect on cyclooxygenase-2 (COX-2) and iNOS expression (Hussein et al. 2012), (d) inhibition of matrix metalloproteinase-9 (MMP-9) (Majtan et al. 2013).

Another aspect of honey related to its anti-inflammatory potential is immunomodulation. Infact the anti-inflammatory and the immunomodulatory actions are intricately balanced to produce the desired effect. Honey can stimulate the expression of certain key mediators of immune system like TNF-a, IL-1b and IL-6. While TNF-a promotes macrophage activation, stimulates angiogenesis and re-epithelialization during wound healing; IL-1b stimulates the release of certain growth factors helpful in wound healing. IL-6 helps in proliferation of keratinocytes and attracts neutrophils. Honey also stimulates production of antibodies, T and B lymphocytes, neutrophils, monocytes, and natural killer (NK) cells during primary and secondary immune responses.

Honey is being indoctrinated as a novel dressing material with a strong backing from recent research. Molan had stated in 2002:

> "Dressing wounds with honey, a standard practice in past times, went out of fashion when antibiotics came into use. Because antibiotic resistant bacteria have become a widespread clinical problem, a renaissance in honey use has occurred."

Traditionally, honey has been used in acute and chronic non-healing wounds and ulcers. The wide spectrum of wounds includes burns, boils, venous and diabetic foot ulcers, pilonidal sinuses, malignant wounds, pressure sores, etc. Clinically, topical application of honey has been found to facilitate wound healing due to its antimicrobial, antioxidant and anti-inflammatory actions. Moreover, it also helps deodorize and debride deep dirty wounds and provides stimulus for tissue growth to heal wounds. As a bioactive dressing, honey surpasses or at least is equally effective, when compared with various standard treatments of modern times. It has heals superficial burns quicker than polyurethane film (OpSite™) and silver sulfadiazine (SSD) 1% ointment, the current treatment of choice in prevention of infection in burns (Pruitt 1987). Other advantages are its soothing effect on application and lesser scarring. Similarly, studies have shown honey to be a superior to conventional dressings in postoperative wounds and chronic complicated wounds like burst abdomen, venous ulcers, etc. (Cooper et al. 2001; Phuapradit and Saropala 1992). The use of some medically certified honey has become a standard practice in Europe. Medihoney, which is FDA approved for such use, is one such example. As certain microbial spores like Clostridium botulinum exist in our environment (soil, air dust, etc.), sterilization with gamma irradiation is a must before use. The use of Medihoney has been extended to fragile and immunocompromised patient population like wound management in cancer patients on chemotherapy, wound care in premature neonates and protection of catheter entry sites in chronic kidney disease patients on hemodialysis.

15.3.5 Gastrointestinal Diseases

Oral administration of honey can help in prevention and treatment of various gastrointestinal conditions like gastritis, duodenitis, gastroenteritis, diarrhea, peptic ulcers, constipation, jaundice, hepatitis, colorectal cancer, inflammatory bowel disease, hemorrhoids, anal fissure, perianal abscesses and many others (Tallet et al. 1977; Haffejee and Moosa 1985; Somal et al. 1994; Topham 2002; Alnaqdy et al. 2005). Honey is used in treatment of both NSAID-induced and *H. pylori* induced gastric ulcers. The mode of action is the interplay of antimicrobial, anti-inflammatory, and cytoprotective properties of honey. Besides this, honey is a versatile prebiotic and probiotic agent. One of the etiologies of colonic cancer and inflammatory bowel diseases is fiber-deficient diet, thus SCFA-replete diet. When ingested orally, partially and nondigestible sugars in honey reach colon and undergo fermentation with the aid of gut microbiota, resulting in production of short chain fatty acids (SCFAs). These SCFA have local effect on enterocytes or can reach blood stream by absorption across gut epithelium. SCFAs have anti-inflammatory, antimicrobial, immunomodulatory, and antitumorigenic actions. Two important SCFA-signaling pathways are well described: inhibition of histone deacetylases (HDACs) and activation of G-protein-coupled receptors (GPCRs).

15.3.6 Dermatological Conditions

The need for new agents to treat various dermatological conditions stems from the bucketful of adverse reactions of the available remedies. Honey has attracted concerned researchers due to its antimicrobial, anti-inflammatory, immunomodulatory, and regenerative properties. Besides a dressing for wounds and burns, it has been used in pityriasis, tinea, contact dermatitis, atopic dermatitis, dandruff, seborrhea, cutaneous leishmaniasis, and psoriasis (McLoone et al. 2016). It also acts as an emollient, humidifier, hair conditioner, and soothener in various cosmetic formulations. The ability of honey to inhibit proliferation of tumor cells and induce apoptosis has found a role in skin cancers like melanoma (Pichichero et al. 2010). Certain components of honey impart protective effect against ultraviolet radiations (Ahmad et al. 2012).

15.3.7 Oral Health

Honey has found its use as a cost-effective treatment in various oral and dental conditions like oral ulcers, stomatitis, oral candidiasis, dental caries, and periodontitis (El-Haddad and Al-shawaf 2013; Nayak et al. 2010; El-Haddad et al. 2014). Peridontitis starts as acute inflammatory response secondary to bacterial infection which progresses to chronic stage resulting in destruction of gums and supporting tissues around the teeth. The protective effect of honey comes into play by virtue of its antimicrobial, antioxidative, anti-inflammatory, and tissue-healing properties. Similarly, due to its inhibitory effect on Candida species, honey is used as an effective treatment for oral thrush.

15.3.8 Ophthalmological Conditions

Globally honey is finding its place as a the treatment modality for various ophthalmological conditions like conjunctivitis, blepharitis, corneal diseases like keratitis, corneal ulcerations, bullous keratopathy and thermal and chemical burns to eyes (Shenoy et al. 2009). The protective effect of honey in senile cataract is also well documented (Golychev 1990).

15.3.9 Diabetes

Honey has a low glycemic index (GI) which makes it beneficial in type I and type II diabetes. Besides this, honey stimulates insulin secretion, decrease blood glucose levels, elevates hemoglobin concentration and improves lipid profile (Al-Waili and Haq 2004). With its regular use, honey helps correct dyslipidemia, reduces blood homocysteine and C-reactive protein (CRP) levels in normal and hyperlipidemic subjects (Al-Waili 2004a, b).

15.3.10 Cardiovascular Diseases

Diabetes, hypertension, obesity, and dyslipidemia are important risk factors for cardiovascular diseases. These are clustered together as an entity known as metabolic syndrome. There are many preclinical and human studies supporting protective effects of honey in metabolic syndrome. The pathophysiology of cardiovascular diseases starts with an endothelial intimal injury due to predisposing factors like hypertension, age-related changes in vessel wall and oxidant stress. This is followed by cascade of thrombotic and inflammatory changes ultimately resulting in deposition of atherosclerotic plaque at the site of initial intimal insult. This process is favored by proatherogenic agents like oxidized LDL, pro-inflammatory cytokines (TNF-a, IFN-y, IL-1 and IL-6) and angiotensin 2, which cause endothelial dysfunction. There is simultaneous inefficiency of nitric oxide (NO), which is a potent vasodilator. As the cellular and lipid content continues to swell the plaque, followed by calcium deposition, there occurs narrowing of vascular lumen. These changes lead to impaired perfusion to target organs resulting in ischemia. For example, myocardial ischemia due to impaired blood supply to cardiac musculature. Honey reduces the risk of cardiovascular disease by its anti-inflammatory, antioxidant, antithrombotic, vasodilatory, and anti-atherogenic properties.

Polyphenols and flavonoids in honey exert their therapeutic effects by reducing the risk factors such as improving endothelial function, inhibition of LDL oxidation, reduction in blood pressure, correction of dyslipidemia, obesity and hyperinsulinemia and antiplatelet action.

15.3.11 Anti-neoplastic

The anticancer property of honey is extensively researched. Various phenolic compounds like Caffeic acid, Kaempferol, Chrysin, Galangin, Quercetin, Apigenin, etc. have been shown to impart honey its anticancer property (Jaganathan and Mandal 2009a, b). The possible mechanisms involved are apoptosis, antiproliferation, antioxidation, anti-inflammation, and immunomodulation. Polyphenols in honey cause depolarization of mitochondrial membrane of cancer cells resulting in apoptosis. Other actions of honey polyphenols are due to increased expression of p53, caspase 3, and proapoptotic protein Bax. Honey also downregulates the expression of antiapoptotic protein like Bcl-2. Various in vitro studies and clinical trials have shown certain cancer cell lines to be sensitive to honey polyphenols. It has also been shown to potentiate action of certain chemotherapeutic drugs like 5-fluorouracil and cyclophosphamide (Abubakar et al. 2012; Jaganathan and Mandal 2009a, b). Examples of human cancers where antitumor action of honey has shown promise include leukemia, breast, colorectal cancer, renal, prostatic, endometrial, cervical, oral and skin cancers (Tsiapara et al. 2009; Gribel and Pashinski 1990; Jaganathan and Mandal 2009a, b; Samarghandian et al. 2011; Ghashm et al. 2010).

15.3.12 Pediatric Use

Honey has been used in treatment of pediatric wounds and burns. Despite limited clinical data, positive role in difficult postoperative wounds in normal and immunocompromised pediatric population has been found in few studies (Vardi et al. 1998; Simon et al. 2006). Oral intake after tonsillectomy has been found to decrease pain and thus lessened the need for postoperative analgesia (Ozlugedik et al. 2006). Honey is a good antitussive probably by virtue of its sweet syrupy nature per se. Due to issues of safety and efficacy with the currently available drugs like dextromethorphan, diphenhydramine, codeine and others, the use of honey has been revisited in this regard and has held promise as a safe and effective alternative (Paul et al. 2007; Shadkam et al. 2010). Other indications are acute gastroenteritis, gastritis, constipation, etc. Besides a blanket antimicrobial cover against the enteritis-causing group of bacteria, its composition makes it a good oral rehydration solution.

15.4 Toxicity of Honey

Every molecule used in clinical practice has some adverse action and honey is no exception to it. The adverse effects of honey are as under:

Plant Toxins: There are examples of honey containing plant toxins which can have deleterious effects on humans. For example, honey produced from the nectar of Rhododendron ponticum, also called "maddening honey" due to its action on central nervous system. Other examples are honey produces from plants like Kalmia, Andromeda, Datura, Hyoscamus, etc. Symptoms of poisoning may include dizziness, vomiting, headache, convulsions, respiratory distress, palpitations, or even death.

Hydroxymethylfurfural (HMF): HMF is a cyclic aldehyde produced as a result of sugar degradation. It is usually absent in fresh and untreated sugar rich foods but the concentration increases due to long-term storage or heat treatment. HMF is thus a parameter for quality and freshness of honey. At high concentrations, HMF has been found to be cytotoxic. It can cause irritation to eyes, skin, respiratory tract, and other mucous membranes. Further, carcinogenic potential of HMF has been studied in animal studies.

Infantile Botulism: Infantile botulism is caused by ingestion of spores of Clostridium botulinum. Since this bacterium is ubiquitous in nature, the untreated natural honey is considered to be a commonly implicated dietary source of Clostridium spores. The child presents with constipation, muscle weakness, difficulty swallowing and breathing, excessive drooling, slow or no reflexes.

Allergy: Allergic reactions due to honey are very uncommon and range from milder reactions to anaphylaxis. The cause for these reactions is proteins produced by bees or proteins derived from plant pollens. Individuals allergic to pollen of certain plants are more likely to have allergies to honeys produced from nectar of such plants.

15.5 Conclusion

Backed by recent research regarding nutraceutical and pharmaceutical properties, the list of clinical uses of honey seems to be ever expanding. The central theme of honey being used as a medicine revolves around its antimicrobial, antioxidant, anti-inflammatory, and immunological properties. In fact, this field of medicine has been named as apitherapy. Modern medicine acknowledges the beneficial effects of honey ranging from nutrition to wide array of infective pathologies (bacterial, viral, fungal) to life style disorders like obesity, hypertension, diabetes, cardiovascular diseases to diseases of skin, genitals, eyes, and oral cavity to various malignancies. Honey is like an elixir that keeps us healthy, prolongs our lives and acts as a shield against wear and tear and degenerative processes involving central nervous system, bones and joints. Other advantages of honey as a drug are low cost and safety. Given the economic impact, adverse reactions or emerging resistance of present day conventional pharmacotherapy, more research is needed to illustrate complete clinical profile of honey.

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Chinese Honey Composition, Production, Trade, and Health Benefits

16

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Abstract

Honey is regarded as the first and most primitive source of sweet food used by human beings. Apart from carbohydrates, honey also contains proteins, enzymes, polyphenols, minerals, amino acids, trace elements, vitamins, and fragrance compounds. Honey has been shown to have antimicrobial, antiviral, antioxidant, antimutagenic, antitumor, antiparasitic and anti-inflammatory properties that could favor and benefit the human health. Chinese honey consists of more than 180 components, most of which are altered during maturation process. Chinese honey consists of 81% sugars, water 17%, and 2% of volatile, nonvolatile compounds, enzymes, phenolic compounds, and flavonoids which determine its medicinal properties. The production, consumption, import, and export of honey vary among different countries. China serves as a main source

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_16

of honey production all over the world. The production of Chinese honey accounts for over 20% of the overall honey production worldwide. Various Chinese scriptures including that of Shen Nong's Herbal Classic and Compendium of Materia Medica have documented the medicinal properties of honey.

Keywords

Honey · Production · Composition · Medicinal properties

16.1 Introduction

Honey "a natural sweetener substance processed from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants by honey bees, which they collect and process by combining them with their own substances to dehydrate and deposit in the honey comb to ripen and mature" (FAO 1987). Honey is fundamentally a supersaturated solution of sugars in water. This is a sticky liquid and naturally sweet substance created by bees that is made when certain particular honey bees gather and deposit nectar and sugar from plants into the honeycomb. The honey is subjected to maturing process which involves the transformation of nectar into honey by honey bees (*Apis mellifera* L.), elimination of water and addition of a few enzymes. Depending upon the source from where nectar is obtained, honey may be unifloral or multifloral, unifloral honey is originated from single flower which is regarded as more valuable due to their good quality and pure flavor in China while as multi-floral honey is obtained from various types of flower species. Hence, market prices in china or in any country are determined by its botanical origin, and the increased value of some modifies the major proportion of sugars in the nectar (Ball 2007). About 3 days of time is required to convert honey from nectar (Ball 2007). Bees fills the honey comb –cells until the cells are full of honey and subsequently cap the filled cells by newly produced bee wax which may take a week or more depending on the period of flowering, environment, size of bee colony, and other factors.

16.2 The Health Benefits of Consuming Honey

Honey is regarded as the first and most primitive source of sweet food used by human being (Ghorbani and Khajehroshanaee 2009). Apart from carbohydrates, honey also contains proteins, enzymes, polyphenols, minerals, amino acids, trace elements, vitamins, and fragrance compounds. Honey has been shown to have antimicrobial, antiviral, antioxidant, antimutagenic, antitumor, antiparasitic, and anti-inflammatory properties that could favor and benefit the human health. Consuming natural honey in general imparts diverse and numerous health benefits to human body. Above all, honey is a great source of energy. Its dry matters are largely constituted by carbohydrates, mainly fructose and glucose. And there are in total 25 saccharides (Bogdanov et al. 2008). As an important source of energy, honey played a significant role in *Homo sapiens*' diet since their beginning. Some anthropologists claimed that it could furnish essential energy to boost human brain and allow it to out-compete other species (Crittenden 2011).

According to nutritionists, higher doses intake of honey will have a range of beneficial nutritional and health effects (Bogdanov et al. 2008), such as, the jujube one of the Chinas most commonly consumed honey has been shown to have antioxidant and preventive potential on alcohol-induced hepato injury in mice (Cheng et al. 2014). Another study highlighted the ability of the honey in preventing obesity (Samat et al. 2017).

16.3 Composition of Chinese Honey

Honey a natural sweetener, converted by enzymatic reactions from nectar or honey dew by removal of water and decomposition of sucrose to simple sugars (Ball 2007) because of its antioxidant and antimicrobial functions and for the enhancement of immunity and anticarcinogenic properties it has found its use in ancient medicine (Fukuda et al. 2010). Chemically honey comprises around 80% sugars such as glucose, fructose, sucrose, maltose, and some other sugars, 19% of the water and 1% other compounds (Majtan et al. 2014). The honey has pH value 3.4–6.1. Proline is the common amino acid in honey, which contributes for nearly 70% of the total amino acids present in it (Ruckriemen et al. 2015). The total amino acid constitutes 1/1000 of the total dry matter (Pätzold and Brückner 2006). In addition there are also different flavoring compounds and pigments present in honey (da Silva et al. 2016). 5-hydroxymethyl-2-furadehyde (5-HMF) a heterocyclic compound is typically synthesized in honey when stored for longer durations or exposure to high temperature or sometimes both. The production of 5-HMF has been shown to be responsible for reducing the consistency of the honey and creating polymers such as pigment (Aslanova et al. 2010). The production of 5-HMF in honey was assumed to be due to a condensation reaction whereby acids initiate sugar reduction and degeneration (Capuano and Fogliano 2011). This was derived from the synthesis of 5-HMF in organic or amino acid sugar solutions. Chinese honey consists of more than 180 components (da Silva et al. 2016), most of which are altered during maturation process (Vyviurska et al. 2016). Chinese honey consists of 81% sugars, water 17%, and 2% of volatile, nonvolatile compounds, enzymes, phenolic compounds, and flavonoids which determine its medicinal properties (Sushil et al. 2019). Volatile compounds are essentially responsible for flavor and aroma which increases its aesthetic value and appreciates its consumer's acceptance. The Chinese born *Apis cerana* (Acc) is an important ecotype of the eastern honey bee. With more than three million colonies, Acc has importance not only for the beekeeping industry in China but also in Asia for honey production, crop pollination, and ecosystem preservation.

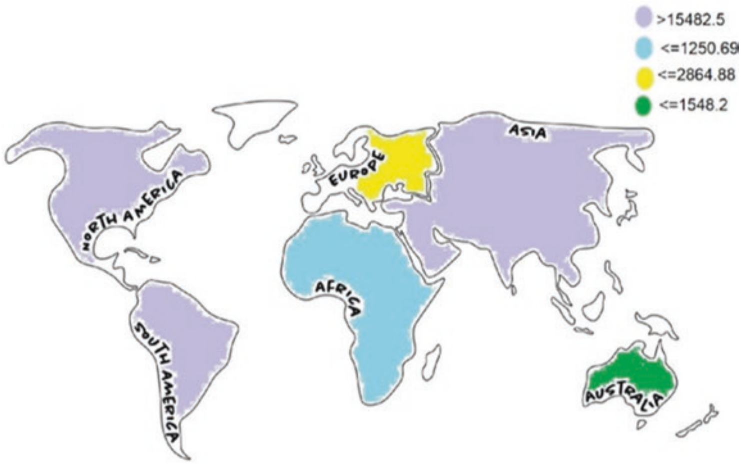


Fig. 16.1 Global honey production density, total average from 2001 to 2016, data source FAO

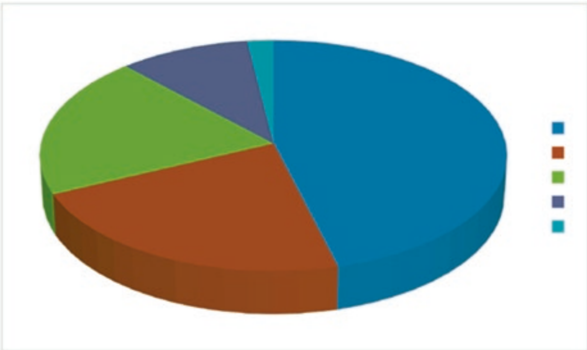


Fig. 16.2 Global production share of honey by region data source FOA

16.4 Honey Production in China

The production, consumption, import, and export of honey vary among different countries. The FAO has pictured the world honey production distribution from the average production density (Fig. 16.1) to the average production shares among different continents (Fig. 16.2). According to FAO, the top ten honey producing countries are China, Turkey, Argentina, Iran, U.S., Ukraine, Russian federation, India, Mexico, and Ethiopia (Fig. 16.3). China serves as a main source of honey production all over the world (Guoda and Chun 2003). For instance, one European article interpreted that Europe could not satisfy its growing demands of honey without China (Tamma 2017).

China exports honey as raw material like other developing countries, while as developed countries export honey as packaged products (CBPA (China Bee Products

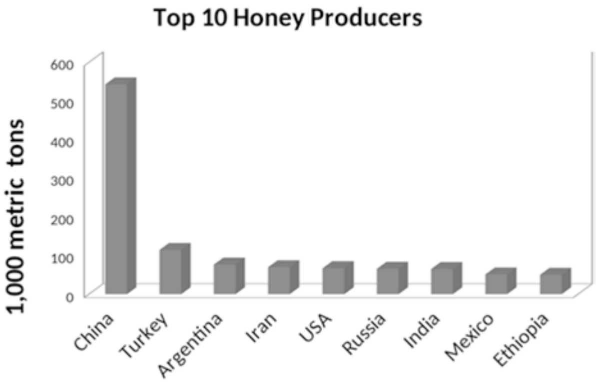
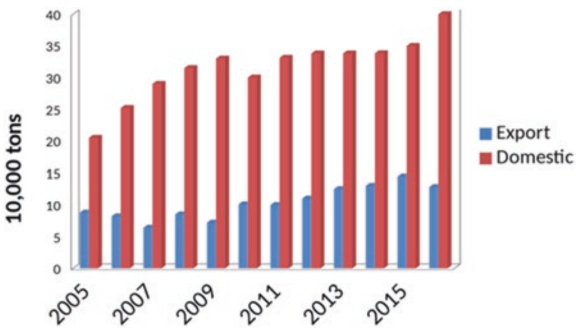


Fig. 16.3 Top ten honey producing countries of the world data source FOA

Fig. 16.4 Domestic consumption of honey and its export from China (2005–2016)



Association) 2013) there has been an unusual trend in the honey related trade in china from last few years. From 2000 to 2002, the amount of honey exported from the China dropped sharply. This could be explained by the fact that Chinese honey was banned or heavily taxed by many countries since 2000 when adulteration, impurities and pollution of heavy metal and antibiotics had been reported (Wu et al. 2015). After 2004, however, the exporting amount was again increasing slowly but steadily, because the ban was lifted shortly.

16.5 Honey Consumption in China

Domestic consumption of honey in China has a fast growing trend which is quite clear from Fig. 16.4 such trend was captured by comprehensive report of China Bee Products Association (CBPA) as well. The report emphasized that china has become world's largest nation in honey consumption. As far as the data from China Bee Products Association (2008) is concerned, the volume of honey consumed domestically is greater than three quarters of its annual production.

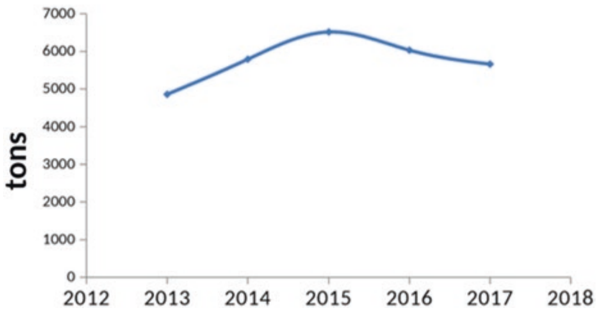


Fig. 16.5 Honey imports to China from 2012 to 2017 data source FOA

According to literature from china, the increase in raw honey consumption in China is up to 10% per year (Zheng et al. 2011). The reasons for increased demand of honey consumption in China include increased population, increased employment, health consciousness, and honey product diversification. From the perspective from honey consumption per capita, it grew on 0.11 kg per capita in 2001 to 0.3 kg per capita in 2012 (CBPA 2013). Compared with the developed countries, however, it is still a small figure that has a big potential to increase. For instance, the annual honey consumption in Germany, Austria, and Switzerland ranges from 1 to 1.8 kg per capita (Bogdanov et al. 2008). Notably, the amount of honey imported is also growing (Fig. 16.5). Imported honey prices in China are three to ten times higher than Chinese domestic honey, often even more, still, higher prices do not guarantee the good quality. There were cases of adulteration or contamination in imported honey as well, which were reported by Entry-Exit inspection Quarantine Bureau in recent years (CBPA (China Bee Products Association) 2013; Sun et al. 2017). Given increased importance and preference in China toward imported honey, the agricultural ministry in collaboration with other departments of the country organized World honey and Bee products show in Beijing in 2017 and repeated the same event in 2018 with a view to aware the people about the importance of honey consumption as well as to exhibit the honey products at national as well as international level.

16.6 International Trade Status of Chinese Honey

The price of honey is growing as well, which was captured in the price research of honey. During 2012–2015, enormous research work has been performed in china for determining the market status of honey in different cities of country and it was observed that there was acute rise in price of honey as well as demand of honey products. During 2012–2015, the average honey price increased from 55.6 to 79.8 renbinmi (RMB) per kilogram. The reasons behind this trend were as a result of rising costs and higher income and health awareness of consumers (Gao and Zhijun 2016). Chinese consumers express serious concerns over food scares (Table 16.1).

Table 16.1 Consumer images of Chinese-brand honey, honey from local bee keeper, honey imported from EU and their comparisons

	Chinese-brand honey (Mean ± SD)[a]	Honey from local bee keeper (Mean ± SD)[a]	Honey imported from EU (Mean ± SD)[a]	*p*-value
Healthy	3.92 ± 1.02	4.07 ± 1.04	3.57 ± 1.14	<0.001**
Safe	3.95 ± 1.07	4.05 ± 1.01	3.59 ± 1.11	<0.001**
Tasty	3.94 ± 1.02	4.08 ± 1.00	3.58 ± 1.11	<0.001**
Authentic	3.87 ± 1.05	4.09 ± 1.01	3.55 ± 1.14	<0.001**
Sustainable	3.89 ± 1.05	4.01 ± 1.06	3.49 ± 1.13	<0.001**
Environment friendly	3.87 ± 1.05	4.02 ± 1.05	3.56 ± 1.12	<0.001**
Affordable	4.13 ± 1.04	4.15 ± 1.01	3.32 ± 1.16	<0.001**
Value for money	3.93 ± 1.07	4.03 ± 1.03	3.24 ± 1.14	<0.001**
Trustworthy	3.87 ± 1.05	4.06 ± 0.98	3.55 ± 1.12	<0.001**
Free of hazards	3.78 ± 1.07	4.05 ± 1.01	3.59 ± 1.09	<0.001**

[a]Denotes that values were measured in a 5-likert scale. Reproduced from thesis "Consumer Attitude and Behavior Towards Honey in China" by Zhang Minzhu for award of International Master of Science in Rural Development from Ghent University (Belgium) 2018
**Highly significant

Food safety has been the highest priority for consumers in China with regard to healthy beverages (Lee et al. 2014a, b). One extensive survey study in china showed that about 83% of the respondents had a high degree of concern and understanding of food hazards risk (Liu 2014).

Families with children are more likely to show higher concern over food safety. For instance melamine contamination of milk in 2008 had greatly plummeted milk consumption among house holders, particularly those with young children. However, most Chinese consumers had little knowledge of the food safety law that actually came into effect 3 months ago (Qiao et al. 2010, 2012). A literature review paper examined the decision-making process on safe food for Chinese consumers with respect to healthy. And they suggested that Chinese consumers have a level of awareness but little knowledge of healthy foods, and they have low recognition of labels. Hence, Chinese consumers have little capacity to recognize healthy foods (Liu et al. 2013). Their conclusion was in line with many other papers. A survey in Beijing food consumers concluded that less than 20% of the respondents were aware of HACCP, a management system aiming at reducing food safety risks. The same study revealed that respondents after receiving information of HACCP were ready to spend extra price for HACCP-certified products (Wang et al. 2008).

Honey exports from china are interesting because of the important role China plays in global development and commerce. The production of Chinese honey accounts for over 20% of the overall honey production worldwide (FAO 2010). Meanwhile, there is growing evidence emerging from many countries which shows that safety standards for honey have been changed by several major countries (Gu and Zhang 2003; Yang and Zhen 2007), but as of now no verifiable data have surfaced with regards to quantitatively examined the effect of changing safety standards in other countries on Chinese honey exports. Hence, exporting honey from

Table 16.2 The MRL of chloromycetin in honey and honey import (million US$ in 2000 constant) from China 1996–2009[a]

Item	1996–2001	2002	2003–2004	2005–2009
The MRL of chloromycetin (ppb)				
EU	10	0.1	0.1	0.3
Japan	5	5	0.3	0.3
US	5	5	0.3	0.3
Canada	–	–	–	0.3
South Korea	–	–	–	0.3
Others[b]	–	–	–	–
Annual honey import from China (US $million)				
EU	32.3	6.8	1.6	22
Japan	31.3	48.3	38.2	43.2
US	19.6	7.8	30.7	13.2
Canada	2.4	1.0	2.9	0.7
South Korea	0.1	1.3	0.7	1.5
Others[b]	3.2	9.6	15.0	12.0

[a]WTO (2010), Hangzhou Entry-Exit inspection Institute (2007). Reproduced from thesis "Consumer Attitude and Behavior Towards Honey in China" by Zhang Minzhu for award of International Master of Science in Rural Development from Ghent University (Belgium) 2018
[b]Malaysia, Singapore and Hong Kong

China is suitable case for evaluating the effect of food safety requirements on agricultural trade. China's honey export was at peak in 2000 with 103,000 tons, accounting for 42% of total production (246,000 tons). After 2000, though production continued to increase however exports dropped to 88,000 in 2005 and 73,000 tons in 2009, respectively. In 2009 only 18% of the total production was exported. In conclusion, the rise in the honey production in China in recent years is associated with decline in export since the early 2000s. Table 16.2 indicates the substantial decrease in the export of China's honey since 2000 is explained by other factors rather than tariff adjustments. Except for India the tariff rated levied by its major importers have either dropped or remained unchanged (Table 16.2 columns 2 and 3). Some studies have argued that nontariff initiatives and other exporting countries such as Argentina and Canada have weakened China's low price competitiveness (Ying and Zhou 2005; Zhou and Qi 2010). Some scholars suggest that Argentina has broadened its international market share and replaced some of the existing markets in the China (Li and Wu 2009). Some, however, claimed that decline in the export of Chinese honey is linked to food safety requirements imposed by importers. Honey exports in China declined sharply as major importing major countries (EU, the US, and Japan) increased safety standard requirements. Wang and He (2008) reported that the increase in chloromycetine MLRs in the US, Japan, and Germany drastically reduced export of between 2000 and 2005. Given the importation of honey from China by more than 50 countries/regions; most of China's honey goes through limited number of nations. Chinese top five importers, including Japan, the US, Belgium, the United Kingdom, and Spain account for approximately 77% of total China's exports between 2005 and 2009 (Table 16.2). Japan was the leading importer of the China's honey between 2005 and 2009. Its import from China accounted for 46% of China's overall export. China's honey export to the US during this period was around 14% of China's overall export. Together Belgium, the United Kingdom, and Spain imported around 16% of China's total exports. Around 2005 and 2009, Chinas top 16 importers accounted for almost 95% of China's overall honey export. Throughout the past decade there has been a noticeable diversified trend on China's honey exports. Japan, Belgium, South Korea, Singapore, Poland and India increased their imports of honey significantly during 1996 and 2009. Certain importers such as the US, the United Kingdom, Spain, Dutch, Germany, Hong Kong, Canada, and France recorded negative annual growth rates from China during the same time.

16.7 Food Safety Standards and Honey Export

Food health regulations have become more critical in the honey trade over the last decade. As one of the main measure for quality food protection in importing countries, honey related sanitary and phytosanitary (SPS) measures were put in place now and then by main importing countries and the number of SPS warnings has increased. These increasing alerts on honey came from the EU, Japan, the US, Canada, South Korea, Poland, and India. Between 2001 and 2009, South Korea's alerts were released at least once in a year. The EU, Japan and India have regularly received SPS alerts. SPS notification trend shows that honey protection requirements attract rising attention from importing countries. Another essential honey health criterion of China concerned by these countries is the maximum residual limit (MRL) of chloromycetine (Ch). Ch. is a bacteriostatic antibiotic used against broad range of gram + and gram negative bacteria including many species (Falagas et al. 2008).Ch. is commonly used for treating bee diseases (Katznelson 1950). The Ch. therapy results in bone marrow toxicity and occurs in two different forms: one is the suppression of bone marrow a direct toxic effect of the drug and is reversible and aplastic anemia which is rare, impredictable, and independent of dose and often lethal (Rich et al. 1950). Most countries therefore have set Ch. MRL on food items to take care of human health. The smaller MRL Ch has resulted in more strict safety standards. Table 16.2, reveals the MRL of Ch. that had been changing in four major honey importing countries (EU, Japan, the US, Canada, and South Korea) between 1996 and 2009, and honey safety standards have become more stringent in these countries.

16.8 Therapeutic Properties of Honey

Meda et al. (2004) documented that honey is becoming appropriate to traditional medical practitioners and the general public as an acceptable and effective therapeutic agent. Various Chinese scriptures including that of Shen Nong's Herbal Classic and Compendium of Materia Medica have documented the medicinal properties of the use of *A. cerana* (multifloral honey bee).

16.9 Antibiotic Effects of Chinese Honey

The jujube honey, commonly used honey in China has been proved its antioxidant and preventive potential on alcohol-induced liver damage of mice (Cheng et al. 2014). An antimicrobial effect of Chinese honey is well documented in Chinese literature since centuries. Chinese honey serves as an essential therapeutic tool for combating infection. Zaghloul et al. (2001) revealed that honey has powerful antimicrobial effects including both pathogenic as well as nonpathogenic microbes even against those which develop antibiotic resistance to many antibiotic drugs. Depending upon the concentration used, antimicrobial properties of honey can be bacteriostatic or bactericidal. However certain factors such as high osmolarity (low water activity), low pH and hydrogen peroxide and non-peroxide compounds have been associated to antimicrobial activity (Taormina et al. 2001; Tanih et al. 2009). In addition, honey being a highly saturated sugar solution; such sugar compounds dispel high attraction to water molecules sparing less or no water to sustain the microorganism's production. The microorganisms thus get dehydrated and ultimately die (Malika et al. 2004). In addition, many pathogens get inhibited by natural acidity of honey. Most of pathogens typically have pH around 4.0–4.5. The main antimicrobial potential of honey is attributed to hydrogen peroxide (Temaru et al. 2007), which is synthesized by glucose oxidation a reaction carried by glucose-oxidase enzyme during the dilution of honey (Iurlina and Fitz 2005). On decomposition hydrogen peroxide produces various free radical species that kill the bacteria. Hydrogen peroxide activity in honey can be diminished by heating or by the action of catalase. Given this, other honeys possess different mechanism to that of peroxide effect, exhibiting a strong and robust antibacterial action (non-peroxide activity), (Alvarez-Saurez et al. 2009) and are referred as non-peroxide honeys. Manuka honey (*Leptospermum scoparium*) and jelly bush (*Leptospermum polygalifolium*) from New Zealand and Australia are non-peroxide honeys that, in addition to hydrogen peroxide production, are postulated to possess unidentified active components. Honey also possess other bacterial inhibiting compound, non-peroxide inhibins also referred as phenolic compounds, aromatic acids, and other phytochemicals (Lee et al. 2014a, b).

16.10 Role of Chinese Honey on Liver Disease

The damage to liver by consuming alcohol is obvious and alcoholic liver disease is known to be the most prevalent cause of avoidable morbidity and mortality liver disease worldwide (Mathurin and Bataller 2015; Addolorato et al. 2016). Within the liver, the metabolic center of the human body, ethanol is oxidized by alcohol dehydrogenase to acetaldehyde and subsequently to acetic acid in a reaction catalyzed by acetaldehyde dehydrogenase (Liu 2014; Mello et al. 2008). This cycle produces hepatic cytochrome P4502EI (CYP2EI0 and generates reactive oxygen species (ROS), resulting in enhanced microsomal ethanol oxidation system (MEOS) activity and increased hepatic injury (Cederbaum et al. 2015; Neuman et al. 2015; Lu and Cederbaum 2016). Therefore, oxidative stress reduction must be a primary factor for preventing alcoholic damage to the liver. Acute alcohol consumption is a common way to drink alcohol, which accounts for the majority of alcohol consumption and has resulted in a specific chronic alcohol related liver damage. Most of the people who consume alcohol suffer from alcoholic associated liver injury (Stahre et al. 2014).

Honey is important for its established role as antioxidative effects against free radicals and antimicrobial activity (Alvarez-Saurez et al. 2009). Honey's key ingredients are saccharides such as fructose and glucose, water, and few other compounds viz. trace elements, some proteins, vitamins, organic acids, phenolic, and volatile compounds (da Silva et al. 2016; Solayman et al. 2016). Phenolic compounds have specifically established a class of biochemically active compounds which play their role as antioxidants and scavenge free radicals (Can et al. 2015; Sousa et al. 2016). A number of in vivo studies have shown that honey improves serum antioxidant function by enhancing oxidative stress defenses (Gheldof et al. 2003; Cheng et al. 2014, 2015). As the research on honey has progressed, it has been documented that honey has possible hepatoprotective role against chemically induced liver damage and large number of researchers have demonstrated protective role of honey (Yıldız et al. 2013; Wang et al. 2015; Saral et al. 2016). *Apiscerana fabricius* (*A. cerana*) bee reared for honey making is a multifloral honey produced from the nectar of flowers of various plants of honey source which are distributed all over the mountains in China. *A. cerana* honey has found its application as a traditional medicine thousands of years prior to the introduction of *A. mellifera* in China. Traditionally, *A. cerana* due to its longer nectar cycle and the large variety of nectar sources cerena honey is more nutritious than other honey species. Zhao et al. (2017) conducted a study on hepatoprotective role of chines honey made by *A. Cerana* honey Bees, where they demonstrated curative role of honey on chronic alcoholic liver injury that was previously documented by Cheng et al. (2014). Zhao et al. (2017) concluded that polyphenols in *A. Cerana* honeys resulted in enhanced antioxidant properties in vitro. The research work was conducted on mice using honey from Qingling Mountains of China for 12 weeks which resulted in serum antioxidant inhibition, depleted liver index, despondent dehancement of serum amino transferases, improved hepatic MDA output, SOD and GSH-Px activities and increased TGF-β expression. Therefore they revealed *A. cerana* has a hepato protective role in mice because of its antioxidant and prevention of oxidative stress potential. Wang et al. (2015) conducted a study where they selected 14 vitex honey from China for investigation to assess the antioxidant and hepatoprotective potential against paracetamol-induced liver damage in mice and concluded that vitex honey has a prominent role against the hepatic damage by its antioxidant activity.

16.11 Conclusion

Honey is the natural bee-derivative consumed by human civilizations since ancient times as sweet food as well for medical purposes owing to its innumerable health benefits. Honey contains a broad spectrum of valuable phytocomponents that make it a potent candidate in healthcare system. Chinese honey consists of more than 180 components including sugars, water, volatile, nonvolatile compounds, enzymes, phenolic compounds, and flavonoids which determine its medicinal properties. Volatile compounds are essentially responsible for flavor and aroma which increases its aesthetic value and appreciates its consumer's acceptance. China tops the world in the production of honey and exports large amounts to Europe. The Chinese honey serves as an essential therapeutic tool for combating infection, exerting hepatoprotective effects, and so on. In addition to serving the science of medicine, honey production has boosted economy all over the world, especially in China. Further, extensive research work is needed on honey worldwide including Chinese honey to unravel and confirm its effective therapeutic role in wide range of diseases including tumors, diabetes, and the current Covid-19.

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The Gut–Brain Axis, Cognition and Honey

17

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and Mohammad Mahamood

Abstract

Honey has been trusted in traditional healing and wellness in most of the civilizations. Modern medicine has accepted wound healing properties of honey in burns and ulcers. There is tremendous evidence regarding antioxidant, antibacterial, anticancer, antimicrobial, and anti-inflammatory properties of honey. There are some experimental—in vitro and in vivo data and a little clinical data demonstrating role of honey in reversing the effects of neurodegenerative disorders and cognitive amelioration. Despite all the proven and assumed goodness, honey has not been able to establish to its full potential as a brain tonic under modern science. A casual search on PubMed and Cochrane databases reveals that there is not enough research on the neuroprotective aspects of honey. However, dissection of key components of the composition of honey has deciphered many compounds which are individually appreciated for their role in improvement of cognition and neurodegenerative disorders. The recent acceptance of gut–brain axis and role of microbiome in the development and modulation of neuronal functions has led to new insights; growing data recognizes honey as a prebiotic. It may be concluded that improvement in cognitive functions is a cumulative effect of the unique chemical composition of honey and it may not be identical for all types of honey. More longitudinal research is required to establish honey as a brain tonic.

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_17

Keywords

Honey · Gut-brain axis · Diet · Cognition · Gut microbiota · Nootropic agent · Prebiotic agent

17.1 Introduction

Honey has been used for therapeutic purposes since time immemorial by popular civilizations in Arab, Egypt, India and Greece. Most of the ancient cultures and philosophies have advocated use of honey for health including Islamic medicine called Tibb-e-Nabwi, Ayurvedic, Unani, etc. Modern medicine has also kept honey on a high pedestal. A large number of in vitro and in vivo studies have proven its antioxidant, antitumor, antimicrobial, anti-inflammatory, antilipidemic, antidiabetic and antiviral properties. There is accumulating evidence in favour of positive cardiovascular, respiratory, nervous and gastrointestinal activities of honey. The recent biomedical literature widely supports its use in wound-dressing and as a healing substance particularly in ulcers and burns. The heterogeneously complex molecular events are still under research and debates play myriad role in neurodegenerative disorders that initiate mild cognitive impairment which may lead to dementia. Uncontrolled dementia is a hallmark of Alzheimer's disease. The current chapter would try to unfurl the potential role of honey in learning and memory besides preventing the trigger leading to mild cognitive decline.

There are various kinds of honey depending on the species of bee, health of the bee, floral source/s, season and geographic location. There are notable differences in colour, flavour, sensory perception and medical response of various kinds of honey. There are three types of honey according to botanical origin (Cianciosi et al. 2018). Honey is classified as floral if it is derived from nectar of a flowering plant; again it can be further distinguished on the basis of its multifloral or monofloral source of nectar. It is classified as honeydew (non-floral) if the bee species obtains nectar by sucking sap of non-flowering plants like acacia. Mixed honey has nectar from both sources, floral and non-floral (Fig. 17.1).

17.2 Nutritional Value of Honey

Honey is a rich source of carbohydrates, proteins, essential minerals, vitamins, enzymes, and organic acids (Figs. 17.2 and 17.3). No two honeys are same; they have different pH and chemical signatures depending on floral source, climate, geographical location and bee species. The botanical origin of honey makes difference in quality of honey depending on the presence of phenolic compounds (phenolic acids and flavonoid) content of the nectar (Cianciosi et al. 2018). There are as many as 600 volatile organic compounds in honey. As a general rule, dark coloured honey is qualitatively better due to its rich phenolic and flavonoid content. The

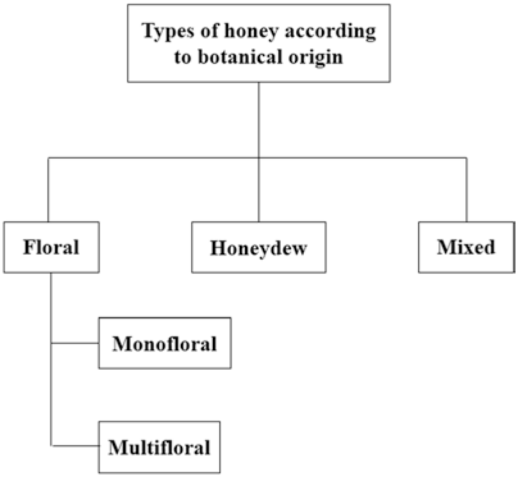

Fig. 17.1 Various types of honey depending on the botanical origin

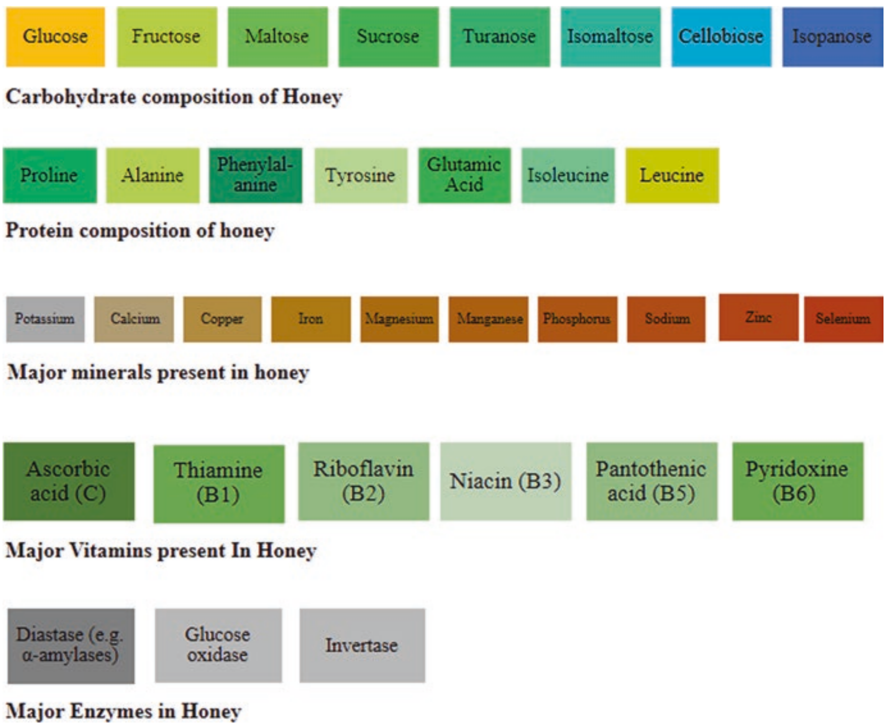

Fig. 17.2 Major carbohydrate, protein, minerals, vitamins and enzymes present in honey

polyphenols present in honey scavenge free radicals. Honey of the same botanical origin may turn out to have different properties depending on season and geography (Castro-Várquez et al. 2010).The volatile compounds contribute to sensory and aromatic properties of honey and just like fingerprints, they are mostly helpful to

Gluconic acid	Pyruvic glyoxylic acid	α-Hydroxyglutaric acid	Aspartic acid	Citric acid
Formic acid	Fumaric acid	Galacturonic acid	Glutamic acid	Butyric acid
Acetogalactur-onic acid	Gluconic acid	Methylmalonic acid	Quinic acid	Shikimic acid
Propionic acid	2-hydroxybutyric acid	Isocitric acid	Lactic acid	Malic acid
Malonic acid		Succinic acid	Tartric acid	Oxalic acid

Fig. 17.3 Major organic acids present in honey

differentiate different honeys. The methods of storage and heating also contribute to some changes in volatile organic compounds composition of honey. Floral markers, along with volatile organic compound analysis, help us in identifying specific honey (Manyi-Loh et al. 2011). Purity of honey has recently been certified using antioxidant activity as biomarker (Dżugan et al. 2018). Standard tests of honey reveal presence of certain microbes. Post-harvest handling of honey and non-hygienic sanitary conditions may lead to contamination of honey with certain yeast and bacteria, which sometimes lead to adverse effects on human health (Snowdon and Cliver 1996).

In a comparative study on various properties of monofloral honey, it was found that the colour parameters of honey had direct correlation with phenoloic content and antioxidant capacity. It was reported that dark coloured honey samples had higher phenolic content levels and antioxidant activity than the light coloured honey samples. High level of magnesium was reported in all samples. Cornflower honey sample had the highest phenolic content (645.85 mg/100 g) while antioxidant activity of cedar honey sample was found at the highest level, thorn honey sample showed the least antioxidant activity (Ozcan and Olmez 2014). In another study, it was found that acacia honey was the most acidic while pineapple honey had least moisture. Both high acidity and low moisture content ensure that the honey can resist microbial activity (Moniruzzaman et al. 2013). Proline (an amino acid) is present in all kinds of honey. The concentration of proline is highest in pineapple honey (Moniruzzaman et al. 2013).

17.3 Some Famous Botanical Types of Honey and Associated *Apis* Species

Apis sp. and Meliponini sp. (*Scaptotrigona* sp. also known as stingless bees) are popularly called as 'lebah kelulut' in Malaysia. The honey of Meliponini sp. has higher moisture content, acidity and low sugars when compared to that of *Apis* sp.

(Chuttong et al. 2016). Manuka honey, a monofloral honey derived from the manuka tree (*Leptospermum scoparium*) found in New Zealand, has greatly attracted the attention of researchers for its biological properties, especially its antimicrobial and antioxidant capacities. Tualang honey is derived from trees of *Koompassia excelsa* found in forests of Malaysia and Thailand by the bee, *Apis dorsata.* Tualang honey, amber in colour has the highest concentration of phenolic compound, flavonoids, DPPH, FRAP and lowest AEAC values making it a very strong honey (Moniruzzaman et al. 2013).

17.4 Dissection of Key Components of Honey (Figs. 17.2 and 17.3) and Their Individual Role in Memory and Neurodegenerative Disorders

The proline content in honey varies between 240 and 848 mg/kg (Moloudian et al. 2018). Amino acid composition of honey should be given more attention than phenolic content as high proline content was found to be more effective in free radical scavenging (Meda et al. 2005). The neuroprotective effect of proline rich polypeptide led to the recovery of monoaminergic system in a model of Alzheimer disease like rat model (Yenkoyan et al. 2018). Amino acid, tyrosine a precursor of dopamine is also a chemical component of honey. Transcranial direct current stimulation of human brain using Tyrosine in healthy humans has led to improvement of working memory (Jongkees et al. 2017).

Selenium, a microelement is also found in honey and neuroprotective effects of selenium are well recorded (Zafar et al. 2003). Zinc and copper homeostasis plays a crucial part in the maintenance of body including nervous system. There is a very narrow range for the functioning of both the metals. Accumulation or lack of these metals may lead to the development of neurodegenerative disorders. Zinc, besides being involved in neuronal glutamate signalling, is a cofactor of more than 250 enzymes and metallothioneins in our body. Supplementation with zinc has shown to enhance memory and was able to reverse age-dependent increase in plasma copper in animal study (Sandusky-Beltran et al. 2017).

Vitamin C, B, iron, zinc, copper, selenium, and a protein rich diet have been shown to be part of the nutrition strategies that improve cognition by optimizing brain function (Martínez García et al. 2018); interestingly, they are all present in honey. Similarly, manganese (Mn) and magnesium (Mg) are also components of honey. The dynamics of magnesium and manganese homeostasis has a narrow range. Besides, their role in learning and memory is also getting deciphered (Hoane 2011; (Pfalzer and Bowman 2017). Calcium and potassium have crucial established roles but data regarding role of their homeostasis in microcircuits of astrocytes in leading to Alzheimer's disease is under study (Osborn et al. 2016).

Butyric acid in honey has shown to be neuroprotective in in vivo studies (Sun et al. 2015; Garcez et al. 2018). Honey is a major source of organic acids particularly butyric acid (Pauliuc et al. 2020). High content of galacturonic acid is present in honey. Recently, glycoproteins derived from Chinese *Panax ginseng*, which was found to be neuroprotective had high content of glucose and galacturonic acid (Luo et al. 2018).

17.5 Effect of Honey on Brain Cells, Astrocytes and Microglia

In cultured astrocytes honey prevented cellular death in a dose-dependent manner (Ali and Kunugi 2019). The pro-inflammatory cytokines TNF-alpha and IL-1Beta were inhibited along with reduced markers for reactive oxygen species (ROS) and reactive nitrogen species when microglial cells were exposed to honey flavonoid markers (Candiracci et al. 2012). In streptozotocin-induced diabetic rats, natural honey prevented neuronal cell death in various areas of hippocampus (Jafari et al. 2014). Tualang honey has shown to improve architecture of brain, reduce brain-derived neurotrophic factors (BDNF) and reduce acetylcholinestrase concentration in homogenate (Othman et al. 2015).

17.6 Effect of Honey on Memory of Ovariectomised Rats and Menopausal Women

Tualang honey led to the enhancement of anti-depressive effects in stressed and ovariectomised rats through increase in BDNF levels through restoration of hypothalamic pituitary axis (Al-Rahbi et al. 2014a, b, c). Tualang honey has anti-anxiolytic effect; it helped ovariectomised rats to overcome stress by reducing free radical stress (Al-Rahbi et al. 2014a, b, c). A study conducted on postmenopausal women demonstrated improvement in their immediate memory when they were given 20 g/day of tualang honey (Othman et al. 2011).

17.7 Effect of Honey on Various Neurological Markers Induced by Chemical as Well as Noise Induced Stress in Animal Models

In a controlled study conducted on Sprague-Dawley rats, long-term replacement of sucrose with honey gave very promising results by decreasing anxiety levels and increasing spatial memory in ageing rats (Chepulis et al. 2009). Honey reversed oxidative stress leading to deficit in cognitive performances induced by exposure to lead acetate by ameliorating oxidative stress markers in experimental rats (Wahab et al. 2016). The tualang honey protected the male Sprague-Dawley rats against kainic acid-induced excitotoxicity which is also a hall mark symptom of major neurodegenerative disorders in pyriform cortex by reducing the free radical marker thiobarbituric acid reactive substances (Sairazi et al. 2017). Tualang honey also protected experimental rats against paraquat (PQ) induced dopaminergic neurotoxicity in midbrain and lungs of rats (Tang et al. 2017). In a study conducted on rats, it was concluded that 200 mg/kg body weight of tualang honey was able to attenuate the effect of noise stress which lead to depressive symptoms in rats (Azman et al. 2015). In the subsequent year, another study on rats concluded that 200 mg/kg body weight of tualang honey when given to rats exposed to noise of 100 dB, 4 h daily for 14 days protected against memory decline through enhancement of neuronal proliferation in the medial prefrontal cortex (mPFC) and hippocampus, decline in brain oxidative stress and/or upregulation of BDNF concentration and cholinergic system (Azman et al. 2016).

17.8 The Gut–Brain Axis, Dietary Habits and Cognition

The accumulating evidence from last two decades finally led to the proposal of brain-gut-microbiota axis (Kowalski and Mulak 2019). The healthy brain is developed as a result of pre- and postnatal molecular signals emerging from the gut. The molecular signals from gut emerge from its microbiome. Human gut microbiome is a collection of around 1000 microbial species distributed across 7000 communities representing various strains of biota Human Microbiome Project Consortium (2012). They range from the dominant bacterial and archeal populations to relatively less dominant viruses, fungi and eukaryotes. Significant studies on evolutionary history demonstrates that helminthes and many eukaryotes were previously part of human gut (Lloyd-Price et al. 2016). The Human Microbiome Project Consortium (2012) project indicates *Firmicutes* and *Bacteriodetes* as dominant groups in human intestine. MetaHit (Qin et al. 2010) and Human Microbiome Project Consortium (2012) have revealed that regular ecological interaction between different microbial communities across the kingdoms leads primarily to healthy gut besides skin, vagina, lungs and brain. There is geographical variation in gut microbiome of human populations. The precipitation of gut microbiome is influenced by early-life stimuli including first and subsequent diet (breast feeding), mode of delivery normal or C-Section (Sharon et al. 2016). The functional profile of microbiota particularly in gut is established early in life. Key neurodevelopmental events coincide with changes in the maternal and neonatal gut microbiome. In adulthood, the microbiome reaches a steady state in terms of bacterial strains, and does not change significantly under stable environmental or health conditions. The high functional diversity of different taxa is an evidence of healthy human body particularly gut. Use of germ-free mice and antibiotic-induced gut dysbiosis are two methods to study gut–brain relationship (Fröhlich et al. 2016).

17.9 Role of Microbiota in Neurodegenerative Disorders

Recent studies have demonstrated that gut microbiota has substantial role in neurogenerative processes like formation of blood–brain barrier, myelination (Hoban et al. 2016), neurogenesis, microglia maturation and animal behaviour (Sharon et al. 2016). There is mounting evidence from both animal (Bonfili et al. 2017) and human studies (Zhuang et al. 2018) that any fluctuation in gut microbiome leads to

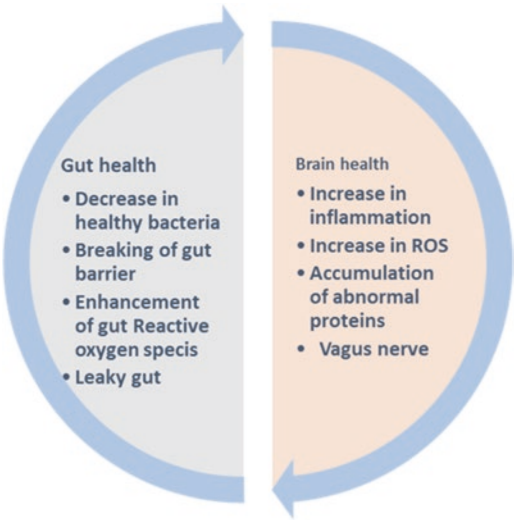

Fig. 17.4 There is direct communication between gut and brain, any alteration in gut microbiota population sends impaired signals to brain which reflects in poor brain health

structural and functional alteration in brain functions. The two way exchange of information between brain and gut has already been accepted by researchers (Zhu et al. 2017) (Fig. 17.4).

17.10 Cognitive Function and Diet

A number of studies have claimed that cognitive function can be improved by diet (Romo-Araiza et al. 2018). Increasing rodent and animal studies claim that the modern diet based on high sugar (Hsu et al. 2015), refined food and high fat (Ledreux et al. 2016) leads to cognitive decline (Chong et al. 2019) accompanied by inflammation of hippocampus (Tsai et al. 2018; Noble et al. 2017). In an animal study on early Alzheimer's stage, researchers were able to reverse the cognitive decline by treatment of mice with probiotics leading to alteration in gut microbiota and their metabolites through restoration of two neuronal proteolytic pathways (Bonfili et al. 2017).

17.11 Probiotics, Prebiotics, Gut Microbiome and Cognition

Pre and probiotics alter intestinal microflora in favour of human mental health (Liu et al. 2015). Recent trends show populations of probiotic microbial strains of genera *Lactobacillus* and *Bifidobacterium* residing in gut are influenced by oligosaccharides of prebiotic food, honey (Mohan et al. 2017). Fluctuation in numbers of healthy microbiota in gut produces reactive oxygen species (Jones et al. 2012), which directly and indirectly enhances free radical stress in brain (Fig. 17.5).

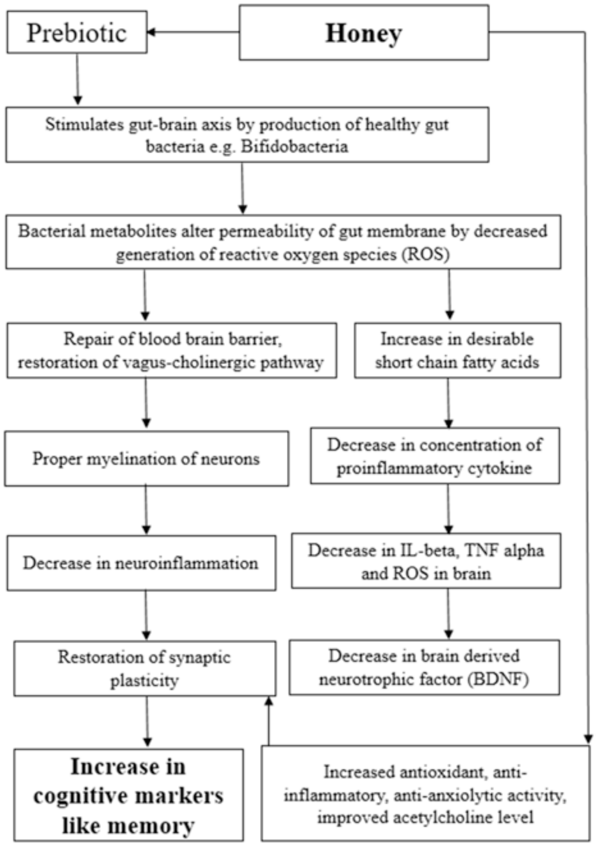

Fig. 17.5 Proposed mechanism for action of honey as a nootropic agent

Age-related neuronal loss, neuronal inflammation, loss of synaptic plasticity and accumulation of free radicals inside brain also contributes to age associated loss of memory. Many studies have suggested supplementation with prebiotics and probiotics to altering gut microbiota as a possible treatment for age-related cognitive impairment (Romo-Araiza and Ibarra 2020). A systematic review using 14 studies concluded that chronic prebiotic interventions lasting more than 28 days led to improvement of verbal episodic memory (Desmedt et al. 2019). Experimental studies on treatment of mice to antibiotics from weaning onwards led to depletion of gut microbiota, coupled with impact on anxiety and cognition (Desbonnet et al. 2015).

17.12 Honey Is a Nootropic Agent

A randomized, controlled, double blind 5 year pilot study conducted in Iraq concluded that one daily teaspoon of honey controlled dementia and cognitive decline (Al-Himyari 2009). It is proposed (Fig. 17.5) that honey stimulates brain through gut–brain axis by altering the bacterial population of favourable species like bifidobacteria. They act by decreasing free radical stress of gut along with release of short chain fatty acids. These events lead to proper myelination of neurons, repair of blood brain barrier, decreased release of inflammatory cytokines IL-beta and TNF-alpha besides decrease in BDNF. All these events restore synaptic plasticity of brain leading to increase in memory. Besides, honey is a source of vitamin C, B, iron zinc, selenium and various microelements, amino acids including proline, flavonoids and other organic acids particularly butyric acid. These constituents individually have well established neuroprotective record. There is tremendous support of honey as a nootropic agent (Azman and Zakaria 2019). The high oligosaccharide content of honey promotes healthy gut microflora (Ali and Hendawy 2018). The presence of 4 hydroxybenzaldehyde in buckwheat honey regulates growth of healthy gut bacteria, bifidobacteria restricts the growth of pathogenic bacteria in gut (Jiang et al. 2020). Additionally, 4-Hydroxybenzaldehyde marker in Buckwheat honey selectively promotes growth of bifidobacteria in the gut promoting health (Jiang et al. 2020). Honey is also a source of acetyl/butyl cholinesterase inhibitors, making therapeutic value of honey high (Baranowska-Wójcik et al. 2020).

17.13 Conclusion

Every year millions of people are losing cognitive functions, starting from mild cognitive impairment which progresses into full-blown dementia or Alzheimer's disease with age. The markers are in favour of honey as a brain tonic. The scientific fraternity should seriously consider candidature of honey as nootropic agent by conducting human trials and improve life of millions of patients and caregivers of Alzheimer's disease.

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18 Antiproliferative and Apoptotic Activities of Natural Honey

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Abstract

Cancer is a dreadful disease characterized by uncontrolled proliferation of cells in tissues or organs. It is one of the main reasons of death and kills thousands of people every year. Currently several treatments like chemotherapy, radiotherapy and surgery are available but still there is no cure when it is detected at late stages. Chemotherapy involves the use of anticancer drugs to eradicate the cancer cells by apoptosis. It is defined as programmed cell death, a mechanism used by body to maintain homeostasis or to kill cancer cells. Current research suggests a putative role of dietary agents in taming apoptosis of cancer cells. Honey, one of the victuals from natural treasure is a rich source of antioxidants and has greater implications in cancer prevention and treatment. Research done globally has proved the anticancerous and proapoptotic mettle of honey under various experimental set ups ranging from cancer cell lines to animal models. This chapter will provide insights about the role of honey in regulating antiproliferative and proapoptotic mechanisms in human cancers and also endorse honey as a promising candidate against cancer.

Keywords

Honey · Antiproliferative activity · Antitumor · Apoptosis · Cancer

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_18

18.1 Introduction

Globally cancer kills more than seven million people and approximately 11 million people are affected. Carcinogenesis is a gradual process that starts from a single transformed cell. It involves rapid cell division and can occur due to various factors like tumor promoters, carcinogens, and inflammatory agents (Shishodia et al. 2003). Besides transcription factors, antiapoptotic proteins, cyclooxygenase-2 (COX-2), proapoptotic proteins, cell-adhesion molecules, cell cycle proteins, protein kinases, and diverse molecular targets have deeper implications in the regulation and modulation of cancer (Wang et al. 2000; Aggarwal and Shishodia 2006). At present cancer can be treated through radiotherapy, chemotherapy, and surgery. Harmful radiations and chemical drugs employed in the treatment often have serious side effects (Chari 2008). Currently scientific community focuses on developing target specific anticancer drugs with least or no side effects (Goldman 2003). Natural gifts like honey possess marked anticancer effect (Othman 2012). Honey contains, flavonoids, amino acids, sugars, phenolic acids, and various other compounds. Honey ingredients vary as per floral sources and origin (Gheldof et al. 2002). Studies show it possesses pharmacological activities like antimicrobial (Sherlock et al. 2010), antitumor (Swellam et al. 2003; Tomasin and Cintra Gomes-Marcondes 2011), anti-inflammatory (Cooper et al. 2001), antimutagenic (Wang et al. 2000), and antioxidant (Al-Mamary et al. 2002). Reports suggest that the phenolic contents of honey exhibit antileukemic activity (Abubakar et al. 2012). In addition, its antiproliferative potential is reported in prostate (Tsiapara et al. 2009), breast (Fukuda et al. 2011), colorectal (Jaganathan and Mandal 2009b; Ghashm et al. 2010), renal (Samarghandian et al. 2011), cervical (Fauzi et al. 2011) and endometrial (Tsiapara et al. 2009) cell lines. Honey augments the anticancerous potential of chemotherapeutic agents like cyclophosphamide and 5-luorouracil (Gribe et al. 1990). Here it is noteworthy that honey has shown anticancer potential in experiments involving tissue cultures (Jaganathan and Mandal 2010) and in vivo studies (Orsolić et al. 2003). Phenolic compounds of honey represent the main factors that bestow the honey with anticancer activities (Jaganathan and Mandal 2009a, b).

18.2 Composition of Honey

Being a rich source of carbohydrates, honey majorly contains two sugars i.e., levulose and dextrose in addition to small proportions of 22 other complex sugars. It is collected from beehives wherein bees live and carry out their life processes. They make honey from the nectar which they collect from millions of flowers. As per reports, a single bee requires to collect nectar from two million flowers by traveling a distance of approximately 55,000 miles just to make one pound of honey (Todd and Vansell 1942). The physical appearance and behavior of honey is determined by the type of sugars contained in it. Furthermore, a diverse range of vitamins like, thiamine, riboflavin, pantothenic acid, niacin, and vitamin B6 are also present in honey. Essential minerals including copper, calcium, magnesium, iron, phosphorus,

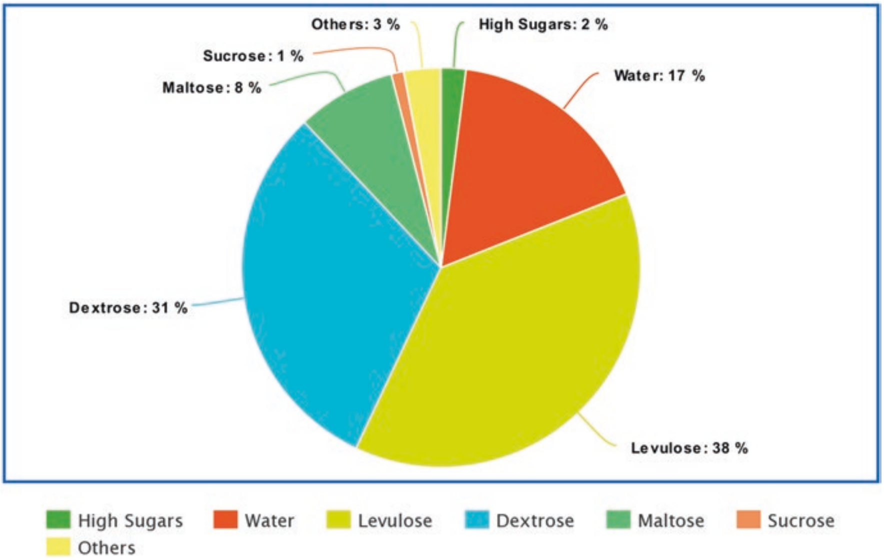

Fig. 18.1 Pie diagram showing different components of honey

manganese, potassium, zinc, sodium, and several amino acids are also present in honey. Each type of honey has a different nature of constituents.

The mostly commonly reported phenolic constituents of honey include apigenin, chrysin, pinocembrin, caffeic acid, galangin, acacetin (Jaganathan and Mandal 2009a, b) p-coumaric (Jaganathan et al. 2013), eugenol (Jaganathan et al. 2011). These compounds have evolved as potent anticancer agents in many studies conducted over a period of time. Most studies confer the antiproliferative and apoptotic activity to honey by virtue of such phenolic compounds in it (Jaganathan and Mandal 2009a, b). The percentage of several constituents that constitute honey is depicted in the Fig. 18.1.

18.3 Honey and Its Apoptotic Activity

Cancer is characterized by two important features i.e., abnormal cell proliferation and restricted apoptosis (Nicholson 2000). Many drugs which induce apoptosis are used as anticancer drugs (Earnshaw 1995). Apoptosis also termed as programmed cell death has three important stages namely (1) induction stage, (2) effector stage, and (3) degradation stage (Susin et al. 1998). Induction stage triggers apoptotic pathways via death-inducing signaling cascades while the effector stage is committed to cause cell death though mitochondrion. Degradation stage involves various events that occur in cytoplasm as well as nucleus. Events that occur inside nucleus include nuclear and chromatin shortening, cell contraction, DNA breakdown, and blebbing of cellular membranes (Earnshaw 1995; Susin et al. 1998). Activation of protein cleaving enzymes known as caspases takes place inside cytoplasm. At first cell is splitted into small apoptotic bodies which are later engulfed due to the action of macrophages (Earnshaw 1995; Susin et al. 1998). The process of apoptosis can take place via two cascades including mitochondria cascade or Caspase-9 mediated and death-receptor cascade or Caspase-8 mediated (Andersen et al. 2005).

Honey stimulates mitochondrial membrane depolarization thereby causing apoptosis of uncontrollably proliferating cells (Fauzi et al. 2011). The high level of tryptophan and phenolic content in honey confers it the property to activate caspase 3, an executioner enzyme having prominent role in apoptotic activities. Honey also activates poly (ADP-ribose) polymerase (PARP) cleavage as well in colon cancer (Jaganathan and Mandal 2009a, b). Apoptosis is induced by honey also via increasing the levels of proapoptotic and antiapoptotic proteins in colon carcinogenesis (Jaganathan and Mandal 2010). Reports show that Honey downregulates the expression of antiapoptotic protein Bcl2 and upregulates the expression of proapoptotic protein Bax, caspase 3 and p53 (Fig. 18.2). Formation of reactive oxygen species (ROS) due to honey activates p53 and the later alters expression of several proteins including Bax and Bcl-2 (Jaganathan and Mandal 2010). Combination of *Aloe vera* and honey decreases levels of Bcl-2 in rat model and elevates the expression of Bax (Tomasin and Cintra Gomes-Marcondes 2011). These two proteins are antagonistic in action, former being antiapoptotic while the latter being proapoptotic. One of the type of honey named Manuka imposes its proapoptotic effect on cancer cells by inducing caspase 9 which is the activator of executor protein, caspase-3. Manuka also triggers PARP activation, DNA fragmentation, and expressional loss of Bcl-2

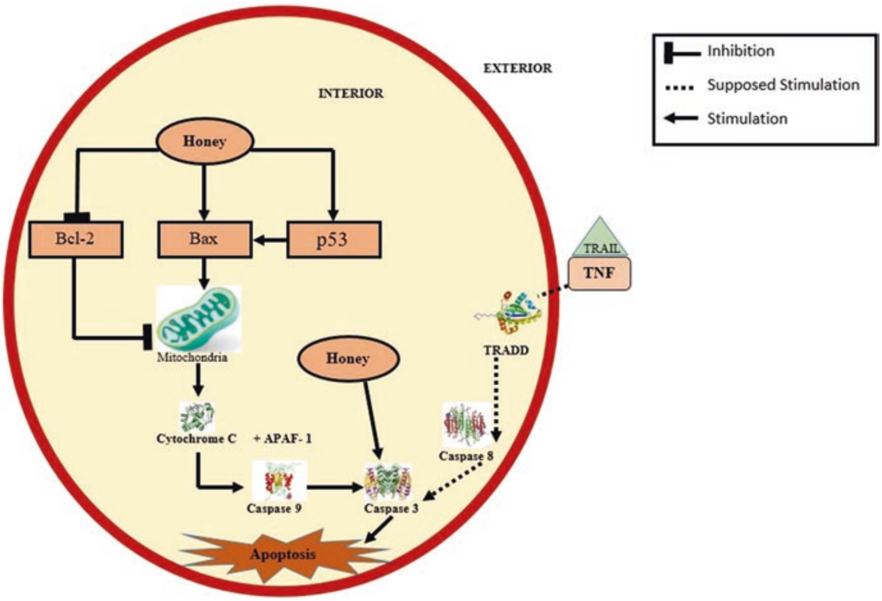

Fig. 18.2 Figure showing the role of honey in activation of apoptosis

(Fernandez-Cabezudo et al. 2013). This prominent proapoptotic efficacy of honey highlights the scope of honey as a source for anticancer agent since many drugs used currently are activators of programmed cell death.

18.4 Honey and Its Antiproliferative Activity

There are four discrete steps in cell division process viz. G1, S, and G2 and M. Several proteins play important role in regulating various events in each phase of cell cycle. These proteins include cyclins and cyclin-dependent kinases which act as controls panel of cell cycle. Transition from G1 phase to S phase is a critical control point in cell cycle where a cell decides whether to go for proliferation, quiescence, apoptosis, or differentiation (Diehl 2002). Deregulated levels of cellular proteins like cyclin-dependent kinases (CDK) and cyclin-D1 are associated with tumorigenesis (Diehl 2002). Ki-67, one of the important nuclear proteins is used for probing growth of cells during proliferation phase. Ki-67 expression is observed during the all phases of cell cycle (viz G1, S, G2, and M) except the resting phase (G0) (Scholzen and Gerdes 2000). Cell cycle arresting property of honey is a well-documented event. Marked decrease in the level of Ki67-LI in tumor cells exposed to combination of *Aloe vera* and honey. It indicates that honey therapy has potential to alleviate tumorigenesis by blocking cell cycle (Tomasin and Cintra Gomes-Marcondes 2011). Phenolic and flavonoid components of honey are reported to arrest cells in sub-G1 stage during colon carcinogenesis (Jaganathan and Mandal 2009a, b), glioma, melanoma (Pichichero et al. 2010) and (Lee et al. 2003). This blocking effect on tumorigenesis involves the downregulation of several cellular proteins like ornithine decarboxylase, tyrosine, cyclooxygenase, and kinase (Oršolić et al. 2010). The outcomes of the trypan blue exclusion assay and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay validated honey exhibits a concentration dependent inhibition of cell proliferative during cancers. Therefore, components of honey block tumor cell proliferation arrest my modulating cell cycle. Honey can also regulate expression of tumor suppressor protein p53 during cancer (Jaganathan and Mandal 2009a, b).

18.4.1 Mechanism of Honey-Induced Apoptotic Cell Death in Cancer

Studies available in literature suggest that honey potentiates programmed cell death in wide range of malignant cells. In particular, the role of reactive oxygen stimulated mitochondrial cascade is central. Honey alters the potential of the mitochondrial membranes during colon and breast cancer. Honey markedly elevates production of ROS in colon cancer cells. In addition, there was initiation of PARP cleavage and p53 activation in colon cancer cells. Honey arrested cell cycle in cancer cells in sub-G1 phase. It also upregulated expression of executioner caspase-3 in liver, colon and breast, cancer cells (Jaganathan and Mandal 2009a, b). Furthermore,

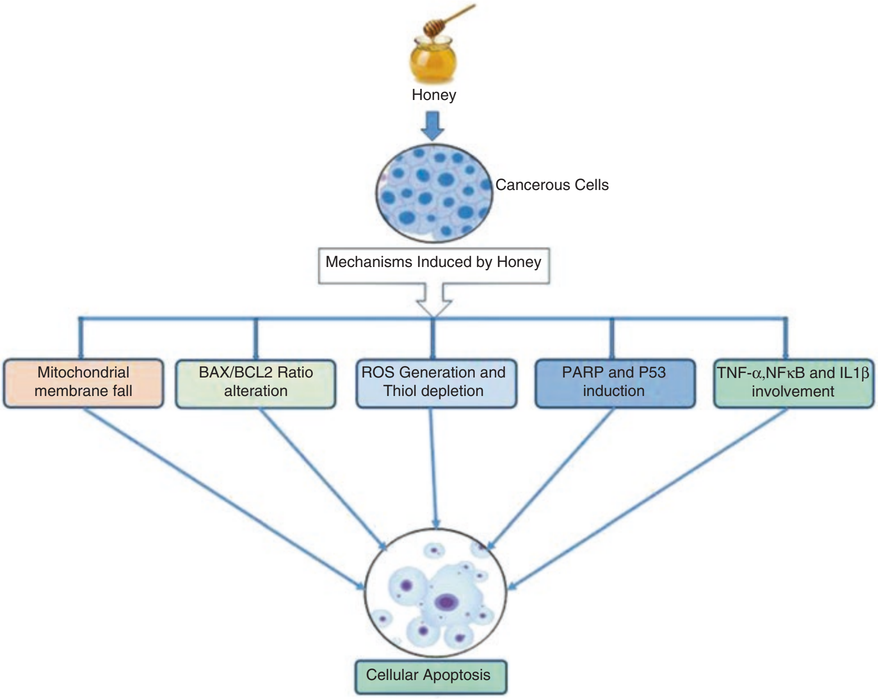

Fig. 18.3 Different mechanisms induced by honey in cancer cells for apoptosis

honey modulated levels of cytokines viz. TNF-α, IL-1β, and NF-κB that ultimately lead to apoptosis (Fig. 18.3).

Caffeic acid, a phenolic compound contained in honey is known to suppress the liver metastasis by inhibiting MMP-9 and NF-κB activity (Chung et al. 2004). Caffeic acid initiates programmed cell death in colon cancer cells via mitochondrial cascade (Jaganathan 2012). Chrysin triggers apoptosis by downregulating of Akt and p38-MAPK and upregulating caspase-3 in cancer cells (Woo et al. 2004). Apigenin causes downregulation of Akt and stimulates of Bim and Bax in cancer cells (Shao et al. 2013). Quercetin inhibits leukemia cancer cells by blocking the tyrosine protein kinase as well as the protein kinase-C. Quercetin also downregulates the expression of Ki-ras & c-myc oncogenes (Csokay et al. 1997; Robaszkiewicz et al. 2007). Acacetin activates Bax and p53 thereby inducing apoptosis whereas galangin activates caspase 3 thus arresting cells in sub-G1 phase (Hsu et al. 2004; Bestwick and Milne 2006). Treatment with pinocembrin increases levels of caspase-3 as well as cytochrome-C and Bax in colon cancer cells (Kumar et al. 2007). Constituents of honey like eugenol and p-coumaric acid induce programmed cell loss in colon cancer via oxygen free radical stimulated mitochondrial cascade (Jaganathan et al. 2011, 2013). Thus constituents of honey act through diverse mechanisms to show antiproliferative property.

18.5 Honey and Cancer

Constituents of honey have an inbuilt property to suppress abnormal cell proliferation. Honey has proved its anticancer mettle in diverse studies ranging from oncogenic cell lines to in vivo cancer models. Herein, we will try to provide collective knowledge regarding the role of honey against cancers of lung, colon prostrate, liver, blood, breast, bladder, skin, and kidneys. Honey also exerts a potent antitumor effect in various animal models.

18.5.1 Lung Cancer

As per reports the mortality rate because of lung cancer highest among all types of cancers and it affects both the genders. Lung cancer also termed as lung/pulmonary carcinoma involves an abnormal growth of lung tissue (Horn et al. 2012). Aliyu and coworkers tested the efficacy of acacia honey on the growth pattern of NCI-H460 cell line. The study revealed that lower concentration of acacia honey was not effective in inhibiting proliferation of NCI-H460.They observed smaller levels of cytokines (IL-1β and TNF-α) at lower dose of honey (2–4% honey) but there was a dramatic increase in cytokine expression at higher dose. The attributed this cytokine surge responsible for honey's proapoptotic activity in NCI-H460 cell line. Treatment with Acacia honey upregulated the levels of calcium in the cultured supernatant. This could probably be due to the generation of cytokines notably IL-1β which has a definite role in the release of Ca^{2+} from the lumen of smooth endoplasmic reticulum. The released calcium plays important role in inducing apoptosis. NCI-H460 cells treated with honey got blocked in sub-G1 stage of cell cycle and there occurred a concurrent arrest of S and G2/M phase. Furthermore, they observed a noticeable reduction in the level of Bcl2 and p53 due to acacia honey. Thus overall they found acacia honey exerts antiproliferative potential due to its capacity to persuade cell cycle blockade, upregulate cytokine-mediated calcium ion concentration and downregulate of p53 and Bcl2 (Aliyu et al. 2013).

18.5.2 Prostate Cancer

Prostate gland is a minute gland that secretes seminal fluid which sustains and carries sperms. Since this gland is present only in males therefore only males are affected by prostate cancer. This cancer usually develops in males over 50 years of age. In males, prostate cancer stands sixth leading cause of mortality. Aliyu and his coworkers evaluated the antiproliferative action of acacia honey in prostate cancer. They employed PC-3 cell line to investigate the action of acacia honey against prostate cancer. Outcomes from MTT assay showed that acacia honey at the concentration of 1.9% restrains 50% proliferation of PC-3 cells while in normal NIH/3T3 cell line it exhibited IC_{50} value of 3.7%. They observed surge of calcium ions in the supernatant of cells treated with honey (between 24 and 48 h) thus indicating its possible role in calcium mediated apoptosis. Role of two major cytokines viz. IL-1β and TNF-α was investigated to elucidate the function of IL-1β and TNF-α in acacia honey triggered death of PC-3 cell line. Calorimetry was used to measure the consequence of varying concentrations of honey on level of these cytokines at 24- and 48-h time intervals. At lowest dose of honey marked rise in the level of TNF-α after 24 h incubation. Level of these cytokines declined with the increasing honey concentration. There was a drastic reduction in the expression of TNF-α following 48 h incubation. IL-1β expression increased considerably at lowest dose and decreased at higher concentration after 24 h thereby potentiating its the anti-inflammatory nature. Surprisingly, the level showed an increasing trend when the concentration of honey was increased following 48 h incubation. This rise might be due to the fact that TNF-α stimulation leads to IL 1β production which has a role in the release of Ca^{2+} from the lumen of endoplasmic reticulum. In addition, levels of prostate specific antigen (PSA) decreased at higher doses of honey. Further acacia honey modulates the fraction of PC-3 cell line in sub-G1 phase concurrent halt of S as well as G2/M phase. Overall it seems acacia honey stimulate programmed cell death in PC-3 cell line is mediated though mechanisms like Ca^{2+} secretion, alteration in cytokines level, regulation of G0/G1 phase and down regulation of PS (Aliyu et al. 2012). Tsiapara and coworkers found that among several extracts of Greek honey (fir honey, thyme and pine) the thyme honey cause marked cell inhibition of prostate cancer cells (PC-3 cells). Furthermore, they also conclude that the outcome noticed was directly related to the elevated content of phenolic compounds in thyme honey (Tsiapara et al. 2009).

18.5.3 Liver Cancer

Liver cancer or hepatic cancer is invasive tumor that develops on the surface of liver or inside it. As per reports, out of all the cancer-related deaths about 23,000 people died only because of hepatic cancer and about 33, 190 new cases were also reported in 2014. Researchers used HepG-2 cell line to investigate the potential of honey against liver cancer. Time as well as dose-dependent evaluation was conducted to test the antiproliferative activity of honey. At the same time, artificial honey was formulated to keep out the effects of honey's acidity and osmolarity on HepG-2 cells. Honey inhibited HepG-2 cells both in concentration as well as time-dependent manner. Furthermore, no changes were observed on the growth rate of HepG-2 cell line following exposure to varying concentrations of artificial honey at varying time intervals. They concluded constituents besides the sugars are actually involved for providing anticancer effect to honey. They also noticed a considerable reduction in deacetylase enzyme action with higher concentrations of honey after exposure for 48 h. In addition, intracellular TNF-α and MDA levels were also found downregulated both in concentration- and time-dependent manner. NFκB, an important mediator for cell survival was found considerably reduced in honey-treated HepG-2 cells in comparison to control (untreated). Honey induced growth reduction in HepG-2 cell line could presumably occur through various mechanisms including altering the cytokines expression, diminishing lipid peroxidation and minimizing HDAC action (Ismail et al. 2013). Jubri and coworkers tested the potential of gelam honey on the growth of HepG-2 cell line. They observed that lower concentrations of honey could not restrict HepG-2 cells proliferation and that the gelam honey was found to inhibit 50% cancer cell growth at a dose of 25%. They also investigated gelam honey's anticancer efficacy against WRL-68 cancer cell line. At lower doses, gelam honey was not effective but its higher concentration caused considerable inhibition of WRL-68 cell growth. At 70% dosage gelam honey was able to inhibit 50% growth of WRL-68 cell line which is appreciably greater than the HepG-2 cell line potentiating the non-toxic effect of gelam honey on the survival of normal cells. The IC 50 value of gelam honey for the WRL-68 was found to be 70% against normal cells which is comparatively higher than that of HepG-2 cells thereby potentiating the protective property of this very variety of honey. This indicates that low dose of Malaysian gelam honey reduces the growth rate of hepatic cancer cells without affecting the normal liver cells (Jubri et al. 2012). Another study by Ismail and coworkers tested a combined formulation of gelam honey and Tinosporacrispa on the survival rate of WRL-68 and HepG-2 cell lines. They observed that this combination could inhibit 50% cell growth at a dose of 42.67% in case of HepG-2 cell line while it showed no inhibition on WRL-68 cell line. Their findings showed that the apoptosis-inducing capacity of this honey mixture could be due to its ability to downregulate IGF-1R and to activate caspase-3 enzyme (Ismail et al. 2013).

18.5.4 Oral Squamous and Osteosarcoma

This cancer develops due to unrestrained proliferation of squamous cells of skin epidermis (Berman 2004). Ghasm and coworkers tested the effect of honey against human osteosarcoma (HOS) cells and oral squamous cell carcinoma (OSCC). At beginning they examined the morphological changes linked with honey exposure at varying time intervals of 24 and 48 h following treatment to 2 and 10% doses. They observed cell membrane shrinking as well as the reduction in cell growth thus potentiating its proapoptotic role. MTT test showed honey can successfully inhibit the growth of HOS and OSCC cancer cells. There was inhibition of both the cell lines in concentration- and time-dependent approach. Dose of 3.5 and 4% of honey was able to inhibit 50% growth of HOS and OSCC cancer cell lines, respectively. Highest restrain of HOS and OSCC cell growth i.e., ≥80% was obtained at 15%. Moreover, they also excluded the chance of pH, osmolarity and H_2O_2 in the honey stimulated apoptosis of HOS and OSCC cancer cell lines. They also investigated the programmed cell death via PI staining and annexin-V staining of HOS and OSCC cell lines. Immediate apoptosis was apparent and the fraction of apoptotic cells elevated in time as well as and dose-dependent manner in HOS and OSCC cells. Overall they promulgated honey a good source of compounds for the treatment of osteosarcoma and oral squamous cancer. They also pressed for the urge to conduct more research for revealing the molecular machinery stimulated by honey for initiating apoptotic death of HOS and OSCC cancer cell lines (Ghashm et al. 2010).

18.5.5 Leukemia

Leukemia involves uncontrolled proliferation of immature leukocytes is a type blood cancer (Mathers et al. 2001). Morales and coworkers evaluated the potential of Spanish honey on blood pro-myelocytic leukemia cells (HL-60). They examined the antiproliferative potential by employing various honey types honeys viz. polyfloral, heather and rosemary. Doses of honey less than 25 mg/mL could not diminish viability of HL-60 cell. Higher dose of 50 mg/mL for 72 h diminished the HL-60 cell viability upto 60% in case of polyfloral honey and 70% for heather. Morales and coworkers also employed artificial honey to exclude the possible role of sugars in the anticancer property of honey. Artificial honey exhibited insignificant antiproliferative potential in the HL-60 cells in comparison to all the honey varieties examined in the study. Chromatin condensation experiment showed that the fraction of cells undergone apoptosis at 50 mg/mL dose of polyfloral honey or heather for a period of 48 h was 70.4–78.5% which is comparable to some known antiproliferative medicine like etoposide. Moreover annexin/V staining assays also verified the apoptosis induction due to honey and also inferred that 50 mg/mL dose of honey type (containing abundance of polyphenolic constituent) like poly floral and heather could raise cell loss in HL-60 cells upto almost 74%. Furthermore they investigated the programmed cell death by exposing the cells to NAC for 1 h prior to treating the cells to 50 mg/mL dose of honey for 48 h. Fascinatingly the outcome showed an increase in the apoptosis fraction and they promulgated that oxidative stress has no involvement in the honey-induced programmed cell death of HL-60 cells (Morales and Haza 2013).

18.5.6 Bladder Cancer

Bladder is an expanded cone shaped organ that stores urine. It is positioned in pelvic area of human body. Abnormal cell proliferation of bladder is known as bladder cancer. It generally develops from the cells constituting the inner lining of bladder. Tarek and coworkers evaluated the efficacy of honey in inhibiting bladder cancer. Initially they evaluated the antiproliferative potential of honey against RT4, MBT-2T24 and 253J cell lines. They found honey quite useful in blocking bladder cancer cells. The growth of MBT-2 and T24cell lines was declined by 1–25% honey while the 253J and RT4 cell lines needed 6–25% honey. Cell cycle study showed reduced S-phase fraction, and lack of a euploidy compared to control cells. Furthermore they administered honey via oral or intralesional route in implantation set up like MBT-2 bladder cancer model. They observed a considerable distinction in the tumor volume of the intralesion (IL) honey-treated groups and the IL saline group (control) (Swellam et al. 2003).

18.5.7 Melanoma

Melanoma involves the abnormal proliferation of melanocytes cells of skin (Swellam et al. 2003). Also as per WHO globally about 48,000 people die each year due to melanoma-related diseases (Lucas et al. 2006). Pichichero and coworkers evaluated the efficacy of honey against melanoma cells. They chose murine B16-F1 melanoma cell lines and human A375 cell line to evaluate the anticancer potential of honey. Honey was found to affect the metabolic activity of the both cell lines in both time- and dose-dependent manner. At a dose of 0.02 g/mL (IC 50) of honey they observed that both murine and human cell growth was declined to 50%. Honey exerted antiproliferative effect in A375 cells and it was observed to be 42, 57, and 68%, 35, 43, and 53% and 20, 32, and 46% inhibition at doses of 0.025, 0.02, and 0.01 g/mL following 24, 48-, and 72-h exposure, respectively. MTT and trypan blue assay-based cell cycle analysis was also performed that clarified noticeable cytotoxic consequences of 0.1 and 0.2 g/mL doses. Following 24-h exposure of honey, flow cytometry was performed that revealed about 90% inhibition of A375 and B16-F1 cell lines cells in the sub-Go phase. When exposed to an IC 50 concentration, A375 and B16-F1 cell lines showed concentration dependent blockade of cell progression in G0/G1. Parallel studies using chrysin showed noteworthy antiproliferative potential and alterations in the cell cycle of both A375 cells and B16-F1 melanoma cell lines. Therefore, they concluded that the antiproliferative effect of honey was primarily due to the presence of chrysin in honey (Pichichero et al. 2010). One more study by Cabezudo and coworkers tested the antitumor potential of manuka honey against melanoma mouse model. Earlier they had used antiproliferative test of honey against murine B16-F cells, breast cancer MCF-7 cell line and colorectal carcinoma (CT26) cells. They found that manuka honey inhibits the proliferation of these melanoma cancer cell lines both in time- and dose-dependent fashion at lower concentrations such as 0.6% w/v. Furthermore, evaluation of pro-apoptotic activity of honey using melanoma cell line namely B16-F was also performed. Honey triggered apoptosis by activating caspase-3 with the parallel decline of Bcl 2 and stimulating the DNA breakdown. Manuka honey when used in combination with paclitaxel showed no combinatorial effect. Furthermore, they investigated the efficacy of intravenously-injected manuka honey as well as its combination with paclitaxel via in vivo syngeneic mouse model. Intravenous injection of manuka honey produced no changes in the hematological values of treated mice indicating nontoxic nature of the honey. Manuka honey successfully inhibited tumor cell proliferation and the percentage inhibition shown by manuka honey alone was 33% whereas it was 61% by paclitaxel alone showed or in combination with manuka honey. Though there was an elevation in the host survival in co-treatment group when compared to untreated mouse group (Fernandez-Cabezudo et al. 2013).

18.5.8 Renal Carcinoma

Renal cell carcinoma also known as kidney cancer develops in the lining of tubes which carry glomerular filtrate (generated in the glomerulus) to descending limb of nephron (Mulders et al. 2008). Samarghandian and coworkers studied the apoptotic effect of honey in renal carcinoma. They tested honey against the kidney cancer cell line namely ACHN. In the beginning they used MTT assay to inspect the antiproliferative efficacy of honey which plainly showed the time- and dose-dependent inhibition of ACHN cells. They observed IC 50 values of 1.7 ± 0.04% and 2.1 ± 0.03% μg/mL against the ACHN cell lines after 48- and 72 h time intervals respectively. Inverted microscope-based morphological examination of honey-treated cells showed no significant changes after 24 h. Higher concentration of honey (20%) showed apoptosis with clear signs of cellular contraction. Annexin-V/PI assay based examination of 2.5 and 20% honey-treated cells exhibited that the proportion of the early-stage apoptotic cells elevated considerably from 2.0 to 19.0%, whereas percentage of late-stage apoptotic cells elevated markedly from 10.0 to 43.8% thus verifying the cell death triggering capability of honey in kidney cancer (Samarghandian et al. 2011).

18.5.9 Antitumor Property of Crude Honey Against Other Cancers

Initially, a study by Fukuda et al. found antitumor and immunomodulatory activity of Jungle honey (JH) in mice. They selected female C57BL/6 mice for the experiment from which they collected peritoneal cells (PC). They observed increase in PC concentration in JH-injected honey. They also identified neutrophils in the JH-injected mice through flow cytometry as a result of which they concluded chemotactic activity of JH has for neutrophils. They also revealed that the tumor incidence, weight was decreased due to JH and ROS were generated from activated neutrophils which were related to the antitumor activity of JH. Additionally, it was also found that the effective component in JH was having a molecular weight of 261 KDa (Fukuda et al. 2011). Another study by Jaganathan et al. revealed the antitumor activity of honey against Ehrlich ascites and solid carcinoma. In this study, they used two varieties of honey, one with low phenolic content and another with rich phenolic content. It was demonstrated from this study that honey rich in phenolic content has inhibitory effects on the growth of enrich ascites compared to other (Jaganathan et al. 2010). Another study demonstrated antimetastatic effects of honey when supplemented before injecting tumor cell (Orsolić et al. 2003). Another interesting study made by Gribel and Pashinskiĭ, demonstrated that honey is having the moderate antitumor and significant antimetastatic activity in animal models. They also concluded that antitumor activity of certain drugs such as cyclophosphamide and 5-fluorouracil were also enhanced by honey (Gribel and Pashinskiĭ 1990). Recently an investigation found the connection between consumption of honey and gastric cancer. During the study, they performed a thorough investigation with 62 subjects, and found a positive correlation between the apoptosis and consumption of honey (Ghaffari et al. 2012). However, they also demanded detailed view and interventional research to further explore these findings more specifically.

18.6 Conclusion

Besides having very ill effects on the quality of people's lives, cancer continues to be a major economic burden. This dreadful disease not only implies physical trauma for the family but also a monetary burden. To solve this huge mission of eradicating cancer, there is an urgent requirement to manufacture safe and cost effective drugs. Although a number of dietary agents have been studied, honey represents one of the gifted natural sources for preventing and managing of cancer. Honey is composed of various biologically active compounds which have been thoroughly studied for their therapeutic potential. In this chapter, we summarized the anticancerous activity of honey against various cancers and promoted it as an effective candidate against cancer. Even though it has potent anticancerous ability, it is also believed to have some negative effects in humans. But the plentiful positive outcomes of honey certainly overweigh the few negative effects of honey. Thus honey is believed as a potent candidate against various cancers. Although honey has been widely studied against various types of cancers, still more preclinical trials are required, which may provide us some new insights. Besides focusing on animal trials, there is a huge demand for studies involving some orthotopic mouse models, which is very necessary to take honey to the next level of development. Moreover, more and more clinical trials on larger groups of participants who are at high risk for cancer are also required to validate the anticancerous effect of honey and to provide more precise information about the protective effect of honey against various cancer biomarkers.

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19 Heath Benefits of Phenolic Compounds in Honey: An Essay

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Abstract

Honey comes with a legendary history of being used as an indigenous medicine to cure a number of diseases. Honey is an essential source of phenolic molecules such as flavonoids and phenolic acids. The most abundant flavonoids present in honey include flavones, flavanones, and flavonols. Flavonoids show diverse activities such as non-inflammatory, antiallergenic, antiviral, antimalignant, antimicrobial, however, the antioxidant activity has been studied widely. Honey also possess a diverse molecules of phenolic acids including p-coumaric, ferulic,

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_19

caffeic acid, acetophenones, phenylacetic acids, syringic, vanillic, gallic acid, and so on which endow it with the therapeutic activities against pathogens, inflammation while at the same time shows antioxidant and healing properties. The phenolic compounds owing to their medical properties make honey a very critical and attractive prophylactic entity for the prevention of chronic diseases associated with oxidative stress including cancer, cardiovascular disease, diabetes, respiratory disease, hypertension, neurodegenerative diseases etc. In this chapter, a discussion has been made on classification, structure, and medicinal and health benefits of phenolic compounds.

Keywords

Honey · Phenolic compounds · Antioxidant activity · Anticancer activity · Anti-inflammatory activity · Antidiabetic activity · Antimicrobial activity

19.1 Introduction

Over a thousands of years, honey was utilized as an indigenous therapeutic agent to treat a number of diseases by diverse ancient civilization such as Egyptian first dynasty, ancient Greeks, Vedic, and Islamic texts (Uthurry et al. 2011; Hossen et al. 2017). From the last few decades a number of studies have investigated the medical effects of honey especially against microbial pathogens, neutralizing inflammatory mediators and other healing activities (Alvarez-Suarez et al. 2013). However, recently it has been reported that honey is a novel antioxidant and may plays a curative function in the treatment of chronic noncommunicable ailments correlated with oxidative stress including, cardiovascular disease, malignancies, diabetes, respiratory disease, hypertension, etc. (Erejuwa et al. 2012; Albright 2008).

Honey is an aqueous gel of carbohydrates which contributes about 96% of its composition (Uthurry et al. 2011). D-fructose and D-glucose (31%), are the most predominant carbohydrates of honey constituting about 31% and 38%, respectively, apart from maltose that is present around 3% and sucrose that rarely exceeds 1% (Uthurry et al. 2011; Khalil and Sulaiman 2010). Besides the carbohydrates that constitute about 95% of the dry weight of honey, it also contains several other bioactive molecules like enzymes, phenolic compounds, vitamins, organic acids etc. (Manyi-Loh et al. 2011). Among the enzymes, invertase, amylase, catalase, and glucose oxidase are present along with amino acid proline that constitutes approximately 50% of the total amino acid content (Uthurry et al. 2011).

Honey is an essential source of phenolic compounds that possesses the antioxidant and anti-inflammatory properties (Schramm et al. 2003a; Viuda Martos et al. 2008). Phenolic compounds are secondary metabolites having a phenolic ring bearing at least one hydroxyl group and synthesized by mainly via shikimate and acetate pathways (Uthurry et al. 2011). Depending on the floral source, geographical and climatic conditions the phenolic concentration varies in honey (Uthurry et al. 2011). Furthermore, research reveals that phenolic composition plays as essential role in functional properties of honey including the color and flavor (Hossen et al. 2017). In other words, the overall quality of honey depends on the concentration of carbohydrates and phenolic compounds (Cheung et al. 2019).

19.2 Classification of Phenolic Compounds from Honey

Honey is considered as one of the most important sources of nutrients since from ancient times (Uthurry et al. 2011). It consists of approximate 200 substances including the phenolic compounds that are considered an important constituent of honey (Cianciosi et al. 2018). Studies have reported that honey consumption effectively increases the antioxidant level in plasma (both enzymatic and non-enzymatic antioxidants) (Hossen et al. 2017; Erejuwa et al. 2012; Uthurry et al. 2011). Phenolic compounds promote the non-enzymatic antioxidant activity of honey and are considered as the most important compounds for its antioxidant activity (Hossen et al. 2017; Uthurry et al. 2011; Cheung et al. 2019). On the basis of chemical nature and structure phenolic compounds of mainly categorized into the flavonoids and phenolic acids (Cheung et al. 2019; Cianciosi et al. 2018). Flavonoids and phenolic acids are secondary metabolites characterized by their complex configurations due to the presence of specific phenolic groups (Cianciosi et al. 2018). These are widely known for their antioxidant activity of the honey and stabilize the free radicals as they donate their hydrogen from one of their hydroxyl group therefore, are involved be associated with the ability of free radical scavengers.

19.2.1 Flavonoids

Flavonoids are one of the important groups of secondary metabolites with skeleton of polyphenols. Due to the powerful antioxidant property, they may be used as a best remedy for oxidative stress-related noncommunicable disorders like cancer, cardiovascular, and respiratory diseases (Cianciosi et al. 2018). They have low molecular weight and are mainly water soluble. They have a general structure of 15-carbon phenylpropanoid core (C_6-C_3-C_6 system) formed by two phenyl rings (A and B) linked by a heterocyclic pyran ring (C) (Jiang et al. 2016). Flavonoids are further divided into various sub-groups depending on the degree of oxidation of the C-ring. Flavones, flavanones, and flavonols are the most abundant flavonoids of the honey. Flavonoids show various biological activities such as anti-inflammatory, antiallergenic, antiviral, anticancer, antimicrobial, however, the antioxidant activity has been studied widely (Pietta 2000).

Flavones: Flavones are subclass of flavonoids having 2-phenylchromen-4-one backbone. The molecular formula of flavone is $C_{15}H_{10}O_2$ containing 3-rings in its chemical structure C6-C3-C6. The IUPAC name of flavones is 2-phenyl-1-benzopyran-4-one. Flavones consist of three functional groups, hydroxy, carbonyl, and conjugated double bond and exhibit the characteristic reactions to these functional groups. These compounds are soluble in water and ethanol. Flavones cross the cell membrane by interacting with membrane lipids. It also exhibits interactions with DNA and proteins (Jiang et al. 2016). Some of the important subclasses of flavones present in honey are as under:

Apigenin: The IUPAC name of apigenin is 4′5, 7-trihydroxyflavone. It has been used as a traditional medicine since from the ancient times due to its strong antioxidant, anti-inflammatory, antiviral, and anticancer properties (Shukla and Gupta 2010). It inhibits activity of α-glycosidase and increases insulin secretion, thus, exhibits antidiabetic property (Salehi et al. 2019). Apigenin reduces the arterial blood pressure by upregulating the ACE2 expression in kidney (Salehi et al. 2019). However, its role in anticancer activities have been recently investigated and it has been found that apigenin shows tumor suppressor efficacy among various cancers such as colorectal, breast, liver, lung, prostate cancers (Shukla and Gupta 2010). Apigenin prohibits the cell proliferation by inducing apoptosis and also induces the immune response against the cancer cells. (Cardenas et al. 2016). It induces the cell cycle arrest at G1/S-phase and G2/M-phase by regulating the expression of CDKs and other genes involved (Salehi et al. 2019).

Chrysin: The IUPAC name of chrysin is 5, 7-dihydroxy-2-phenyl-4H-chromen-4-one. Chrysin shows a number of pharmacological activities including antioxidant, anti-inflammatory, anticancer, antidiabetic, and antiviral activities (Mani and Natesan 2018). Usually, chrysin induces apoptosis and inhibits the cell division besides exhibiting a strong antioxidant and anti-inflammatory properties (Salimi et al. 2017). Chrysin is found in abundant amount in honey. It shows cancer preventive activity particularly in leukemia cells where they act via caspases activation and Akt signaling inhibition. It reduces the cell proliferation, metastasis and induces the process of apoptosis to suppress the tumors' growth in a wide variety of cancers including thyroid, breast, skin, lung, and hepatic cancers (Mani and Natesan 2018).

19.2.2 Flavanones

Flavanones consist of the most important group of flavonoids that are considered as an important dietary component due to their critical role in maintaining healthy blood vessels and bones. In flavanones, double bond is absent in c-ring of Carbon 2 and 3. Therefore, in flavanones C-2 consists of one hydrogen atoms apart from phenolic B-ring and two hydrogen atoms at C-3. Some of the important subclasses of flavanones in honey are as under:

Pinocembrin: The IUPAC name of pinocembrin is 5,7-dihydroxy-2-phenyl-2,3-dihydro-4H-chromen-4-one. Pinocembrin exhibits antibacterial activity with three kinds of Gram-negative bacteria (*E. coli, P. aeruginosa, and K. pneumoniae*) and three kinds of Gram-positive bacteria (*B. subtilis, S. aureus, and S. lentus*) via inducing the cell lysis (Rasul et al. 2013). Pinocembrin is also well known for its antimicrobial, anti-inflammatory, antioxidant, and anticancer activity (Rasul et al. 2013). In colon cancer cell line (HCT116), Pinocembrin exhibits cytotoxicity by upregulating the activity of caspase-3 and -9, heme oxygenase, and mitochondrial membrane potential (Rasul et al. 2013). A study conducted by Kumar et al. (2007) revealed that in colon cancer cells, pinocembrin initiates Bax-dependent mitochondrial apoptosis.

Genistein: Genistein is an isoflavone, mainly known for inhibiting a number of tyrosine kinases involved in chemotactic signaling (Wu et al. 2019). It possesses a number of chemopreventive and therapeutic properties such as anticancer, antioxidant, and antimicrobial activities (Kim et al. 2014). Studies have reported that genistein regulates the apoptosis, cell cycle, and angiogenesis and inhibits the metastasis. Besides acting as antioxidant, genistein act as a potent anti-inflammatory substance in atherosclerosis (Zhao et al. 2009; Kim et al. 2010). Genistein is reported to inhibits the angiogenesis and uncontrolled cell growth and regulates the cell cycle in various cancers like prostate, cervix, gastric, breast, and colon (Tuli et al. 2019). The IUPAC name of genistein is 5,7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one.

19.2.3 Flavonols

Flavonols constitute a class of flavonoids having the back bone of 3-hydroxy-2-phenylchromw-4-one. The subclass of flavonols differs in positions of one or more phenolic-OH groups. The IUPAC name of the flavonol is 3-hydroxy-2-phenylchromen-4-one. Some of the important subclasses of flavonols found in honey are as under:

Kaempferol: The IUPAC name of kaempferol is 3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one. It consists of several pharmacological properties including anticancer, antioxidant, anti-inflammatory activities (Calderon-Montano et al. 2011). It exhibits therapeutic applications in a number of diseases such as diabetes, cancer, neurological, and cardiovascular diseases (Imran et al. 2019). It possesses the antitumor activity by inducing the process of apoptosis and cell cycle arrest at G2/M phase of cell cycle (Marfe et al. 2009). A number of studies have reported that Kaempferol significantly reduces the risk of tumor development in a wide variety of human cancers such as breast, blood, brain, cervical, colon, kidney, liver, lung, etc. via regulating the expression of several cancer-related genes. Furthermore, studies have revealed that kaempferol targets selectively the cancerous cells without affecting healthy ones (Imran et al. 2019).

Myricetin: The myricetin is a member of flavonol class, with a potential antioxidant activity 3, 5, 7-trihydroxy-2-(3, 4, 5-trihydroxyphenyl)-4-chromenone. It exhibits a number of pharmacological activities such as anticancer, anti-inflammatory antimicrobial, and anti-allergic activities (Semwal et al. 2016). Myricetin showed protective effect against the cardiovascular diseases, diabetic, CNS Disorders, and hypertension (Wang et al. 2010). Studies have reported that myricetin inhibits the initiation and development of cancer by downregulating a number of enzymes involved in tumorigenesis (Semwal et al. 2016; Wang et al. 2010). It exhibits the cytotoxic properties toward a broad spectrum of human cancer cell lines, including hepatic, skin, pancreatic, and colon cancer cells (Semwal et al. 2016). Myricetin

(100 μM) displayed a vasculoprotective effect through transcriptional changes in human umbilical vein endothelial cells, as determined by microarray gene expression profiling (Semwal et al. 2016; Wang et al. 2010).

Quercetin: The IUPAC name of quercetin is 2 (3,4-diydroxyphenyl)- 3,5,7-trihy droxy-4H-chromen-4-one. It is classified as an antioxidant carrying out a number of biological activities such as anticarcinogenic, antiviral, and anti-inflammatory as well as boosting immunity (Li et al. 2016). Quercetin has a protective role against the wide range of diseases including osteoporosis, cardiovascular, cancer, and pulmonary (Boots et al. 2008). It prevents the formation of lipopolysaccharide-induced tumor necrosis factor-α in macrophages and inflammation producing enzymes cyclooxygenase and lipooxygenase (Li et al. 2016).

19.2.4 Phenolic Acid

Phenolic acids (phenol carboxylic acid) consist of a phenolic ring including one or more carboxylic acid as functional group. They may be divided according to their structure: C6-C3 (e.g., p-coumaric, ferulic and caffeic acid), C6-C2 (e.g., acetophenones and phenylacetic acids) and C6-C1 structure (e.g., syringic, vanillic, and gallic acid). Usually, most of these compounds are bound to the structural components of the plant (cellulose, lignin), but also to other types of organic molecules such as glucose, other sugars, or flavonoids. Some of the important phenolic acids found are given in Table 19.1.

19.3 Structure of Phenolic Compounds of Honey

Honey contains a wide range of phenolic compounds. Chemical structures of common phenolic compounds present in honey are depicted in Fig. 19.1.

19.3.1 Antioxidant Activity of Phenolic Compounds of Honey

Oxidative damage, caused due the imbalance of oxidants and antioxidants, impairs several physiological functions of cellular components. Studies report that the high level of oxidants or low levels of antioxidants were prevalent among the individuals with chronic diseases like cancer, diabetes, hypertension, atherosclerosis (Shibata and Kobayashi 2008; Kadenbach et al. 2009). Honey is considered a rich source of phenolic compounds and therefore plays an essential role in antioxidant activity (Khalil et al. 2011; Erejuwa et al. 2012). The antioxidant effect of honey may exert a protective effect against the risk of numerous chronic diseases. Research suggests that gastrointestinal tract, liver, pancreas, kidney, and reproductive organs are susceptible to oxidative damage and beneficial effect of phenolic components of honey on their oxidative stress has been documented (Erejuwa et al. 2012). Studies show that honey supplementation significantly reduces hepatic damage by decreasing the

Table 19.1 Biological properties of various phenolic acids

S. no.	Phenolic acid	Molecular formula	IUPAC name	Biological Properties	References
1.	Caffeic acid	$C_9H_8O_4$	(2E)-3-(3,4-Dihydroxyphenyl) prop-2-enoic acid)	Exhibits antioxidant, anti-inflammatory and anticancer effects. It regulates apoptosis, metastasis and angiogenesis in tumor cells including the antiproliferative effect against colon cancer	Lee et al. (2003)
2.	Ellagic acid	$C_{14}H_6O_8$	2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde] chromene-5,10-dione	Possesses the strong antioxidant properties related to the number of health benefits including cancer and cardiovascular diseases, it exhibits the antiproliferative and anti-inflammatory property	Kang et al. (2016)
3.	Syringic acid	$C_9H_{10}O_5$	4-Hydroxy-3,5-dimethoxybenzoic acid	Exhibits antioxidant, antimicrobial, anti-inflammatory, anticancer properties in therapeutic properties in a number of diseases including diabetes, cancer, cardiovascular defects	Srinivasulu et al. (2018)
4.	Gallic acid	$C_7H_6O_5$	3,4,5-Trihydroxybenzoic acid	It includes multiple therapeutic effects such as antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. It possesses the therapeutic effects in a variety of diseases including cardiovascular, diabetes, cancer, and gastrointestinal diseases	Kahkeshani et al. (2019)
5.	Cinnamic acid	$C_9H_8O_2$	(2E)-3-Phenylprop-2-enoic acid	Shows antioxidant, antimicrobial properties including antibacterial, antifungal, and antiviral activities in several diseases like diabetes, cardiovascular, and other neurodegenerative diseases like Alzheimer's disease	Sova (2012)
6.	Coumaric acid	$C_9H_8O_3$	(2E)-3-(4-Hydroxyphenyl) prop-2-enoic acid	Exhibits a wide range biological activities including antioxidant, anti-inflammatory, antidiabetic, anticancer and antiulcer	Ilavenil et al. (2016)
7.	2-Hydroxycinnamic acid	$C_9H_8O_3$		As a strong antioxidant act as a therapeutic agent in a number of diseases related to oxidative stress such inflammatory injury, atherosclerosis, cancer, and cardiovascular disease	Teixeira et al. (2013)

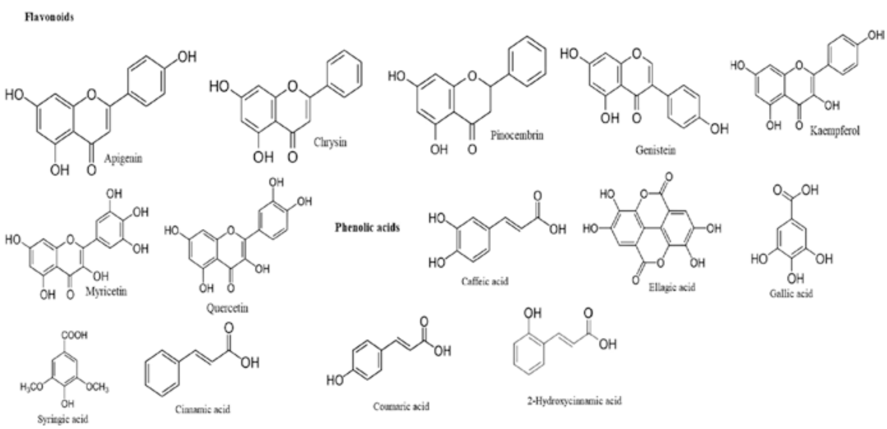

Fig. 19.1 Structure of the flavonoids and phenolic acids commonly found in honey

levels of various oxidants of liver (Kilicoglu et al. 2008). Furthermore, honey supplementation is also significantly associated with increased or restored activities of a number of antioxidants in liver (Petrus et al. 2011; Yao et al. 2011). The pancreatic β cells are highly susceptible to oxidative damage due to the low expression and free radicles scavenging enzymes (Grankvist et al. 1981). However, evidences suggest that honey supplementation potentially reduces the raised MDA levels and restored SOD and CAT activities in pancreas of rat and protects against any oxidative damage (Erejuwa et al. 2010). Recently, it has been reported that honey supplementation strengthens the antioxidant defense mechanisms and prevents the formation of some oxidants in Kidney in spontaneous hypertensive rats (Erejuwa et al. 2012). Furthermore, research data reveals that honey may have beneficial effects on oxidative stress of the reproductive system (Erejuwa et al. 2012). It has been reported that 8-weeks supplementation of honey significantly reduces the oxidative stress by increasing the seminal levels of SOD, CAT, TAS and it is associated with decreased concentration of IL-1β, IL-6, IL-8, TNF-α ROS, and MDA levels (Tartibian et al. 2011).

The oxidative stress is caused due free radicals of reactive oxygen species and reactive nitrogen species that damage the various cellular components such as lipids, proteins, and DNA (Ahmed and Othman 2013). The antioxidant effect of honey is catalyzed via iron and copper that leads to the inhibition of free radical formation (Manyi-Loh et al. 2011). The phenolic compounds readily lose hydrogen atoms to the free radicals along with the remaining phenoxy radicals which also react with other radicals and terminate the chain of propagation (Uthurry et al. 2011). Honey consists of a number of phenolic compounds that demonstrates an important role as antioxidant agent including many of them having pharmacological significance such as caffeic acid, caffeic acid phenyl ester, chrysin, galangin, quercetin, acacetin, kaempferol, pinocembrin, pinobanksin, and apigenin (Jaganathan and Mandal 2009). Gheldof and Engeseth (2002) reported the antioxidant activity of phenolic compounds in honey significantly inhibits the oxidation of serum lipoprotein.

Furthermore, investigations reveal that antioxidant activity of honey has a positive correlation with color, in other words darker the honey higher the antioxidant capacity (Khalil and Sulaiman 2010). Yemeni honey has a high concentration of phenolic components, therefore has a high potential of antioxidant therapeutic value followed by Malasiyan, Gelam, and coconut honey (Uthurry et al. 2011).

19.3.2 Anticancer Activity of Phenolic Compounds of Honey

Numerous in vitro and in vivo studies have investigated the potential effects of honey on cancer. It is found that honey may have a defensive effect on the occurrence and development of cancer and may be used as indigenous product in prevention and treatment of cancer therapy. Phenolic compounds are the most significant ingredients in honey that is responsible for its anticancer activity (Jaganathan and Mandal 2009; Abubakar et al. 2012). Phenolic compounds of honey possess many antitumor activities and inhibit the incidence and progression of cancer by reducing the proliferative ability of cells, promoting apoptosis, regulation various cancer-related signaling pathways and prevention of oxidative stress and DNA damage (Hossen et al. 2017).

Studies show that honey and its phenolic components inhibit the cellular proliferation of tumorous through different means. The abnormal uncontrolled cell division of colon, glioma, and melanoma cancer cells lines at G0/G1 phase that leads to the downregulation of various cancer-related signaling pathways through tyrosine cyclooxygenase, ornithine decarboxylase, and kinase (Lee et al. 2003; Pichichero et al. 2010; Oršolić et al. 2010). Quercetin is an important phenolic compound that blocks the cell division of prostate cancer cells either by reducing the expression of cdc2/cdk-1, cyclin B1, phosphorylated pRB, Bcl-2, Bcl-XL or by increasing the expression of p21, Bax, and caspase-3 (Hossen et al. 2017). In colon cancer SW480 cell lines, quercetin exerts antitumor effect by decreasing the transcriptional activity of β-catenin/Tcf via blocking the β-catenin/Tcf signaling pathway as well as by impeding the PI3K-Akt/PKB pathway (Park et al. 2005; Gulati et al. 2006). Apigenin, another phenolic compound inhibits the proliferation of pancreatic cells by arresting the cell cycle at G2/M phase by decreasing cyclin A and B and ultimately phosphorylation of cdc2 and cdc25 (Hossen et al. 2017).

Another important phenolic compound with anticancer activity is chrysin consisting of antiproliferative effect in human (A375) and murine (B16-F1) melanoma cell lines where it induces the cell cycle arrest at G0/G1 (Pichichero et al. 2010). In C6 glioma cells of rats, phenolic compound Chrysin checks abnormal cell division by activating p38-MAPK through the accumulation of p21Wafi/Cip1. In this case chrysin increases the expression of p21 significantly along with the reduced activity of CDK2, CDK4 in a dose-dependent and time-dependent manner (Weng et al. 2005). Galangin induces apoptosis and inhibits the cell division in human myeloid cell lines (K562 and KCL22) and in imatinib mesylate-resistant myeloid cells (K562-R and KCL22-R). It increases the activity of imatinib in both imatinib-sensitive and imatinib-resistant Bcr-Abl+ cell lines by reducing the Bcl-2 levels and therefore act as a potential candidate and can be evaluated as a treatment of imatinib-resistant leukemias (Tolomeo et al. 2008).

Phenolic compound in honey induces the apoptosis in colon cancer cell lines by mitochondrial depolarization and increasing the expression of caspase 3 and poly ADP-ribose polymerase cleavage (Jaganathan and Mandal 2009). These compounds by their antagonistic action target the estrogen receptor activity in breast and endometrial cancers (Hossen et al. 2017). Investigations reveal that Manuka, Pasture, Nigerian, Jungle, and royal jelly honeys exhibits tremendous anticancer activities by stimulating potential immune response through the activation of antibodies, lymphocytes, neutrophils, monocytes, eosinophils, and natural killer cells (Al-Waili 2003; Attia et al. 2008). Phenolic compounds of honey can be regarded important source of interesting for cancer treatment, and therefore can be considered to have a functional value in the cancer field.

19.3.3 Anti-inflammatory Activity of Phenolic Compounds of Honey

Inflammation is generally considered as regular response in cases of injury and infection, however, the excessiveness and protraction that inhibits the process of curing and leads to further impairment becomes the matter of concern. One of the most harmful effects of excessive inflammation is the formation of free radicals that triggers the breaking down of lipids, proteins and nucleic acids and damages the normal functioning of the cells (Manyi-Loh et al. 2011). Inflammation is positively correlated with cancer as excessive and prolonged inflammation damages the cells and prevents their healing process (Ahmed and Othman 2013). Investigations have reported that honey reduces the inflammatory response by decreasing edema and pain through inhibition of NO and formation and release of prostaglandin E (Erejuwa et al. 2012). The antibacterial and antioxidant properties of honey are responsible for its anti-inflammatory effect that plays a vital role to wound healings.

The anti-inflammatory effect of honey is induced by phenolic compounds (Ahmed and Othman 2013). Guardia et al. (2001) reports some of the anti-inflammatory activities of quercetin, rutin, and hesperidin in rats as they are effective to reduce the acute and chronic phases of inflammation in rat model having adjuvant arthritis. Honey reduces the inflammation in various diseases without having any major side effects (Vallianou et al. 2014). Several studies report that honey exhibits inflammatory response in several clinical trials, animal models and cell cultures (Al-Waili 2003; Candiracci et al. 2012; Leong et al. 2012; Bilsel et al. 2012).

Apigenin exerts anti-inflammation through various mechanisms such as suppression of lipopolysaccharide, inhibition of nitric oxide, decreasing the TNF-α levels, suppression of intracellular adhesion molecule-1 and by downregulating the gene and protein expression of E-selectin (Lee et al. 2007). Kaempferol, another polyphenol commonly present in honey possesses the anti-inflammatory activity by reducing IL-1β, malondialdehyde, and TNF-α levels, upregulating activity of superoxide dismutase and downregulating expression of E-selectin, ICAM-1, VCAM-1, and monocyte chemotactic protein-1 (Kong et al. 2013).

The inflammation process is induced in various chronic and acute diseases like cancer, atherosclerosis, diabetes mellitus, and cardiovascular diseases. Various chemical and biological processes are responsible for induction of inflammation for example the COX-2 enzyme, pro-inflammatory mediator metabolizes arachidonic acid to prostaglandin that initiates the process of inflammation (Ahmed and Othman 2013). The pro-inflammatory activity of COX-2 is inhibited by phenolic compounds of honey via reducing the migration of NF-κB transcription factor to the nucleus which in turn leads to down regulation of pro-inflammatory gene expression (Vallianou et al. 2014).

19.3.4 Antidiabetic Activity of Phenolic Compounds from Honey

Diabetes mellitus (DM) is one of multifactorial chronic metabolic disorder in which the body loses control over glucose level. Every medicine has some side effects and antidiabetic medicine is one of them. So, scientists are much interested to discover alternative remedies. Antidiabetic active polyphenols have been observed in some plants that were traditionally used and are used still in controlling diabetes (Patel et al. 2012; Gray and Flatt 1997). Dietary polyphenols present in natural food are helpful in treatment and management of diabetes mellitus proved by many experimental studies. Fortunately, nature provided us many such ingredients through the blessing of honey. It is found to maintain the blood glucose level that is blood glucose homeostasis. Fructose found in honey improves the efficiency of glucose uptake by liver in dose-dependent manner and enhances glycogen synthesis in hepatocytes. Thus, fine tuning the glycemic control in diabetes mellitus. Flavonoids, one of the class of polyphenolic compounds found in honey elevates the glucose tolerance of body by improving the cellular absorption of glucose and maintaining a healthy glucose level (Amalia 2015) there is a wide range of polyphenols contained in honey, only a few of them like quercetin, apigenin, luteolin, catechin, rutin, and kaempferol, were found to possess antidiabetic effects and they target on different biological processes to maintain a healthy glucose level.

Luteolin: Luteolin, a common honey flavonoid reduces blood glucose level by improving the property of pancreatic beta cells to release insulin (Zarzuelo et al. 1996). Studies found luteolin and its modified form luteolin-7-0-glycoside reduces glucose absorption in intestines through its deactivating effects on sugar degrading enzymes like α-amylase and α-glucosidase (Jong-Sang et al. 2000). Use of Luteolin by patients of type1 diabetes alleviates their heart related complications due to neutralization of reactive oxygen species (Wang et al. 2012). Use of Luteolin by Type2 diabetic patients minimizes insulin resistance by stimulating adipocytes to release specific cytokines called adipokines that act through peroxisome proliferator activated receptor gamma (PPAR-γ) to improve sensibility for insulin (Ding et al. 2010).

Rutin: Rutin and Quercetin are well-known honey polyphenolic compounds that may improve the property of insulin-resilient muscle and fat tissue to take up glucose (Lee et al. 2012). In rats that were treated with streptozotocin to induce diabetes, inclusion of Rutin in their diet facilitates elevation of free radical scavenging enzyme levels and insulin hormone in their pancreatic beta cells (Esmaeili and Sadeghi 2009). For example it is streptozotocin-induced diabetic rats fed with 25–100 mg of Rutin per kilogram of body weight show important antidiabetic effects like elevated insulin release and decreasing fasting blood glucose concentration (Kamalakkannan and Prince 2006).

Apigenin: Apigenin, a honey flavonoid when administrated to diabetic rats at a level of 50 mg/kg of body weight normalizes glucose level by enhancing insulin release from pancreatic beta cells (Cazarolli et al. 2009). Apigenin negatively regulates pancreatic cell amplification by suppressing expression of cyclin A, cyclin B and the phosphorylated forms of cdc2 and cdc25, thereby blocks the G2 to M phase transition of the cell cycle (Ujiki et al. 2006), thus have a role as anticancer agent. It is found that apigenin enhances learning and memory faculties in diabetic rats by reducing oxidative species and inhibiting nitric oxide synthase pathway thereby protects brain from neurotoxic effect of nitric oxide. Thus it indicates apigenin alleviates diabetes-associated cognitive decline (DACD) in rats (Mao et al. 2015). Apigenin lowers lipid peroxidation level by enhancing free radical scavenging defense enzymes (superoxide dismutase and catalase) which may reduce severity of Type2 diabetes mellitus. Apigenin protects cellular profile of our vital visceral organs that are mostly damaged by hyperglycemic condition observed in streptozotocin-treated rats which triggers pancreatic beta cell apoptosis. However, it did not show any detrimental effect to visceral organs of normal rats. Furthermore, apigenin treatment in diabetic rats increases GLUT4 translocation and also preserves beta-cell integrity. Apigenin elevates the NAD+ level within the cells by inhibiting the activity of NAD+ degrading enzyme (CD38), improving glucose homeostasis and metabolic syndrome related diseases like Type2 diabetes mellitus, obesity, and obesity-related disorders. So apigenin may be considered as a potential antidiabetic agent in coming time and it may save vital organs from hyperglycemia shock that is certainly one of the reasons for multiple organ dysfunction observed in diabetes mellitus (Hossain et al. 2014).

Kaempferol: There are reasonable evidences of Insulin hormone cell damage triggered by consistent hyperglycemia shock which in long run leads to deterioration of glucose homeostasis and overt diabetes mellitus. A study conducted on obese diabetic mice found that kaempferol discourages peripheral insulin resistance while improving the sensibility of Insulin releasing cells toward glucose. Another experimental dose-dependent study conducted on mice (maximum effect at 10 μM kaempferol) found that it facilitates insulin cell viability discourages programmed cell death through blockade of caspase-3 enzyme activity in pancreatic beta cells experiencing consistent hyperglycemia shock (Zhang and Liu 2011). Alkhalidy et al. (2018) observed that kaempferol administrated orally 50 mg/kg of body weight per day in diabetic mice (streptozotocin-induced) maintained healthy glucose and reduced disease severity from 100 to 77.8% even in advanced stage. Kaempferol actually enhance glycogen synthesis and prevent liver glucose while encourages muscles to harness more and more glucose in energy production. However its effect is indifferent toward body mass index, calorie utilization by our body and blood glucose homeostasis hormone levels. Kaempferol treatment directs glucose in glycolytic pathway by promoting the activity of hexokinase in liver and muscle cells while at the same it discourages gluconeogenesis by decreasing the activity of pyruvate carboxylase. These findings show that kaempferol promotes oxidation of glucose and discourages de-nova synthesis of glucose (gluconeogenesis) in the hepatocytes.

19.3.5 Antimicrobial Activity of Honey Phenolic Compounds

Although since from the ancient times, honey was used as a therapeutic substance against several number of infections, however, antibacterial activity of honey was first reported by van Ketel in 1892 (Dustmann 1979). Honey kills or inhibits the growth of various microorganisms by either peroxide mediated activity such as H_2O_2 that is produced from glucose by glucose oxidase enzyme on dilution of honey (White Jr et al. 1963) or by non-peroxide activity such as high osmolarity (low water activity) that is honey is highly hygroscopic that dehydrates the microorganisms, bactericidal activity of methylglyoxal compound and immune-protective peptide called defensin-1 that are originated from hypopharyngeal gland of honey bee and then ultimately mixed with honey (Kwakman and Zaat 2012). The high content of oligosaccharides in honey stimulates the growth of beneficial gut microbiota especially Lactobacilli and Bifidobacteria species that protects the gut from pathogenic bacteria and yeasts and acts as a prebiotic dietary formula. Lactobacilli protects from salmonellosis caused by salmonella typhimurium, on the other hand, Bifidobacteria discourages the overgrowth of yeasts and bacteria and deceases the risk of colon cancer by competing with putrefying bacteria producing toxic carcinogen (Kleerebezem and Vaughan 2009; Mohan et al. 2017).

Honey processed by bees from the nectar of *leptospermum scoparium* flower (Manuka Honey) in New Zealand possessed best bactericidal properties. On analysis of manuka honey by HPLC (high performance liquid chromatography), it contains high quantity of methylglyoxal. Manuka medical grade honey has high demand in market as clinical medicine. It was first introduced as γ-irradiated product in the market. To assess the concentration of methylglyoxal in manuka honey called Unique Manuka factor (UMF). Unique manuka factor actually gives us an idea of how much methylglyoxal is present in honey which is a powerful antimicrobial agent. UMF indicates equivalents of a phenol solution yielding a certain inhibition by inoculating on the pure culture of *Staphylococcus aureus* (Allen et al. 1991).

In addition to above mechanisms of antimicrobial action of honey, there are some well-known phenolic compounds that show antimicrobial activity for example pinocembrin, syringic acid, etc. (Agbaje et al. 2006). Sousa et al. (2016) observed that honeys with highest total phenolic content (TPC) have highest antimicrobial activity.

Antibacterial Activity: Many studies found that relation between phenolic content of honey and antibacterial property of honey. For example, Silici et al. (2010)

found that the phenolic compounds present in Rhodendron Honey of Turkey partially contribute to its antibacterial activity (Silici et al. 2010). The antimicrobial bioactivity of each molecule in honey is either increased or decreased based on rest of composition in honey (Silici et al. 2010). It is known that antibacterial activity of honey depends on many factors such as source of flower nectar and factors where this flower grows like precipitation, temperature, etc. *Staphylococcus aureus* is present in skin and mucous membrane of healthy individuals but is a causative agent of most common bacterial infections like bacteremia, infective endocarditis, skin and soft tissue infections, osteomyelitis, etc. (Saad et al. 217). The maximum response achievable against microbial species like *Staphylococcus aureus* was found to differ with different honey at different concentration. Since as the honey gets ripened, its hydrogen peroxide mediated antimicrobial activity gets decreased but other antimicrobial entities like flavonoids becomes more pronounced (Bizerra et al. 2012). It was found that the Honey which contains high phenolic content inhibits growth of bacterial strains at smallest concentration that is having lowest minimum inhibitory concentration (MIC) value. One such good example is Juazeiro honey (Brazil) that possessed stronger antagonistic property microbes due to its high content of Rutin, catechin, and chrysin flavonoids. Flavonoids have property of disrupting the structure and transport of bacterial membranes that becomes highly permeable to protons, ultimately leading to the loss of cell integrity with leakage of cytoplasmic content (Kirnpal-Kaur et al. 2011). After measuring MIC (minimum inhibitory concentration), Salmonella sp. is found most sensitive followed by *S. aureus, E. coli/P. aeruginosa,* etc. (Sousa et al. 2016).

Some other studies on Honey polyphenols revealed different mechanism of action, for example, Takaisi-Kikuni and Schilcher (1994) found that pinocembrin, galangin, and caffeic acid phenethyl ester inhibit bacterial RNA polymerase while (Cushnie and Lamb 2005) suggested galangin induces degradation to bacterial plasma membrane leading to leakage of potassium ions and following cell autolysis. Furthermore, Mirzoeva et al. (1997) found that quercetin that is a flavonoid increase bacterium membrane permeability leading to electric potential dissipation and decrease in the synthesis of ATP. Kirnpal-Kaur et al. (2011) fractionated Tualang honey into polar, acidic, and basic fractions using the solid phase extraction technique (SPE) in order to evaluate their antibacterial properties against wound and enteric bacteria, and found that the acidic fraction enhanced the antibacterial properties of this honey. In an effort to identify the main phytochemicals with antibacterial activity present in the acidic fraction, the need for further investigations was acknowledged, although preliminary evidence indicated that these compounds could be polyphenols.

Antiviral Activity: Many people observed that some flavonoids present in honey inhibit the proliferation of virus for example apigenin, acacetin and chrysin blocks the human immunodeficiency virus (HIV-1) via interfering with its process of transcription (Critchfield et al. 1996). Other researchers have demonstrated that the inhibitory effects of chrysin shows antiviral property toward herpes simplex virus type 1 (HSV-1) and HIV-1 in acutely infected H9 lymphocytes (Lyu et al. 2005; Hu et al. 1994) while as Apigenin shows antiviral property toward influenza virus

(H3N2) in vitro (Liu et al. 2008). It is also observed that apigenin possesses an effective antiviral property toward herpes virus type-2 (HSV-2), adenovirus (ADV-3), hepatitis B surface antigen (HBsA), and hepatitis B e antigen (HBeA) (Chiang et al. 2005; Al-Waili 2004). Honey polyphenolic compounds may improve the mean healing time in some viral diseases such as Herpes. Labial herpes mean healing time is improved around 43% and 59% in Genital herpes as compared with conventional treatment by Acyclovir and in some cases remission of disease take place after honey treatment (Al-Waili 2004). Zeina et al. (1996) found that infected monkey kidney cell with Rubella virus shows anti-Rubella property after treatment with honey.

19.3.6 Cardioprotective Effects of Honey Phenolic Compounds

Honey polyphenolic compounds such as quercetin, caffeic acid, kaempferol, and apigenin minimizes the deleterious effects of factors leading to cardiovascular disease like hypertension, hypercholesterolemia, obesity, etc. (Ulbricht and Southgate 1991). Polyphenolic compounds ameliorate cardiovascular diseases through various mechanisms. Most of polyphenolic compounds possesses the property of scavenging reactive oxygen species (ROS) generated during normal metabolism (Rein et al. 2000a; Stein et al. 1999), blocks intrinsic platelet aggregation that has important role in (thromboembolism) blood clot formation (Stein et al. 1999; Rein et al. 2000b), hypertension (Taubert et al. 2007; Desch et al. 2010), reduces the chances of atherosclerotic plague formation through the improvement of endothelial cell function and LDL oxidation prevention (Heiss et al. 2003, 2005). It reduces an inflammatory response which is found to be an important triggering event in many noncommunicable diseases including atherosclerosis (Mao et al. 2002; Schramm et al. 2003b). It also decreases oxidative stress (Urquiaga and Leighton 2000; Solayman et al. 2016) and improve coronary vasodilatation (Benavente-Garcia and Castillo 2008).

Therefore, these phenolic compounds found in honey act as game changer for treatment of cardiovascular diseases. Many studies conducted on rats and mice proved their property of reducing oxidative stress and protect endothelium from induced injury and dysfunction.

Quercetin: Regular consumption of foods containing high content of bioflavonoids belonging to polyphenols reduces mortality and the incidence of ischemic heart diseases. It is referred as “French paradox” first observed in France. It is found that bioflavonoids may negatively regulate smooth muscle cell proliferation induced by Ang (angiotensin) II-induced MAP kinase activation in rat aortic smooth muscle cell line (RASMC).For example, Quercetin found in high content in natural foods was found to discourage vascular smooth muscle proliferative signaling mediated through inhibition of the Ang II (angiotensin)-induced JNK (c-jun N-terminal kinase) enzyme but it should be noted that quercetin does not affects ERK1/2 and p38 activation. It was found that Quercetin blocks the angiotensin II triggered interaction Shc protein with p85 that is a regulatory subunit of phosphatidylinositol 3-kinase in rat aortic smooth muscle cells. Further research revealed that LY294002, a quercetin derivative, blocks the Angiotensin II-mediated activation of JNK as well as phosphorylation of protein kinase B (Akt) by PI 3-Kinase. It was found that quercetin blocks Ang II-stimulated enlargement of vascular smooth muscle cells (hypertrophy) that was assessed by reduced 3H-leucine utilization for protein synthesis. When the process was further investigated at the molecular level, it was found that Quercetin actually blocks the phosphorylation on Shc protein and phosphotidylinostol-3-kinase stimulated phosphorylation of JNK in vascular smooth muscle cells. Thus, Quercetin keeps JNK in off-state which in turn keep c-jun proliferative factor in off-state, thus preventing the undesirable proliferation of VSMC that is one of the event in development of atherosclerosis (Yoshizumi et al. 2001). Quercetin alleviates systolic blood pressure and decreases plasma oxidized LDL level by inhibiting myeloperoxidase enzyme in obesity affected persons that is a root cause of many disorders (Egert et al. 2009; Kostyuk et al. 2011). Quercetin does not allow oxidized LDL to change phenotype that may develop later on into foam cells (Al-Awwadi et al. 2005). Quercetin facilitates coronary vasodilatation by normalizing endothelin-1 mRNA transcription (endogenous vasoconstrictor) thus improving vascular function (Romero et al. 2009). Quercetin blocks superoxide anion (O_2) formation and oxidative stress, reduce NADPH oxidase enzyme activity through negative effect on expression of neutrophil cytosol factor 1 and maintains healthy vasodilation mediated through improvement of (eNOS) endothelial nitric oxide synthase activity in hypertensive rats (Sánchez et al. 2006).

Apigenin: Apigenin commonly found flavonoid present in honey possesses anti-inflammatory, apoptotic properties thus neutralizing the agents that promote inflammation. Apigenin blocks initial event of bacterial induced-inflammation mediated through inhibition of bacterial induced cyclooxygenase-2 (COX-2) expression. Apigenin discourages inflammation of cells especially endothelial cells by normalizing tumor necrosis factor-alpha inflammatory signaling that increases VCAM and ICAM-1 adhesive receptors. This decreases the migration of inflammatory cells across the endothelial layer considered important event in the development of atherosclerosis. This was proved human umbilical vein endothelium experienced decreased monocytes attachment on application of apigenin. Apigenin down regulates E selectin transcription to basal levels that discourages adhesion and rolling of white blood cells on endothelium of blood vessels (Lee et al. 2007). These effects of apigenin might not allow accumulation of atherosclerotic plaques within the blood vessels. Studies conducted on bone marrow and peritoneal derived macrophages packaged with oxidized LDL experience proapoptotic character like upregulation of Bax protein, catalyze caspase-3 cleavage and downregulated expression of anti-apoptotic genes (Mcl-1,Bcl-2) on treatment with apigenin. These results suggested that the anti-atherosclerotic effects of apigenin are associated with the upregulation of apoptosis in Ox-LDL-loaded MPMs (Wright et al. 2013).

Chrysin: Chrysin is one of the key flavonoid possessing protective properties that scavenge reactive oxygen species (ROS) generated during cell metabolism and discourages liberation of inflammatory substances which are formed due to external or internal agents. Chrysin acts as a chelating agent of transition metal ions and blocks the activity of prooxidant enzymes that are instrumental in the generation of reactive oxygen species within the blood vessels. Chrysin decelerates process of lipogenesis while at the same time canalizing the excess lipids toward metabolism thereby normalizing lipid profile of blood (Patel et al. 2016). Chrysin minimizes the vasoconstriction by improving nitric oxide bioavailability and thus maintains normal blood pressure. Chrysin blocks development of atherosclerotic plaques on the vessel walls and vascular inflammation mediated through its inhibitory effect on NF-kB signaling pathway (Berliner et al. 1995; Missassi et al. 2017). Chrysin discourages any vascular injury stimulated smooth muscle cell proliferation and blood clot formation (thrombogenesis). All these observations suggest that chrysin may be indigenous prophylactic medicine to discourage the thickening and narrowing of blood vessels especially coronary arteries due to the accumulation of atherosclerotic plague. Though the free radical scavenging effect is the dominant protective function of chrysin, it was found that chrysin modifies the cell signaling pathways within macrophages, endothelial cells, VSMC by targeting transcriptional factors like TNF- α,NF-kb,MMP-1,MMP-9 related with the development of inflammation and cardiovascular diseases (Veerappan and Senthilkumar 2015; Farkhondeh et al. 2019).

Catechin: The cardio protective property of honey is due to catechin which scavenges free radical species (ROS) liberated at uncoupled mitochondrial electron transport chain, encounter of inflammatory cells with pathogens and activity of oxidative enzymes e.g., xanthine oxidase (Babu and Liu 2008). Catechin chelates redox active transition metal ions, blocks the interaction of redox sensitive transcription factors, blocks prooxidative enzyme activity while stimulating antioxidative enzymes (Mihm et al. 2001; Frei and Higdon 2003). Catechin, a honey flavonoid has healthy effects on most f the events that are responsible for development of cardiovascular diseases. It negatively affects the inflammatory signaling, proliferative signaling within vascular smooth muscle tissue and lipoprotein oxidation. All these events do not allow vascular injury and thus prevents platelet aggregation on the walls of blood vessels. Regular consumption of catechin containing foods like honey maintains a healthy lipid profile (LDL, HDL, fatty acids) and stabilizes vascular reactivity (Pham et al. 2010). Many observational studies found elevated blood LDL cholesterol (hypercholesterolemia), triglycerides (hyperlipidemia) is one of the dominant risk factor for accumulation of atherosclerotic plaques and increased incidence of myocardial infarction. Catechins present in honey maintain a healthy blood cholesterol level which lowers its chances of accumulation in vital organs like heart and liver. The pathogeneses and clinical manifestations of cardiovascular diseases are aggravated by low bioavailability of nitric oxide (NO), an important vasodilator molecule within vascular smooth muscle tissue. Many experimental studies conducted on human population and animal models (rat) found catechin improved bioavailability of nitric oxide mediated through enhanced expression and activity of endothelial nitric oxide synthase (eNOS) (Pham et al. 2010).

19.3.7 Neuroprotective Effects of Honey Phenolic Compounds

Most of the biochemical insults are triggered by oxidative stress. Common biochemical insults to neural cells are aging, neuroinflammation and cytotoxicity of neurotoxins such as lead metal. Since brain neurons are most of the time active, they require abundant oxygen supply all the time to work smoothly. The presence of high amount of polyunsaturated fatty acids (PUFA) in neural cells makes them highly susceptible to oxidative damage (Schmitt-Schillig et al. 2005). Many studies conducted on free radical scavenging phytochemicals (phenolic acids, flavonoids) found them helpful in activation of important endogenous free radical scavenging enzymes within brain, thus protects the brain from lethal effects of oxidative stress (Esposito et al. 2002; Lau et al. 2005). Many studies conducted on honey polyphenols proved neuroprotective effect that promotes mental faculties of memory and learning while at the same time neutralizes the effects of neurooxidative and neuroinflammatory external agents that cross blood–brain barrier. Many neuroprotective compounds are found in honey but here we are discussing some of them.

Caffeic Acid: Caffeic acid is a well-known ingredient of coffee, some fruits, and also found in honey. Many studies conducted on neuronal cells found its neuroprotective effects mediated through lowering inflammation (Jeong et al. 2011). Caffeic acid protects brain tissue from some neuroinflammatory agents like aluminum-induced over activity of 5-lipoxygenase (5-LOX enzyme which is a key enzyme in generation of inflammatory molecules (leukotrienes) (Yang et al. 2008). It promotes memory and learning faculties of brain slowing down neural degeneration in cerebrum and hippocampus (Yang et al. 2008). Also by the in vivo treatment of caffeic acid, similar effect is also seen in the cerebellum, hypothalamus, and pons. From the above observations, it brings our attention toward the possibility of caffeic acid cross talk with cholinergic signaling and antineurodegenerative effects (Anwar et al. 2012).

Chlorogenic Acid: Chlorogenic acid is generated by the esterification of caffeic acid and is another antioxidant molecule commonly found in honey. Chlorogenic acid shows a dose-dependent protective effect by preventing apoptotic induced damage in pheochromocytoma-12 (PC12) cell lines treated with methyl mercury compound. The protective activity of chlorogenic acid is mediated by alleviating oxidative stress through neutralization of reactive oxygen species and slowing down Caspase-3 activated apoptosis especially within the neural cells (Li et al. 2008). A research conducted on mice using various behavior assessment tests (Y maze, Morris water maze test) found that chlorogenic acid promotes “free will” that is drastically reduced by treatment with scopolamine substance (Kwon et al. 2010). The results of all the tests found as improved memory in the mice. Later on, many ex vivo and in vitro studies on animal nervous system memory recovery acetyl cholinesterase activity in hippocampus and frontal cortex (Kwon et al. 2010). Chlorogenic acid in honey protects our brain neurons from neuroinflammatory and neurotoxicity injuries by decreasing levels of TNF-α and nitric oxide respectively. It is found to be an anti-inflammatory analgesic agent against carrageenan-induced inflammation (dos Santos et al. 2006)

Ferulic Acid: Ferulic acid is another neuroprotective phenolic acid present in honey. It has been observed that ferulic acid decreases neural cell death induced by brain infarction or reperfusion injury while at the same time promotes brain neuron viability in rats. Ferulic acid treated rats experienced lowered neural macrophage proliferation (microglia) along with reduced apoptosis and downregulation ICAM-1 miRNA transcription. The underlying is the reduced level of neuroinflammation and free radical generation lowers the extent of programed neural death (Cheng et al. 2008). Another study found favorable effects of ferulic acid on brain infarction or reperfusion induced apoptosis. For example, ferulic acid possesses neuroprotective property toward nitric oxide (NO) triggered apoptosis that is mediated through p38 protein activated by mitogen-activated protein (MAP) cell signaling (Cheng et al. 2010). It is also seen in the same study that ferulic acid prevents proapoptotic protein translocation like Bax protein, liberation of cytochrome c from intermitochondrial membrane space and does not allow MAP kinase induced p38 protein phosphorylation and increases the number of GABAB1 receptors on brain neurons (Cheng et al. 2010). Thus it may be effective against aging-related neurodegeneration. The cognitive deficiencies in rats are minimized by the treatment with ferulic acid that is found to inhibit acetyl cholinesterase and elevates superoxide dismutase activity (SOD).This treatment also decreases the concentration of glutamate and malondialdehyde in the hippocampus of rats. These studies proposed that the antioxidant properties of the honey polyphenolic compounds may either contribute to the enhancement of the cholinergic signaling system within neuron network of brain or to protecting from neural injury caused due to excitatory neurotransmitters (glutamate) (Luo et al. 2012). Ferulic acid reverses trimethyltin-induced cognitive decline in mice that is added in their diet as well as enhancing the activation of key enzyme involved in acetylcholine synthesis that is choline acetyltransferase (ChAT) in dementia (Kim et al. 2007).

Gallic Acid: Gallic acid inhibits the apoptotic death of cortical neurons in vitro by blocking amyloid beta (25–35)-induced glutamate release and the production of reactive oxygen species (ROS) (Ban et al. 2008). Gallic acid possesses an anti-anxiolytic activity that is proved by its memory-improving effect since anxiety is associated with memory disturbance (Dhingra et al. 2012). Al Mansouri et al. (2012) observed the memory-improving effects of gallic acid. He found that gallic acid protects memory associated neurons from neurotoxicity caused by 6-hydroxydopamine and cerebral oxidative stress that induces memory deficits. Gallic acid improved memory by increasing total antioxidant activity, total thiol pool and glutathione peroxide activity that decreases lipid peroxidation in the hippocampus and striatum (Mansouri et al. 2013). However, we cannot claim that these biochemical findings are entirely responsible for the improvements in memory.

Myricetin: Myricetin is another well-known flavonoid present in honey that has also been reported to be found in honey. Reduction in calcium stimulated oxidative metabolism in brain neurons is found when rats are supplemented with myricetin at a concentration of 3 nM or greater (Oyama et al. 1994). Human neuroblastoma cells experiences neuroprotection from retinoid-induced apoptosis due to retinoid-induced oxidative stress when myricetin is applied to them. The neuroprotective effect of myricetin was reported to be associated with a reduction in lipid peroxidation, myricetin decreases retinoid-stimulated hydrogen peroxide (H_2O_2) generation, and superoxide radical generation (O_2^-) that may cause neural lipid peroxidation. It elevates of the glutathione redox status (Molina-Jiménez et al. 2004). Myricetin is also found to significantly reduce D-galactose-stimulated cognitive damage. The results of this study also found that cognitive impairment is most likely mediated by the extracellular signal-regulated kinase- (ERK-) cyclic AMP response element binding protein (CREB) signaling pathway in the hippocampus (Lei et al. 2012).

Naringenin: Naringenin phenolic compound in honey shows neuroprotective property against some agents lethal to human neurons. Interestingly is a common manifestation of both amyloid beta- and quinolinic acid is free radical-stimulated oxidative stress causing neurotoxicity which is neutralized by antioxidant property of naringenin. For example, it prevents quinolinic acid-stimulated excitotoxicity due to high intracellular calcium levels, NO-mediated neurotoxicity, and, therefore blocks neurodegeneration and cognitive deficits (Braidy et al. 2010). The neurotoxicity produced due to accumulation of amyloid beta protein stimulating free radical generation is also minimized by naringenin (Heo et al. 2004). Intracerebroventricular administration of streptozotocin (Apoptosis inducing agent) mediated impairment in cognitive abilities, memory and learning is found to be reversed by the administration of naringenin (Baluchnejadmojarad and Roghani 2006). Naringenin treatment is found to elevate the levels of endogenous antioxidant enzymes and therefore decreasing the levels of reactive oxygen species (ROS) in neurons. It increases the pool of reduced glutathione (GSH) and the activities of glutathione peroxidase, glutathione reductase, glutathione-S-transferase, SOD, and choline acetyltransferase in the hippocampus in a rat model of Alzheimer's disease (AD-) type neurodegeneration with cognitive impairment (AD-TNDCI). It lowers the degeneration of Choline acetyltransferase (ChAT) positive neurons and impairments in spatial learning and memory (Khan et al. 2012).

19.4 Conclusion

Honey has innumerable health benefits due to the broad spectrum of vital components especially flavonoids and phenolic acids. These phenolic compounds exhibit antimicrobial, anti-inflammatory, and antioxidant properties that make honey a safe, economical, potential therapeutic agent for preventing a wide range of diseases including cancer, diabetes, brain disorders, cardiovascular defects, gastrointestinal disorders, respiratory infections, and arthrosclerosis. Therefore, we recommend for further exhaustive research on honey to unravel its novel therapeutic potentials.

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Role of Honey for Enhancing Performance in Endurance Sports

20

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Abstract

Endurance events are becoming popular globally and at the same time demands higher energy levels to sustain the performance in the form of carbohydrates. Different carbohydrate loading at various stages like pre-competition, and during the exercise has been prescribed for athletes depending upon the nutritional demands of that particular sport. Honey is a natural source of energy rich in carbohydrates and other minerals and vitamins. Carbohydrates like fructose and glucose present in honey and low glycemic index makes it eligible for consumption for endurance athletes. There are limited studies that show the

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M. U. Rehman, S. Majid (eds.), *Therapeutic Applications of Honey and its Phytochemicals*, https://doi.org/10.1007/978-981-15-6799-5_20

honey improves the physical performance among endurance athletes and it seems promising to be ingested as an alternate health drink for such athletes.

Keywords

Endurance · Honey · Performance · Fructose

20.1 Introduction

Endurance events are becoming popular globally and at the same time demands higher energy levels to sustain the performance in the form of carbohydrates. Different carbohydrate loading at various stages like pre-competition, and during the exercise has been prescribed for athletes depending upon the nutritional demands of that particular sport. Honey is a natural source of energy rich in carbohydrates and other minerals and vitamins. Carbohydrates like fructose and glucose present in honey and low glycemic index makes it eligible for consumption for endurance athletes. There are limited studies that show the honey improves the physical performance among endurance athletes and it seems promising to be ingested as an alternate health drink for such athletes.

20.2 Endurance Athletes

Endurance events have become popular globally and participation in such events has also increased, with 3.5 million participants worldwide. Endurance sports these days is not confined to standard marathon races, other races as color runs, obstacle course races and mud runs have also become the part of the sports (Brand-miller and Arcot 2005). To encourage the beginner athletes to take up endurance sports various events are being organized that may last upto 30 min to 2 h (Saris et al. 2003). Additionally, ultra-endurance sports with event duration of 4 h (Costa et al. 2019) to 6 h (Nikolaidis et al. 2018) are also gaining popularity.

Prior studies have demonstrated that endurance exercise put forth the challenges on the human body that may include fatigue, suboptimal nutrition, energy deficit (Costa et al. 2019; Nikolaidis et al. 2018), and potential medical complications. To meet the nutritional demands of endurance among athletes, several studies have been done but the voids in the literature are still there as it seems to be a complex topic for related healthcare professionals, scientists, and nutritionists. European Commission came up with PASSCLAIM with a goal to devise a set of procedures/methods for evaluating scientifically function-enhancing and health related claims for foods and food components (Saris et al. 2003).

20.3 Physiological Demands and Nutritional Demands of Endurance Sports

Carbohydrate (muscle glycogen and blood glucose) are essential for muscle functioning/contraction (Romijn et al. 1993). There are different viewpoints among medical community regarding the ideal intake of carbohydrate among endurance athletes. The Dietitians of Canada (DC), Academy of Nutrition and Dietetics (AND), and the American College of Sports Medicine (ACSM) jointly advocates that 5–7 g per kilogram of bodyweight per day (g/kg/day) of carbohydrates is required for moderate exercise (1 h/day), while 6–10 g/kg/day of carbohydrate is required for moderate to high-intensity exercise (1–3 h/day). Ultra-endurance athletes (4–5 h of moderate to high-intensity exercise every day) with extreme levels of commitment to daily activity demand carbohydrate up to 8–12 g/kg/day (Jäger et al. 2017). Carbohydrate in the form of blood glucose and muscle glycogen is capable of generating more ATP per volume of oxygen (O_2) in comparison to fat (Spriet 2014). However, fatigue, impaired concentration, and reduced work is experienced during prolonged exercise and is often associated with depletion of muscle glycogen and reduced blood glucose concentrations (Jäger et al. 2017; Jeukendrup 2014; Getzin et al. 2017). Athletes very often describe it as a feeling of "hitting the wall," or "bonking." Therefore, high pre-exercise muscle and liver glycogen concentrations are recommended for optimal performance, although it is unlikely that any of these factors alone limits prolonged exercise performance. Glycogen depletion is not considered the only reason of fatigue, there are other factors such as lactate utilization and increased capability to oxidize fat are also proposed (Burke et al. 2011). Dehydration can also lead to impaired endurance performance (Sawka et al. 2007). In order to dissipate the heat generated during exercise sweating takes place. Hence, the nutritional challenge is to prevent major dehydration (>2–3%) and thus contribute to the prevention of fatigue (Shirreffs and Sawka 2011). The American College of Sports Medicine has recommended that excessive dehydration (>2–3% of body weight) should be prevented and at the same time has also warned against drinking in excess of sweating rate (Sawka et al. 2007) to prevent hypernatremia.

20.3.1 Pre-competition

For the events that last more than 90 min, glycogen supercompensation or carbohydrate loading before 36–48 h may improve the performance by 2–3% as compared to low to normal glycogen loading (non-supercompensated) (Jeukendrup 2014). However, events lasting less than 90 min, less or no performance benefit was found after glycogen supercompensation. In supercompensation model one has to deplete glycogen stores with high-intensity exercise bouts prior to high carbohydrate intake in order to double glycogen stores (Bergstrom et al. 1967). Highly trained endurance athletes have shown to achieve supercompensation and may not require going through the depletion phase prior to loading (Burke et al. 2011). It is recommended to load the athletes with carbohydrate rich diet not less than 6 g/kg (Getzin et al.

2017) or at maximum 7–12 g/kg (Jäger et al. 2017) 24 h prior to the event. A single dose of carbohydrate (1–4 g/kg) is recommended for the final 1–4 h prior to the event in order to supercompensate the glycogen stores (Jäger et al. 2017). There are some studies that have shown adverse effect on performance among the athletes when carbohydrate was served 30–60 min before the event performance, the reason could be hypoglycemia and hyperinsulinemia (Kofi Ayittey et al. 2020).

20.3.2 Carbohydrate Ingestion During Exercise and Performance

Endurance exercises that last longer than 2 h may require ingestion of carbohydrate in order to improve exercise capacity and performance, although the exact mechanism is not known (Jeukendrup 2014; Burke et al. 2011). Carbohydrate consumption during endurance exercises of high intensity (>75% VO_{2max}) that last less than 1 h have shown improvement in exercise performance and the underlying mechanism is not only metabolic but it is related to positive afferent signal capable of modifying motor output (Gant et al. 2010).

20.4 Honey

European Communities legislation has defined honey as "the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature" and classification is based on origin or means of production. Honey is considered energy food due to its biochemical properties and nutritional value. Its main composition is of carbohydrates (CHO) i.e., 95–99% with glycemic index (GI) ranging between 32 and 87 (Robert and Ismail 2009), water content ranges from 13.6 to 19.2%, vitamins and minerals. Its composition may vary depending upon the variety of flowers it has been extracted by bees, and climatic variation of that particular region.

General composition (Table 20.1) and Physical properties (Table 20.2) of honey

1. Each 100 g of honey consists of monosaccharides like fructose around 30–45 g, followed by glucose 24–40 g, and sucrose 0.1–4.8 g. Disaccharides around 2–8 g per 100 g of honey which includes maltose, trehalose, etc. It also includes trisaccharides (0.5–1 g), erlose (0.5–6 g), and melezitose <0.1 (Bogdanov et al. 2008).
2. Minerals present in mg/kg of honey e.g. Fe, K, As, Al, Zn, Na, P, Cu, etc. are listed in Table 20.1 (Vanhanen et al. 2011).
3. The pH of honey ranges between 3.67 and 5.04, with conductivity 0.21–1.20 ms/cm (Vanhanen et al. 2011). Phenolic acids and flavonoids present in honey ranges from 199.2 ± 135.23 mg/kg, 46.73 ± 34.16 mg/kg, respectively

Table 20.1 Chemical composition of honey

1. Carbohydrates (g/100 g)	
• Fructose	30.0–5.0
• Glucose	24.0–40.0
• Sucrose	0.1–4.8
• Other disaccharides (trehalose, maltose, etc.)	2.0–8.0
• Melezitose	<0.1
• Erlose	0.5–6.0
• Other trisaccharides	0.5–1.0
2. Minerals (mg/kg)	
• K	34.8–3640
• Ca	7.21–94.3
• B	0.05–0.49
• As	0.04–0.17
• Fe	0.67–3.39
• Cu	0.09–0.70
• Cd	0.01–0.45
• As	0.04–0.17
• Al	0.21–21.3
• Mg	7.52–86.3
• S	13.4–93.9
• P	29.5–255
• Zn	0.20–2.46
3. Others	
• pH	3.67 ± 0.02–5.04 ± 0.01
• Conductivity (mS/cm)	0.21 ± 0.01–1.20 ± 0.02
• Pfund color (mm)	16.4 ± 0.9–100.9 ± 1.1
• Phenolic acids (mg/kg)	199.2 ± 135.23
• Flavonoids (mg/kg)	46.73 ± 34.16
• Antioxidants and anti-inflammatory (invertase, diastase, glucose oxidase, phosphates, catalase)	0.1–3.3%
• Glycemic index (GI)	32–87

Table 20.2 Physical properties of honey

Characteristic	Value
Specific heat (17.4% moist 200 °C)	2.26 kJ/kg/K
Viscosity (17.1% moist 250 °C)	150 P
Specific gravity (17% moist 200 °C)	1.423
Thermal conductivity (17% moist 210 °C) (17% moist 710 °C)	5.36×10^{-5} W/MK 5.95×10^{-5} W/MK
Water activity (aw)	0.5–0.6
Freezing point (15%soln.)	−1.42 to −1.530 °C

(Moniruzzaman et al. 2014). Honey is also having anti-inflammatory and anti-oxidant properties due to the presence of invertase, diastase, glucose oxidase, catalase, etc. (da Silva et al. 2016) (Table 20.1).
4. The glycemic index of honey ranges between 32 and 87 (Robert and Ismail 2009),
5. Physical properties of honey (viscosity, specific gravity, specific heat, thermal conductivity, etc.) are mentioned in Table 20.2 (Arcy et al. 1999).

20.5 Honey as Supplement for Endurance Athletes

Honey is rich in carbohydrates like fructose (30–45%), glucose (24–40%), other monosaccharides, disaccharides, and trisaccharides (Schneider et al. 2013; Bogdanov et al. 2008; Ahmed and Othman 2013). There has been found a variation in the composition of honey and simultaneously significant variation in glycemic index (GI) (refer Table 20.3) of honey and that may further lead to variation in post-prandial insulinemic response. GI values reported in honey ranges from ~32 to 87 depending upon the source of extraction (Bogdanov et al. 2008) is considered due to relative concentration of fructose, higher the fructose to glucose ratio lower is GI values and vice versa. Acacia honey is a fructose rich variety with low GI values ≤55 or moderate GI values 55–69 (Atkinson et al. 2008; Deibert et al. 2010). Carbohydrates serve as a primary source of energy and are essential required for exercise (Jeukendrup 2014). It has been found that higher muscle glycogen loading improves the physical performance during the endurance events lasting for >60–90 min (Close et al. 2016; Hawley et al. 1997). In addition, carbohydrate loading just before or during the event has also shown to sustain and prolong the

Table 20.3 Glycemic index (GI) and glycemic load (GL) for a serving (25 g) of honey (Brand-miller and Arcot 2005; Foster-Powell et al. 2002)

	Honey origin	Fructose g/100 g	GI	AC g/ serving	GL (per serving)
Red gum	Australia	35	46 ± 3	18	8
Yellow box	Australia	46	35 ± 4	18	6
Iron bark	Australia	34	48 ± 3	15	7
Acacia (black locust)	Romania	43	32	21	7
Stringy bark	Australia	52	44 ± 4	21	9
Yapunya	Australia	42	52 ± 5	17	9
Salvation June	Australia	32	64 ± 5	15	10
Commercial blend	Australia	38	62 ± 3	18	11
Honey of unspecified origin	Canada		87 ± 8	21	18
Pure Australia	Australia		58 ± 6	21	12
Commercial blend	Australia	28	72 ± 6	13	9
Average		55	55 ± 5	18	10
Glucose			100		
Sucrose			68 ± 5		

AC available carbohydrate

performance during the endurance events (Coyle et al. 1986; Burke et al. 2011). Carbohydrate consumption could enhance the exercise capacity by sparing glycogen (stored in muscle and liver), blood glucose and acting on central nervous system by facilitating motor output. This effect has been observed in short-term exercise performance when CHO are just swilled around the mouth (Jeukendrup 2014; Burke et al. 2011).

Honey has a reputation of being a rich source of CHO content that may serve as source of energy for sports persons and other exercise population (Bogdanov et al. 2008). The variety of carbohydrates i.e., monosaccharides, disaccharides, and trisacharrides present in honey makes it a favorable source of energy for endurance athletes. Added advantage of honey is because of its low GI value when compared to other health/sports drink available in the market can be consumed by athletes participating in intermittent sports (Hills and Russell 2017; Stevenson et al. 2017). The honey is absorbed at different rates by human body because of variable ratio of fructose to glucose. The fructose having a low GI value gets slowly and evenly absorbed than glucose (high GI) and can act as dietary source of energy for endurance sports when taken before the event. The glucose on the other hand with high GI value is readily absorbed and enters the blood stream at a rapid pace (Abbey and Rankin 2009).

Earnest et al. (2004) compared the effect of honey (0.97/g/kg bw/day), dextrose and placebo gel supplement drink. Subjects performed 64 km cycle time trail (TT) and ingested the assigned respective drinks after every 16 km i.e., 15 g of low GI honey (GI-35), and dextrose of high GI (GI = 100). They found no significant difference between groups for 64 km TT however, the both groups showed an improved performance when compared to the placebo especially in the last 16 km both. Earnest et al. (2004) also supported that low GI honey (1.9 g/kg bw/day) improves the performance (distance covered in time trail) in comparison with placebo. The subjects were asked to perform 60 min run at 65% $VO_{2\,max}$ and during 2 h recovery ingested honey drink and performed a 20 min time trail after that. The amount of CHO administered in this study was low as the pre-exercise prescribed dose of CHO ranges from 1.3 (Kirwan et al. 1998) to 2.67 g/kg bw/day (Nybo 2003). The presence of fructose and glucose in honey may have led to the improvement in the time trails in spite of administered at lower quantity that has shown to improve the total oxidation rate of CHO when compared to glucose alone (Lecoultre et al. 2010). Carbohydrates with low GI has shown to improve athletics performance in long duration events (>20 min) in comparison to shorter version of high-intensity exercise events (Sherman 1998) the reason has been attributed to the sustenance of optimum blood glucose level (Henry and Manickavasagar 2010). During prolonged exercise events low GI CHO releases glucose at steady and slow pace that delays utilization of muscle glycogen (Wu and Williams 2006). In a nutshell ingestion of low GI (e.g., honey) food reduces the glycogen oxidation and favors fat oxidation when compared to intermediate or high GI diet (Bonen et al. 1989), which is considered beneficial during endurance events (Klein et al. 1994; Wolfe et al. 1990). To further support the concept, ingestion of intermediate GI honey yielded no performance benefits in soccer players. Abbey and Rankin (2009) conducted the study the effect of acute intervention of honey (1 g/kg bw/day), sports drink and placebo in soccer players. Subjects ingested the prescribed drinks before and during the soccer match at two intervals (30 min before soccer match and at half time). The observation was attributed to low frequency of CHO ingestion which failed to maintain the blood glucose level especially in the second half of match play where hypoglycemia sets in and glycogenolysis was favored over fat oxidation (Abbey and Rankin 2009). Similarly, Zeederberg et al. (1996) observed no skill enhancement in soccer players after ingestion of CHO beverages (6.9% glucose polymer) before 15 min of the event and during halftime). It was hypothesized that higher rate of ingestion of CHO would be would suffice the energy requirement for the activity (Zeederberg et al. 1996). However, Russell et al. (2014) did not observe any improvement in performance after ingestion of high frequency feeding of CHO (15 min). It seems evident that not only frequency of consumption but also GI value of the administered CHO should be taken into account for improving the endurance among the athletes. To sustain a prolonged activity among athletes honey ingestion seems to prevent hypoglycemia, improves CHO oxidation, and improves endurance.

Honey as nutritional supplement and exercises in combination have shown to regulate hormonal and mineral functions necessary for bone health. There are some studies suggesting improvement in bone health (bone mass, mechanical properties, and enzymes) after consumption of honey (0.37–1 g/kg bw) in conjunction with exercises (Ooi et al. 2011). Presence of vitamin D and K, and minerals (calcium, magnesium, and phosphorous) in honey are considered to enhance bone health by facilitating osteoblastic activity and enhancing cortical architecture (Reid et al. 2014).

20.6 Conclusion

Honey serves as a source of energy rich in carbohydrates, vitamins, and minerals and can be administered to improve the performance in athletic population. Also due to its low GI value and fructose/glucose ratio it can serve as a better fuel for endurance events like marathons, ultra marathons, etc. Low GI honey has shown promising results to enhance the physical performance in endurance athletes as compared to intermediate/high GI honey. There are many questions yet to be answered like when and what amount of honey should be served for different forms of endurance events. At present there are limited studies to support the fact and more research is needed to set the dosiometry besides to study more about its mechanism of action.

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