Dhiraj Kumar
Mohammad Shahid *Editors*

Natural Materials and Products from Insects: Chemistry and Applications

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Preface

Since ancient time, humans are attracted to insects due to their beneficial natural products. Several economically important and traditional insects such as silkworm, honey bee, lac and drosophila, termites, etc. were also used as the best invertebrate model. Additionally, insect-derived products are widely used in biomedical and biotechnology industries. This present book is a compilation of writings focused on natural products from different insects and their general, biomedical, and industrial applications. Chapter "Polyphenols and Flavonoids from Honey: A Special Focus on Diabetes" of the book highlights the action of polyphenols and flavonoids of honey for human wellness, especially diabetes. Chapter "Chemistry and Applications of Lac and Its By-Product" deals with processing, chemistry, and application of lac and its by-products. Chapter "Silk: An Amazing Biomaterial for Future Medication" discusses relevance of silk protein-based engineered biomaterials for the biomedical industries. Chapter "Insect Chitin and Chitosan: Structure, Properties, Production, and Implementation Prospective" illustrates various applications of insect-derived chitin and chitosan along with their chemistry and possibilities of commercial production. Chapter "Recent Advances in the Insect Natural Product Chemistry: Structural Diversity and Their Applications" shows the structural diversity of natural products from arthropods and their potential applications in modern medicinal therapies, validation of their medicinal properties, textile and staining applications along with their identification, and characterizations. Chapter "Silkworm: A Unique Creature for Natural Products" summarizes the usages of silkworms and their by-products with their chemical constituents, biological activities, and biomedical applications. Chapter "Present and Future Prospects on Nutritious Feeding Using Insects" deals with nutritional performance of insect feed/edible insects and their current environmental aspects with socioeconomic reflections. Chapter "Insect Pheromones and Its Applications in Management of Pest Population" attempts to delineate the details of insect pheromones and further elaborate the present status of their implications in management of pest population in different agricultural systems. Chapter "Non-protein Chemical Compounds from Lepidopteran Insect Cocoons", deals with nonprotein chemical compounds from lepidopteran insect cocoons. The content of book will provide a common platform for the entomologist, material scientists, and natural product researchers to develop novel, significant, and accessible products for the mankind and biomedical industries. We strongly hope that this textbook would become a seminal milestone in this research fields and its relevant research domains.

We feel elevated to express our deep sincere regards and a profound sense of gratitude to Prof. Sangeeta Shukla, Vice-Chancellor, Jiwaji University, Gwalior, India, and Prof. Ravindra V. Adivarekar, Head, Department of Fibers and Textile Processing Technology, ICT, Mumbai, for their inspiration and constructive and critical suggestions throughout the tenure of our book editing.

It is a great privilege to express our humble gratitude to all contributing authors of the chapters in the book, who provided support, read, wrote, offered comments, and allowed us to quote their remarks and assisted in the editing, proofreading, and design.

The University Grants Commission, India, is also being greatly acknowledged for granting us editors, Dr. Dhiraj Kumar (No.F.4-2/2006 (BSR)/BL/17-18/0549) and Dr. Mohammad Shahid (No.F.4-2/2006 (BSR)/CH/18-19/0074), Dr. D. S. Kothari Fellowship.

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About the Editors

Dhiraj Kumar holds a doctorate in Animal Science from B.B. Ambedkar University, India and a post-doctorate from Soochow University, China. Currently, he is working as a Dr. D.S. Kothari fellow at Jiwaji University, India. He also served as an Assistant Professor at the Department of Zoology, Guru Ghasidas Vishwavidyalaya, India. Dr. Kumar has published 29 research papers in leading international journals, two books (15000+ edownloads), five book chapters, three international proceedings and one patent (2 filed). He is also a reviewer for numerous SCI journals. Dr. Kumar has received several national & international awards for his contributions in biological science. Dr. Kumar's current research focus is metagenomics, natural products, novel wound healing patches and biomedicines.

Mohammad Shahid received his Ph.D. in Organic Chemistry from the Jamia Millia Islamia, New Delhi (India). He has contributed to 34 publications, including two books and three book chapters. His publications have attracted more than 1500 citations to date. He also coordinated the publication of a special issue of the Research Journal of Textile and Apparel. Dr. Shahid has been the recipient of several prestigious fellowships for his doctoral and postdoctoral research, such as the UGC Central University Ph.D. fellowship, China postdoctoral fellowship (Soochow University, China), Marie Curie individual fellowship (University of Glasgow, UK) and Dr. D S Kothari postdoctoral fellowship. He is currently involved in a project focusing on superhydrophobic finishing of nanocolored textiles at the Department of Fibers and Textile Processing Technology, Institute of Chemical Technology, Matunga, Mumbai. His research interests include natural dyes and dyeing, the chemistry of textile dyeing and functional finishing, functional coatings, and analytical methods for historical dye analysis.

Polyphenols and Flavonoids from Honey: A Special Focus on Diabetes

Visweswara Rao Pasupuleti and Chandra Sekhar Arigela

Abstract Honey is a matrix of vegetal origin processed by various types of bees. Besides physical properties honey constitutes of several minor chemical constituents such as polyphenols and flavonoids. Phenolic compounds or polyphenols are basically products of the secondary metabolism of plants and so a major group of compounds occurring in honey. The colour intensity of honey defines the percentage of polyphenols present in it. The polyphenols are classified into different types of groups due to the presence of a number of phenol rings and their binding capacity to the structural elements. Phenolic acids and flavonoids are the main classes of the polyphenols. Flavonoids may be categorized into several types including flavonols, flavanones, flavones, anthocyanidins and isoflavones due to their dietary significance. The phenolic compounds show a great extent of biological activities such as antioxidant, antimicrobial, anti-diabetic, anticancer and so on. Honey is one of the natural products which helps in decreasing oxidative stress by cleaning up the oxygen free radicals and also decreases blood sugar level. The rise in reactive oxygen species production depends on various factors. One of the factors is the glucose absorption by muscle cells and adipose tissue which contributes to oxidative stress and, thereby, rise in glycogen synthesis and glucose uptake by cells. Insulin resistance is also one of the important aspects that occur through oxidative stress by disturbing the insulin pathway. The honey found to have the mechanistic properties that ameliorate the damages occurred in diabetic condition and thereby provide benefits to the human beings. This chapter clearly sheds light on the action of polyphenols and flavonoids of honey for the human wellness especially on diabetes.

Keywords Honey · Polyphenols · Flavonoids · Anti-diabetic · Cancer · Biological activity

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1 Introduction

The prevalence of chronic diseases including atherosclerosis, cancer, diabetes mellitus, hypertension and oxidative stress-related Alzheimer's is increasing and has become the main cause of death worldwide (Erejuwa et al. 2014). Oxidative stress is an imbalance between the production of extremely reactive molecules and antioxidant defences and causes structural and functional alterations to the proteins, lipids and nucleic acids which again lead to various types of biological problems including carcinogenesis, aging and atherosclerosis (Droge 2002; Beckman and Ames 1998). Therefore, exogenous antioxidants from diet can counteract the toxic effects of free radicals, reducing oxidative damage (Bach-Faig et al. 2011). Indeed, many epidemiological studies indicate that a polyphenol-rich diet is often associated with a reduced prevalence of several chronic pathologies, such as obesity, infections, cardiovascular and neurological illnesses and cancer (Chu et al. 2002). Honey has been used in the long human tradition not only as an alternative natural sugar but also as a drug for its medicinal properties; it has been used in many cultures, including as a remedy for burns, cataracts, ulcers and wound healing (Bogdanov et al. 2008). Only recently, scientific research focused on its attention on the therapeutic effects of honey particularly on its capacity to protect against cardiovascular diseases (Yaghoobi et al. 2008), cancer (Jaganathan and Mandal 2009) and microbial infections (Kwakman and Zaat 2012).

Honey is widely classified as nectar honey or blossom honey (those acquired from plant nectars) and honeydew honey (mainly insect Hemiptera sucking plant exudations from living plant components) (Bogdanov 1997). Furthermore, honeydew honey can also be described as a type of honey generated by crop sap-sucking insects as flower nectar aphids. Honey can also be grouped as mono- or multifloral honey that relies on pollen types. Pollen quantification is based on four methods such as the first one is Dominants pollen types (obtained from total pollen grains greater than 45%, %); the second one is major Secondary pollen types (16–45%); the third one is Minor pollen types (3–15%); and the fourth method is Minor pollen types (<3%). The honey is regarded as monofloral honey if it possessed a dominant type. If the honey is made up of other pollen types, it is grouped as multifloral honey (Feás et al. 2010). Honey includes enzymes like peroxidase, diastase, glucose oxidase, catalase and invertases. Honey also consists of other enzymes bioactive constituents such as ascorbic acid, vitamins, organic acids, amino acids, trace elements of proteins and Maillard reaction products (Song et al. 2012).

The flavour and fragrance just as the constituents of nectar relies upon the plant sources and climatic conditions. The primary part of nectar is dry load as sugars with fructose having most extreme constituent around 32–38% and then glucose in addition to other disaccharides and oligosaccharides; different constituents of nectar incorporate minerals, organic acids, various nutrients and proteins (Bogdanov et al. 2008; Rao et al. 2016; Solayman et al. 2016; Saba et al. 2013). There is also a fluctuating amount of basic minerals which are approximately 0.2% of its dry weight, which changes as indicated by its plant source, condition and preparing strategies.

The significant minerals in the honeys are calcium, magnesium, copper, manganese, potassium, phosphorus, iron, sodium, selenium and zinc. Honey is made out of little amounts of nutrients such as ascorbic acid (vitamin C), thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic corrosive (vitamin B5) and pyridoxine (vitamin B6) (Alvarez-Suarez et al. 2013). Most of complex B is chiefly from pollen and together with ascorbic acid. Different processes including filtration and oxidation processes may influence the honey carried out by glucose oxidase (Ciulu et al. 2011). The proteins of honey are mainly made up of enzymes which are derived from the pollen, nectar and bees. The major enzymes present are diastases, glucose oxidases and invertases. Diastases which are also considered as amylolytic enzymes include α-amylases that are liable for the hydrolysis of the starch chains. The hydrolysis of the starch chains leads to the production of β-amylases and dextrin which are responsible for maltose formation, which is one of the vital factors of honey quality. The second important enzyme present in honey is glucose oxidase which breaks down the glucose into two different products which are gluconic acid and also hydrogen peroxide (H2O2), which is accountable for honey's antimicrobial activity. The invertase can hydrolyse sucrose in glucose and fructose (White 1980). As per the recent predictions, by 2022 the global honey market will reach 2.4 million tons (White and Doner 1980). Turkey, Mexico, the United States, Argentina and China are the main makers of honey.

Phenolic Compounds: Polyphenols are a specific class of chemical compounds which are divided into (a) flavonoids and (b) phenolic acids.

These are secondary plant metabolites which differ from primary and these compounds are characterized by the presence of multiple phenolic groups which are represented by complex structures. Floral origin generally describes the structure of phenols and this can be used as authentication and also classification instrument, particularly in the event of unifloral varieties. Table 1 shows the most prevalent flavonoids and phenolic compounds available in honey (Chan et al. 2013; Kečkeš et al. 2013; Campone et al. 2014; Ranneh et al. 2018; Petretto et al. 2015; Hamdy et al. 2009; Kuś et al. 2016; Ferreres et al. 1994; Campillo et al. 2015; Arráez-Román et al. 2006; Akalın et al. 2017).

Flavonoids are low molecular weight water-soluble natural chemical compounds. These compounds usually indicate at least two phenolic groups (OH), associated with sugars (glycosides), primarily xylose, galactose, rhamnose, arabinose, rutinoside and glucorhamnose; flavonoids are defined as aflycones when they are not associated with sugars. The flavonoids are then classified according to the degree of oxidation of the C ring in flavanols, flavones, flavanonols, flavonols, flavanones, isoflavones, anthocyanins and anthocyanidins. Flavones, flavanols and flavonols are the most abundant in honey (Moniruzzaman et al. 2014). Phenolic acids (phenol carboxylic acids) have a phenolic ring and at least one organic carboxylic acid function; they can be split by composition: C6-C3 (e.g. p-coumaric, ferulic and caffeic acid), C6-C2 (e.g. acetophenones and phenylacetic acids) and C6-C1 (e.g. syringic, vanillic and gallic acids). Most of these compounds are usually associated with the plant's structural elements (cellulose, lignin), but also with other kinds of organic molecules such as glucose, other sugars or flavonoids (Padayachee et al. 2012).

Table 1 The common flavonoids and phenolic compounds in honey (Cianciosi et al. 2018)

Flavonoids		
Apigenin	$C_{15} H_{10} O_5$	AH, TH, STH
Catechin	$C_{15} H_{14} O_6$	TH, PH
Chrysin	$C_{15} H_{10} O_4$	MH, AH, TH, HH, THH, RH H
Galangin	$C_{15} H_{10} O_5$	MH, AH, STH, H
Genistein	$C_{15} H_{10} O_5$	AH
Isorhamnetin	$C_{16} H_{12} O_7$	MH
Kaempferol	$C_{15} H_{10} O_6$	MH, AH, TH, STH, THH, RH
Luteolin	$C_{15} H_{10} O_6$	MH, AH, TH, STH, THH, RH
Myricetin	$C_{15} H_{10} O_8$	AH, HH, THH
Pinobanksin	$C_{15} H_{12} O_5$	MH, AH, STH, RH
Pinocembrin	$C_{15} H_{12} O_4$	MH, AH, STH, RH
Quercetin	$C1_5 H_{10} O_7$	MH, AH, CH, THH
Rutin	$C_{27} H_{30} O_{16}$	STH
Phenolic acids		
2-*cis*,4-*trans*-Abscisic acid	$C_{15}H_{20}O_4$	STH
2-Hydroxycinnamic acid	$C_9H_8O_3$	TH
Caffeic acid	$C_9H_8O_4$	MH, AH, TH, THH
Chlorogenic acid	$C_{16}H_{18}O_9$	AH, HH, THH
Cinnamic acid	$C_9H_8O_2$	TH, STH, CH, HH, THH
Ellagic acid	$C_{14}H_6O_8$	HH
Ferulic acid	$C_{10}H_{10}O_4$	MH, AH, HH, THH
Gallic acid	$C_7H_6O_5$	MH, AH, TH, HH, THH, PH
p-Coumaric acid	$C_9H_8O_3$	MH, AH, TH, HH, THH, RH, PH
p-Hydroxybenzoic acid	$C_7H_6O_3$	CH, HH
Protocatechuic acid	$C_7H_6O_4$	HH, PH
Sinapic acid	$C_{11}H_{12}O_5$	HH
Syringic acid	$C_9H_{10}O_5$	MH, AH, TH, STH, HH, THH
Vanillic acid	$C_8H_8O_4$	AH, HH

MH Manuka honey, *AH* acacia honey, *TH* tualang honey, *STH* strawberry tree honey, *CH* clover honey, *HH* heather honey, *THH* thyme honey, *RH* rosemary honey, *PH* pine honey

1.1 Bioavailability and Metabolism of Honey Polyphenols

Polyphenols in honey play an important role in health beneficiary effects. Currently there are very few studies on bioavailability of honey polyphenols in humans and hence the mechanism of action of honeys has become vital.

A research revealed that after consumption of 1.5 g of honey/kg body weight of two kinds of honey in 40 tropics, the total phenolic plasma content ($P < 0.05$) similar to antioxidant and reduced plasma capacity ($P < 0.05$) (Schramm et al. 2003) endorsed the idea that phenolic honey antioxidants are bioavailable and boost plasma antioxidant activity by enhancing the defence against oxidant stress. While the honey used in this study is supplied with 4-hydroxybenzoic and 4-hydroxycinnamic acids per kg of body weight, HPLC assessment could not verify the plasma concentration of these acids. According to the authors, this could be due to (i) the absorption of less than one-third of these compounds, (ii) the rapid distribution of these compounds into non-plasma body compartments or (iii) the first pass metabolism of the monophenols in the human body.

The absorption of flavonoids, however, appears to be much more complicated, mainly owing to their chemical properties. The existing literature indicates that not only the bacterial enzymes in the intestine (Day et al. 1998) are accountable for beta-hydrolysis of sugar molecules in the flavonoids of O-glycosides. Two endoglycosidases are capable of flavonoid glycoside hydrolysis were also defined in the small intestine of humans, namely, lactase-phlorizin hydrolase (LPH) acting as an alternative hydrolysis step within the epithelial cells of the small intestine (Spencer et al. 1999) and cytosolic glucosidase (CBG) as an alternative hydrolysis in the epithelial cells (Sugihara et al. 1999). LPH has a wide substrate specificity for flavonoid-O-D-glucosides and the released aglycone can then enter the epithelial cells due to its enhanced lipophilicity and closeness to the cell membrane (Day et al. 2000). It was also suggested that for the occurrence of CBG-catalysed hydrolysis, the polar glycosides should be transferred to the epithelial cells, potentially with the participation of the active sodium-dependent glucose transporter 1 (SGLT1) (Gee et al. 2000). Bioavailability and pharmacokinetics studies have shown that some flavonoids can inhibit non-Na + −dependent monosaccharide diffusion in intestinal epithelial cells (Kimmich and Randles 1978). This benefits for monosaccharaides the parallel concentrative Na + −dependent ATPase transport (Sharma et al. 1981). Therefore, the two possible routes by which the glycoside conjugates are hydrolysed and the resultant aglycones cross into enterocytes are LPH/diffusion and transport/CBG (Del Rio et al. 2013). Therefore, LPH/diffusion and transport/CBG are the two possible paths through which the glycoside conjugates are hydrolysed and the resulting aglycones pass into enterocytes (Del Rio et al. 2013). In the case of honey, the presence of the glycosidase enzyme in the bee salivary glands (Sabatier et al. 1992) should also be added producing a hydrolysis of the glycosylated flavonoids and releasing the aglycon form. This partly illustrates the fact that, unlike other phenolics found in foods or drinks, flavonoids in honey were recognized mostly as aglycons and not in their glycosylated form. Phenolic aglycons are more readily absorbed through the gut barrier than their corresponding glycosides by passive diffusion (Scalbert and Williamson 2000) and, therefore, flavonoids present in honey may be more readily bioavailable.

Established Biological Roles of Polyphenols: Oxidative stress from reactive oxygen and nitrogen species has been a cause of many diseases. Antioxidant activities of fruits are due to the phytochemicals such as polyphenols rather than vitamin c and also flavanol constituents with highly conjugated systems in phytochemicals and their hydroxylation products such as the 3-hydroxy groups in flavanols are considered important to exhibit the antioxidant activities either as free radical scavengers or metal chelators (Wang et al. 1996).

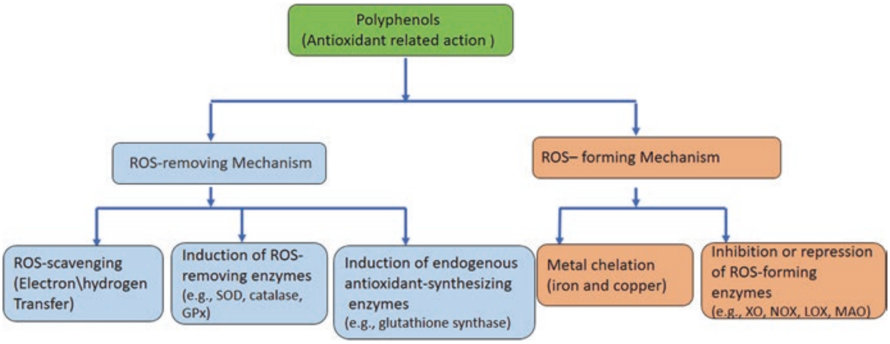

Fig. 1 Antioxidant action of polyphenols from honey (ROS removing and ROS forming mechanisms)

Polyphenols can act as antioxidants via two major modes of action:

(a) ROS removing stage (Fig. 1) consists of a ROS scavenging system, primarily owing to the existence of benzene ring-bound hydroxyl groups capable of donating either one hydrogen atom or one electron to the ROS, thus stabilizing the reactive species (Bors et al. 1990). This mechanism also helps with free radicals like hydroxyl (HO-), superoxide (02-), nitric oxide (NO-) and alkoxyl and peroxyl radicals and non-radicals like peroxynitrite (OONO-) and hypochlorite (ClO-) (Bors et al. 1990). As a result, a polyphenol radical phenoxyl is produced and a stable quinone structure is created after reaction with a second radical (Amic et al. 2007).

(b) ROS formation mechanism: The ROS formation mechanism usually inhibits the production of free radicals which are metal dependant such as superoxide and hydroxyl as well as enzymes producing ROS owing to their direct inhibitory action of polyphenols. It is a systematic process starting with the superoxide dismutase.

ROS-removing enzymes including superoxide dismutase (SOD), catalase (reduction of hydrogen peroxide), glutathione peroxidase (reduction of hydrogen peroxide and lipid hydroperoxide) and endogenous regenerating enzymes such as glutathione reductase (reduction of oxidized glutathione) and also the thioredoxin reductase (reduction of oxidized thioredoxin) play an important role in the antioxidant activity. A well-known mechanism by which polyphenols could be applied to in vitro antioxidant action relates to the capacity of some of these compounds to up-regulate the signalling pathway through activation of Keap1/Nrf2/ARE (Kelch ECH-associated protein 1/NF-E2-associated factor 2/antioxidant reaction elements). Similarly, some polyphenols can stimulate certain stage I and phase II enzymes through the Nrf2 pathway that are involved in the detoxification of xenobiotics (possibly pro-oxidant) (Tsuji et al. 2013). The increase in the activity of all these enzymes follows an NRf2-mediated increase in the expression of their respective coding genes. Cells comprise an amount of endogenously synthesized antioxidant molecules, such as glutathione (GSH) dihydrolipoic acid and ubiquinol, in addition to the above-mentioned ROS-removing enzymes. Among these, the capacity of polyphenols such as quercetin, kaempferol or apigenin to improve the transcription of the gene coding for glutamate cysteine ligase through the Keap1/Nrf2/ARE pathway for elevating the intracellular levels of this thiol has been documented (Sandoval-Acuna et al. 2014).

2 Actions Promoted at the ROS Formation Level Metal Chelation

In addition to the significant known enzymatic sources of superoxide generation (e.g. membrane-bound NOX, cytosolic xanthine oxidase (XO) and I, II and III mitochondrial complexes), redox-active transition metals such as copper and iron can catalyse to form hydroxyl radicals. The free response of iron in mitochondria may happen under circumstances that lead to increased superoxide output. For example, neurotoxins used to generate experimental PD models during the mitochondrial dysfunction of dopaminergic neurons in Parkinson's illness (PD) or those caused by MPP+ or rotenone. In specific the [4Fe–4S] cluster-containing enzymes aconitase (one cluster) and NADH-ubiquinone dehydrogenase (eight clusters) are affected by an intramitochondrial release of iron mediated by superoxide. Some flavonoids (e.g. baicalein, quercetin, myricetin) and some non-flavonoids (e.g. gallic, 2,3-dydroxybenzoic and protocatechuic acids) are particularly involved in chelating iron and copper ions, making them inactive to engage in free radical reactions. The SAR tests of iron-related polyphenols were usually produced for compounds containing catechol and pyrogallol. In the case of flavonoids in specific, it has been suggested that some molecular elements are prevalent and significant to their metal-chelating properties (Serrano et al. 2016) despite the big variations in the metal-chelating ability of various congeners. However, the metal-chelating property of a specified polyphenol becomes important in terms of its antioxidant capacity only if the latter becomes redox inactive in addition to the sequestration of a specified metal. Otherwise, the shaped metal-flavonoid complex could catalyse the development of free radicals, thus acting as a pro-oxidant.

Total evaluation of antioxidant activities of polyphenols is an outcome of the experimental model system. They have helped to comprehend how polyphenols work as antioxidants and play a crucial part in the human system with confined techniques according to studies. Both the advantages and disadvantages of the experimental in vitro chemical antioxidant animal models were discussed evidently and compared for suitability (Prior et al. 2005).

Future polyphenol study is moving in the same direction. Polyphenols are thought to be powerful antioxidants and defences against oxidative stress induced by excessive reactive oxygen species (ROS) produced in vitro after a progressive disease with antioxidant vitamins and enzymes.

3 Anti-diabetic Properties of Honey and Their Molecular Functions

Diabetes mellitus is one of the complex syndromes that has affected around 424.9 million world population in 2017 (https://www.escardio.org/Sub-specialty-communities/European-Association-of-Preventive-Cardiology-(EAPC)/News/global-statistics-on-diabetes). It is characterized by insulin insensitivity causing rise in the blood glucose level. This chronic non-communicable disease also affects the other organs of the body such as heart, eyes, kidneys and majorly the tissue repair process contributing to 10% of adult mortality (Hillage 2010). Acute complications in this disorder may include hyperosmolar, diabetic ketoacidosis and hyperglycaemic state, which may lead to death (Kitabchi et al. 2009). Many studies have supported the anti-diabetic and the hypoglycaemic capacity of honey because of its antioxidant ability. Oxidative stress is closely related to pathogenesis of diabetes developing ROS in various organs and tissues (Folli et al. 2011). The reason is increase of glucose absorption by adipose tissue and muscles resulting in ROS and hence oxidative stress mechanism that affects the synthesis of glycogen and glucose uptake (Fig. 2). Insulin resistance is due to alteration in the insulin signalling pathway caused due to oxidative stress. Honey helps in restoring the glycogen pathway and increases the pancreatic oxidative stress (Erejuwa et al. 2010a).

Research has reported that the honey is a potential diabetic agent due to certain clinical trials on animal models. The range of concentrations tested (0.2, 1.2 and 2.4 g/kg/day body weight) resulted in improved antioxidant effect exerting a hypoglycaemic in streptozotocin-induced diabetic rats (Omotayo et al. 2010). Similarly, glucose level in type 2 diabetes mellitus was also seen reduced when 60% (W/V)

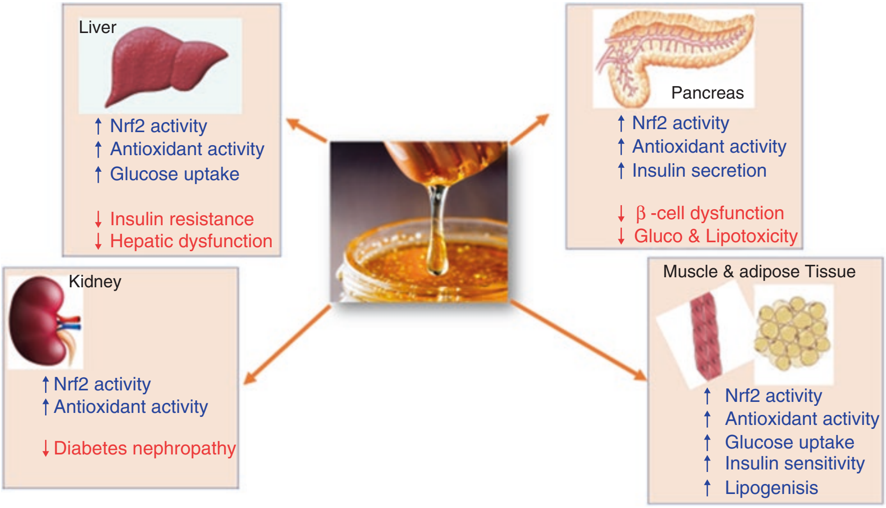

Fig. 2 Effects of honey on various types of tissues including liver, pancreas, kidney, muscle and adipose tissues

honey was administered by inhalation (Al-Waili 2003). The presence of fructose describes the anti-diabetic or hypoglycaemic effect of honey (Erejuwa et al. 2012). Fructose assists to regulate the insulin-response system, resulting in controlled blood glucose level; the main sugars present in honey responsible for glycaemic control are glucose and fructose. Fructose as also shown decreases in the level of hyperglycaemia when tried on various models in diabetic research (Vaisman et al. 2006; Kwon et al. 2008). Evidence suggests that fructose controls blood sugar levels by slowing digestion (Moran and McHugh 1981), prolonging gastric emptying and slowing down the rate of intestinal absorption (Kellett et al. 2008). Glucose is the second major sugar constituent in honey after fructose. Intestinal absorption of fructose is improved in the presence of glucose (Jones et al. 2010) due to a significant collaboration between these molecules which actively influence their absorption. Fructose and glucose have different transporters, GLUT5 (and/or GLUT2) and SGLT1, respectively (Wright et al. 2007). The recruitment of GLUT2 carrier to the brush border membrane caused by increased intestinal fructose may contribute to the synergistic effect of glucose on the absorption of fructose (Jones et al. 2010).

The transport of two sodium ions and one glucose molecule takes place in the brush border membrane of the enterocyte which is responsible for absorption of glucose and fructose. The energy produced by sodium electrochemical potential gradient across the brush border membrane is used to facilitate the glucose accumulation inside of enterocyte against its concentration gradient. Na/K pump in the basolateral membrane helps in the transportation of sodium ion along with glucose, hence maintaining the driving force of glucose transport.

A driving force to transport glucose from cells into the blood via GLUT2 is the accumulation of sugar into enterocytes. A part of the intracellular glucose seems to be taken up into endosomes, as glucose-6-phosphate, and then released into the blood by exocytosis through the basolateral membrane (Uldry and Thorens 2004). Otherwise, after ingestion of fructose, unlike glucose, an increase in the expression levels of GLUT5 mRNA was found (Miyamoto et al. 1993); studies have also stated that there may be a disaccharide-related transport system which considers both fructose and glucose production from the enzymatic hydrolysis of sucrose (Riby et al. 1993). Other evidence suggests that fructose is absorbed via a carrier in the absence of glucose, while in the presence of glucose, fructose is absorbed via a disaccharide-related transport system (Riby et al. 1993). Besides this, passive diffusion across the intestinal epithelium has also been proposed as a possible mechanism (Riby et al. 1993). Studies have shown that glucose improves the transportation and absorption of fructose but not vice versa, increasing the amounts of fructose that reach the liver. According to the results of the investigations exposed above, the potential role of honey against diabetes mellitus is at an early stage, where more specific researches are needed to understand the mechanisms by which it can exert its hypoglycaemic action. Even if these studies are still scarce, they have shown that honey is preferable to the most common sugars or sweeteners, because it is more tolerable both in healthy subjects and in patients with diabetes mellitus.

A particular hypoglycaemic function of the fructose in the liver can also regulate glucose levels. Fructose stimulates phosphorylation enzymes, such as glucokinase, hepatic glucose phosphorylation (Van Schaftingen and Davies 1991). Glycogenolysis is inhibited by the inhibition of these enzymes. Therefore, fructose is regulated throughout the whole metabolism of glycogen and glucose, which show its essential regulatory function for the control of hyperglycaemia (Youn et al. 1987). Another suggested mechanism illustrates that the impact of honey on hypoglycaemic effect can be caused by the role of honey in modulating the pathway for insulin signalling pathway (Batumalaie et al. 2013). The PI3 K/Akt (Ferreres et al. 1994) is a major element in the signals of insulin. It is renowned for its role in multiple substrates modulating features that control cell cycle development, cell survival and cell development. Honey extracts have been recently explored in the pancreas under the hyperglycaemic condition for Akt-activated insulin signalling pathway. Increased levels of NF-kB, MAPK and the serine acid receptor substrate 1 (IRS-1) were found to characterize the development of insulin resistance (Fig. 3). The expression of the Akt and the contents of insulin have been significantly decreased. This research has shown that honey and quercetin extract pretreatment increases insulin resistance and insulin levels. Honey treatments enhance Akt expression and decreased IRS-1 phosphorylation, NF-kB and MAPK expression (Vincent et al. 2013).

Stingless bee honey (SLBH) showed hypoglycaemic effect in partial insulin deficiency rats caused by the combined administration of STZ-nicotinamide, was reported by Aziz et al. (2017). Rats treated 1.0 and 2.0 g/kg bw/day with SLBH for 28 days have shown a significant reduction of fasting blood sugars (FBS) in the amount of the untreated diabetic rats due to a substantial increase in serum insulin level. In the immunohistochemical assessment analysis also considerably improves the expression of catalase antioxidant enzyme (CAT), reducing oxidative stress in pancreas and promoting pancreatic healing (Arráez-Román et al. 2006). SLBH assessment by means of liquid chromatographic mass spectrometry, which was shown to be accountable for stimulating insulin release and improving glucose tolerance in diabetic rats, showed L-phenylalanine in honey (Aziz et al. 2017).

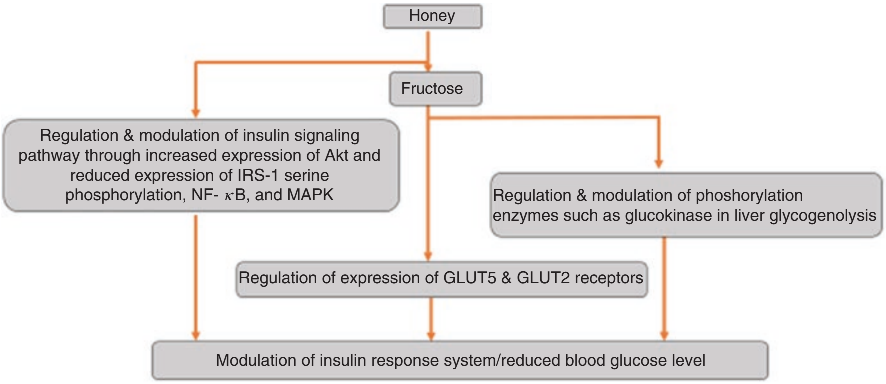

Fig. 3 Mechanisms of anti-diabetic effects of honey. MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor kappa B; Akt = altered PI3 kinase; IRS-1 = insulin receptor substrate 1

Tualang honey (TH) is Malaysian multifloral jungle honey made from *Apis* (or rocky bees), found mainly in the tropical rainforest in hives built in the high branches of *Koompassia excelsa* (known locally as tualang tree) (Ahmed and Othman 2013). The impact of TH on the pancreas of diabetic rats induced by the STZ has been researched. An important down-regulation of pancreas superoxide dismutase (SOD) and MDA ($p > 0.01$) with high pancreas CAT activity ($p < 0.04$) in comparison to diabetic controlling rats was observed with TH (1.0 g/kg/day) provided in diabetic rats over 28 days. The antioxidant effects of TH shielded oxidative damage in the pancreas of diabetic rats, resulting in a substantial enhancement of FBS in diabetic rats compared to diabetic (median (IQR) control: 8.8 (5.8) and 17.9 (2.6) mmol/L) (Erejuwa et al. 2010b). Other types of honey, such as Nigerian honey, also exert a similar hypoglycaemic effect. When given to alloxan-induced diabetic rats for 21 days with a dose of 1.0 and 2.0 g/kg/day, diabetic rats fed with honey had significantly reduced FBS compared with the diabetic control ($p < 0.05$) (Erejuwa et al. 2016). Shorter duration of honey supplementation for a period of 7 days to alloxan-induced diabetic rats also reported a similar trend although the results were not statistically significant (alloxan + honey vs. alloxan alone; FBS mean ± SD; 8.44 ± 1.66 vs. 11.05 ± 2.11 mmol/L, respectively; 2-h postprandial glucose level: 11.57 ± 2.22 vs. 16.45 ± 3.11 mmol/L, respectively) (Bilkisu et al. 2011).

Due to the inherently small expressions and operations of free radical enzymes, the pancreas β-cells are extremely susceptible to oxidative stress (Grankvist et al. 1981). It is well known that antioxidants have a positive impact in defending the pancreas against oxidative stress and harm (Palsamy and Subramanian 2010). We have demonstrated that honey can safeguard the pancreas from oxidative stress and damage. Honey add-ons considerably lowered high MDA concentrations and restored SOD and CAT operations in diabetic rat pancreas (Erejuwa et al. 2010b). The impact on oxidative stress in diabetic rat pancreas was explored and contrasted with that of glibenclamide and honey alone (Erejuwa et al. 2011). The information show that the oxidative stress in the pancreas of diabetic rats was not improved by glibenclamide. In comparison, glibenclamide-honey combined pancreas of diabetic rats has improved CAT activity and restored (Erejuwa et al. 2011) the high concentrations of plasma glucose, and glycosylated haemoglobin was shown to be decreased by antioxidants (Palsamy and Subramanian 2010). Honey supplementation decreased hyperglycaemia in STZ-induced diabetic Sprague-Dawley rats (Erejuwa et al. 2009).

3.1 Anti-diabetic Properties of Flavonoid Compounds and Their Molecular Functions

Honey flavonoids and their action into various biological activities are represented in Fig. 4. The significant bioactive flavonoid in berry is expected to be quercetin and its derivatives to activate MPPA and promote glucose uptake in muscle cells.

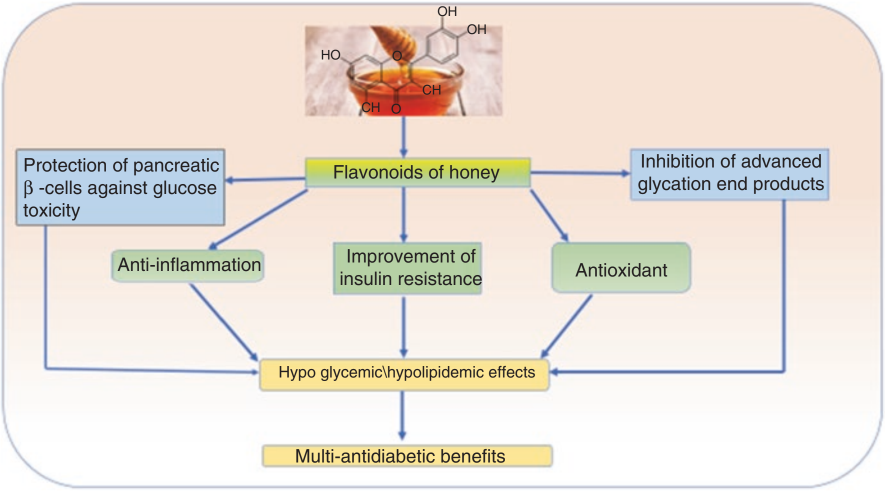

Fig. 4 Schematic representation of anti-diabetic properties of honey flavonoids

Quercetin's anti-diabetic impact has been studied also in streptozotocin (STZ)-caused diabetic mice. Quercetin drug treatment has reduced GLUT 4 and glucokinase stimulation, enhanced liver glucose absorption and reduced gluconeogenesis and hepatic glycogenolysis (Xu et al. 2014). Quercetin injection in STZ-induced diabetic rats resulted to lower hyperglycaemia and increased glucose tolerance, increased activity in hepatic glucokinase activity and reduced plasma cholesterol and triglycerides (Vessal et al. 2003). Both quercetin and the derivative glycoside enhanced glucose-stimulated insulin secretion by controlling NF-kB and ERK 1/2 to protect cells and clonal pancreatic β-cells from oxidative stress. Combined, quercetin is a biomolecule which is efficient to inhibit the digestion of intestinal starch and liver glucose by increasing the absorption of glucose in the skeletal muscle and protecting the islet from pancreatic damage.

Quercetin, apigenin and luteolin inhibited pancreatic β-cell damage caused by cytokine by inhibiting the activation of the nuclear factor kappa B in RINmF5 cells (Kim et al. 2007). Quercetin showed protective impacts by reducing oxidative stress with the protection of streptozotocin (STZ)-induced pancreatic β-cell integrity in rats (Coskun et al. 2005). Isolated rat islets subjected to epicatechin (0.8 mmol/L) or quercetin (0.01 mmol/L) showed increased insulin secretion in the presence of 20 mmol/L glucose by roughly 44%–70% (Hii and Howell 1985).

Through phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3 K) and mitogen-activated protein kinase (MAPK) pathways, rutin was shown to impact glucose uptake in the rat soleus muscle (Kappel et al. 2013). Plasma glucose, haemoglobin A1C (HbA1c, a glycated (beta-N-1-deoxy fructosyl) haemoglobin) and cytokines, including IL-6 and TNF-α, were also reported to decrease rutin. In the STZ-treated diabetic rats fed high-fat diet (HFD/STZ) the flavonoid also resulted in the reestablishment of antioxidant and serum lipid profile (Niture et al. 2014).

In another research, oral isorhamnetin administration did not only substantially inhibit the levels of serum glucose but also decreased the accumulation of sorbitol in STZ-induced red blood cells, lenses and sciatic nerves (Lee et al. 2005). Recent research shows that isorhamnetin glycosides have anti-diabetic action to reduce endoplasmic reticular stress markers and lipid metabolism (An et al. 2011).

Kaempferol obtained from the leaves of *Bauhinia forficata* lowered hyperglycaemia and increased glucose absorption in the rat soleus muscle similar to insulin (Jorge et al. 2004). In vitro outcomes show that kaempferol treatment (10 μM) promotes continued cell viability in β-cells and human islets subjected to hyperglycaemic circumstances, repressed cell apoptosis and decreased caspase 3 activities. These defensive impacts were linked to improved anti-apoptotic AKT expressions (also called protein kinase B (PKB) or Bcl-2), enhanced cAMP signalling and improved insulin secretion and synthesis in ß-cells (Zhang et al. 2011). In addition, the PI3 K and protein kinase C (PKC) pathways and the fresh glucose transducer synthesis in rat soleus muscles stimulated kaempferol. In addition, kaempferol also decreased both TNF-α and IL-1β expression and lipid peroxidation, leading to improved body weight and antioxidation of diabetic rats (Al-Numair et al. 2015). Blood glucose and serum HbA1c concentrations reduced significantly and an enhanced insulin resistance (Coskun et al. 2005) was administered orally with kaempferol. An assessment of the gene expression showed that in liver cells kaempferol reduced the expression of PPAR-β and SREBP-1c. Kaempferol was controlled by SREBP-1c and PPAR-β modulation through AMPK activation for anti-obese and anti-diabetic characteristics (Zhang and Liu 2011).

The glucose-insulin index was decreased by myricetin injected intravenously into genetically obese diabetic rats. Myricetin therapy resulted in an enhanced expression to GLUT4 (Erejuwa et al. 2012; Vaisman et al. 2006) and reduced AKT and insulin receptor substrate phosphorylation 1 (IRS1) (Tzeng et al. 2011). The activity of the synthase I hepatic glucose glycogen and 6-phosphate has also been stimulated by myricetin and adipocytic glucose uptake in rats and an enhanced lipogenesis influenced by elevated insulin levels (Ong and Khoo 1996).

3.2 Anti-diabetic Properties of Phenolic Compounds and Their Molecular Functions

Non-flavonoid phenolics comprise constitutive carbon frameworks, including hydroxycinnamic (C6C3) and hydroxybenzoic (C6C1). Phenolics are well-known compounds with potential antimicrobial activity and, however, their dietary values and anti-diabetic effectiveness have also been examined. Anti-inflammatory activities in various T2DM models were discovered to be associated with decreasing production of IL-1β, IL-8, MCP-1 and COX-2 or iNOS. Some phenolics, like retinopathy, cardiopathy and nephropathy, were also reported to prevent secondary T2DM complication.

Phenolic Acids This class of compounds includes the derivatives of benzoic acid. Hydroxybenzoic acid, cinnamic acid, gallic acid, ellagic acids, caffeic acid, ferulic acid and p- coumaric acids are major compounds of this class. 4-Hydroxybenzoic acid was found to increase peripheral consumption and, therefore, decreased the plasma glucose levels without affecting serum insulin and liver glycogen contents. Hydroxycinnamic acid derivatives, i.e. cinnamic acid (PubChem CID: 637542), p-coumaric acid (PubChem CID: 637542), caffeic acid (PubChem CID: 689043), ferulic acid (PubChem CID: 445858), chlorogenic acid (PubChem CID: 1794427) and rosmarinic acid (PubChem CID: 5281792), possess potent antioxidant and anti-inflammatory properties. These derivatives have been reported to inhibit infiltration of macrophage and activation of NF-μB, reducing expression of type-1 (PAI-1) TNFα, MCP-1 and plasminogen activator inhibitor. These derivatives also prevent differentiation of adipocytes in experimental animals and decrease the lipid profile. Therefore, in diabetes, hyperlipidaemia and obesity, these derivatives are useful (Lau et al. 2013).

Cinnamic acid was also reported to inhibit HMG-CoA reductase and ACA T activities in high cholesterol-fed rats and, hence, reduced the TG and cholesterol levels. It was reported to stimulate the adiponectin secretion and AMPK phosphorylation and, therefore, improved the insulin sensitivity (Tedong et al. 2010). Cinnamic acid also reported to normalize the lipase and angiotensin-converting enzyme (ACE) in high-fat diet rats and increase the diameter of aorta and aortic arch and avoid vasoconstriction comparable to standard drug glibenclamide. Cinnamic acid derivative, i.e. hydroxycinnamic acid, was also reported to inhibit the PTP1B, a major negative regulator of insulin signalling pathway (Alam et al. 2016). Therefore, it exhibits anti-diabetic, anti-obesity and anti-hypertension properties.

Gallic acid (PubChem CID: 370) was reported to increase the expression and secretion of adiponectin by increasing the adipocyte differentiation. It was found to increase the expression of PPAR-γ target proteins and fatty acid binding protein-4. Gallic acid and its derivatives have been discovered to considerably decrease symptoms of depression and oxidative stress.

These findings suggest that gallic acid exerts anti-diabetic effects through adipocyte differentiation and scavenging the free radicals and peroxynitrite (Nayeem et al. 2016). Caffeic acid has been observed to modulate the expression of Nrf2 and thus cause the nuclear translocation of NF-kB and the downstream expression of endothelial adhesion molecule 1 and restore antioxidant concentrations by up-regulating Nrf2/EpRE in human endothelial cells subjected to glucose. It was also observed that caffeic acid affects the apoptosis pathway by down-regulating the expression of caspase enzymes and increasing the Bcl-2 phosphorylation in high glucose-treated cells (Oboh et al. 2015). Caffeic, chlorogenic, ferulic, p-coumaric and cinnamic acids were reported to inhibit triglyceride accumulation in Hepg2 cells and, therefore, useful to treat non-alcoholic fatty liver disease (Oboh et al. 2015). It has also been recorded that ferulic acid acts in the same way as caffeic acid and up-regulates the expression of Nrf2 and inhibits the expression of TNF-α and

IL-1β by inhibiting dose-dependent activation of NF-μB and preventing cell apoptosis and reducing oxidative stress. Ferulic acid treatment also increases the glucose uptake, insulin sensitivity and tolerance in T2DM animal models. It was found to decrease the activity of GS and GK and increase the activity of PEPCK and G-6Pase and restore the glucose levels in a similar fashion of metformin. Moreover, ferulic acid and feruloylated arabinoxylan mono−/oligosaccharides were reported to inhibit GLUT-2 expression by impairing the interaction between SREBP1c, HNF1α, HNF3β and GLUT2 gene promoter and modulate GLUT-4 activity. Ferulic acid was reported to have anti-diabetic properties and significantly reduced the glycated haemoglobin (HbA1c) and lipid peroxidation. It was reported to increase Na+/K + -ATPase levels in high glucose-treated rats and, hence, prevents T2DM. It was also reported to reduce oxidative stress by inhibiting protein carbonylation in GSH-depleted hepatocytes and can be used to inhibit or decrease glyoxal and methylglyoxal-induced hepatotoxicity (Ghosh et al. 2017). Overall, ferulic acid improves the sensitivity of insulin and hepatic glycogenesis and inhibits gluconeogenesis and oxidative stress to maintain homeostasis of glucose in T2DM.

P-Coumaric acid (PubChem CID: 637542) has been discovered to inhibit adipogenesis, GPDH activity and the expression of PPARγ, C/EBPα and leptin and up-regulated the expression of adiponectin in 3 T3-L1 adipocytes. At the same time, it was reported to decrease the cholesterol and triglyceride levels in plasma. It was also reported to inhibit TNF-α-mediated increase in MCP-1, PAI-1 and ROS in adipocytes. Therefore, it increased the secretion of adiponectin and expression of SOD, GSH, GPx and GST in TNF-α-treated adipocytes. Moreover, LD50 was very high and therefore, an increased dose may increase the biological activates. However, its low intestinal absorption is a disadvantage in its utilization (Pei et al. 2016). Chlorogenic acid (PubChem CID: 1794427) had adverse connections with fasting blood glucose, haemoglobin glycated and C-reactive protein. As with other phenolics, it also decreases the activity of HMG-CoA reductase and improves plasma LPL activity, thus reducing plasma cholesterol and triglyceride levels. It was noted that by enhancing GLUT 4 translocation and AMPK and Akt phosphorylation (Meng et al. 2013), glucose transport in the skeletal muscle was stimulated similar to the anti-diabetic drug rosiglitazone.

4 Conclusion

Honey acts as a natural source of cure to many diseases. The medicinal features of polyphenols in honey also lead to antimicrobial, antioxidant and anti-diabetic properties and it stimulates the release of cytokines that help repair tissues in wound healing. Honey plays important part in food industry right from being as a natural sweetener to the food and preservation. Various scientific evidences suggest that the use of honey in disease management and more diversified research to discover innovative medicinal properties as well as its underlying mechanisms of honey.

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Chemistry and Applications of Lac and Its By-Product

K. K. Sharma, A. Roy Chowdhury, and S. Srivastava

Abstract Lac is a natural resin secreted by Indian lac insect, *Kerria lacca* (Kerr), which thrives on the tender twigs of specific host trees. Lac is collected as the minor forest produce and cultivated since time immemorial in India, and lac cultivation is an important source of income for livelihood of the forest and sub-forest dwellers of central and eastern India. Lac still enjoys the monopoly of non-toxic, biodegradable, natural and safe resin and used for applications in pharmaceuticals, cosmetics and surface coatings for variety of uses in industries. India is the largest producer, processor and exporter of the lac, having average production of 20, 000 tons for the last 5 years. Organized research on lac was started in India after realization of its commercial potential worldwide. Research as well as documentation on all aspects of production, processing, product development and use diversification of lac has led to generation of authentic information. During processing of lac, various economically important by-products are obtained. The major component of lac is resin (65%) followed by two important by-products, *viz*. lac dye (1%) and lac wax (5–6%). The lac resin is processed into various value-added forms as per the need of the different industrial applications. Similarly, the dye and wax find its application in various sectors such as pharmaceutical, textile, etc. This chapter covers processing, chemistry of lac and its by-products and industrial and other applications, and also on outlining the future scope of the work.

Keywords Lac resin · By-product · Lac dye · Lac wax · Chemical constituents · Application

1 Introduction

Lac is derived from the Sanskrit word '*laksha*' which means 100,000 and refers to the vast swarms of insect larvae that cover twigs of host trees during brood season. Lac is a general term used in the trade for all forms of natural resin, which is secreted

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by the tiny lac insects on certain host trees, principally found in India, Thailand, China and Indonesia. Lac history can be traced back to Vedic period (c.1500–500 BC). Atharva Veda devotes a chapter containing vivid description of ‘*laksha*’ (lac insect) and its use in invigorating potion and wound healing. In the great epic, Mahabharata, Purochak constructed ‘Lakshagriha’ (house of lac) at the instance of Kauravas for killing Pandavas. The works of a number of foreign travellers reveal the trade of lac from India to a number of countries during post-Vedic and medieval periods.

Lac is a natural resin secreted mainly by Indian lac insect, *K. lacca* (Kerr), which thrives on the tender twigs of specific host trees, *viz. palash* (*Butea monosperma*), *ber* (*Ziziphus mauritiana*), *kusum* (*Schleichera oleosa*), *Flemingia semialata*, *Ficus* spp., etc. *Rangeeni* and *kusmi* are the two strains of lac insect which are classified based on (i) preference of the insect for specific host plants, (ii) time taken to complete one life cycle and (iii) quality of the resin produced. Each strain gives two crops in a year. Raw lac is the source of three valuable products, i.e. resin, dye and wax. The major crop obtained from *rangeeni* host principally from *palash* and *ber* in summer is called the *baisakhi* crop. The subsequent crop from these hosts maturing and collected in October–November is called *katki* crop. Similarly, the crops obtained from *kusum* trees in June–July and January–February are called *jethwi* and *aghani respectively.*

On the basis of survey in the markets of different lac-producing districts, the estimated national production of sticklac during 2015–2016 was approximately 18,746 tons comprising *rangeeni* (7597 tons) and *kusmi* (11,149 tons) sticklac. Among the lac-growing states, Jharkhand ranks first followed by Chhattisgarh, Madhya Pradesh, Maharashtra and Odisha. These five states contribute around 93% of the national lac production. Contribution of Jharkhand in national lac production is about 53% followed by Chhattisgarh (17%), Madhya Pradesh (12%), Maharashtra (8%) and Odisha (3%). Among the different cropping seasons, *jethw*i crop was ranked first with the contribution of 32% followed by *aghani* (27%), *baisakhi* (24%) and *katki* (17%) in total lac production (Yogi et al. 2018).

Lac encrustations on twigs of host trees are removed either by scrapping manually or by machines. Lac resin, thus, obtained is known as sticklac. It contains resin, wax, dye, insect body, bark of host trees as well as other impurities. This is, therefore, refined to obtain purified material of commerce (called shellac). The lac resin is a polyester complex of long-chain hydroxy fatty acids and sesquiterpenic acids. Indian lac dye is a mixture of at least five anthraquinone derivatives called laccaic acids. Lac wax is a complex mixture of long-chain acids, alcohols, esters and hydrocarbons.

In the present chapter, we are mainly dealing with the chemistry and various applications of lac and its by-products.

2 Processing of Lac

The process of purification of lac, in general, consists of two steps. The first step involves grinding and washing of sticklac or raw lac to remove sand and wood chips, followed by drying of resin. This semi-refined form is called seedlac. The pure resin, shellac, is then obtained by hot filtration, either by the country (*bhatta*) process or in mechanized factories. The purified lac in the form of button obtained from the country process is called the button lac. Shellac obtained/processed in mechanized factories is called machine-made shellac. The lac can be used in other forms like dewaxed decolorized lac (DDL) and bleached lac. Dewaxing of lac is usually done by dissolving it in cold alcohol (optimum 10%), in which wax is insoluble, filtering to separate wax and reclaiming lac after distilling of solvent and for preparation of decolourized lac; refluxing the lac with suitable solvent and pure and dry grade of activated carbon was found quite effective. Bleached lac is one of the most important lac-based products for which there is a constant demand in the western markets. The number of methods of bleaching of lac is available in the literature, but the most commercially adopted methods involve bleaching with sodium hypochlorite (NaOCl) with some recent modification (Srivastava et al. 2015). During the processing of sticklac into seedlac and then seedlac to shellac, certain valuable by-products are obtained and presented in Fig. 1. They contain a good amount of lac resin. Several methods have been developed for the utilization of these by-products. The main by-products are *molamma*, *kiri*, *passewa*, etc. These are also exported at a comparatively lower price than seedlac and shellac. The by-products obtained during washing of sticklac are lac dye and lac wax. Both of these are valuable by-products of lac industries having different applications (Goswami and Sarkar 2010a, b).

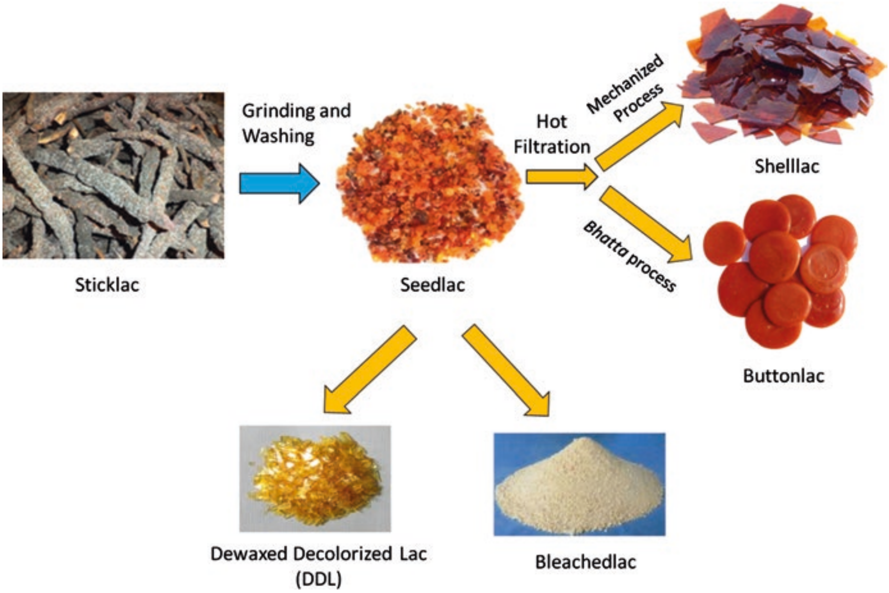


Fig. 1 Schematic diagram of processing of lac and its related products

Table 1 Constituents present in different forms of lac

Constituents	% in sticklac	% in seedlac	% in shellac
Resin	68	88.5	90.9
Dye	10	2.5	0.5
Wax	6.0	4.5	4.0
Gluten	5.5	2.0	2.8
Foreign bodies	6.5	–	–
Impurities	4.0	2.5	1.8

Source: Hatchett as cited by Bose et al. (1963)

3 Chemical Constituents of Lac

Raw lac (sticklac) contains resin, wax, dye, insect body, bark of host trees as well as other impurities. This is, therefore, refined to obtain purified material of commerce (called shellac). The chemical composition and constituents of shellac have been discussed elsewhere. The lac resin is a polyester complex of long-chain hydroxy fatty acids and sesquiterpenic acids. Indian lac dye is a mixture of at least five anthraquinone derivatives called laccaic acids. Lac wax is a complex mixture of long-chain acids, alcohols, esters and hydrocarbons. On the basis of the earlier literature, the constituents present in sticklac, seedlac and shellac have wide variations and are presented as follows (Table 1).

4 Chemistry of Lac Resin

The chemistry of lac resin is very complex and at the same time extremely fascinating (Bose et al. 1963). Commendable results could only be achieved in the 1960s with the techniques such as column, paper, thin layer and gas-liquid chromatography and spectroscopic as well as conventional methods.

It is believed that the resin on an average has five free hydroxyls, one free carboxyl, one aldehyde partly free and partly combined and a point of unsaturation, and linkages present are esters, acylal, acetal and ether. The oxidation of the resin with periodic acid (Sengupta 1964) has shown that one-third of the vicinal hydroxyl groups, in terms of aleuritic acid (a major constituent acid), is free and the rest are in combined form. The oxidation affords in the case of aleuritic acid, ω-hydroxyheptan-1-al and azelaic semi-aldehyde. But after oxidation (Sengupta 1964; Madhav et al. 1967) of the resin no appreciable amount of either of the degradation products could be isolated. This suggested that most probably in this part of aleuritic acid, the carboxyl and the primarily hydroxyl groups are not free. The rest of the aleuritic acid appears linked up at least through one of the vicinal hydroxyl groups.

The resin is not a chemical entity and that it is composed of at least seven components (Sengupta 1970) was made clear by its successful resolution into its components by thin layer chromatography over silica gel. It can be broadly resolved into two fractions by diethyl ether, an insoluble solid mass (~75%) called hard resin and a soluble soft mass (~25%) called soft resin. Sukh Dev and coworkers (Upadhye et al. 1970; Singh et al. 1974) succeeded for the first time in isolating a few pure fractions by fractional precipitation from hard and soft resins. Sankaranarayanan and Kunhunny (1962) isolated a neutral fraction from soft resin, which was believed to be a lactone with one free hydroxyl group having empirical formula $C_{21}H_{37}O_3$. Later, Banerjee and Sengupta (1965) established that the neutral fraction, m.p.49 °C, is not a chemical entity but a mixture of at least seven components. Prasad and Sengupta (1972, 1975) have fractionated hard resin into six fractions through the formation of urea complexes. A few of these appeared to be essentially pure.

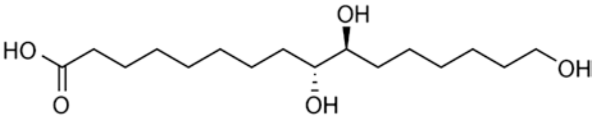
The constituent acids of the resin and its fractions fall under two categories: (i) hydroxy fatty acids and (ii) hydroxy terpenic acids having the rare cedrene skeleton. The former was least soluble and the latter was highly soluble in water. The most successful method of isolation of these constituent acids is by alkaline hydrolysis. A certain portion of them was found present in uncombined state (Bose et al. 1963). The investigators have taken either shellac, hard resin or its various fractions such as soft resin, pure and neutral fractions for study and the results obtained are described regarding isolation and characterization of various constituent acids.

4.1 Aleuritic Acid

This is readily obtained as sodium salt from alkaline hydrolysate. The acid (m.p. 100–101 °C) was first isolated by Tschirch and Farner (as cited in Bose et al. 1963) and was characterized as threo-9,10,16-trihydroxypalmitic acid (Fig. 2) by researchers. The structure was re-examined by Sengupta (1970) by chromatographic techniques after subjecting the acid to oxidation with periodic acid. The resulting acidic semi-aldehyde and the neutral semi-aldehyde after further oxidation and methylation were identified by GLC as dimethyl azelate and pimelate, respectively, thus confirming the envisaged structure (I). The first conversion of the natural threo acid to erythro isomer (m.p. 126 °C) was through the tribromo derivative (as cited in Bose et al. 1963).

The lac resin is composed of aleuritic acid and the hydroxy terpenic acids. There are terpenic acids in the form of butolic acid, shellolic and related acids, jalaric and laccijalaric acids and other minor acids presented in variable quantities. The brief details of the terpenic acids are given as follows.

Fig. 2 Aleuritic acid



4.2 Butolic Acid

Butolic acid (m.p. 58-590C), first isolated by Sengupta and Bose (as cited in Bose et al. 1963), was believed to be 6-hydroxypentadecanoic acid. Later, Christie et al. (1963) and Wadia et al. (1969) independently proved it to be 6-hydroxytetradecanoic acid.

4.3 Minor Acids

Christie et al. (1964) isolated and identified a number of acids, which are present in minor quantities in lac hydrolysate. These are tetra-, hexa- and octa-decenoic acids, 16-hydroxyhexadec-cis-9-enoic acid, threo-9,10-dihydroxytetradecanoic acid and threo-9,10-dihydroxyhexadecanoic acid.

Aleuritic, butolic and all other non-hydroxy, hydroxy- and oxo-fatty acids except 9,10-dihydroxyhexadecanoic acid were isolated from soft resin by Agarwal et al. (1976). Another dihydroxy acid was also isolated which was confirmed to be 10,16-dihydroxyhexadecanoic acid. All the above non-, mono-, di- and tri-hydroxy acids along with the oxo-acid were found present in free state in shellac/seedlac (Sengupta 1964) to the extent of nearly 6%.

4.4 Shellolic and Related Acids

Shellolic acid was first isolated by Harries and Nagel (as cited in Bose et al. 1963) and is an unsaturated dihydroxy dibasic hydroaromatic acid having molecular formula $C_{15}H_{20}O_6$. Yates and Field (1960) correctly proposed that it is a sesquiterpene with rare cedrene skeleton.

An isomer of shellolic acid was also isolated (as cited in Bose et al. 1963) and may be similar to the one mentioned by Kirk et al. (as cited in Bose et al. 1963). It was later identified as the epimer of shellolic acid and named epishellolic acid (as cited in Bose et al. 1963).

4.5 Jalaric Acid

An aldehydic acid was isolated by Sengupta (1954) from shellac as well as from soft resin which he believed to be lactonic in nature. Almost simultaneously Kamath and Potnis (1955) also separated a similar acid from jalari (*Shorea talura*) seedlac which they named as jalaric acid. It is believed to be present to the extent of nearly 1% in the free state (Sengupta 1954). Pure jalaric acid (m.p. 178–180 °C) was later obtained by Wadia et al. (1969) and has the molecular formula $C_{15}H_{20}O_5$. It contains one carbonyl, one carboxyl and two hydroxyl groups and an α,β-unsaturated carboxyl group.

4.6 *Laksholic and Epilaksholic Acids*

During the preparation of shellolic acid in large scale by the method of Cookson et al. (1962a, b), Wadia et al. (1969) noticed the presence of two more new acids which after isolation were termed laksholic (m.p.181–183 °C) and epilaksholic (m.p. 201–203 °C) acids. Both the acids were analysed for $C_{15}H_{22}O_5$ and titrated for monobasic acids. In addition to OH groups, the α,β-unsaturated carboxyl function was also present in the structure.

4.7 *Laccijalaric, Laccishellolic, Laccilaksholic, Epilaccishollolic and Epilaccilaksholic Acids*

Singh et al. (1969) isolated two more acids (m.p.s. 196–198 °C and 235–236 °C) whose dimethyl esters were analysed for $C_{17}H_{24}O_5$ (m.p.s. 85–87 °C and 94–95 °C). Based on spectral data it was deduced the structures of these two acids, the first acid being the epimer of the second and termed them laccishellolic and epilaccishollolic acid, respectively.

The cedrene skeleton for shellolic acid (and related acids) was well established by confirmatory degradative and X-ray studies. The cedrene skeleton for laccijalaric acid and related acids has also been well established by Sukh Dev (1974).

5 Structure of Lac Resin

Though at present almost all of the constituent acids of lac resin are known, it is not yet fully known how these are linked up to form this versatile resin. Earlier investigators have put forward some arbitrary structures but none satisfy chemical and physical properties of the resin. An attempt was made by Sukh Dev (1974) to put forward structures of some pure components of the resin. They had isolated a pure fraction (m.w. 2095 ± 110) in a yield of ~12% from hard resin. By means of alkaline silver oxide hydrolysis/oxidation, esterification, formylation and GLC analyses of this, they found that for every one mole of laccijalaric acid approximately three moles of jalaric acid and four moles of aleuritic acid are generated (Sharma et al. 2014). Thus they concluded that in pure lac resin there are aleuritic acid and terpenic acids (jalaric/laccijalaric acid and derived dicarboxylic acids) in a ratio of 1:1.

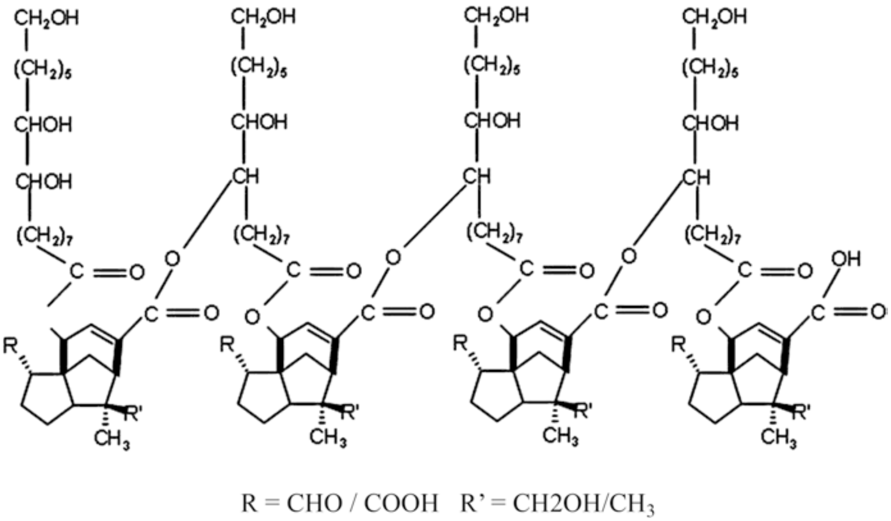

R = CHO / COOH R' = CH2OH/CH$_3$

Fig. 3 Proposed structure of lac resin

Of the four terpenic acids, there are three jalaric/epishellolic acid and one laccijalaric/epilaccishollolic acid (Fig. 3). This molecule should have a molecular weight of 2194, which was in close agreement with the observed value.

6 Applications of Lac Resin

Lac possesses innumerable applications in various fields; the diversity is unmatched by any single resin natural or synthetic. This consists of traditional fields, which are being practiced by artisans through centuries, and researchers around the world have developed many more (Goswami and Sarkar 2010a, b).

More than 50% consumption of shellac is in the surface coatings. The age-old applications of shellac in surface coatings are believed to be due to the following qualities:

- Its ability to produce smooth, decorative and durable films from alcoholic solutions, which dry rapidly.
- Excellent adhesion and bonding material for a wide variety of surfaces, exhibiting high gloss, hardness.
- Ultraviolet resistance of films.
- Excellent compatibility with cellulosic materials and hence capable of yielding laminated products.
- Superb thermal plasticity, ready fusibility coupled with ability to absorb large amount of fillers.
- Ability of film formation of alkaline solution.

Table 2 Major applications of shellac

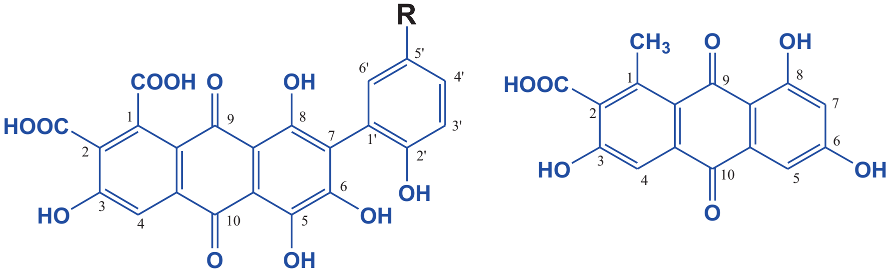
Field of application	Specific application area
Paint and varnishes	Wood finishing, metal foil, sealers, leather, rubber, car tyres
Printing ink	Felt pen inks, binder for flexographic inks for paper, cardboard, non-toxic printing ink for food packaging
Electronics	Lamps, fluorescent lamps, insulating agent for parts, insulating varnish Insulation, adhesion, laminate, lamp manufacture, glue for joining metal with glass, isolator coating, sockets of electrical lamps, PCB coating, etc.
Polishes	Fruits, furniture, floors, shoes, stain sealer, wallboard primer, knot and sap sealer on wood
Tapes	Masking, adhesive paper tape
Pharmaceuticals	Coating for tablets; enteric (digestive fluid proof) coating for tablets, pills, etc. Removing agent for medicinal odour. Enteric pills for sustained release medication
Food	Chestnuts, healthy foods, glazing agent for chocolates and sweets, protective coating for oranges, lemons, apples, etc. Binding agent for stamp inks for lemons, eggs and cheese. Barrier coating for feeds and seeds. Protective candy coating or glazes on candies. Coating of apples and other fruits. Coating of food materials
Cosmetics	Additive and binder for manicure, mascara, eye shadow and conditioning shampoo for personal care. Setting agent for hairspray, microencapsulation for perfumes for longer stay
Rubstones	Grinding wheel
Adhesive	Crystals, precious stones, lenses
Leather	Protection, polishes for leather finishing and stiffening of leather
Wood products	Finishes, primers, polishes and sealers for the harmful gases (formalin, etc.) that evaporate from building materials and protective coatings for wood
Others	Felt hats, pyrotechnics, gunpowder, strippable paints, cards, stiffening felt in hat manufacture and also in textile industries

- Unique dielectric properties.
- Non-toxic nature and renewable resources.

Major applications of shellac in different fields are depicted as follows (Table 2).

7 Lac Dye

Lac dye is secreted by the lac insects, *Kerria* spp. (family, Tachardiidae; order, Homoptera), feeding on the sap of specific host tree, allegedly to protect themselves from harmful UV radiations of the sun. It is obtained from sticklac. Laccaic acid is the principal colour imparting component of lac dye. It has been popularly used for dyeing silk and leather and in cosmetics in ancient Chinese and Indian civilizations.



Laccaic acid A, B, C & E

A: R= $CH_2CH_2NHCOCH_3$
B: R= CH_2CH_2OH
C: R= $CH_2CH(NH_2)COOH$
E: R= $CH_2CH_2NH_2$

Laccaic acid D

Fig. 4 Structure of laccaic acid

Lac dye is now legally registered as a natural food additive in a number of countries; the Chinese National Standard, CNS, No. is 08.104; the Korean Food and Drug Administration, KFDA, No. 13, and the Japanese no. 394. In India, Indian Institute of Natural Resins and Gums, Ranchi has transferred technology for manufacturing for technical grade lac and also developed a plant for production of food grade lac to expand the value-added product for more export (Srivastava and Baboo 2010).

Pure dye is sparingly soluble in water and is orange red in acidic pH and reddish violet in alkaline pH. In alkaline solutions, it decomposes rapidly (Srivastava et al. 2013). Its principal colour imparting component is laccaic acid, a hydroxyanthraquinone carboxylic acid (Fig. 4). Lac dye is a mixture of at least five closely related pure compounds all being anthraquinone derivatives designated as laccaic acid A, B, C, D and E (Chairat 2009; Pandhare et al. 1966, 1967, 1969; Rama Rao et al. 1968; Bhide et al. 1969; Mehandale et al. 1968; Venkataraman and Rama Rao 1972).

7.1 Extraction of Lac Dye

The scraped lac is processed (crushed and washed in desired uniform size) to get refined product known as seedlac. During this crushing and washing, the lac dye gets solubilized in water which in turn is processed to obtain the crystallized dye. The red colour of the resin comes from the insects. Lac dye is obtained during washing of sticklac for preparing seedlac. Pure dye is sparingly soluble in water, is orange red in acidic medium and reddish violet in alkaline medium. In alkaline solutions, it decomposes rapidly (Srivastava et al. 2010).

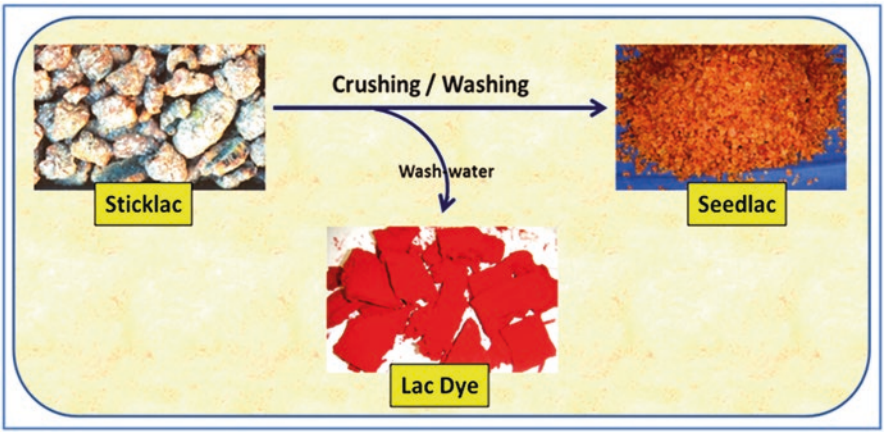


Fig. 5 Extraction of technical grade lac dye

7.1.1 Technical Grade Lac Dye

Sticklac wash water is acidified with sulphuric acid/hydrochloric acid (0.1% on the vol. of water) and the precipitate is allowed to settle in tanks. The supernatant clear liquor is collected by filtration. The acid precipitated sludge is separately extracted thrice with boiling water for removal of dye and washings are mixed with previous collections (Srivastava et al. 2010).

The mixed filtrate is then treated with calcium carbonate or quicklime till the liquor becomes almost colourless. The calcium salt of dye, which separates out, is collected by filtration and washed with water. The cold suspension of calcium salt of lac dye in water is acidified with 10% aqueous acid solution and kept for 7–10 days at room temperature when the dye crystallizes out (Ghosh et al. 1964; Ghosh and Sengupta 1977). It is filtered, washed and dried (yield approx. 0.5% on the weight of sticklac, dye content 80–90% depending on the quality and age of sticklac (Fig. 5).

7.1.2 Pure (Food) Grade Lac Dye

Technical grade dye is further purified by dissolving it in mild alkali, filtering the insolubles, acidifying the filtrate and, finally, keeping it for 8 days for crystallization. The crystallized dye is filtered, washed and dried (yield approx. 0.25% on the weight of sticklac, purity 99% and above). The purified lac dye is bright red. It is sparingly soluble in cold water (0.13–0.14%) but completely soluble in dimethyl formamide. It can be used for colouring food materials as it gives deep orange-red colour in aqueous solution like colour of beverages and cold drinks available in the market (Pandhare et al. 1969).

7.2 Uses of Lac Dye

Lac dye is non-toxic, principal red dye, hence incorporated in the manufacture of skin creams, food and drug manufacturing and textile industries. The use of lac in India probably dates back many centuries. From ancient times, lac dye has been employed in India as a skin cosmetic and for the dyeing of wool and silk, while China has a tradition of usage for leather dyeing. The colour of the lac dye can be modified by the appropriate choice of mordant from violet to red and brown.

Lac dye is used in classical Persian carpets and to dye silk, yielding a range of colours from rose to purple. During the eighteenth century India exported large quantities of lac dye (Hunter 2000), exporting 901,649 kg in the year 1868–1969. It is also known as Natural Red 25 (CI Number 75450) in international trade (Tanaka 1997). In China, pure lac dye (obtained from technical grade) is extensively used in a number of food products as per the national food safety standards, Health Ministry of China (GB 2760–2011; Ling 2003; Lu et al. 2007; Zheng et al. 2003). These products include fruit beverages, vegetable juice (pulp), fruit-flavoured beverages, soda pop, compound seasonings, jam, cocoa products and fillings for bakery wares, imitation wine, chocolates and chocolate products. It is in the approved food additive list of China (CNS No. 08.104), Japan (Natural additive 394) and Korea (Natural Additive 13). To be used as food additive, lac dye must conform to the requirements of standard specification of respective countries, e.g. GB 2760–2011 in China; Japan external trade organization specifications and standards for foods, food additives, 2011; and KFDA in Korea (KFDA 2019). Recently methyl derivatives of lac dye have been reported as potent antifungal and antibacterial agents (Srivastava et al. 2017b).

As per the reports from Directorate of Economics and Statistics, MoA, Govt. of India, India produced about 17,000 tons of lac in the year 2014–2015 (Yogi et al. 2017). Processing of this raw lac may yield around 200 tons of lac dye, majority of which is lost during washing. Some processors use the partially pure form (technical grade) of dye for utilization as textile dye, while other processing industries dispose the sludge of washing as manure to villagers. Thus, an enormous potential exists for utilization of this by-product of lac factories. Even if half of the potential is exploited, then it will be possible to turn trade of lac dye into profitable business of specialty product with assured foreign market. Furthermore, because of the ban on potentially carcinogenic red dyes Sudan IV, Sudan I and few others, there is a great demand of red colour dyes in food industry. The manufacturers are working hard to meet new laws of safety approval by recognized agencies such as JECFA, EFSA and others, to avoid use of unsafe pigments. Being natural and biodegradable, lac dye has great potential to replace currently used synthetic dyes.

Fig. 6 Lac wax

8 Lac Wax

Lac wax is a constituent of natural resin lac. It is secreted by the lac insect along with the resin, in the form of thin white filaments. It is generally found to the extent of nearly 4–5% in seedlac, 3–5% in shellac and slightly higher percentage in sticklac (5–6%).

Lac wax is recovered directly during processing of sticklac and also during the preparation of solvent-based dewaxed shellac generally called shellac wax (Fig. 6).

8.1 Chemical Constituents of Lac Wax

Lac wax is complex mixture of long-chain acids, alcohols, esters and hydrocarbons. Alcoholic components are mixtures of n-primary alcohols, 26–34 carbon atoms (Srivastava et al. 2010).

Tschirch and Schaffer (1926) observed that about 85% of the wax is soluble in boiling alcohol and wax melts at 80–81 °C. An alcohol, m.p.79 °C, and acid, m.p.78 °C, were separated from the wax. Based on the investigations of Tschirch and Schaffer (1926), the following were the conclusions:

1. About 85% of lac wax fraction is soluble in boiling alcohol and consists of an alcohol $C_{25}H_{51}OH$ and an acid $C_{26}H_{52}O_2$.
2. A boiling alcohol-insoluble fraction, consisting about 15%, is an ester of alcohol $C_{32}H_{66}O$ and acid $C_{32}H_{64}O_2$.
3. About 2% of wax is reported to be a hydrocarbon $C_{25}H_{52}$.

It was found that yields of pure products were small in most cases, indicating that there was a large portion of the material left uninvestigated. Warth (1956) suggested following composition (Table 3).

Table 3 Composition of resin-free shellac wax

Esters of wax acids	80–82%
Ceryl lignocerate (m.p. 79 °C)	
Ceryl cerotate (m.p. 84 °C)	
Lacceryl lacceroate (10–12%)	
Ceryl aleuritate (1% or less)	
Free wax acids	10–14%
Lacceroic acid (m.p. 95–96 °C)	
Cerotic acid	
Free wax alcohols	Less than 1%
Neoceryl alcohol (tachardiacerol, m.p. 80.1 °C, Tschirch)	
Lacceryl alcohol	
Hydrocarbons	2–6%
Pentacosane (tachardiacerin) 2%	
Heptacosane	
Lac resins	2–4%

Further, composition of lac wax was recorded: acid component 3.1%, alcoholic component 26.4%, anhydrohydroxy acid 1.6%, esters 66.8% and hydrocarbons 2.1% of which acids are cerotic, myristic and palmitic acids. Alcoholic components are mixtures of n-primary alcohols, 26–34 carbon atoms (C_{26} alcohol, 0.5%; C_{28} alcohol, 4.9%; C_{30} alcohol, 14.9%; C_{32} alcohol, 4.1%; C_{34} alcohol, 2.0%).

The esters are derivatives of C_{26} to C_{32} alcohols and C_{14}, C_{16} and C_{26} to C_{34} acids. Acids appear to be a mixture of at least 15 different esters with a mean molecular weight of 718.5 and molecular formula of $C_{49}H_{98}O_2$. The hydrocarbon is likely to be a C_{27}, n-aliphatic hydrocarbon. The most important observation is the presence of myristic esters. Myristic acid itself has been fully identified and characterized as its anilide.

8.2 Uses of Lac Wax

Principal use of shellac wax has been reported in electrical industry, though it is of general use in formulations where use of a natural wax of a hard type is advantageous. True shellac wax commands high price, and the demand for refined product exceeds supply. High melting point of shellac wax and its high electrical insulation value favour its use as electrical potting compounds and appliances. In shoe creams, addition of a little shellac wax to an otherwise ordinary formula containing montan and paraffin waxes is a means of producing a high lustre. Shellac wax sold for polishes is said to be frequently admixture with Chinese insect wax, tallow, resin and montan wax. It also has application in floor and car polishes, food, confectionery and tablet finishing, lipsticks, crayons and tailor's chalk. Emulsifiable wax was formulated consisting of candelilla wax, shellac wax, lignite wax, stearic acid and zinc stearate. For making water in oil (W/O) polishes, wax stock is then emulsified with white mineral spirit containing little ammonia or an amine and a mild abrasive. An aqueous wax emulsion polish was a developed polish of the ‘dry bright’ type using alkali-soluble resin selected from the group consisting of rosin, shellac wax, casein, phenol formaldehyde resins, etc.; upon application of the polish, the wax is deposited and, on drying, leaves a hard surface having a bright lustre (Chapman and John 1958 - US patent no. US2964487A).

Shellac wax has also been utilized in manufacture of radio condensers because of its high melting point. Radio condensers require waxes of a more exacting specification than do stout electrical conductors or ordinary size wire. Condenser in the small radio set in service becomes readily heated, therefore necessitating the use of a wax that will withstand at least 160 °F (~71 °C) of heat without softening or partially melting. Miniature assemblies require even higher melting point waxes. Waxes such as shellac wax, esparto wax, candelilla wax, and fibre wax, when plasticized by an adequate amount of softer wax of high melting point, are suited for the purpose.

Shellac wax has been utilized in making of finishing waxes for boot soles, because of its hard and lustrous nature. Alkaline solution of shellac was used in preparing liquid finishes. Liquid finishes were also prepared from shellac dissolved in an organic volatile solvent, containing a small amount of carnauba and other waxes. Shellac wax has also been used in making shoe pastes.

Recently, it has been reported in the literature that the long-chain fatty alcohols like triacontanol, dotriacontanol, octacosanol and hexacosanol are well known for promoting plant growth regulatory activity whereas triacontanol is a natural plant growth regulator and used commercially also. It is naturally found in epicuticular waxes and responsible for plant growth promotion activities like growth, yield, photosynthesis, protein synthesis and uptake of water and nutrients. The lac wax policosanol which is a mixture of octacosanol (C28), triacontanol (C30), dotriacontanol (C32), hexacosanol and other lower fatty alcohols was expected to show the PGR activity (Srivastava et al. 2017a, Indian Patent Application no. 201631013579).

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Silk: An Amazing Biomaterial for Future Medication

Dhiraj Kumar, Sadhana Shrivastava, Chengliang Gong, and Sangeeta Shukla

Abstract Silk is one of the famous natural materials since ancient time due to its elegance and diverse applications. Two key proteins are hydrophilic: sericin and hydrophobic fibroin. It has unique properties like biodegradation, oxidation resistance, antibacterial and UV resistance which attract researchers. The variety of silk proteins has helped in the development of novel biomaterials and successful functioning in the treatment of various diseases. Silk proteins play an important role in the development of human tissues, skin development, regeneration of eye lenses, intervertebral disc, stem cells, nerve cells, ligament and biocompatible implants for sleep disc including anticancerous stuff. In the future, we can see more silk and its proteins based highly on advanced engineered biomaterials for the biomedical industries.

Keywords Silkworm · Silk · Protein · Biomedical products · Diseases

1 Introduction

India is the second largest silk-producing country after China and plays a significant role in the economy of textile industries of the nation. Silk is one of the best natural fibres categorized under animal fibres. Silk has been used by the textile industry since long period of time by the emperor due to its admirable physical and chemical properties including biomedical applications. Glistening, mechanical strength, flexibility and most importantly biocompatibility properties made different to silk from other natural fibres (Omenetto and Kaplan 2008; Omenetto and Kaplan 2010). Silk consists of protein fibres, typically produced by silkworms and spiders (Huby et al. 2013; Wongpanit et al. 2012). *Bombyx mori* silk has been tested and established as sutures since several millennia. Basically, silkworm *Bombyx mori* silk is a natural

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Table 1 Silks engineered by various types of silkworms

Sl. No.	Name of silk	Produced by	Origin
1	Mulberry silk	*Bombyx mori*	China
	Wild silk		
2	Tropical tasar silk	*Antheraea mylitta*	India
3	Oak tasar silk	*Antheraea yamamai*	Japan
		Antheraea pernyi	China
		Antheraea compta	India
		Antheraea proylei	
		Antheraea frithi	
4	Muga silk (golden silk)	*Antheraea assama*	India
5	Eri silk (ahinsa silk)	*Philosamia ricini*	

polymer which is primarily made up of two types of proteins, i.e. sericin and fibroin. The sericin acts as an adhesive between two fibroin filaments to form cocoon shell and silk (Mondal 2007). In last decades, researchers have been proved sericin role in biological activities such as antioxidant, antitumour, whitening, antiobesity and antidiabetic (Singh et al. 2011; Zhao and Zhang 2015; Okazaki et al. 2010; Seo et al. 2011). In addition, it decreases the blood glucose levels in diabetic rats but also has significant therapeutic effects on a variety of complications caused by diabetes (Song et al. 2013).

2 Categories of Silkworm Silks and the Process of Silk Production

Among the four varieties of silk produced in 2017–2018, mulberry accounts for 70.1% (22,066 MT), tasar 10.8% (2988 MT), eri 18.6% (6661 MT) and muga 0.56% (192 MT) of the total raw silk production 30,263 MT (*Source: Central Silk Board, Bangalore, India 2018*). There are a variety of silks produced (Table 1) by various animals; among all, mulberry silk is the finest one due to its easy rearing process and quality. After the eggs are laid by the female moth, the newly hatched out silkworm larvae fed on fresh mulberry leaves and pass from various metamorphosis stages to construction of a protective silk cocoon (Adachi et al. 2006; Iizuka et al. 1983; Zhao et al. 2005, 2007). Afterwards, green cocoon stifled through various processes to kill the pupa and reel the cocoons to make lustrous silk. (Gulrajani et al. 1997; Freddi et al. 2003) (Fig. 1).

3 Chemical Composition and Structure of Silk

As previously explained, various types of silkworms (mulberry, *Bombyx mori*; tasar, *Antheraea mylitta*; muga, *Antheraea assamensis*; oak, *Antheraea pernyi*; and eri, *Philosamia ricini* or *Samia cynthia*) produce lustrous silk made up of natural

Fig. 1 Natural silk formation by silkworm *Bombyx mori*

proteins (Fig. 2). Silk proteins are exclusively biosynthesized in the middle region of silk gland which consists 25–30% sericin and 60–70 fibroin of total silk fibre mass. Sericin coats fibroin threads to glue them together and fills the gaps to enhance the toughness of the silk fibre (Mondal 2007).

Silk consists of two main proteins, sericin and fibroin. The structural centre of the silk linked with certain macromolecules like polysaccharides and lipids including natural colours and mineral matters (Hardy and Scheibel 2009). The colourants include flavones, carotene, xanthophyll and small amount of amino sugars (Sinohara 1979). Both the major proteins are composed of 18 diverse amino acids and main components are serine, alanine and glycine. However, spider silk primarily contains glycine- and alanine-enriched fibroin protein. Additionally, the amino acid sequences of silk proteins differ from species to species, which is responsible for a wide range of mechanical properties of silk yarn. For example, the hexapeptide sequence Gly-Ala-Gly-Ala-Gly-Ser dominates the beta-sheet regions of *B. mori* fibroin, while those of *A. pernyi* and *S. cynthia* are primarily composed of repetitive stretches of polyalanine (Mita et al. 1994; Yukuhiro et al. 1997). Fibroin and sericin can be detached by precipitation (Table 2) and the fraction of silk can be studied by polyacrylamide gel electrophoresis (PGE) with reference to amino acid composition (Tokutake 1980). Details of the wide diversity of structure and chemical nature of silk variants have been extensively reported (Jin and Kaplan 2003; Vollrath and Knight 2001).

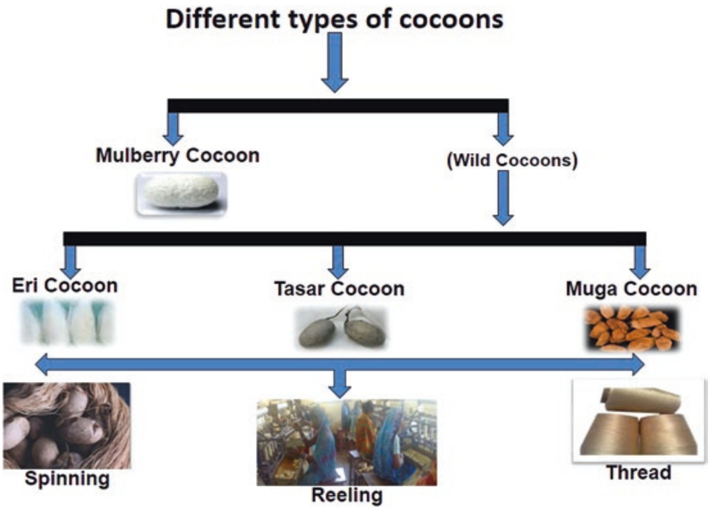

Fig. 2 Different types of cocoon and silk

Table 2 Amino acid composition of silk protein sericin and fibroin (Tokutake 1980)

	Precipitate protein	Supernatant protein
Name of amino acids	Sericin (mol %)	Fibroin (mol %)
Alanine	15.2	31.6
Glycine	24.2	46.4
Serine	18.9	9.48
Aspartic acid	12.9	1.68
Glutamic acid	4.25	0.96
Threonine	5.21	1.17
Tyrosine	4.10	4.98
Valine	3.34	2.04
Arginine	3.02	0.29
Lysine	2.07	0.58
Leucine	1.99	0.22
Isoleucine	1.82	0.28
Histidine	0.98	0.08
Proline	0.69	0.00
Phenylamine	0.69	0.00
Methionine	0.11	0.00
Amino	Trace	0.00
Ethylcysteine		

The fact that there is a readily available source of silkworm silk has facilitated an understanding of its structure and function (Marsh et al. 1955). Fibroin protein is a fibrous protein composed of heavy (H) chain, light (L) chain and glycoprotein linked by disulfide bonds. However, sericin protein is a natural macromolecular protein which works as an adhesive to bind or unite fibroin for making silk cocoons by silkworm.

Silk images of scanning electron microscope (SEM) clearly show that mulberry silk is the finest one compared to the wild varieties of silk (Fig. 3). *B. mori* silk has a core-shell type structure with a large protein called as heavy chain (HC) fibroin (350 kDa) and small protein, i.e. light chain (LC) fibroin (25 kDa) via disulfide bonds. The last small chain is glycoprotein (GP) referred as P25 protein (30 kDa) which is linked with non-covalent hydrophobic interactions (Kundu et al. 2014; Inoue et al. 2005; Tanaka et al. 1999). The ratios of HC/LC/GP is 6:6:1. The crystalline-like structures of the silk thread are due to HC which is hydrophobic in nature; however, LC is hydrophilic and flexible, and the integrity of the complex is governed by P25 protein (Inoue et al. 2005; Zhou et al. 2003). HC, LC and GP proteins are secretes from the silk gland of the silkworm and emerge out from the spinneret to form silk thread (Marsh et al. 1955; Zhou et al. 2003). A straight anisotropic β-sheet-rich nanocrystals appeared and dispersed in an unstructured matrix.

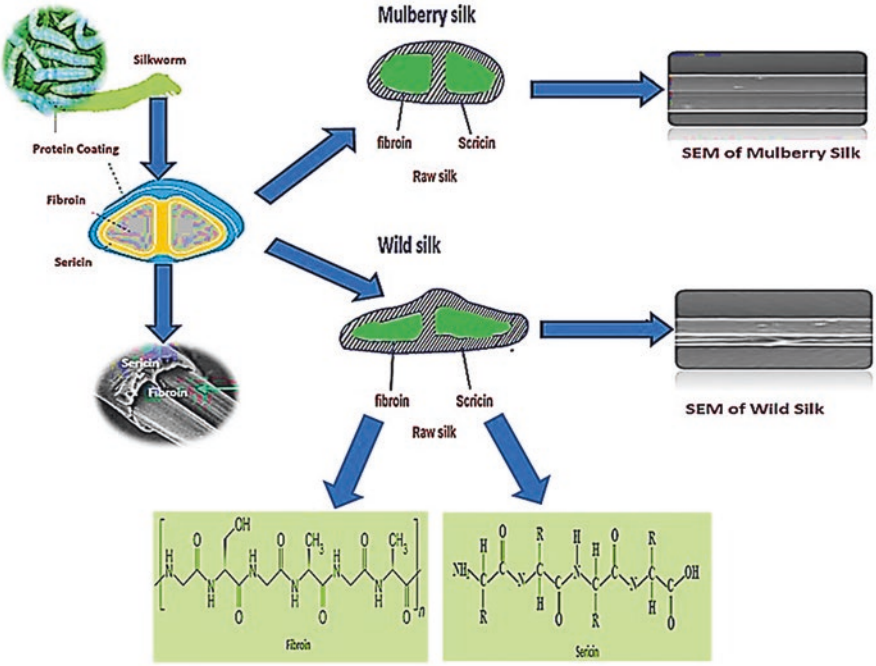

Fig. 3 Chemical and SEM structure of mulberry and wild silk

Silk gland discharges an adhesive-like sericin that covers the fibroin fibres and assures the cohesion of the cocoon by joining the two filaments together (Tsukada et al. 1979; Kaplan et al. 1993). At last, the fibre is covered with a variety of different proteins proposed to ensure the defence against microorganisms and predators (Kludkiewicz et al. 2005; Gauthier et al. 2004).

4 Biomedical Application of Silk Proteins

Silk is a historic natural material and known for its quality and medicinal properties throughout the globe. Silkworms stand out as a matchless class of structural proteins and model biomaterial (Vollrath and Porter 2006). This natural biopolymer has been found suitable for a variety of applications. More than thousands of years, it has been used as suture for stitching of wounds and nacre as dental implants (Altman et al. 2003; Ratner et al. 2004). Being members of a fibrous and natural protein family, silks hold remarkable mechanical strength, which makes silk widely accepted as natural biomaterials (Saric and Scheibel 2019) including outstanding biocompatibility and immunogenicity (MacIntosh et al. 2008; Meinel et al. 2005a, b), limited bacterial adhesion (Cassinelli et al. 2006) and controllable biodegradability (Wang et al. 2008).

Sericin is an important natural protein that can be used in the medical and cosmetic fields such as silk-based artificial hair, mask, powder, silk protein lipsticks, eyeliners, face cream and scrubber due to its good compatibility to human tissues, biodegradation, oxidation resistance, antibacterial properties and UV resistance. Additionally, sericin absorbs and releases moisture readily and exhibits the inhibitory activity of tyrosine and kinase (Zhang 2002; Aramwit and Sangcakul 2007). Silk fibroin has been broadly used in biomedical industries due to its excellent biocompatibility, biodegradability including good oxygen and water vapour permeability (Murphy et al. 2008; Meinel et al. 2005a, b; Horan et al. 2005).

From past to present updates, various scientists developed a wide range of biomedical products based on silk proteins such as suture, wound dressings, scaffold, biomaterials, silk cornea, silk-based eardrum, knitted hernia mesh and silk hydrogel (Fig. 4) (Haupt et al. 2015; Guziewicz et al. 2013; Wu et al. 2012; Yin et al. 2017).

The dynamic culture condition for creating specific tissue microenvironment, organ development, cartilage development, bone tissue engineering and polymeric nanomaterials were successfully invented (Farokhi et al. 2018; Liu et al. 2014). Additionally, vascular grafting, cardiac tissue development, rapid prototyping to develop novel scaffolds for tissue engineering, eye regeneration, skin development, intervertebral disc regeneration, establishment of stem cells, nerve regeneration, ligament regeneration, biocompatible implants for sleep disc and liver tissues were also investigated by various researchers (Kasoju and Bora 2012; DeBari and Abbott 2019; Janani et al. 2017).

In silk-based anticancer drug formation, recently protein-based nanoparticle has been also investigated for rewiring of macrophages (Totten et al. 2019), anticancer therapeutics (Wongpinyochit et al. 2015), drug delivery, silk-based anti-ageing

Fig. 4 Uses of silk proteins in medication

materials and self-assembled nanoparticles (Vootla et al. 2015). However, from silk various significant products have been produced such as electrospinning; injectable silk protein microparticle-based fillers; silk gelatin bio-ink; 3D bioprinting; 2D and 3D composite; fibre-hydrogel composite; silk braided scaffolds for maxillofacial reconstructive surgery; graphene-based biomaterial; production of recombinant silk; antibacterial, anti-inflammatory and antifungal silk patches; anti-scar patches; and electrospun fibre mat (De Vos 2015; Aibibu et al. 2016; Chouhan et al. 2017; Catto et al. 2015; Jiang et al. 2018; Rodríguez-Lozano et al. 2014).

Some other products have been also constructed with the help of silk proteins like silk foam, membrane, adhesive, silk substrate, cell adhesion, silk culture medium for bacteria, silk-elastin-like protein polymer formation, in situ generation of silver nanoparticles, reinforced biomaterials and cell culture medium (Shao and Vollrath 2002; Motta et al. 2002; Panda et al. 2015; Rockwood et al. 2011; Kim et al. 2005; Kapoor and Kundu 2016).

5 Future Prospective

Silk protein has been widely explored and investigators are making efforts to develop new products by harvesting silk proteins. Our research team is also engaged in the development of novel wound healing patch using silk proteins from different varieties of silks. Aside from the biomedical application of this amazing silk, it is playing a vital role in the cottage industries for people's livelihood. Researchers and governments can connect laboratories, biomedical industries and seri-farmers together to produce more silk and discover novel silk-based products which certainly empower rural industries of the country.

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Insect Chitin and Chitosan: Structure, Properties, Production, and Implementation Prospective

Manish Kumar, V. Vivekanand, and Nidhi Pareek

Abstract Chitin is the second most naturally abundant long-chain polysaccharide on the earth after cellulose. Being natural biopolymer chitin and its deacetylated form chitosan (CHS) has enormous applicability in various fields, viz., drug delivery, agriculture, cosmetic, food, and tissue engineering. Chitin and CHS are found in several microbes and invertebrates including insects. Insect's exoskeleton mainly composed of chitin which is replaced periodically to maintain the proper growth and development. At present, chitin and CHS is commercially produced from shrimp shells but due to the remarkable potential applicability of insect chitin and CHS in textile and food industries its commercial exploitation is in demand. In order to develop feasible bioprocess for chitin and CHS extraction from insects, the extensive knowledge of structural and physicochemical aspects of the insects and the extracted products is much required. The study illustrates various applications of insect-derived chitin and CHS along with their structure, properties, as well as the possibilities of commercial production.

Keywords Insects · Chitin · Chitosan · Extraction · Application

1 Introduction

Chitin and its deacetylated product, CHS, is a natural polymer of immense utilization. Continuous research development has resulted in the generation of several beneficial products from chitin and its derivatives. Chitin and its derivatives, i.e., CHS, *N*-acetyl glucosamine (GlcNAc), and chitooligosaccharides (COS), have been

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reported to application in several sectors like medicine, tissue engineering, agriculture, wastewater treatment, drug delivery, cosmetics, and food (Kumar et al. 2018a). The rapid increasing utilization emerges the large-scale production of chitin. Although chitin is reported to be present in several fungi, crustacean shells, mollusks, and insects, commercial-scale exploitation is currently in practice from the aquatic crustacean due to emergence of a gigantic amount of chitin containing wastes from seafood industries (Yadav et al. 2019). However, in order to compete the increasing demand of chitin and CHS, there is urge of alternate sources and insects could be proven critical in it due to large-scale cultivation and harvesting of silkworm and honeybees (Paulino et al. 2006). These practices forecast the possibilities of cultivation of insects for higher level of chitin and CHS extraction. Recent research showed presence of better degree of deacetylation and acetylation in chitin and CHS extracted from insects as compared to chitin and CHS derived from aquatic crustacean (Kaya et al. 2014). Additionally, some studies also revealed that the insect CHS has better antimicrobial and antioxidant activity compared to shrimp shell commercial CHS (Kaya and Baran 2015). This finding provides new insight for chitin and CHS extraction from insects on large scale which is not practiced till date.

Therefore, this article reviews the recent development made for the production process of chitin and CHS from insects that includes exploration of higher producer and efficient extraction methods. Additionally, structural and functional properties of chitin and CHS extracted from insects are studied. The possibility and current status of large-scale production along with potential application of insect's chitin and CHS are also discussed.

2 Chitin

Chitin, the natural polysaccharide, is composed of poly (β-(1 → 4)-*N*-acetyl-D-glucosamine) units. Chitin, the most abundant natural polymer on earth after cellulose, is synthesized by an enormous number of living organisms (Kumar 2000). It is present in fungal and yeast cell wall; in tendons and shells of a lobster, shrimp, krill, and crab; as well as in an insect cuticle (Rinaudo 2006). Due to the versatility of sources chitin occurs in three allomorphs, namely, α, β, and γ forms. The different forms of chitin differ in their crystalline microfibril orientation. In α microfibrils are arranged in antiparallel organization whereas in the β form, microfibrils are arranged in parallel fashion (Anitha et al. 2014). Moreover, γ-chitin contains a mixture of both antiparallel and parallel microfibril sheets. However, some studies suggested that the γ form of chitin is a variant of α-chitin (Atkins 1985). The utilization of chitin is limited due to its high degree of crystallinity and insolubility in aqueous and organic solvents, but its utilization can be augmented by exposing it to interconversion into derivatives like CHS, GlcNAc, and COS that shows better solubility and less crystallinity (Kumar 2000). The conversion of chitin into its derivatives can be done through chemical as well as enzymatic/biological means (Kumar et al.

2017; Husson et al. 2017). Apart from the degradation of chitin into valuable and highly applicable derivatives, chitinolytic enzymes are also beneficial in the growth and protection of plants from phytopathogenic fungi (Kumar et al. 2018b, c). Application of chitin in its deacetylated form is more explored due to its remarkable enhanced biological properties.

3 Chitosan

CHS is a linear polymer of D-glucosamine and *N*-acetyl-D-glucosamine monomers that is linked through β-(1–4) linkages (Kumar et al. 2004). CHS contains 5–30% of *N*-acetylation degree with the molecular weight ranging from 100,000 to 1,000,000 Da. The cationic polymer is generally present in the three different morphological forms, namely, amorphous, semicrystalline, and crystalline. Generally, CHS is insoluble in water, but they can interact with negatively charged molecules such as fatty acids, bile acids, phospholipids, proteins, and anionic polysaccharides (Pillai et al. 2009). CHS is obtained from the deacetylation of chitin so the native source for CHS is akin of chitin. CHS is biocompatible, biodegradable, and renewable and has the ability to mold into different forms like composite, scaffolds, and films that support its biomedical applications, viz., drug delivery, immunologic, antitumoral, hypocholesterolemic, and wound healing (Kumar et al. 2019b).

4 Major Sources of Chitin and CHS

Chitin and CHS occurrence in the nature is quite diverse. They are found in a wide range of organisms belonging to Plantae, Animalia, and fungi kingdoms. At present the majority of chitin and CHS are obtained from sea animals like shrimps, crabs, and lobsters. Chitin is also present in the cell wall of fungi as well as in insects, but their commercial-scale exploitation is still in its primitive stage. Among all the sources for chitin and CHS production, the most exploited means are crustaceans, mollusks, fungi, and insects.

4.1 Crustacean

Chitin is found in abundance in the shell of crustaceans like crabs, lobsters, krill, prawns, and shrimps (Kurita 2006). It is found as a component of the complex system with proteins on which calcium carbonate deposits to form the rigid shell (Younes and Rinaudo 2015). The process of demineralization and deproteinization is required for the extraction of chitin from the complex system (Percot et al. 2003). Different crustaceans' chitin content varies in the range of 20–75%, approximately

(Hamed et al. 2016; Tokatlı and Demirdöven 2018). At present, crustacean shell wastes are the major producer of chitin and CHS on commercial level and approximately about 75% of the total weight of crustaceans is considered as waste (Limam et al. 2011). This huge amount of generated waste from the seafood processing industries without proper waste management is a potential environmental hazard concern. Therefore, the extraction of chitin and CHS from crustaceans' shells not only provides a way to minimize the waste but also assists the generation of valuable chitin and CHS applicable in various fields. In a study, chitin from the crustacean waste was transformed into nanostructured chitin with average size of 240 × 5 × 7 nm, showing potential applications in biomedical products and biometric cosmetics (Morganti et al. 2011). Chitin and CHS extracted from crustaceans' shells has shown several biological activities, i.e., antibacterial, antifungal, antitumor, and antioxidant as an individual or in combination with other agent (Hafsa et al. 2016; Chien et al. 2016; Senthilkumar et al. 2015).

4.2 Mollusks

Although mollusks have not been exploited like crustaceans for the extraction of chitin and CHS, they have been reported as potential source. In several mollusks mantle edge develops a shell which is mainly made up of chitin and conchiolin proteins. Chitin was reported in the cuticle of Solenogastres and Caudofoveata whereas CHS was observed in the tegmentum and articulamentum of the plates of Polyplacophora (Peters 1972). Mollusk shell's organic matrix plays parts in mechanical protection from predators as well as a support for internal organs (Furuhashi et al. 2009). The presence of chitin in mollusk's shell was revealed in early 1980s through the X-ray/electron diffraction studies of insoluble shell organic matrix (Weiner and Traub 1980). In mollusks chitin fibers are arranged with certain mineralogical axes of crystalline calcium carbonate in such a way that stimulate the functional characterization of chitin-forming enzymes (Weiss 2012). Generally, chitin is found in the extracellular coating in mollusks. However, Martin et al. (2007) reported the presence of intracellular granular chitin in the epidermis of nudibranch gastropods that was meant to fill the epidermis cells of the skin and the epithelial cells of the stomach. Recently, chitin and CHS was extracted from apple snail and the carbon nitrogen ratio of the extracted CHS was 5.7 with a degree of deacetylation of 59.69% (Thillai Natarajan et al. 2017). The study also observed 18.44% of chitin composition in the mussel shell. Similarly, 23.91% and 31.14% of chitin were extracted from the shell and operculum of mangrove gastropod *Nerita crepidularia*, respectively (Palpandi et al. 2009). Chitin has also been extracted from the chiton shell with 4.3% of chitin (Rasti et al. 2017). The study also reported CHS with remarkable 90% of degree of deacetylation from the extracted chitin through 45% sodium hydroxide (NaOH) solution that results in the significant antioxidant activity. Osuna-Mascaró et al. (2015) demonstrated the ultrastructure of the interlamellar membrane of the nacre of *Pteria hirundo* through scanning electron microscopy. The study revealed that the minor chitin component consists of very thin fibers in disoriented manner with a high aspect ratio. The fibers were coated with a protein that lead to the formation of complex with chitin and the resulted chitin-protein complex fibers were embedded in an additional proteinaceous matrix (Osuna-Mascaró et al. 2015). Recently, α-chitin was also reported in the skeletons of red sea demosponges, *Acamus wolffgangi*, and *Echinoclathria gibbosa* (Ehrlich et al. 2018).

4.3 Fungi

Fungi are a highly demanding source for chitin and CHS extraction due their better biological properties over the chitin and CHS obtained from crustacean resources (Anitha et al. 2014). CHS is generally prepared from the deacetylation of chitin extracted from different sources. However, CHS can be directly extracted from some fungi as it is found in them in deacetylated form; i.e., no further deacetylation is required (Rane and Hoover 1993). Moreover, fungal chitin is also associated with other polysaccharides that is specific to fungal cell wall. In fungi chitin is widely distributed in a wide range of phylum, i.e., Ascomycetes, Basidiomycetes, Zygomycetes, and Phycomycetes (Ghormade et al. 2017). Chitin is a component of cell walls of fungi as well as of structural membranes of mycelia, stalks, and spores. The amount of chitin present differs between traces to 45% of the organic fraction which depends upon the natural physiological parameters and fermentation conditions (Peter 2005). In order to enhance the production of chitin from *Aspergillus niger*, a successful concurrent approach was developed with the involvement of shrimp shells along with fungal mycelia and the resultant shrimp shell chitin protein content was less than 5%, whereas the fungal mycelia chitin protein content was found to be 10–15% (Teng et al. 2001). CHS production on the mixture of corn steep liquor and papaya peel juice as substrate through Mucorales fungi was evaluated and it was observed that the fungal CHS content was in the range of 5.02 mg/g and 15.63 mg/g in the case of *Phaeoannellomyces heterothallic* SIS 28 and *Cunninghamella elegans* SIS 41, respectively (Berger et al. 2018). In another study, chitin and CHS were produced from a well-known medicinal fungus, *Fomitopsis pinicola*, and about 30.11% of dry weight of the fungus was chitin content (Kaya et al. 2015a). The study also reported 71.75% of CHS yield from the produced chitin. Similarly, 24.01% of chitin was extracted from the mycelia of *A. niger* whereas 13.25% of chitin and 12.49% of CHS were obtained from *Mucor rouxii* mycelia (Wu et al. 2005). Hassainia et al. (2018) extracted chitin from pileus, stipes, and gills of *Agaricus bisporus* and the percentage extraction yield was 7.4%, 6.4%, and 5.9% of dry weight, respectively. Another study showed CHS production from *Auricularia* sp. and was compared with the commercial CHS in terms of antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. The study concluded that the CHS obtained from *Auricularia* sp. exhibited enhanced antibacterial activities against the tested bacterial species (Chang et al. 2019).

4.4 Insects

Insects possess lightweight mechanically strong exoskeleton (cuticle) responsible for their success in nature. The cuticle along with peritrophic matrix is one of the most important organs of the insect's body (Lehane 1997). They are the supermolecular assembly of chitin, protein, minerals, lipid, catechols, pigments, and water that determine the body shape of insects and allow to grow, move, communicate, reproduce, and cope with environmental hazards (Karl and Kramer 1986). Insect exoskeleton consists of different layers of cuticle attached with the epidermis (Fig. 1). In insects chitin is found in the procuticle, foregut and hindgut lining, tracheal tubes, and muscle attachment junctions. The major role of chitin in insects is as scaffold materials that support the cuticles of the epidermis and trachea and peritrophic matrices (Merzendorfer and Zimoch 2003). Thus, the insect's growth and morphogenesis are mainly reliant upon the biosynthesis, turnover, and ability to alter chitin-containing structures (Zhu et al. 2016). Insects molded these chitin-containing structures through the application of different produced chitin-metabolizing enzymes (Kumar et al. 2018d; Muthukrishnan et al. 2012). The beneficial potential of chitin and CHS has resulted in the urge of development of efficient extraction process as well as the high level of producers. At present utilization of crustacean sources is in practice at commercial level but the more beneficial potential applicability of chitin and CHS derived from fungi and insects has turned the attention of scientific community toward the exploitation of these other natural resources. In this regard some work is noteworthy and has shown immense potentiality of utilization of insects for chitin and CHS commercial extraction. Chitin extraction of 12.2%, 5%, and 2.5% was reported from *Schistocerca gregaria*, *Apis mellifera*, and *Calosoma rugosa*, respectively (Marei et al. 2016). The produced

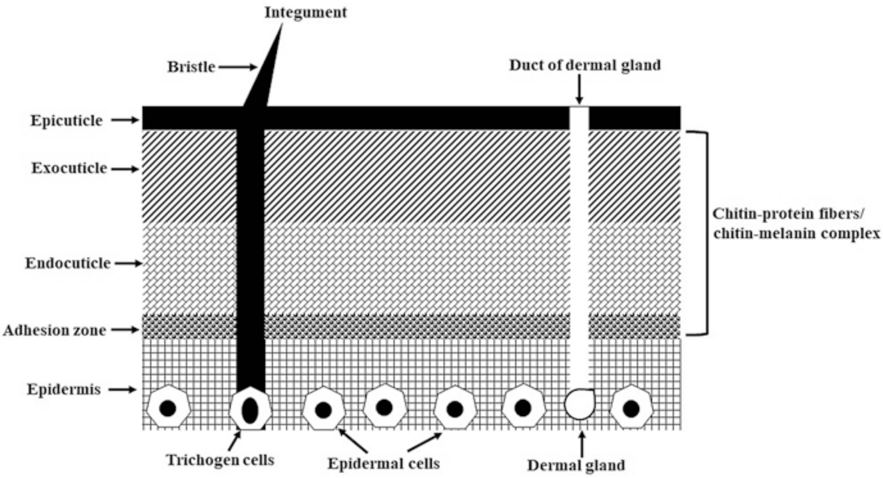

Fig. 1 Simplified illustration of insect's exoskeleton and its organic matrix

chitin was subjected to deacetylation to convert it into CHS and it was found that the degree of deacetylation of CHS derived from the locust, beetles, and honeybee was approx. 95–98% which was better than the shrimp CHS (Marei et al. 2016). Sajomsang and Gonil (2010) extracted α-chitin from cicada sloughs with higher recovery rate with significant percentage of degree of acetylation, i.e., 9.7–10.2%. In another study chitin, CHS, and water-soluble low molecular weight CHS production was reported from the honeybee's corpses (Nemtsev et al. 2004). The produced CHS degree of deacetylation was 80–85% with molecular weight ranging from 200 to 250 kDa. Similarly, α-chitin was isolated from beetle larva cuticle and *Bombyx mori* pupa *exuvia* with ease of deacetylation as 94% deacetylation degree was achieved in one treatment of 40% NaOH in 4 h at 110 °C (Zhang et al. 2000). The establishment of insects as a huge potential source of chitin and CHS has resulted into tremendous outgoing research in the field of their high level of production and biological property determination. In the coming section of the work emphasis has been given on the different biological properties of chitin and CHS extracted from insects and the recent development achieved in their generation.

5 Significant Properties of Insect-Derived Chitin and CHS

Insects are emerging as an alternative source to produce biologically active chitin and CHS. The diverse utilization of chitin and CHS is well documented in literature in the form of patents, books, and articles. Some chitin- and CHS-based products have been developed and in practice in the field of additive and cosmetics. Insects derived chitin and CHS have shown several better properties compared to that of the crustacean sources. For better utilization of CHS degree of deacetylation is considered as one of the major factors and it has been reported that insect-derived CHS showed more degree of deacetylation or less degree of acetylation compared to commercial shrimp shell chitin (Zhang et al. 2000; Sajomsang and Gonil 2010; Marei et al. 2016). Kaya et al. (2014) compared chitin extraction from *Melolontha melolontha* and *Oniscus asellus* and reported that the insect *M. melolontha* chitin content was 13–13% that is much better than the chitin content of crustacean *O. asellus* (6–7%). The study also compared the physicochemical structure and observed that the *O. asellus* chitin nanofibers were adherent to each other whereas the nanofibers in *M. melolontha* were in a nonadherent fashion. Additionally, the number of pores was much higher in chitin from *M. melolontha* compared to *O. asellus*-derived chitin (Kaya et al. 2014). CHS extracted from insects, cicada slough, silkworm chrysalis, mealworm, and grasshopper showed lower viscosity and modulus values than shrimp shell CHS along with the crystallinity index values of 64.8%, 32.9%, 51.9%, 50.1%, and 49.1%, respectively (Luo et al. 2019). Chitin was extracted from beetle *Holotrichia parallela* with a yield of 15% and the properties were comparable to the α-chitin from shrimp (Liu et al. 2012). The produced chitin contains 3.63%, 5.53%, and 11.29% of moisture, ash, and nitrogen content, respectively. The CHS extracted from *Catharsius molossus* L was reported to be superior over commercial medical grade CHS from shrimp in the term of degree of deacetylation, crystallinity, heavy metal content, viscosity, protein residue, ash content, and in vitro adhesion (Ma et al. 2015). Kaya et al. (2015c) established differentiation in the chitin content and surface morphology of chitins derived from male and female grasshopper. The study concluded that the chitin content in male grasshopper was better than female and the male chitin surface structure contained 25–95-nm-wide nanofibers with 90–150 nm nanopores whereas these were absent in the case of females (Kaya et al. 2015c). Chitin was also extracted from millipede Spirobolida and the yield was 35.7% which is comparable to the commercial production of chitin from the other conventional sources (Achur 2018). Another study demonstrated about the similarity in physicochemical properties of chitin and CHS from nymphs and adult stages of *Dociostaurus maroccanus* (Erdogan and Kaya 2016). The study reported chitin contents of the adults around 14% and of nymphs around 12% with the crystalline index values 71% and 74%, respectively. The molar mass of adult CHS was 7.2 kDa whereas the molar mass of nymph was 5.6 kDa (Erdogan and Kaya 2016). Chitin and CHS were also extracted from larval exuvium and whole body of edible mealworm, *Tenebrio molitor*, with average chitin yield of 18.01% and 4.92% of dry weight, respectively (Song et al. 2018). The study also claimed that the average yield of CHS from whole body was 3.65% of dry weight with approx. 90% of degree of deacetylation. Similarly, chitin and CHS with significant level of acetylation and deacetylation were extracted from the *Musca domestica* pupa shells with a yield of 8.02% of chitin and 5.87% of CHS (Kim et al. 2016). Ibitoye et al. (2018) harvested chitin and CHS from *Brachytrupes portentosus* and achieved a yield of 4.3–7.1% and 2.4–5.8%, respectively. In comparing the cricket chitin and CHS to shrimp shell chitin and CHS, the degree of acetylation and deacetylation was found to be similar, i.e., 108% and 80.5%, respectively (Ibitoye et al. 2018). However, the percentage ash content of the cricket-derived chitin and CHS was 1% which was reported to be lower than the shrimp chitin and CHS. CHS derived from *T. molitor*, *Zophobas morio*, and *Allomyrina dichotoma* showed antimicrobial activity against *Bacillus cereus*, *Listeria monocytogenes* and *E. coli* and *S. aureus* (Shin et al. 2019). Similarly, Kaya et al. (2016) showed changes in the different physicochemical properties of chitin of *Vespa crabro* at the larvae, pupa, and adult stages.

6 Current Scenario of Chitin and CHS Production from Insects

The rich content of proteins, fats, and biopolymers with the ease of cultivation possibilities on organic biological wastes and high dry matter percentage makes insects as one of the promising sources for value-added product production (Dossey 2010). The extraction of chitin and CHS from insects involves similar approach of demineralization and deproteinization (Table 1) as used for crustaceans' sources. Some of potential sources for chitin and CHS belonging to insects are *Aiolopus simulatrix*, *A. strepens*, *Duroniella fracta*, *D. laticornis*, *Oedipoda miniata*, *O. caerulescens*,

Table 1 Process involved in the extraction of chitin and CHS from insects

Species	Deproteinization	Demineralization	Chitin yield (%)	Deacetylation	CHS yield (%)	Reference
Brachytrupes portentosus	1 M NaOH	1% (w/v) oxalic acid	4.3–7.1%	50% NaOH	2.4–5.8%	Ibitoye et al. (2018)
	95 °C	Room temp.		121 °C		
	6 h	3 h		5 h		
B. germanica	1% (w/v) oxalic acid	1 M HCl	Nymph (5.4%)	50% NaOH	Nymph (2.6%)	Basseri et al. (2019)
P. americana	Room temp.	100 °C	Adult (6.2%)	100 °C	Adult (2.8%)	
	3 h	24 h	Nymph (8.4%)	4 h	Nymph (4.0%)	
			Adult (15.0%)		Adult (7.4%)	
S. Cingulate	2 M NaOH	2 M HCl	8%	50% NaOH	66%	Zelencova et al. (2015)
	150 °C	80 °C		150 °C		
	20 h	4 h		4 h		
S. gregaria	1 M NaOH	1 M HCl	12.2%	50% NaOH	–	Marei et al. (2016)
A. mellifera	100 °C	Room temp.		100 °C	–	
C. rugosa	8 h	–	2.5%	8 h	–	
			5.0%			
M. domestica	1.25 N NaOH	2 N HCl	8.02%	50% NaOH	5.87%	Kim et al. (2016)
	95 °C	Room temp.		95 °C		
	3 h	3 h		3 h		
C. Barbarous	1 M NaOH	1 M HCl	20.5%	50% NaOH	76%	Kaya et al. (2015b)
O. decorus	90 °C	100 °C	16.5%	130 °C		
	21 h	30 min		2 h		

Pyrgomorpha cognata, *Hydrophilus piceus*, *Ranatra linearis*, *Notonecta glauca*, *Rhinolophus hipposideros*, etc. (Philibert et al. 2017). In insect's cuticle organic matrix chitin containing structures are found in between of epicuticle and epidermis (Nwe et al. 2010). Honeybee matrix contains 23%–32% of chitin (Nemtsev et al. 2004) whereas silkworms are composed of about 20% of chitin (Zhang et al. 2000). Chitin extraction from *Bombus terrestris* was studied by Majtán et al. (2007) and was observed that the obtained chitin had 5% lower degree of acetylation than shrimp shell chitin. The less degree of acetylation makes chitin more soluble and thus the abovementioned insects can be exploited for scale-up process. Soon et al. (2018) optimized NaOH concentration for deproteinization process used for chitin and CHS extraction from *Z. morio* larvae and proposed 0.5 M NaOH as the most suitable concentration for deproteinization in terms of physicochemical characterization. 2 N hydrochloric acid (HCl) and 1.25 N NaOH solution were adopted for chitin extraction from *Gryllus bimaculatus* followed by application of 50% NaOH (w/v) solution to convert into CHS (Kim et al. 2017). The study also concluded that the adult exoskeletons of *G. bimaculatus* could be used for chitin and CHS production which would be applied as additive in industrial animal feeds. Wings of cockroach like *Periplaneta americana* and *Blattella germanica* can also be utilized as a source of chitin extraction (Kaya and Baran 2015; Badawy and Mohamed 2015). Chitin and CHS can also be obtained from *Scolopendra cingulata* which is a Chilopoda and the reported dry weight chitin content was 8% of body weight with 66% of CHS yield (Zelencova et al. 2015). In a study, feasibility of different extraction protocols, i.e., chemical extraction and enzyme-assisted extraction, from *Hermetia illucens* was investigated, and the stepwise protein extraction under mild condition based on Osborne fractionation method was observed to be best (Caligiani et al. 2018). Recently, black soldier flies, *H. illucens*, were also observed to produce chitin. The crystallinity index of the extracted chitin from *H. illucens* adult and larvae were found to be 24.9% and 35%, respectively (Waśko et al. 2016). Silk industry waste in the form of dead silkworm larvae and honeybee is a highly potential biowaste for the extraction of chitin and CHS from insects (Paulino et al. 2006). A lot of work is required to achieve the commercial level of chitin and CHS extraction from insects. In order to develop highly efficient process to extract highly significant chitin and CHS, a lot of research work is needed. In this regard identification of insects with high chitin and CHS content would be primary concern along with the development of proper insect's cultivation conditions and further highly efficient extraction process.

7 Potential Application of Insect's Chitin and CHS

Chitin and CHS derived from crustacean sources has a wide range of application, i.e., biomedical, nutraceutical, agricultural, cosmeceutical, and antimicrobial (Kumar et al. 2019a; Rabea et al. 2003; Prashanth and Tharanathan 2007). However, insect-derived chitin and CHS has been less explored. Thus, there is a little known about their specific applications. So, the current scientific community is paying much more attention on the less explored source of chitin, i.e., insects. Many researches have reported the superiority of chitin and CHS derived from insects over crustacean in terms of degree of acetylation and deacetylation which is one of the crucial factors for the biological activities of chitin and CHS. Zhang et al. (2011) prepared CHS from housefly larvae which were comparable to the food-grade CHS according to the Chinese Fishery Trade Standard SC/T3403–2004. Several studies have been carried out to determine the antimicrobial activity of extracted chitin and CHS from insects. A study extracted chitin and CHS from *P. americana* and *B. germanica* and evaluated their antibacterial activity against gram-positive and gram-negative bacteria as well as antifungal activity against *A. flavus* and *A. niger* (Basseri et al. 2019). The extracted chitin and CHS were effective against all the tested bacterial cultures except *Micrococcus luteus* whereas they are effective against both fungal strains. The antimicrobial effectiveness of CHS can be enhanced by converting them to nanoparticles loaded with other drugs. Marei et al. (2019) synthesized ciprofloxacin HCl-loaded CHS nanoparticles through ionic crosslinking and demonstrated its enhanced antimicrobial activity against *E. coli*, *Bacillus thuringiensis*, methicillin-resistant *S. aureus*, and *Pseudomonas aeruginosa* (Marei et al. 2019). The loaded CHS nanoparticles showed highest MIC of 0.0043–0.01 μg/ml and 0.07–0.14 μg/ml against *E. coli* and *S. aureus*, respectively. Chitin and CHS from *Calliptamus barbarus* and *Oedaleus decorus* were evaluated for antimicrobial and antioxidant activities against pathogenic organisms (Kaya et al. 2015b). The antimicrobial activity evaluation showed significant antimicrobial activity against *Listeria monocytogenes*, *B. subtilis*, *Salmonella* Enteritidis, *Yersinia enterocolitica*, and *Candida albicans* whereas the IC_{50} values were 10.68 and 10.91 mg/ml. Similarly, *Hylobius abietis*, a waste parasitic insect-derived chitin and CHS were transformed into biodegradable films which showed antioxidative as well as antimicrobial activity (Kaya et al. 2019). Recently, CHS extracted from mayfly was reported of antitumor activity (Tan et al. 2018). The cytotoxicity and antiproliferative activity of the extracted CHS was evaluated on L929 fibroblast and HeLa, A549, and WiD cancer and the mayfly CHS showed strong cytotoxic effects at a concentration below 2250 μg/ml leading to the early and late apoptosis in A549 cells with late apoptosis and necrosis in WiDr cells (Tan et al. 2018). Apart from the abovementioned potential applications, insect-derived chitin and CHS can be utilized in textile industries and in silk industries. A huge amount of waste products are generated in the form of dead silkworm larvae which contain approximately 24% of chitin content (Paulino et al. 2006). Use of CHS in textiles industries involves fabrication of manufactured fibers (Ebru and Aksoy 2016), dyeing (Dev et al. 2009), finishing (Lim and Hudson 2004), printing (Bahmani et al. 2000), etc.

8 Conclusion and Recommendation

Chitin, CHS, and their derivatives have gained immense importance due to their properties like mucoadhesive, hemostatic, biodegradable, antimicrobial, and biocompatible, and these highly desirable properties allow them to be used in various sectors, viz., wastewater treatment, drug delivery, medicine, agriculture, cosmetic, and food. Although crustacean source is exploited on large scale for the extraction of biological polymer, alone it is not competing with the increasing demand of the chitin-related product market. Therefore, investigation of alternate sources is progress and regarding this insect is of prime concern due to the better level of biological properties of the polymer derived from them. Several reviews and research papers for the production and application of chitin and CHS derived from insects have been published in various journals, and almost all of them mentioned the significance of insect's chitin and CHS over crustacean-derived chitin and CHS. Despite these substantial progress regarding insect's chitin and CHS, still there is a lot of research required on chitin metabolism in insects, selection and exploration of high chitin producer insects, desired product development from the raw insect's chitin, and implementation of the potential applications. Presently, major challenges of chitin and CHS extraction from insects are the low yield compared to aquatic crustacean sources and difficulties in extraction of chitin complexed with other compounds in insects like melanin. Although in recent years a significant progress has been made in identification of potential chitin producer insects and detailed structural analysis of the extracted chitin and CHS but still there is lack of attempts for the commercial-scale production of chitin and CHS from insects. Overall keeping in mind the interest and recent progress made in chitin and CHS extraction from insects, it can be said that the large-scale production of chitin and CHS from insects will be achieved in the near future.

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Recent Advances in the Insect Natural Product Chemistry: Structural Diversity and Their Applications

Luqman Jameel Rather, Mohammad Fawad Ansari, and Qing Li

Abstract Phylum Arthropoda is the largest phylum of animal kingdom with around one million different species comprising more than 80% of all known animal species. These include insects, spiders, ticks, lice, centipedes, shrimp, and crabs with several less known species as well. Arthropod natural products represent structurally and functionally diverse chemical compounds ranging from fatty acids, polyketide, terpenoids to nucleosides. Most of the chemical compounds from arthropods are of polyacetate or fatty acid origin with highly nonpolar structures. Chemical analysis and spectroscopic identifications of new and fascinating structures have led to analyze the biological roles of identified compounds. More recently, small-molecule metabolites are facilitating for identification of novel types of chemical and ecological interactions. Besides metabolic chemistry and various biological applications, insect natural products are found to be active ingredients of various natural dyes (cochineal and lac dye). The semisynthetic derivative carmine later found its use as a biological staining and as a food coloring agent.

Keywords Arthropoda · Insects · Natural products · Semisynthetic · Environmental interactions

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1 Introduction

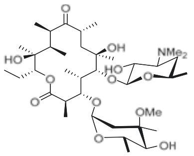
Natural products are defined as the purified organic compounds isolated from natural sources (plants, animals, or microbial) that are produced through their primary or secondary metabolic pathways (Lee 1993). The chemical and structural diversity of natural products from various sources are unparalleled by any synthetic library. From the time immemorial mankind has used plant and animal materials not only as a source of nutrition but also for numerous other purposes (Itokawa et al. 2000). Apart from the chemical compounds necessary for normal growth and metabolism, a vast number of secondary metabolites and identified chemical substances belong to three major chemical classes: terpenes (group of lipids), phenolics (derived from carbohydrates), and alkaloids (derived from amino acids) along with saponins, flavonoids, anthraquinones, terpenoids, coumarins, lignans, polysaccharides, polypeptides, and proteins (Mandal et al. 2007; Ahmad and Beg 2001). However, natural products from insects represent structurally and functionally diverse chemical compounds ranging from fatty acids, polyketide, terpenoids to nucleosides. From the time immemorial the main focus of natural product medicine was kept limited to plants, algae, and fungi. Apart from the medicinal plants, another source of inspiration, namely, entomotherapy, has been kept in practice for a long time (Benítez 2011; Mahawar and Jaroli 2008).

Entomotherapy is the use of insect natural products or whole insect body for medicine and has been found to be an important alternative to modern therapeutics all over the world (Jaroli et al. 2010; Patricia et al. 2015; Alonso-Castro 2014; Mlcek et al. 2014). People believe insects with little benefits except some insect products such as honey (a sweet bee product) as food, silk for clothing, and pollination of plants. Honey is regarded as very good antioxidant and antimicrobial agent and plays its role in defending against heart and skin disorders (Erejuwa et al. 2012; Maddocks and Jenkins 2013; McLoone et al. 2015; Mandala and Mandal 2011). Propolis, a by-product from beehive, has been found to be used extensively for its antioxidant, antimicrobial, anti-inflammatory, cardioprotective, immunomodulatory, and antiangiogenic properties (Daleprane and Abdalla 2013; Freires et al. 2016; Bellik 2015). Venoms from bees and wasps are now considered and studied as an alternative medicine for cancer therapy (Perez-Riverol et al. 2017; Oršolić 2012; Lee et al. 2015). They are unique chemical weapons to ensure full defense of their colonies. Scientists have achieved much success in the identification and isolation of many active biological isolates from insect whole bodies in the form of excretions and secretions. Antimicrobial peptides are another class of natural products, are innate components of insect immunity, and have high potential against fungi, parasites, viruses, and antibiotic-resistant bacteria (Yi et al. 2014; Ntwasa et al. 2012; Li et al. 2012). Cantharidin and other toxins act as defense from the rove and blister beetles have marked high scientific interest as potential anticancer agents (Galvis et al. 2013a, b).

Apart from usual modern-day medicinal applications, insect natural products have been used for coloring of textile surfaces from time immemorial. The quantitative determination of insect dyes by HPLC, diode-array detection, ^{1}H, and ^{13}C NMR was used to recognize color components on historic yarns prepared from *D. coccus*, *K. vermilio* (kermes), *P. polonica*, *P. hamelii*, or *K. lacca*. Earlier research studies have shown the use of plant reds and other tannins as a part of scale insect red dyes with widespread use (Koren 1994; Cooksey 2018). This book chapter focuses on the structural diversity of natural products from various insects and their potential applications in modern medicinal therapies, validation of their medicinal properties, textile and staining applications along with their identification, and characterizations.

2 Challenges in Arthropod Natural Product Chemistry and Their Structural Diversity

Today, even with the advancement of modern technology, our knowledge of the structures and functions of insect secondary metabolites is uneven with most of the insect species such as crustaceans unscrutinized for the presence of various natural products in comparison to other insect species such as ants, beetles, butterflies, moths, and spiders. Even the chemically scrutinized natural products have not been fully explored for their biological role except some sex pheromones. In most of the cases the chemical compounds have been identified as irritant or having any kind of defensive role without a detailed analysis of mechanism of action. But we lag behind in understanding the biochemical pathways and mechanism involved in arthropods compared to other groups such as plants, bacteria, and fungi. However, structure-oriented chemical prospecting has been focused on the elucidation of the detailed mechanisms underlying chemical-ecological interactions (Blum 1996; Seybold 2004). More than 60% of anticancer and anti-infective agents are of natural product origin such as erythromycin A (**1**), penicillin G (**2**), colchicine (**3**), vinblastine (**4**), vincristine (**5**), and paclitaxel/taxol (**6**). Some of the recently approved natural product-based drugs include elliptinium (**7**), galanthamine (**8**) and huperzine (**9**) (isolated from plants), daptomycin (**10**) (from microbes), and exenatide and ziconotide (from animals). Tigecycline (**11**), everolimus (**12**), telithromycin (**13**), micafungin, and caspofungin are some examples of synthetic or semisynthetic drugs based on natural products. Approximately, 33% of all the approved drugs and 68% of all approved antibacterial drugs from 1981 to 2010 are of natural product origin or their derivatives. The most important and well-studied ecological interactions among arthropods include use of pheromones for intraspecific communication, use of venoms/toxic agents for defense, and the employment of antipredatorial other defensive agents. Fossil records of the chemical defense in insects date back to the Early Cretaceous period (Poinar Jr. et al. 2007). The large number of species in phylum Arthropoda adds itself a big challenge to the present-day research with virtually unlimited number of organisms for chemical analysis with very diverse genetic diversity corresponding to the collection of small-molecule metabolites. Moreover, a considerable big challenge for screening different natural products for their biological activities is being posed by the fact that insects in different life stages have different metabolites which require additional collaborators with specific taxonomic expertise.



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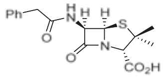

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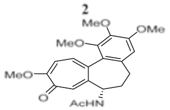

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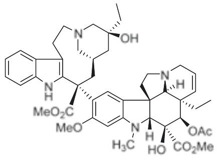

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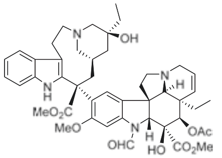

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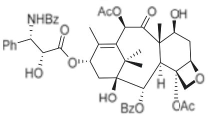

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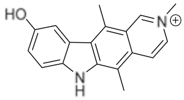

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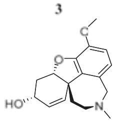

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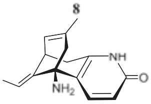

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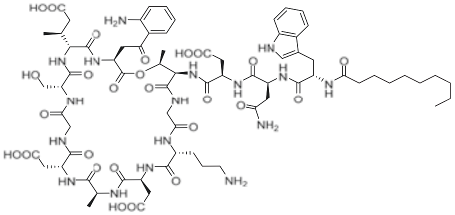

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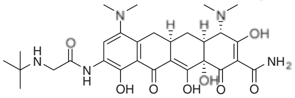

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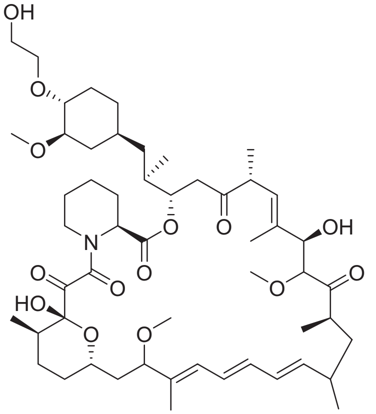
From the recent studies it is certain that insect evolution is widely accepted to be linked with that of the plants. A detailed understanding of the bimolecular mechanisms of plant-insect ecological interactions holds high weightage in many areas such as great environmental benefits associated with the use of blanket pesticide in agricultural pest control (Farrell 1998; Degenhardt et al. 2003; Aldrich et al. 2003). Another example is bacteria-insect symbiosis and symbiont conferred protection with an example of the females of *P. triangulum* who catch and paralyze honey bees for food. Likewise for protecting their offspring, beewolves cultivate the endosymbiont "*Candidatus* Streptomyces philanthi" in antennal glands.

Moreover, most of the arthropod species live in close symbiotic association with various types of microorganisms and it is difficult to assume 100% purity of isolated compounds and whether they have been biosynthesized by the arthropod or other species.



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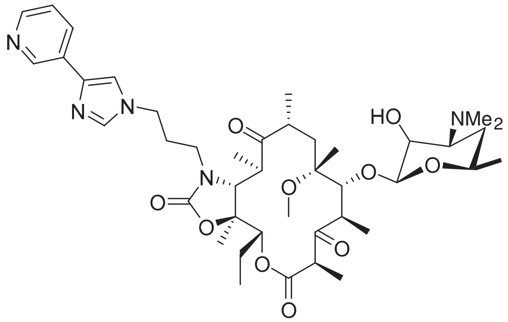

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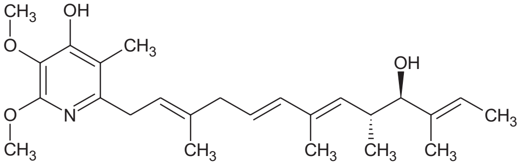
The defensive secondary metabolites identified were piericidin derivatives (e.g., piericidin A1 (**14**) and the chlorinated indole derivative, streptochlorin (**15**)) (Kaltenpoth et al. 2006; Kroiss et al. 2010). Imaging analysis (LDI-TOF) and subsequent GC-MS analyses have revealed the spatial distribution of the antibiotics on the outer cocoon surface and the production of both antibiotics peaked within the first 2 weeks after cocoon spinning (Koehler et al. 2013). Pederin (**16**) and diaphorin (**17**) are the secretion of blister beetles and rove for their defense has shown potential anticancer activity.



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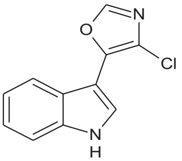

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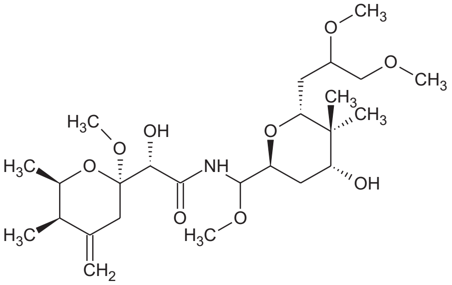

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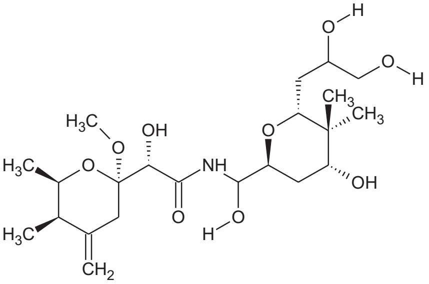

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3 Natural Products from Bees, Wasps, Ants, and Sawflies (Hymenoptera)

The Hymenoptera constitutes one of the largest orders of holometabolous insects. More than 120,000 species have been described, but actual number of species is considerably higher.

3.1 Sawflies, Wood Wasps, and Horntails (Symphyta)

Sawflies, wood wasps, and horntails belong to family Symphyta of order Hymenoptera. It is the smallest family of this order contributing only 7% of the whole order having 8000–10,000 species (Huber 2009). Many foresters consider them as pest, as they feed on leaves and borrow in woods. Yin et al. (2013) reported the isolation of two small molecules macrocarpal (**18**) and grandinol (**19**) from the methanolic solution of Australian sawfly larvae which were further evaluated for their antimicrobial activity against *Bacillus subtilis (*Yin et al. 2013*)*.

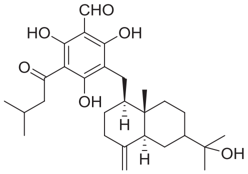
3.2 Ants (Formicidae)

Ants belong to family Formicidae and are one of the most known members of order Hymenoptera. Ants and their by-products are widely used in fork medicines in various parts of the world. Song et al. (2012) isolated three new coumarin compounds (**20–22**) and two known amide alkaloids (**23, 24**) from the adult red ants of the Changbai Mountain *Tetramorium* sp. which is widely used in traditional Chinese medicine, and these compounds were further investigated for their antibacterial activities (Song et al. 2012). Solenopsin A (**25**) isolated from *Solenopsis invicta* and *Solenopsis germinate* fire ants is an active primary alkaloid that exhibits antiangiogenic activity (Heinen and De Veiga 2011). Chen et al. (2012) isolated three new compounds (**26–28**) from the venom of alate queen of fire ants, *S. richteri* (black) and *S. invicta* (red) and their hybrid (*S. richteri* x *S. invicta*) (Chen et al. 2012).

Tang et al. (2014) isolated new dopamine derivatives, (±)-polyrhadopamine A (**29**), (±)-polyrhadopamine B (**30**), and polyrhadopamines C-E (**31–33**), and later, they isolated 13 nonpeptide nitrogen-containing compounds (**34–46**); most of these substances possess pyridine moieties (**34–43**) and three are alkaloids (**44–46**) from *Polyrhachis dives*. Compounds **29** and **30** are dimeric N-acetyldopamine derivatives, **31** is a dopamine analog containing an unusual sulfone group, and **32** and **33** possess a rare benzo[*d*]thiazole moiety (Tang et al. 2014).

3.3 Paper, Potter, and Hover Wasps, Hornets, and Yellow Jackets (Vespidae)

Paper, potter, hover wasps, hornets, and yellow jackets belong to family Vespidae of Hymenoptera characterized for their paper and clay nests and for defense use of sting. Saidemberg et al. (2010) isolated a neuroactive compound, namely, 3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl-3-(1H-imidazol-4-yl)propanimidate (**47**), from the venom of social wasp *P. paulista* (Saidemberg et al. 2010). *P. paulista* contains another class of principal toxins which have been traditionally identified by second-generation proteomic approach (Perez-Riverol et al. 2017).



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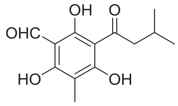

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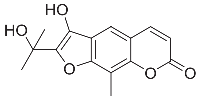

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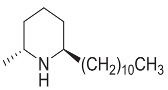

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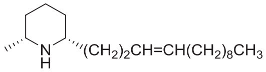

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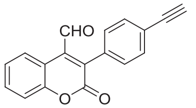

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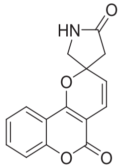

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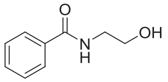

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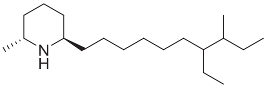

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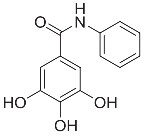

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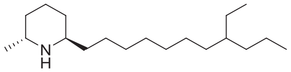

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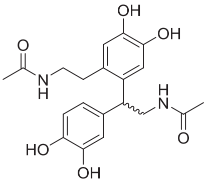

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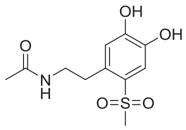

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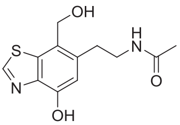

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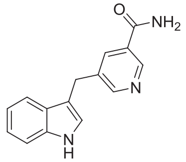

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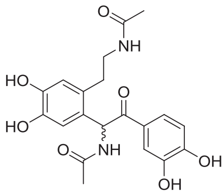

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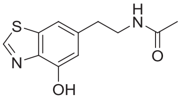

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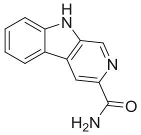

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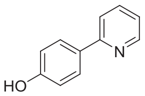

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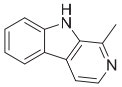

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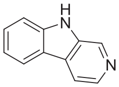

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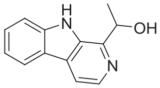

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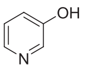

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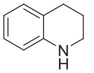

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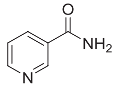

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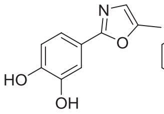

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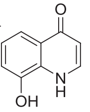

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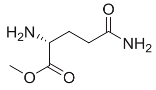

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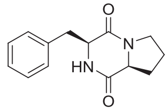

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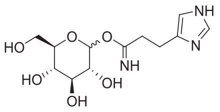

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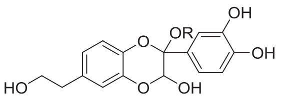
3.4 *Bees (Apidae and Halictidae)*

Bees are one of the important members of Hymenoptera which include honey bees (*Apis)* and bumble bees (*Bombus*). Propolis is gap filling glue used for the construction of beehive; the plant diversity, bee species, and climate decide its chemical composition and play a crucial role on its therapeutic properties. Silva et al. (2012) studied the total phenolic and flavonoid content in the Portuguese propolis from the beehive of *Apis mellifera* from three different places (Bragança, Coimbra, and Beja) and found that Bragança propolis has the largest total phenolic and flavonoid content (Silva et al. 2012). Campos et al. (2014) demonstrated that the propolis extract of this bee contains a number of compounds like aromatic acid (benzoic acid), phenolic acid (dihydrocinnamic acids, cinnamic acids, phenyl, benzyl, long-chain caffeates, and *C*-prenylated coumaric acids), alcohols and terpenes (diterpenic acids and triterpenic alcohols), and sugars. Geopropolis is the propolis collected from the stingless bees of the Meliponini tribe (Campos et al. 2014). Coelho et al. (2015) found that the geopropolis from *Scaptotrigona postica* consisted mainly of pyrrolizidine alkaloids and C-glycosyl flavones, which were collected from the region of Barra do Corda, Maranhao state, Brazil (Coelho et al. 2015). In another study Campos et al. (2015) discovered that the propolis from the stingless bees *Tetragonisca fiebrigi* found in Brazil mainly contains phenolic compounds, alcohol, and terpenes like cinnamyl caffeate, benzyl caffeate, cinnamic acid, hydrocinnamic acid, hydrocinnamic acid ethyl ester, *p*-coumaric acid, 3-phenyl-*p*-coumaric acid, kaurenoic acid, 4-methoxybenzoic acid, retinol, cholesterol, and tocopherol (Campos et al. 2015).

3.5 Beetles

Beetles belong to Coleoptera and are largest insect order with 176 families having 350,000–386,000 species, present almost everywhere. Yan et al. (2015) reported the isolation of four new compounds Blapsols A–D (**48–51**) possessing a 2,3-dihydrobenzo[*b*][1,4]dioxin moiety, along with the five known *N*-acetyldopamine dimers (**52–55**) from *Blaps japanensis* (Yan et al. 2015). Lu et al. (2015) identified three new *N*-acetyldopamine dimers molossusamide A–C (**56–58**) and two known compounds **59** and **60** from *Catharsius molossus* (Lu et al. 2015).



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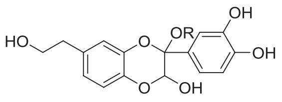
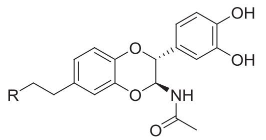
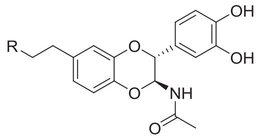
R = -CH_3 (**50**)



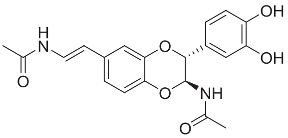
R = -CH_2CH_3 (**49**)
R = -CH_3 (**51**)



R = -CH_2CH_3 (**52**); R = -CH_3 (**54**)



R = -CH_2CH_3 (**53**); R = -CH_3 (**55**)



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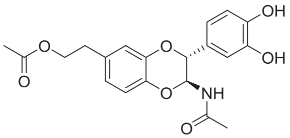

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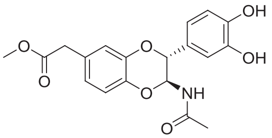

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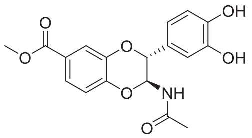

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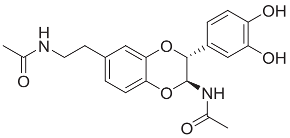

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4 Applications of Insect Natural Products in Various Fields

4.1 Pharmacological Industry

This part of the chapter briefly describes major advancements in developing insect natural products as potential therapeutic agents. From last hundreds of millions of years, insects have utilized enormous range of natural products to service environmental ups and downs or perturbations. In view of that, researchers all over the world believe that insects are a source of untapped natural products. Insect's products especially silk and honey have already been exploited for many thousand years for their use in folk medicine. However, due to the advent of new technologies and strategies it is now possible to manipulate and bioengineer insect natural products into new lead compound for setting up new routes for modern medicines. Utilizing the experience gained from the past knowledge of the uses of insect by-products such as honey and venoms, we can bioengineer insect natural products (silk, cantharidin, antimicrobial peptides, maggot secretions, and anticoagulants) and upscale them to industrial level into therapeutic agents of high values. Concerns and solutions encountered in these endeavors are also described in view of bright future for new insect-derived pharmaceuticals treatments.

4.1.1 Insect Products in Ethnopharmacology (Traditional Medicine)

Despite providing many invaluable natural products including honey, silk, royal jelly, beeswax, pollen, and propolis, insects have not been actively used in modern drugs. Insect secretions have been used in folk medicine all over the globe including China, Bahia, India, Africa, and Mexico (Dossey 2010; Gomes et al. 2011; Pemberton 1999). It is estimated that almost 300 insect species in China and 42 species in Bahia have been used to produce around 1700 traditional medicines (Costa-Neto 2002). However, few insect species have undergone clinical trials to prove their efficacy. In ancient India attraction of ants toward urine was enough to diagnose diabetic patients. Ants were also used to cure lethargy. *Pseudomyrmex* ant venom has been used for the treatment of arthritis (Lockhart 2007).

4.1.1.1 Honey

Honey is one of the complex mixtures of substances with polyphenols (tannins and flavonoids) as major components which are effective in treating a range of human ailments. There are lots of research evidences in favor of the use of honey including various clinical trials which are briefly summarized in tabulated form (Table 1). Apart from mild-to-moderate superficial wound healing ability, honey components are involved in the repair and regeneration of various tissues by stimulating the production of the TNF α-cytokine via TLR4 in human monocyte cultures (Jull et al.

Table 1 Traditional and pharmacological uses of honey and its components

S. No.	Honey and its by-products	Pharmacological uses	References
1	Honey	Wound healing.	Tonks et al. (2007), Kwakman et al. (2011), Cooper et al. (2011), Jenkins and Cooper (2012), Seckam and Cooper (2013), Henriques et al. (2011), Roberts et al. (2012), Brudzynski et al. (2012), Sforcin and Bankova (2011)
		Repair and regenerations of tissues via the production of the TNF α-cytokine.	
		General antimicrobial action because of low pH, high osmolality, H_2O_2 together with defensin-1 and methylglyoxal.	
		Inhibition of the growth of antibiotic-resistant human pathogens (methicillin-resistant *S. aureus*, vancomycin-sensitive and vancomycin-resistant *Enterococci* and *Streptococcus* species).	
		Inhibition of wound-associated gram-negative bacteria (*P. aeruginosa, Stenotrophomonas* species, and *A. baumannii*).	
		Inhibits cell division.	
		Honey in conjunction with hydrogen peroxide is able to degrade bacterial DNA.	
		Wound dressing (alginate in combination with honey).	
		Treatment of *S. aureus*-induced keratitis.	
		Propolis acts as an efficient cariostatic agent to control caries.	
2	Quercetin	Enhance the apoptotic ability of anti-CD95 and rTRAIL (recombinant tumor necrosis) in acute lymphocytic leukemia.	Spagnuolo et al. (2012)
3	Apigenin and acacetin	Induces in vitro caspase-dependent apoptosis in human leukemia cells and also produced apoptosis-mediated inhibition of U937 leukemic cells.	Budhraja et al. (2012)

2008; Lee et al. 2011; Tonks et al. 2007). Low pH, high osmolality, and hydrogen peroxide together with defensin-1 and methylglyoxal are responsible for potent antimicrobial action of honey (Kwakman et al. 2011). Manuka honey is more potent compared to Revamil honey due to the presence of high amounts of methylglyoxal (44 times) (Kwakman et al. 2011). The antibacterial inhibition and wound healing properties of honey have resulted in the development of special wound dressings in the form of ointments and gels in addition to the specific dressings from the mixture of alginate and honey (Seckam and Cooper 2013). Synergetic effects of propolis were found with some antimicrobial drugs in the treatment of experimental *S. aureus* keratitis with the reduction in the antibiotic resistance. There are several reports regarding the anticancer properties of honey products (royal jelly and propolis)

(Ratcliffe et al. 2011). Orsolic (2009) showed immunomodulatory and antitumour activity of honey in various clinical studies (Orsolic 2009).

It is thought that the anticancer activity of honey is attributed to the presence of mixture of polyphenols in honey, propolis, and royal jelly (Abubakar et al. 2012). Polyphenols from propolis have an ability to overcome the resistance of cancer cells to TRAIL-mediated apoptosis with possible use as a dietary supplement in cancer that prevents active strategy (Szliszka and Krol 2013). The killing properties of Manuka honey were discovered on three cancer cell lines via caspase 9-dependent apoptotic pathway. Fernandez-Cabezudo et al. (2013) injected Manuka honey along with taxol into mice implanted with syngeneic melanoma cells and recorded inhibition of tumor growth and host survival. Controls solely with Manuka honey showed 33% inhibition of tumor growth with no synergetic role of taxol. However, the combo increased survival of mice (Fernandez-Cabezudo et al. 2013).

4.1.1.2 Venoms from Bees, Wasps, and Ants

Bee venoms have been used in traditional folk medicine (Chinese, ancient Greece, and Egypt) for many centuries in the treatment of a range of ailments and disease (Chen and Lariviere 2010; Monteiro et al. 2009). Over 75 different chemical compounds are estimated to be a part of this normal complex bee venom mixture with almost 79 different proteins found in parasitoid wasp venoms (Danneels et al. 2010; Hoffman 2010). Hymenopteran venoms have been found to have a wide range of pharmacological activities with high potential of modern drug development. Table 2 represents a list of bee venom components, some of which have been chemically synthesized, characterized, and purified through modern techniques. Among the various chemical components present in bee venom, melittin, a water-soluble α-helical polypeptide molecule composed of 26 amino acids with a tetrameric structure, is an important allergen and principal toxin causing pain, inflammation, and hypersensitivity. Apart from melittin, venom includes low molecular weight terpenes, biogenic amines, peptides, and allergenic proteins such as phospholipases, hyaluronidases, and acid phosphatases (Perez-Riverol et al. 2017). However, melittin alone accounts for 40–60% dry weight of total bee venom (Son et al. 2007; Uçkan et al. 2004).

The cytolytic activity of melittin is attributed to its amphipathic and cationic nature (Hoskin and Ramamoorthy 2008). In order to overcome the cytotoxicity of melittin, scientists have devised various strategies to harness its anticancer/antitumour activity (Table 2). Apart from anticancer properties, bee venom has been found to decrease protein glycation which prevents their secondary structural and functional alterations. It also prevents hemoglobin glycation which decreases diabetes complications (Behroozi et al. 2014). In addition to above, melittin-loaded nanoparticles have high potential for use as topical therapeutic agent for vaginal virucides and have also been shown to inhibit HIV-I (Ahn et al. 2008). Also antimicrobial activity of melittin has been recorded against a wide range of bacterial strains including *E. coli* and *C. albicans* (Ratcliffe et al. 2011).

Table 2 List of some bee venom components and strategies to overcome cytotoxic activities of melittin

S. No.	Insect	Components	Pharmacological activity
1	Honey bee	Adolapin and other polypeptides	Analgesic and anti-inflammatory
2	Bumble bee	Bombolitin peptide	Antimicrobial
3	Wasp	Mastoparan peptide (polybia-MP I, II, III, IV, V, and IV), Polybine-I, Polybine-II, polybioside I, polybia-CP (2, 3), polybitoxins (nPoly p 1 and nPoly p 2), antigen 5 (poly p 5)	Antimicrobial, hemolytic activity and induces mast cells degranulation, antibacterial, chemotactic activity, chemotaxis of polymorphonuclear leukocytes (PMNLs), allergen-hemolytic/CRD and molecular-based IT
4	Ant	Solenopsin alkaloids	Neurological disease and antiangiogenesis
5	Honey bee and wasp	Melittin peptide and apamin peptide	Kills bacteria, cancer cells, anti-inflammatory, muscular dystrophy, and tumors
6	Honey bee and bumble bee	Mast cell degranulation peptide (MCD)	Analogues inhibit IgE binding to mast cells and allergies
7	Wasp and ant	Phospholipases A1 (PLA1) enzyme and antigen 5 polypeptide	Sting diagnosis, Immunotherapy, and anticancer
8	Honey bee, bumble bee, and wasp	PhospholipasesA2 (PLA2) enzyme	Kills cancer cells and Inhibits malaria
9	Wasp, bee, and ant	Kinins, e.g., bradykinin and neurotoxins	Pain control and neurological diseases
10	Honey bee, bumble bee, wasp, and ant	Hyaluronidase enzyme	Enhance cancer chemotherapy

Strategy to overcome cytotoxicity of melittin	**Target cell**
Cancer cells killed by dilutions of melittin	Lung cancer cells in vitro
Point mutation and deletion of specific melittin amino acids	Reduced hemolysis of normal cells but inhibition of bacteria
Synthetic melittin coupled to hecate-CGβ-a as a delivery vehicle	Ovarian, testicular, and adrenocortical tumors in vivo
Melittin coupled to a specific homing peptide	Hepatocellular carcinoma cells in vitro

(continued)

Table 2 (continued)

S. No.	Insect	Components	Pharmacological activity
Gene therapy and transfection of melittin gene into tumors		Human bladder carcinoma cells in vitro	
Use of nanoparticle technology for delivery of melittin to tumor cells		Melanomas in vivo	

Hecate-CGβ-a: the beta chain of human chorionic gonadotropin

4.1.1.3 Silk

Silk is a biological macromolecule which is being produced from last 5000 years with nearly 70% coming from China with 42 billion US $ economic value in 2010. In Chinese medicine, silk has been used for various human conditions (relief from spasms and flatulence) from ancient times whereas silkworms have been used for detoxification of bacterial infections and for curing impotence (Ratcliffe et al. 2011; Ahn et al. 2008). Presently, neither silk nor silkworms are used in conventional medicine. However, whole silkworm extracts are now used to develop modern medicines.

Dimethyl adenosine, a vasorelaxant from *Bombyx mori* pupae, inhibits phosphodiesterase and increases production of NO in endothelial cells. Dimethyl adenosine is a pharmaceutical candidate for the treatment of vasculogenic impotence (Ahn et al. 2008). Due to the advent of synthetic polymers silk fiber/fabric has now lost its values in modern medicine; however, it was previously used for medical sutures. But the ingenuity of scientific research continues to take advantage of silk in the transport and delivery of drugs around human body and tissue engineering (Numata et al. 2009). Silk biomaterials can be prepared in many forms such as tubes, scaffolds, films, coatings, hydrogels, and even nanoparticles with many characteristic properties such as tough, flexible, and biodegradable. Silk is biocompatible in nature, has low degradation rates, and can be chemically modified to a wide variety of structures. Silk fibers have high elasticity and mechanical strength due to which it has been used for bone tissue engineering (Luetchford et al. 2020). The rate of degradation of silk biomaterials can be controlled to regulate the release of carrier drugs in the body (Takeuchi et al. 2019; Yavuz et al. 2019; Quinones et al. 2019). Silk fibroin has been used in conjunction with gelatin, chitosan, and hyaluronic acid for the improvement of its properties (Luetchford et al. 2020). Silk scaffolds loaded with mesenchymal cells have been used for the synthesis of bone and/or cartilage-like tissues enhancing bone healing in vivo (MacIntosh et al. 2008; Meinel et al. 2006). Such technique coupled with the use of immobilized growth factors is an emerging field of research for the development of new bioengineering technologies (MacIntosh et al. 2008).

Table 3 Biomaterial silk products and their applications

S. No.	Materials used	Categories	Applications
1	Silk fibers coated with antimicrobial agents	Hygiene products	Surgical textile fabric, masks, gowns
2	Silk fibroin/collagen	Extracorporeal implants	Artificial kidney and lungs
3	Silk fibroin-loaded drugs	Implantable materials	Gastrointestinal stents
4	PET/silk yarns with sericin coating		Vascular implant (vascular grafts)
5	Modified silk ligature twisted wire		Orthopedic implants (artificial bone joints)
6	*B. mori* silk fibroin net	Non-implantable materials	Soft tissue implants
7	Silk thread or a titanium clip		Surgical sutures
8	Fibrous silk composites		Wound dressings, orthopedic bandages

Numata et al. (2009), Luetchford et al. (2020), Yavuz et al. (2019), Quinones et al. (2019), MacIntosh et al. (2008)

Additionally, silk-based nanoparticle composites containing curcumin have been used for treating breast cancers in vivo (Takeuchi et al. 2019), and gel films made from sericin have been successfully used for wound dressings (Quinones et al. 2019). In general silkworm silk has been approved by FDA for use in human body with never-ending diverse applications as it can be used as glue to close the sutures during surgery (Stewart and Wang 2010). Other biomedical applications of silk and modified silk fibers are given in Table 3.

4.1.2 Cantharidin from Blister Beetles and Other Small Molecules

Blister beetles belong to Coleopteran family Meloidae with around 2500 species producing several toxic defensive secretions which cause blistering when comes in contact with skin. Cantharidin is one such defensive toxic product extracted from the dried bodies of *Mylabris caragnae* commonly used in Chinese folk medicine with around 2000 years of cancer treatment therapy usage and for the removal of warts (Wang 1989). Being toxic to gut and kidneys, its full potential has not been exploited yet, although it has been used for treatment of rabies and impotence (Ratcliffe et al. 2011). Higher doses of cantharidin cause renal failure which has potentially rendered its use as anticancer drug. Increasing interest in the use of cantharidin and its synthetic derivatives have been seen in recent past for the treatment of hepatic, colorectal, bladder, breast, melanomas, and pancreatic carcinoma in addition to leukemia (Ratcliffe et al. 2011).

In response to the higher toxicity of cantharidin, norcantharidins have been synthesized to produce anticancer activity (Wang 1989). A new class of natural compound (norcantharimides) having improved water solubility and enhanced toxicity against human hepatocellular carcinoma cell lines has been discovered from a

Chinese blister beetle, *Mylabris phalerata* (Tseng et al. 2012). Despite the synthesis of lot of low toxic chemical analogues of cantharidin, it is still used in many clinical trials and one such clinical trial (cantharidin mixed with chemotherapy for gastric cancer) was successfully finished (Li et al. 2010). Lissina et al. (2011) in his study showed cantharidin as an effective gene regulator for transcriptional analysis of methyl transferase. Similarly, inhibition of PP1 and PP2 by cantharidin and subsequent apoptosis of cancer cells lead to the design of new gene therapy strategy for killing hepatocellular carcinoma cells (Lissina et al. 2011). Additionally, Dang and Zhu (2013) designed solid liquid nanoparticles of cantharidin with higher solubility, low toxicity, and long half-life period and can be taken orally (Dang and Zhu 2013).

4.1.3 Antimicrobial Peptides (AMPs)

Antimicrobial peptides come in whole body secretion category and have been widely used in traditional Chinese and folk medicine to treat numerous diseases and illnesses including many different types of infections (throat, ear infections, tuberculosis, and influenza) and cancers (Ratcliffe et al. 2011). Many insects thrive in inhospitable environments with many other microorganisms such as dung or rotting corpses which are helpful in improving immune defenses to counter infection. Of the various components of insect innate immunity AMPs are potentially important source of natural products which have come in light of researchers due to the rise of antibiotic-resistant bacteria, such as methicillin-resistant *S. aureus* (MRSA) and *C. difficile*. Antimicrobial peptides have been classified in three subcategories as follows (Fig. 1).

But the urgency and the length of time AMPs have been studied so far, very few molecules have undergone clinical trials (Slocinska et al. 2008). The polypeptides

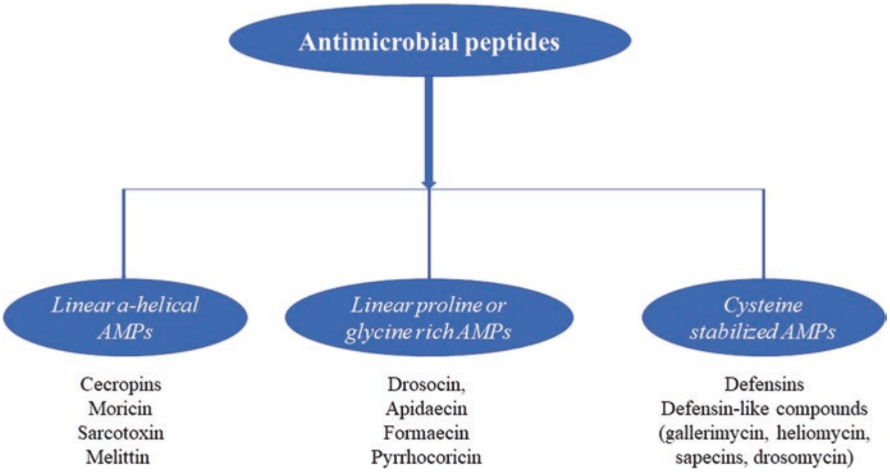


Fig. 1 Classification of antimicrobial peptides

melittin present in bee and wasp venoms are amphipathic and cationic AMPs which have been described previously in earlier section of this chapter. Table 4 gives a brief account of the medicinal uses of antimicrobial peptides (Ratcliffe et al. 2011; Torrent et al. 2012; Ostorhazi et al. 2011; Berthold et al. 2013; Bulet and Stocklin 2005; Ulm et al. 2012).

The cationic AMPs having net positive charge (due to arginine, histidine, and lysine) are thought to interact with negatively charged bacterial cell wall component through electrostatic forces of attraction whereas membranes enriched with zwit-

Table 4 Medicinal uses of different classes of AMPs

S. No.	Antimicrobial peptides	Representative examples	Pharmacological action
1	*Linear a-helical AMPs*	Cecropins, moricin, sarcotoxin, and melittin	Cecropins are active against gram-positive and gram-negative bacteria, viruses, protozoans, fungi, nematodes.
			Cecropins are active anticancer drugs in conjunction with melittin.
			Engineered cecropin A-melittin analogues act as potential as drugs against protozoan parasites such as *Leishmania*.
2	*Linear proline or glycine-rich AMPs*	Drosocin, apidaecin, formaecin, and pyrrhocoricin	Targets proline-rich specific intracellular targets in bacteria.
			Major target of action is believed to be the intracellular chaperone DnaK.
			Proline-rich designer peptide (A3-APO) works against multidrug-resistant bacterial infections in the wounds and lungs of mice.
			Apidaecin (Api88) has shown similar kind of activity as that of A3-APO against pathogenic *E. coli*.
3	*Cysteine stabilized AMPs*	Defensins or defensin-like compounds (gallerimycin, heliomycin, sapecins, drosomycin, spodoptericin, and phormicins)	Mainly active against gram-positive bacteria and fungi in addition being antiparasitic in nature.
			Defensins inhibit MRSA and disrupt myeloma cancer cells.
			Killing of bacteria and modulation of the immune response.
			Invertebrate defensins have higher affinity for bacterial cell wall precursors, lipid II, and inhibit their incorporation into peptidoglycan network.

Ratcliffe et al. (2011), Torrent et al. (2012), Ostorhazi et al. (2011), Berthold et al. (2013); Bulet and Stocklin (2005), Ulm et al. (2012)

terionic phospholipids and cholesterol are refractory to the AMPs. This binding results in the disruption and permeabilization of outer membranes which eventually leads to death (Kang et al. 2012). In the future, AMPs are thought to be potential class of drugs designed to kill the antibiotic-resistant bacteria and cancer cells. The versatility of these potential drugs seems to increase with the use to coat titanium bone implants for infection prevention and inhibition of biofilm formation by these compounds (Kazemzadeh-Narbat et al. 2012; Park et al. 2010).

4.1.4 Maggot Molecules and Maggot Therapy

Mayan Indians and aborigines have used maggot molecules for wound healing practices from time immemorial. However, maggot therapy was properly discovered as a potential wound healing therapy after it was introduced into USA hospitals in the 1920s by Professor William Baer at John Hopkins University which got more popular by the 1930s and 1940s within over 300 USA hospitals. With the discovery of new antibiotics after the 1940s, the enthusiasm for maggot therapy dropped down very quickly until the 1980s with the appearance of antibiotic-resistant bacteria. Nowadays maggot therapy is commonly used for curing diabetic foot wounds, post-operative infections, bedsores, and leg ulcers in many European countries, the USA, and Israel (Ratcliffe et al. 2011; Sherman et al. 2000). The larvae of *L. sericata* are used to kill MRSA although other species (*L. cuprina*, *P. regina*, and *C. vicina*) have also been tried (Sherman et al. 2000). However, maggot therapy involves three stages or processes and are summarized in Table 5.

The anti-inflammatory macrophages secrete fibroblast (bFGF) and vascular endothelial (VEGF) growth factor which mediate mitosis resulting in angiogenesis and eventual healing of wounds. This was also confirmed by maggot secretions to acute skin wounds in rats where the levels of inflammatory cytokines, TNF-ǖFC;, and IL-6 remained significantly low compared to untreated wounds (Li et al. 2013). Cazander et al. (2012) have shown that maggot secretion reduced complement activation by 99.99% in healthy and postoperatively immune-activated patients (Cazander et al. 2012). Additionally, increasing interest has been developed in the antibacterial factors *M. domestica* because of its possible role as a carrier of pathogens. Results confirm that defensin is released by these insects that is upregulated upon bacterial ingestion and is responsible for the antibacterial activity against MRSA and VRE (Park et al. 2010; Chernysh et al. 2012). Alloferons and seraticin are low weight antibacterial factors from calliphorid flies and can be developed as new medicinal drugs.

4.1.5 Insect Anticoagulants

The anticoagulants from the salivary glands of blood sucking insects (Hemiptera, Diptera, Siphonaptera, and Anoplura) can be developed as new potential anticoagulants which can target specific clotting enzymes. Extracts from the salivary glands

Table 5 Characteristic features of maggot therapy

S. No.	Stages of maggot therapy	Characteristics	References
1	*Debridement of wounds*	First stage of wound healing process.	Ratcliffe et al. (2011), Chambers et al. (2003)
		Cleaning of tissue cells and subsequent removal of necrotic debris.	
		Release of chymotrypsin- and trypsin-like serine proteases, aspartyl proteinase, and metalloproteinase.	
		Ammonia secretion by maggots activates serine proteases.	
		All the above enzymes remove the extracellular matrix (debris, fibrin clots, and biofilms), hence allowing healing to begin.	
2	*Wound healing*	This is second stage of wound healing process.	Bexfield et al. (2010), Zarchi and Jemec (2012), Zhang et al. (2010), Li et al. (2013), Cazander et al. (2012)
		The amino acid derivatives and fatty acids from the maggot secretions from *L. sericata* activate fibroblasts.	
		Induction of mitosis in human endothelial cells and activation of angiogenesis.	
3	*Disinfection of wounds*	Maggot secretions can kill bacteria-infecting wounds, including antibiotic-resistant strains such as MRSA.	Ratcliffe et al. (2011), Park et al. (2010), Chernysh et al. (2012)
		Calliphorid flies used in wound healing with lucifensin (AMP).	

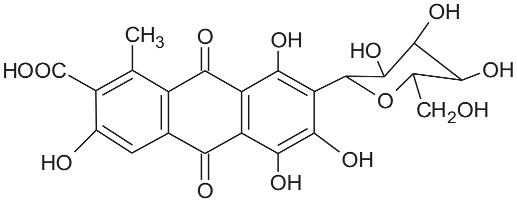
of horse flies have been used for centuries in Eastern medicines in thrombolytic therapy. However, progress in identifying and commercialization of anticoagulants not only from insects but also from leeches (recombinant derivatives of hirudin) and ticks is in process from some years in Europe and the USA (Ratcliffe et al. 2011; Cherniack 2010; Graetz et al. 2011). With regard to the bioactive substances from ticks, intense research is underway with the discovery of variety of molecules with diverse pharmaceuticals functions (Kazimırova and Stibraniova 2013).

However, due to the more complex of insect proteins it was difficult to identify the nature of anticoagulants in their salivary glands. More recently, a unique thrombin inhibition mechanism was discovered in the salivary glands of *Anopheles* mosquitoes which has helped to study the structure and function of thrombin inhibition by anophelin to much greater extent. Additionally, caterpillars (*L. obliqua* and *L. achelous*) have been found to cleave FX similar to endogenous activators, cleave

FV in similar way to that of plasmin and thrombin, and cleave FXa by direct generation of α-thrombin (Sachetto and Mackman 2019; Xu et al. 2016).

4.2 Textile Industry and Staining Applications

Insect natural products or insect dyes have been used in textile industries from ancient times for coloration purposes. Carminic acid (**61**) is a natural colorant from the dried bodies of female insect, *Dactylopius coccus*. Carminic acid (C.I. 75,470) is the main pigment of the cochineal dye (Borges et al. 2012). Scientific investigations in the nature and structure of major chemical components of cochineal dye, carminic acid was given in 1920 by Dimroth and Kämmerer (1920), revised in 1964 by Overeem and Van der Kerk (1964), and confirmed by Schmitt et al. (1984) through ^{1}H and ^{13}C NMR spectroscopy (Dimroth and Kämmerer 1920; Overeem and Van der Kerk 1964; Schmitt et al. 1984). American cochineal mainly contains carminic acid (94–98%) with small amounts of kermesic acid (0.1%) (**62**) and flavokermesic acid (0.4%) (**63**) (Wouters and Verhecken 1989). The carmine dye was used in Central America in the fifteenth century for coloring fabrics and became an important export good during the colonial period. The carminic acid content of a good quality raw cochineal can reach a remarkable 22% of the dry weight of the insects (Lloyd 1980). Cochineal produces range of beautiful crimsons, scarlets, and pinks on fabrics depending upon the mordants used (Perkin and Everest 1918; Tiedemann and Yang 1995). Polish cochineal was identified in Byzantine textiles from the fourteenth century with main chemical components as mono- and di-, C-, and O-hexosides of kermesic and flavokermesic acids or their derivatives. Kermes vermilio contains 75–100% kermesic acid in conjunction with small amounts of flavokermesic acid (Rosenberg 2008).



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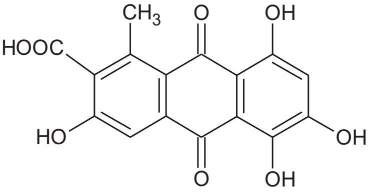
Kermes is a red dye derived from the dried bodies of scale of female insects of genus *Kermes*. Kermes was known from ancient times for its dyeing properties (Verhecken 1989). Kermes was used in Morocco, Turkey, Spain, South France, and G.B. (England and Scotland) for dyeing wool and leather. The insects are collected in the month of June and killed by being exposed to vapors of acetic acid. 5 kg of crude material yields approximately 50–55 g of red pigment (Knecht 1941). The principal coloring matter present in kermes is kermesic acid (C.I. 75460) (Gadgil et al. 1968), which is aglycone of carminic acid (Bingham and Tyman 2000). It is slightly soluble in cold water and soluble in hot water giving yellowish red solution and gives brilliant scarlet shades when dyed on alum mordanted substrates. Crude kermes also contains a second pigment flavokermesic acid (3 g from 5 kg of kermes), the two are being separated due to different solubilities of their sodium salts in sodium acetate solution. Flavokermesic acid produces golden yellow from acid baths and on tin mordant a dull orange shade is obtained.



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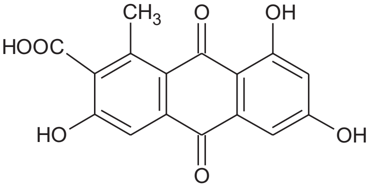

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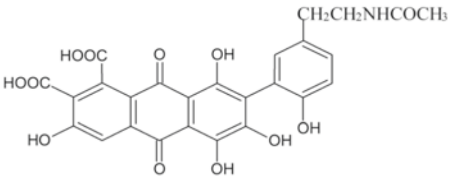
European Food Safety Authority (Italy) has recorded the extensive use of cochineal extract, carminic acid, and carmines E 120 for the coloration of a wide range of food and drink products (Aguilar et al. 2015). In the USA under the Food and Drug Administration, it is required that food and cosmetic products should be labeled as “cochineal extract” if the carminic acid content is >1.8% or “carmine” if the carminic acid content is >50%. Carmine has long history of using as a staining product with first attempt being made by Ferdinand Cohn and Johann Heinrich Robert Göppert in the mid-nineteenth century to stain plant tissues. Dating back to 1851, application of carmine in histology was reported by Le Marquis Alphonse Corti (Corti 1851).

Lac (C.I. 25:75450) is present in stick lac (Burwood et al. 1965), which is a resinous protective secretion of tiny lac insect *Laccifer lacca/Kerria lacca/Coccus lacca* and is probably the most ancient insect red dye. It is native to India and Southeast Asian countries (Thailand and China). The insects secrete a thick resinous fluid which envelopes their bodies and forms hard continuous encrustation over the surface of the twigs. The Natives break off these twigs just before the time of hatching and expose them to sunlight to kill the insects; the product is stick lac, which contains coloring matter and resin (Knecht 1941). For commercial purposes lac dye is obtained by extracting stick lac with water and sodium carbonate solution and precipitating with alum or lime (Mayer and Cook 1943). Lac dye is a mixture of five closely related laccaic acids designated as laccaic acid A, laccaic acid B, laccaic acid C, laccaic acid E, and laccaic acid D having anthraquinonoid-type structures (**64–68**) (Burwood et al. 1967; Pandhare et al. 1966; Pandhare et al. 1967; Rao et al. 1968; Mehandale et al. 1968; Oka et al. 1998; Ferreira et al. 2004). Of these A (>70%) and B (>20%) are the major ones (Ferreira et al. 2004). Lac dye dissolves in hydrochloric acid and is able to dye wool and silk directly giving beautiful orange-red shades which have excellent light fastness property (Klos 2005; Kongkachuichay et al. 2002; Khan et al. 2004). Lac dye is being used as a natural food color and in the coloration of textile materials (Chairat et al. 2005; Kamel et al. 2007; Khan et al. 2012; Rattanaphani et al. 2007).



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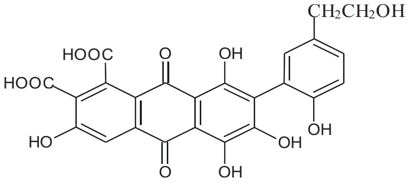

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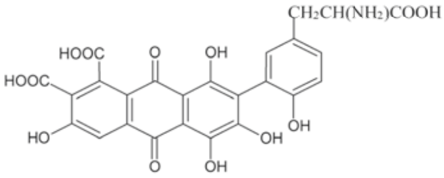

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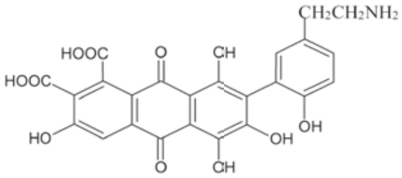

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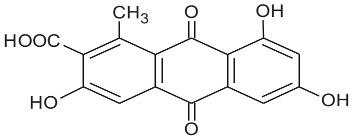

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5 Future Prospectus and Conclusion

Insects have long and rich history for being in use traditional medicine all over the world. Recently, lots of small bioactive molecules have been isolated from a vast number of insects which have potential to be developed in lead compounds for modern medicines. Apart from the medicinal applications, many chemical compounds from insect bodies find extensive use in textile industry as colorant and as staining agent. However, insect-derived natural products are yet to gain recognition and market success compared to their plant-derived counterparts. This is due to the fact that insects are usually mentioned for their negative roles, particularly in the West. Continuous scientific research will definitely overcome the negative public perception related to the use of insect-derived products as drugs and proper attention will hold great promise for the future of natural product drug discovery.

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Silkworm: A Unique Creature for Natural Products

R. Venkatesh Kumar and Devika Srivastava

Abstract Insects are an exemplary source of natural products. However, still they are considered a marginalized source of natural products, although their number is sizable, and have great pharmacological potential of natural products when compared to another group of animals. Among beneficial insects, silkworms and their by-products have emerged as the most valuable creatures due to their incredible medicinal properties and other usages. Apart from production of silk, silkworms are being utilized in several ways, such as a source of nutrients for human consumption, as cattle feed, as an antipollutant, used in the manufacturing of vaccines, and as a bioreactors for the production of recombinant proteins. Further, silkworm pupae, pupal powder, and pupal oil have shown spectacular biological activities such as neuroprotective, antidiabetic, hypolipidemic, antioxidant, anticancer, and antibacterial. This chapter summarizes the usages of silkworms and their by-products with their chemical constituents, proven biological activities, biomedical and pharmaceutical application of silkworm, and their by-products.

Keywords Silkworms · Silkworm excreta · Pupae · Papal oil · Biological activities

1 Introduction

Insects are playing a very significant role as a source of functional food for human beings (Bodenheimer 1951). Silkworm is an economically important insect which belongs to phylum Arthropoda, class Insecta, order Lepidoptera, and family Bombycidae. Silkworms are used in sericulture industry primarily for the production of silk. Apart from silk, many valuable by-products are being produced by silkworms which are neglected part of the silk industry. These by-products are being utilized in several ways. Predominantly, silkworm excreta are used as a natural colorant in the food and pharmaceutical industries. Instant silkworm tea is consumed traditionally in a few countries due to its medicinal value for the benefit of human beings (Vimolmankang et al. 2014). Silkworm pupal oil (Fig. 1g) is used as one of

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(a) Egg (b) Larvae (c) Pupae (d) Moth (e) Pupae powder (f) Cocoon pelade (g) Pupal oil

Fig. 1 (**a**). Egg, (**b**) larva, (**c**) pupa, (**d**) moth, (**e**) pupal powder, (**f**) cocoon palade, (**g**) pupal oil

the key ingredients in the manufacturing of paints, varnishes, and in the pharmaceutical industry (Priyadarshini et al. 2017).

Alpha-linolenic acid (ALA) is one of the omega-3 fatty acids (Fig. 2q) present in silkworm pupae oil and can be utilized for human consumption (Ramappa et al. 2017, Wattanathorn et al. 2012). Chitosan (Fig. 2k) is a derivative of chitin (Fig. 2l) of silkworm pupa and is being used in the removal of toxic pollutants from water (Simionato et al. 2014). The dried form of silkworm larvae (Fig. 1b) is sold as a food product in several Asian countries (Ramakanth and Raman 1997). Silkworm moths (Fig. 1d) are being used in wine preparation (Gui and Zhuang 2000), and the eggs (Fig. 1a) of silkworms are exploited for pharmaceutical application and transgenic researches (Buhroo et al. 2018), (Joy and Gopinathan 1994). In Japan cocoon palade (Fig. 1f) is popular in the name of Shinki fibroin, and it is added in many food preparations as an ingredient, due to its nutritional value (Ramakanth and Raman 1997).

Pharmacological studies show that silkworm pupae (Fig. 1c) and their by-products exhibit various biological activities such as neuroprotective (Vijayakumar 1996), hyperlipidemia (Ryu 2014), antioxidant (Chukiatsiri and Hangtrakul 2018), antidiabetic (Gavia et al. 2003), anticancer, and antibacterial (Ahn et al. 2008).

Utilization of silkworm and its by-product has also enhanced in biomedical and pharmaceutical fields in innovative ways such as using enzymes of silkworm against rheumatism (Rajakumar et al. 2014) and production of low-cost vaccine against the infectious disease of human beings (Datta 1994). Silkworm pupal chitin (Fig. 2l) is being utilized to its full potential in post-operational treatment such as deviatomy,

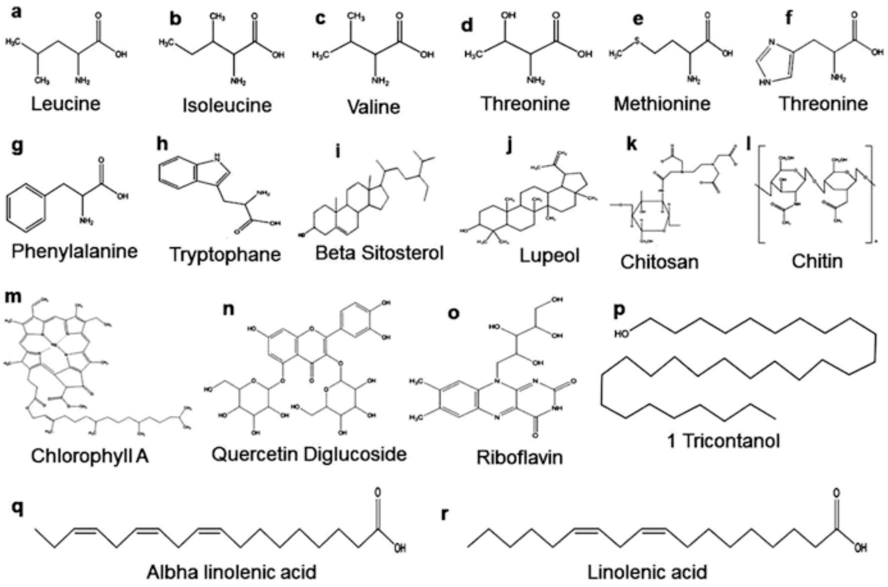

Fig. 2 (**a**) Leucine, (**b**) isoleucine, (**c**) valine, (**d**) threonine, (**e**) methionine, (**f**) histidine, (**g**) phenylalanine, (**h**) tryptophan, (**i**) beta-sitosterol, (**j**) lupeol, (**k**) chitosan, (**l**) chitin, (**m**) chlorophyll A, (**n**) quercetin diglucoside, (**o**) riboflavin, (**p**) 1-tricontanol, (**q**) alpha-linolenic acid, (**r**) linolenic acid

conchotomy, etc. (Buhroo et al. 2018). Sericin is a protein found in a cocoon of silkworm and used for proliferation of mammalian cell line (Terada et al. 2002). Studies have shown that silkworm can be used as bioreactor (Jian et al. 2006). It has been reported that the excrement of silkworm ameliorates the severity of arthritis (Zheng et al. 2018). Artificial blood vessels have been successfully developed using transgenic silkworm-based silk fiber (Iyengar 2010).

In this chapter, we have exclusively emphasized for the usages of silkworm larvae, silkworm chitin, silkworm pupal oil, pupal powder, silkworm excreta, etc., for the human benefits. Among these sources, some of them are considered as a nutritive food source, few of them are used in industries to manufacture goods, and few by-products are exclusively used in pharmaceutical industries due to their therapeutic values. Further, the biological activities of silkworm and its by-products are also elaborated precisely.

2 Uses of Silkworms and Their By-Products

2.1 Silkworm Eggs

Eggs (Fig. 1a) of silkworms are known for albumin, fats, sugars, glycoproteins, and B1 and B2 vitamins. The silkworm eggs are processed into a protein extract, which is the vital ingredient for the preparation of medicines for hepatoprotective, hypolipidemic, and hypoglycemic activities (Nazim et al. 2017). The silkworm eggs have long been used in transgenic studies. In these studies, the gene of interest can be transferred from one organism to another organism egg through recombinant DNA techniques (Joy and Gopinathan 1994).

2.2 Silkworm Larvae

In Hong Kong, China, Korea, and Japan healthy larvae (Fig. 1b) of silkworms are sold as a food product after sterilization and vacuum drying (Ramakanth and Raman 1997). Silkworm larvae are being used in the food industries as supplementary nutraceuticals due to the presence of excellent nutritional value (Nazim et al. 2017). The outcomes of several studies have shown that powder of silkworm reduces blood glucose level; it is also suitable for the immune system. This designates that silkworm powder proved as one of the best protein sources from insects suitable for taikonauts. Another study indicated that silkworm powder reduced plasma glucose level in a streptozotocin-induced diabetic rat model (Rattana et al. 2017). The extract of larvae is one of the critical ingredients for the preparation of an anti-acne cream, which gives significant clinical results (Singh and Jayasomu 2002).

2.3 Silkworm Excreta

In Asian countries, mainly China and Korea, the feces of silkworms have been used as a therapeutic agent in the treatment of infectious diseases, primarily abdominal pain and headache (Tulp and Bohlin 2004). There are some compounds such as pectin, phytol, carotene, and triacontanol which are extracted from feces of silkworms that are used as a therapeutic in various diseases such as acute pancreatitis, chronic nephritis, leukocytopenia, hepatitis, etc. (Singh and Jayasomu 2002). Silkworm excreta produced during different instars of the silkworms are either thrown away or used for the preparation of compost and vermicompost. Due to exemplary burning property and stickiness of silkworm excreta are being used for the development of incense stick, which is additional income for seri-farmers (Dutta et al. 2010). Excreta of silkworms are used to produce biogas yield, which is comparable to another substrate such as cattle, pig, and chicken manure. Fermentation of silkworm excreta and silkworm breeding waste under mesophilic condition produced 167.32 m^2/Mg TS of methanol, 331.97 m^3/Mg TS of biogas, and 256.59 m^3/Mg TS of methane and 489.24 m^3/Mg TS of biogas (Łochynska and Frankowski 2018).

2.4 Silkworm Pupae

India produces 40,000 metric tons of silkworm pupae annually. However, we have not been able to exploit their full potential due to the dearth of proper research and lack of awareness (Mahesh et al. 2015). The pupae of silkworm can also be used in medicines due to the presence of high-quality protein (Chukiatsiri and Hangtrakul 2018). Pupae of silkworm are used as organic manure as they are a good source of nitrogen, calcium, phosphorus, magnesium, zinc, and iron. They are also known as a source for poultry and cattle feed as they contain abundant protein (Priyadarshini et al. 2017).

In a study investigated that quails were fed with a pupal meal as a substitute of fish meal and significant results were observed in egg production and feed conversion in quails (Rahmasari et al. 2014).

Pupae of silkworm are used to make fine powder called pury, which is rich in protein, essential amino acids, polyunsaturated fatty acid, carbohydrates, vitamins, and minerals. Human bodies can easily absorb silkworm pupal powder (Fig. 1e) as it contains natural proteins (Ryu et al. 1997).

2.4.1 Pupal Oil

Among by-products of the silkworm, the pupal oil (Fig. 1g) is considered as the most valuable by-product due to its multifaceted uses. Silkworm pupae are a valued source of pupal oil containing 25–28% on dry weight and 6–7 % on a wet basis (Iyengar 2007). Silkworm pupal oil contains 28.8% of saturated fatty acids, 27.7% of monounsaturated fatty acids, and 43.6% of polyunsaturated fatty acids. Alpha-linolenic fatty acid (Fig. 2q) is prominent fatty acid in pupal oil (Tomotake et al. 2010). Pupal oil of non-mulberry silkworm (eri silkworm) can be produced and utilized as cost-effective alternative edible oil. It has been reported that pupal oil lowers serum cholesterol and triglyceride and enhances high-density lipoprotein cholesterol (Longvah et al. 2012). Extracted pupal oil is used in the manufacturing of paints, soaps, cosmetics, etc. After extraction de-oiled pupa is left, which is considered as nutritious poultry and fish feed. Interestingly outcome of research shows that not only pupal oil but the de-oiled pupa is remarkably useful (Iyengar 2007).

2.4.2 Chitosan of Silkworm Pupae

The outer exoskeleton and internal organs such as tracheae and spiracles of silkworm pupae are lined by chitin (Fig. 2l). Chitosan (Fig. 2k) is acquired from chitin after deacetylation. It has full application in the removal of pollutants from water. The textile industries discharge effluents in water bodies and rendering water bodies polluted and toxic to aquatic flora and fauna. The chitosan derived from silk chrysalides is used as an alternative source for the treatment of remazol contaminated textile effluents (Simionato et al. 2014).

Aluminum sulfate is used to purify the drinking water by removing particulate, colloidal, and other dissolved substances through coagulation. However, accumulation of aluminum is itself very toxic and harmful for aquatic animals, especially in fish; it causes respiratory disorders. In humans, it leads to neurological disturbances, particularly Alzheimer's, etc. It has been studied that chitin and chitosan from silkworm chrysalides successfully adsorbed the aluminum from effluent-contaminated water (Simionato et al. 2006).

2.5 Cocoon Palade

Palade (Fig. 1f) is the innermost layer of the cocoon produced by the silkworm. In China and Japan, it has now become a valuable ingredient for human food. Palade is obtained from the reeled cocoon after removing the pupae. It possesses the same property of silk chemically. From 10 kg of the reeled cocoon, one kg palade can be obtained. The palade is utilized for the extraction of protein and amino acid. By enzyme treatment; this protein is hydrolyzed from the waste silk fiber and refined chemically for use in food item preparations. 1–5% of "Shinki fibroin" is added to the foods. It is recommended at the rate of 5 gm per day (2 g/cup) in milk and coffee (Ramakanth and Raman 1997).

2.6 Silkmoth

Usually, silk moths (Fig. 1d) are not known for their applications after egg laying, as they survive only for a few days. However, several studies have taken advantages with the short duration of their adult stage that healthy moths are being used in the preparation of wine, and these are also used in making medicines (Vijayakumar 1996). The oil which is obtained from silkworm moth can be used in making textile dyes and soaps (Gui and Zhuang 2000).

3 Chemical Composition and Their Role in Silkworm and Their By-Products

3.1 Proteins and Amino Acids

The scleroprotein present in the eggshell of the silkworm is a significant constituent and helps for the structural stabilization of eggshell (Kawasaki et al. 1970). Besides this, the eggs (Fig. 1a) have also been reported to contain chorionins which are the sulfur-containing substances and cysteine proteinase (Xia et al. 1989).

Eighteen different types of amino acids have been reported from mulberry and non-mulberry silkworm larvae and pupae, namely, cysteine (CYS), methionine (MET), aspartic acid (ASP), threonine (THR), serine (SER), glutamic acid (GLU), glycine (GLY), alanine (ALA), valine (VAL), isoleucine (ILE), leucine (LEU), tyrosine (TYR), phenylalanine (PHE), lysine (LYS), histidine (HIS) (Fig. 2f), arginine (ARG), proline (PRO), and tryptophan (TRP), out of these, eight amino acids are essential amino acids (EAA), namely, ILE (Fig. 2b), VAL (Fig. 2c), THR (Fig. 2d), LYS, MET (Fig. 2e), PHE (Fig. 2g), and TRP (Fig. 2h) (Ji et al. 2016; Zou and Han 2006; Tomotake et al. 2010; Mahesh et al. 2015). Essential amino acids present in the silkworm pupae are identical in whole egg protein except tryptophan, which is exclusively present in pupal protein (Rao 1994).

Cocoon palade (Fig. 1f) is consisting of sericin and fibroin (Zhao et al. 2007). Sericin is used in the biomedical field such as antitumor and anti-inflammatory agent (Kunz et al. 2016). Silk fibroin is being exploited for clinical application by utilizing it as a scaffold for the preparation of biomedical products such as hydrogels and microspheres (Lawrence 2014). Lipophilic peptide (VAP) is a unique type of peptide contained in the head region of male moths of the silkworm (Imai et al. 1996).

3.2 Carbohydrates

Olumuyiwa and Omotoso (2015), while conducting a comparative study on the nutritional composition of larval and pupal stage of *Bombyx mori*, reported that silkworm larva comprises about 40.93% of carbohydrates whereas 38.47% of carbohydrate is contained in the pupae of silkworm (Olumuyiwa and Omotoso 2015). Glycogen, which is a multibranch polysaccharide of glucose and trehalose, which is a sugar consisting of two molecules of glucose, has also been reported from fat body hemolymph of silkworm larvae (Yellamma 2018).

Silkworm pupae are a significant source of chitin as this substance forms a major portion of pupae's exoskeleton. It is a polysaccharide, consisting of a linear homopolymer of β-1-4-linked N-acetylglucosamine (Fig. 2l) (Suresh et al. 2012). Chitin is used for the preparation of its derivative such as chitosan (Fig. 2k) (ß-(1,4)-N-acetyl-D-glucosamine) which is derived after deacetylation of chitin and is being exploited for its antipollutant activity in residual water (Suresh et al. 2012; Paulino et al. 2006).

3.3 Fatty Acids

It has been reported that the fat content of the larva was 17.57% while that of the pupa was 19.90% (Olumuyiwa and Omotoso 2015). Silkworm larva contains fatty acids such as palmitic, myristic, oleic, linolenic, linoleic (Fig. 2r), eicosenoic, vaccenic, gamma linoleic, and palmitoleic acid (Ji et al., 2016). Silkworm pupae contain 32.2% total lipid count by dry weight. Silkworm pupal oil contains saturated fatty acids (28.8%), monounsaturated fatty acids (27.7%), and polyunsaturated fatty acid (43.6%). Silkworm pupae and pupae oil contain alpha-linolenic acid (Fig. 2q) and linoleic acid (Fig. 2r) (Dev et al. 2017). These polyunsaturated and essential fatty acids are non-synthesizable in the human body; thus it must be obtained from the diet.

3.4 Vitamins and Minerals

Minerals such as calcium, iron, zinc, sulfur, sodium, and magnesium were present in silkworm larval powder (Ji et al. 2016), whereas minerals reported from silkworm pupae were sodium, potassium, calcium, magnesium, zinc, iron, lead, manganese, copper, phosphorus, nickel, and cobalt (Olumuyiwa and Omotoso 2015). Silkworm pupae also have been reported to contain vitamin B1, riboflavin (vitamin B2), and vitamin E (Singh and Jayasomu 2002; Kwon et al. 2012).

3.5 Other Compounds

Zhang et al. reported that decarboxylated xanthommatin and xanthommatin are the primary ommochrome pigments found in silkworm eggs (Zhang et al. 2017). Silkworm larva contains chemical constituents such as adipokinetic hormone (AKH), chymotrypsin inhibitors, insulin-like growth factor-II (IGF II), DOPA, and sex pheromone bombykol [(10E, 12Z)-10,12-hexadecadien-1-ol] and b-N-acetylglucosaminidase (Singh and Jayasomu 2002).

Silkworm pupae have also been reported to contain quercetin diglucoside (Fig. 2n and o) [23]. Quercetin diglucoside is a derivative of quercetin, a flavonoid, which has been reported to exhibit biological activities against brain disorders, renal injury, cardiovascular disease, diabetes mellitus, etc. (Muhammad et al. 2018). Silkworm pupae contain 1-deoxynojirimycin (DNJ), which is known for alpha-glucosidase inhibition activity (Tomotake et al. 2010).

Silkworm excreta have been reported to contain pharmacologically active compound such as 1-triacontanol, β-sitosterol, and lupeol (Vimolmankang et al. 2014). 1-Triacontanol (Fig. 2p) is a very long-chain fatty alcohol known for its growth stimulant properties in plants (Marks index), β-sitosterol is a phytosterol which is used against benign prostatic hyperplasia (Wilt et al. 2000), and lupeol is a triterpenoid having the chemoprotective property (Gallo and Sarachine 2009). In addition to this silkworm, excreta contain chlorophyll derivatives (CPD-A (Fig. 2m), B, C, and D) (Soumya et al. 2017).

4 Biological Activities of Silkworm By-Products

4.1 Neuroprotective

Generation of free radicals is a typical result of aerobic cellular metabolism. Our built-in antioxidant system protects us from any loss due to free radicals. However, defect in the defense mechanism of antioxidants, overproduction of free radicals, or integration of free radicals from the environment in the human body leads to neurodegeneration (Uttara et al. 2009). Pupae of silkworm (*Bombyx mori*) are used in curing numerous neurological disorders related to oxidative stress. In one of the studies, it was confirmed that silkworm pupae possess cognitive enhancing effect via increasing cholinergic function; it also possesses neuroprotective effects by decreasing oxidative stress. This study indicates that silkworm pupae might be proved useful to protect against Alzheimer's disease (Wattanathorn et al. 2012). Pupal oil is a rich source of eicosapentaenoic acid (EPA) and docosahexaenoic (DHA); these can enhance the nucleic acid synthesis and monoamine neurotransmitters in the brain, thereby improving the mental health.

4.2 Hyperlipidemic Control Activity

Hyperlipidemia is a condition characterized by elevation of serum total cholesterol (TC) and triglyceride (TG) or reduced high-density lipoprotein (HDL) cholesterol that makes the body susceptible to the development of atherosclerosis (Liu et al. 2010). Consumption of silkworm pupae accelerated the fat metabolism and reduced the concentration of serum lipids in an animal model, and it also reduced the fat storage which is known to be effective in suppression of metabolic syndrome (Ryu 2014). Hence, it reduces the chances of developing hyperlipidemia and, thereby, decrease the risk of developing atherosclerosis.

4.3 Antioxidants

Antioxidants are substances that prevent damage to cellular components caused by a chemical reaction involving free radicals (Young and Woodside 2001). The antioxidant activity has been observed in silkworm pupae (Chukiatsiri and Hangtrakul 2018). Several researchers evaluated the antioxidant activity of by-products derived from the pupae of silkworm such as pupal powder, chitosan, and pupal oil. Results have confirmed the presence of antioxidant property in pupal powder (73%), chitosan (76%), and papal oil (64%), respectively (Pachiappan et al. 2016).

4.4 Antidiabetic

Diabetes is a disorder caused by inefficient or failure of insulin secretion from beta cells of the pancreas or defect in insulin action that leads to high blood sugar level with impairment of carbohydrate, fat, and protein metabolism. Pupal oil contains DNJ, which is a potent alpha-glucosidase inhibitor used to treat diabetes (Priyadarshini et al. 2017). Pupal oil facilitates the prostaglandins to uphold the equilibrium with effects of improving the function of beta cells which produce insulin, reinstating the fatty acid desaturase activity of cells in diabetic patients (Gavia et al. 2003).

4.5 Anticancer

Silkworm pupae and pupal oil are the rich sources of alpha-linolenic acid (ALA) which is an essential omega-3 fatty acid (Tomotake et al. 2010). ALA has been reported to hamper the growth of several cancer cell lines, such as breast, cervical (Deshpande et al. 2013), colon (Chamberland and Moon 2014), gastric (Dai et al. 2013), and myeloma (Sravan and Das 1997).

4.6 Antibacterial

It has been reported that silkworms have been used as an antibacterial in the treatment of sore eyes, swollen throat, and loss of speech (Ahn et al. 2008). Dev et al. analyzed the antibacterial property of mulberry silkworm pupal oil and reported that pupal oil inhibited the growth of *Staphylococcus sciuri* strain CD97 (Dev et al. 2017) (Fig. 3).

5 Biomedical and Pharmaceutical Application of Silkworm and Its By-Products

5.1 Serrapeptidase

Serrapeptidase is an enzyme which is secreted in the intestine of silkworm. This enzyme helps in breaking down the walls of the cocoon. This enzyme is used as an alternative to the nonsteroidal anti-inflammatory agents which are used to treat osteoarthritis and rheumatoid. It is also reported that serrapeptidase plays a vital role in the prevention and removal of arterial plaque (Rajakumar et al. 2014).

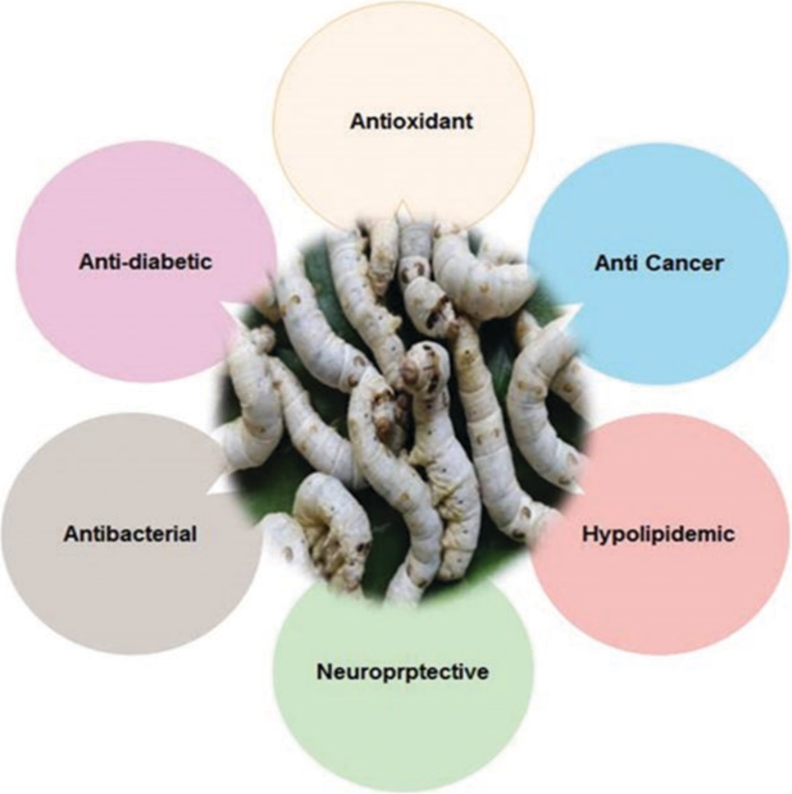

Fig. 3 Biological activity of silkworm and by-products

5.2 Vaccine

Larvae of silkworm can be used as a bioreactor to produce a vaccine against infectious disease at low cost (Datta 1994). Nerome et al. (2015) successfully developed a mass production protocol for an influenza virus-like (VLP) vaccine in silkworm pupae (Nerome et al. 2015). A group of researchers has successfully prepared large amounts of Japanese encephalitis virus-like particle Nakayumma-BmNPV (JEV-NNPV) vaccine in silkworm pupae through the construction of codon-optimized chimeric DNA and recombinant JEV-NNPV generation (Matsuda et al. 2017).

5.3 Post-Operational Treatment

Pupal skin is composed of chitin. It has a full application such as in post-operational treatment such as deviatomy, conchotomy, and polypectomy due to its wound healing and pain relieving properties (Buhroo et al. 2018).

5.4 Sericin for Cell Line Proliferation

Sericin is a protein obtained from the silkworm cocoon. It has full application in biomedical and pharmaceutical fields. When sericin was added to the mammalian cell line cultures, it accelerated the proliferation of cell lines including murine hybridoma 2E3-O, human hepatoblastoma HepG2, human epithelial HeLa, and human embryonal kidney 293 cells (Terada et al. 2002).

5.5 Bioreactor

One of the studies investigates the role of silkworm pupae as a bioreactor. Silkworm pupae were selected to express the protein of interest by using *Bombyx mori* nucleo polyhedron virus (BMNPV). Expression, purification, and characterization of human granulocyte macrophage colony stimulatory factor (GM-SCF) were done using pupae of silkworm *Bombyx mori* and revealed that pupae of silkworm could be more useful for heterologous protein expression as a bioreactor (Jian et al. 2006).

5.6 Antiarthritic Activity

In china, silkworm excrement (SE) is used traditionally as an antirheumatic medicine. The research was conducted to investigate the potential of silkworm excrement to reduce adjuvant-induced arthritis (AA) in the rat model. The result showed that silkworm excrement notably ameliorated the AA severity, such as body weight loss, paw swelling, histological changes, and biochemical index levels in a rat model. This study suggested that SE can be used in the treatment of arthritis (Zheng et al. 2018).

5.7 Artificial Blood Vessels

Transgenic silkworm produces silk which possesses a high level of cell adhesion. NIAS generated this silkworm line by incorporating a gene that contains a cell self-multiplication protein. Using a combination of plaiting of silk threads and winding of cocoon filaments artificial blood vessels have been developed. These vessels were tested in rats through graft operation. The blood vessels were removed after at different times after grafting and sections were observed. The result showed that in artificial blood vessels collagen portion found increase instead of fibroin fibers as time passed (Iyengar 2010).

6 Future Prospective

Among economically beneficial insects, silkworm placed as most pragmatic insect, as it contains high prospective to contribute in many dimensions for the use of human welfare, and the same has been proved through several studies. However, the potentiality of silkworm and its by-products has not been entirely exploited due to lack of awareness and unmethodical ways of research. The current chapter will indeed draw the attention of many researchers who are working in interdisciplinary areas of life sciences and natural product research.

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Present and Future Prospects on Nutritious Feeding Using Insects

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Abstract Limited and challenging food resources initiate opening of new vistas of new food items and can be served to the human diet without any precedence. There is a long historical relationship between insects and human civilization that extends back to antiquity. Several developed as well as developing countries such as US, UK, Europe, Asia, etc., utilize edible insects as food which represent a novelty or snack food to a considerable extent evidenced by their food products. Insects meet the human nutritional requirements and are high in protein and mono- and polyunsaturated fatty acids. Certain carbohydrates mainly chitin and minerals such as K, Na, Ca, Cu, Fe, Zn, Mn, and P and vitamins are also favoring edible insects as a brilliant nutrition diet of humans. In this chapter, the nutritional performance of insect feed/edible insects and their current environmental aspects with socioeconomic reflections are highlighted.

Keywords Edible insects · Food · Insect feeding · Nutrition

1 Introduction to Edible Insects

Arthropods from the phylum Euarthropoda are the animals having an exoskeleton, a segmented body, and paired jointed appendages which include insects, arachnids, myriapods, crustaceans, etc. The insects are a class of animals within the arthropod group that have a chitinous exoskeleton, a three-part body (head, thorax, and abdomen) with two antennae, mainly compound eyes, and three pairs of jointed legs. In addition, insects are the most diverse groups among the animals on the planet: there are more than 1 million described species, which is more than half of all known living organisms (Bodenheimer 1951; Choe and Crespi 1997; van Huis et al. 2013). Insects have been provided many valuable natural substances since thousands of years including honey, silk, and other products (i.e., jelly, wax, etc.). Insect's secretion and/or whole bodies thereof have commonly been used as an important folklore medicine around the globe, for example, countries of Asia, Europe, Africa, and

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America (Yusuf 2018; DeFoliart 1999). Anciently, the evolution has produced a wide variety of arthropod species, more likely "insects" adapted to ecology in millions more believed to exist. All of them directly interlinked with human civilization either beneficial or detrimental role (many insect species considered as harmful to crops, livestock, or humans along with a large number of derived products). Commonest categories have been studied and documented in the nineteenth century: (i) insects producing silk; (ii) insects producing honey, wax, etc.; (iii) insects as sources of dyes; (iv) insects producing manna (sap, pulp, or juice exuded by a plant pricked by an insect); (v) edible insects; (vi) insects as sources of medicine; and (vii) insects as ornament applications. In all cases, there is a close linkage with ecological prospects and the foregoing categories are not mutually exclusive because many useful insects fit into many classes (Bodenheimer 1951; Coleman 2006). Contrary to the most popular faith, of the one million insect species that have been described, only 5000 of them can be considered harmful to crops, livestock, or human beings (DeFoliart 1997; van Lenteren 2006).

The practice of eating insects is known as entomophagy, derived from the Greek words "*éntomon* and *phagein*" which refer to "insect" and "to eat," respectively. The eggs, larvae, pupae, and adults of certain insects have long been eaten by humans from prehistoric times to the present day as a regular part of their diets. The number of ethnic groups noticed to practice entomophagy is around 3000 all over the world (Niassy and Ekesi 2017). Entomophagy is common to several cultures in most parts of the world, including the nations of Africa, Asia, Central and South America, Europe, Australia, etc. An approximate of 80% of the total consumed edible insects contains about 2000 species globally. The most commonly edible insect groups include caterpillars, bees, wasps, ants, beetles, grasshoppers, flies, dragonflies, locusts, crickets, cicadas, leaf and planthoppers, scale insects, true bugs, termites, etc. (https://cisr.ucr.edu/entomophagy.html). However, insect consumption is not a new concept for several divisions of the earth. In Africa and Australia ants to beetle larvae are eaten by tribes as a part of their irregular diets. However, African peoples have never considered preference to edible insects as pests or a nuisance. About 12 sub-Saharan countries in Africa provided tentative names for edible termites with 30 ethnic groups of insects, i.e., *Inswa*, *Iswa*, *Sisi*, *Ishwa*, *Tsiswa*, *Chiswa*, *Chintuga*, or *Esunsun*, which could be used to market termite-based products (FAO 2006, 2012, 2013, 2015). Thailand is known for the popular insect marketed crispy fried locusts, beetles, and other insects. In tropical countries, more than 1900 insect species have been identified as edible. Why are insects consumed more frequently in tropical countries? In this regard, the answer is very likely because, in the tropic regions, edible insects are easier to harvest than in temperate zones: insects are larger and they are available in clumped populations throughout the year. Another real fact is that the world population is continuously increasing with a growing requirement for food and lengthening food shelf-life or food chain has also been a response to the growing need for food. Therefore, improvements in the food production systems are also a big challenge toward the need of food due to intensive farming policies as well as the development of new genetically modified organisms that reduce environmental sustainability (Belluco et al. 2013; Johnson 2010; Makkar et al. 2014). It should be noted that the global fish reserves are dwindling, which are the sources of fishmeal. The use of insects in animal feed is a potential path to improve the sustainability of animal diets and meet the growing global demand for livestock products (Verbeke 2015; Yusuf 2018).

2 Historical Perspectives

There is a long historical relationship between insects and human civilization that extends back to antiquity. How do insects become human food? This question cannot be determined with any accurate option. We can speculate that our primitive's predecessors initially developed a trial-and-error basis and eventually used entomophagy for a successful strategy to survive. Conversely, one plausible scenario which may appear since early men is that harvesting and eating wild insect-derived products such as honey led to the collecting and consumption of bee brood. Still a little thing in honey production there is also deterioration of bee body parts, eggs, larvae, and pupae in the hive as a source of proteins. However, the eating of insects appears to be culturally universal, only varying with location, insect populations, and climate factors. Wherever forest insects are part of the human diet, they have generally been collected from the wild (Xiaoming and Ying 1999). In most cases, minimal management of forest vegetation has been practiced in association with the exploitation of forest insects, and actual domestication of insects thus far has been limited to only a few species such as silkworms and bees. The most commonly eaten insect forms are larvae and pupae, usually with little or no processing of the insects before they are consumed.

Probably, ancient humans had no significant tools to hunt or farm; insects may have represented an important part of their diet. Evidence inferred that evolutionary precursors of *Homo sapiens* were entomophagous (Tommaseo-Ponzetta 2005). Little evidences have been found after analyzing coprolites from caves in the USA and Mexico. For example, coprolites in caves in the Ozark Mountains were found to contain ants, beetle larvae, lice, ticks, and mites. Cave paintings in Altamira, north Spain (Approx. 30,000 to 9000 BC period), have suggested a possibly entomophagous society that consumes insects and wild bee nests. In Africa, progenitors of modern humans ate insects as part of their routine diet and they believed to consume certain insects. These were presumably adopted, over time, as normal, ritual, or emergency food sources (Johnson 2010; Shockley and Dossey 2014). In the Shanxi province of China, from 2000 to 2500 years BC, cocoons of wild silkworm (*Triuncina religiosae*) were found with large holes in them, suggesting the pupae were eaten. Thus, the trends from many ancient entomophagy practices have created the development of modern traditional entomophagy (Capinera 2004).

Every continent except Antarctica, evidences came into existence from Europe is meagre and generally restricted to its more southern and eastern regions (Tommaseo-Ponzetta 2005). Historically, there are several written records that evident European populations and European-derived populations in North America have entomophagous eating practices. For example, several subsequent entomophagy culinary guide cookbooks have been published by Gabriel Martinez, including *Insectes à croquer* (Insects to Munch), produced by the Montreal Insectarium, and *Cuisine des insectes: À la découverte de l'entomophagie* and Ronald Taylor's book *Butterflies in My Stomach* (1975) and a recipe guide, *Entertaining with Insects* (1976) (Coleman 2006; Choe and Crespi 1997; Shockley and Dossey 2014). A stunning photographic collection of Peter Menzel and Faith D'Aluisio was published (Menzel and D'Aluisio 2010) in which authors included featuring portraits of 80 people are presented collectively based on the food they eat in one day 30 countries around the world. The Yukpa Indians living in Colombia are noticed to prefer some traditional insects as foods with fresh meat (Paoletti and Dufour 2005). Therefore, insects and other small invertebrates make a significant contribution to human civilization in their diets; some groups are included in the human diet throughout the year as well as during occasional time periods.

3 Nutritional Aspects of Insects

The insects are known to have several nutritive contents. Their nutritional values vary because of the diversities and various development phases, i.e., the stage of metamorphosis, origins of the insect, and its diet. In a similar way, the nutritional value also varies on the basis of prior processing before consumption, i.e., drying, frying, cooking, etc. A detailed and comprehensive study on the nutrient contents of 236 edible insects was published by Rumpold and Schlüter (2013). The study shows that many insects are rich in micro-minerals which play a valuable role in human nutrition such as iron, copper, magnesium, manganese, selenium, and zinc, as well as vitamins such as riboflavin, pantothenic acid, biotin, and folic acid. Some species of edible insects contain a reasonable amount of minerals (K, Na, Ca, Cu, Fe, Zn, Mn, and P) as well as vitamins such as B group vitamins and vitamins A, D, E, K, and C. However their content is seasonal and dependent on the feed (Finke 2002; Johnson 2010; Makkar et al. 2014). From the hygienic point of view, it should be pointed out that some insects may produce or contain toxic bioactive compounds. They may also contain little residues of pesticides and heavy metals. Thus, adverse human allergic responses may be associated with edible insects and could be a possible hazard to a significant extent (Rumpold and Schlüter 2013; Kouřimská and Adámková 2016). Furthermore, Payne et al. (2016) studied the nutritional compositions between many insect species and observed high diversity among them. The nutritional value of palm weevil, cricket larvae, and mealworm was found healthier than in the case of beef and chicken meat. Six tested insects were statistically proved to be healthier than meat.

Insects meet the amino acid requirements of humans and are high in protein and mono- and polyunsaturated fatty acids. Insects contain significant protein content as well as fatty residues. Protein contents vary from 20 to 76% of dry mass while the fatty content variability is large 10–50% of dry mass which depends on many factors like the type and development stages of the individual insect species (Paoletti and Dufour 2005). Similarly, Barragan-Fonseca et al. (2019) reported a high macronutrient content combined with a low P/C ratio which positively affects the protein as well as fatty contents in black soldier fly *H. illucens*. In the study, diet no. P17:C55 supports the highest larval and adult performance and resulted in a high larval body protein and intermediate crude fat residues. Additionally, some edible insects were reported to have medicinal properties used for curing purposes since ancient times. Chitin is a polysaccharide derived from the outer shells of crustaceans, i.e., crabs, lobsters, and crayfish. Sufficient amount of dietary fibers was reported along with trace of minerals such as Ca, K, Na, etc. Moreover, insects contain a variety of vitamins such as retinol, β-carotene, vitamin B12, etc., in selected species. Cyanocobalamin is abundantly found in larvae of the yellow mealworm beetle (*T. molitor*) and house cricket (*Acheta domesticus*) (Finke 2002). Retinol and β-carotene were identified in *Imbrasia oyemensis*, *I. epimethea*, *Nudaurelia oyemensis*, and *Ichthyodes truncat*. Vitamin E and tocopherol were reported in red palm weevil *Rhynchophorus ferrugineus* and silkworm *Bombyx mori*, respectively (Tong et al. 2011).

Table 1 depicts the variation of protein contents in several stages of insects on the basis of % dry mass. In Table 2 the comparison in protein/amino acid (g/100 g) composition among insect fresh weight (silkworm pupae) and common animal foodstuff fresh weight is presented. Nutritional performance of fatty residues is shown in Table 3. The nutritional requirements summarized in Tables 1, 2, and 3 show that insects have high value protein contents and fatty residues and may provide a great alternative to human nutritional requirements. Carbohydrates combined with protein and fat and their compounds have important physiological functions which are important nutritive elements in the human body (Xiaoming et al. 2010). However, edible insects have rich in protein and fat composition with less carbohydrate contents chiefly polysaccharides. The whole body and outer skin of edible insects contain chitin, a polysaccharide which is variable percentage at different forms of edible insects. For example, *Bombyx mori* L. dried pupa has 3.73% chitin while defatted pupa contains 5.55% chitin content. Moreover, *Dendrolimus houi* Lajonquiere pupa and adults have 7.47% and 17.83% chitin content, respectively (Xiaoming and Ying 1999; He et al. 1999).

Table 1 Variation of protein contents in several stages of insects on the basis of % dry mass (Xiaoming et al. 2010; Xiaoming and Ying 1999; He et al. 1999; FAO 2006, 2012, 2013, 2015)

Stage of insect	Contents % (dry mass)	Class of insect
Adults and larvae	23–66	Coleoptera
	42–74	Hemiptera
Adults and naiads	46–65	Odonata
Adults and nymphs	23–65	Orthoptera
Pupae and larvae	14–68	Lepidoptera
Adults, larvae, and eggs	45–57	Homoptera
Adult, pupae, larvae, and eggs	13–77	Hymenoptera

Table 2 Comparison in protein/amino acid (g/100 g) composition among insect fresh weight (silkworm pupae) and common animal foodstuff fresh weight (FAO 2006, 2012, 2013, 2015)

Protein/amino acid residues	Beef meat	Chicken meat	Silkworm pupae
Protein	21.35	19.40	15.8
Aspartic acid	2.07	1.91	1.54
Threonine	0.87	0.95	0.75
Serine	0.86	0.88	0.82
Glutamic acid	3.61	3.23	2.03
Proline	0.89	0.73	1.02
Glycine	1.08	0.97	0.78
Alanine	1.32	1.15	0.97
Cystine	0.23	0.29	0.08
Valine	1.04	1.02	0.84
Methionine	0.61	0.62	0.36
Leucine	1.82	1.64	1.04
Isoleucine	0.92	0.98	0.69
Phenylalanine	0.86	0.82	0.82
Histidine	0.82	0.69	0.42
Lysine	1.94	1.79	1.03
Arginine	1.30	1.34	0.69

Table 3 Fatty residues on the basis of % dry mass (Tong et al. 2011; Kouřimská and Adámková 2016)

Common name of insects	Scientific name	Stage of insect	Contents % (dry mass)
Yellow mealworm	*Tenebrio molitor*	Larvae	36
Giant mealworm	*Zophobas atratus*	Larvae	40
Silkworm	*Bombyx mori*	Pupae	29
Western honey bee	*Apis mellifera*	Brood	31
African migratory locust	*Locusta migratoria*	Nymph	13
Wax moth	*Galleria mellonella*	Caterpillar	57
Jamaican field cricket	*Gryllus assimilis*	Nymph	34

4 Environmental Reflections

4.1 Land, Feed, and Water Consumption Benefits

The environmental impact of edible insects is increasingly being forefront. Land and water are two primary needs of civilization to survive. In particular, water is essential and used to grow animals, humans, and crops directly or indirectly. The report published by FAO (2012) predicted that at least 1.8 million people will be living in regions with inadequate freshwater supplies and a further two-thirds of the global population will be in areas under pressure from dwindling water resources by 2025. Furthermore, by 2050 some 60% more food – up to 100% in developing

Table 4 Amount of consumables (land, feed, and water) needed to produce 1 kg of live animal weight

Categories / % edible part	Land	Feed	Water
Big mammals (Cow/Beef/ Buffaloes etc.) / 40 %	▲▲▲▲▲▲ ▲▲▲▲▲▲	★★★★★	▼▼▼▼▼
Small mammals (Pig, Goat etc.) / 55 %	▲▲▲▲▲	★★★	▼▼
Birds (Chickens etc.) / 55 %	▲▲	★	▼
Edible Insects (Grasshopper etc.) / 80 %	▴	⋆	▾

countries – will be needed to feed the world while agriculture will continue to be the largest user of water globally, accounting in many countries for around two-thirds or more of supplies drawn from rivers, lakes, and aquifers (http://www.fao.org/news/story/en/item/283255/icode/). Data collected from literature revealed that big mammals consume largest part of the consumables (land, feed, and water) followed by small mammals, birds, and insects, out of which least (80%, live animal weight and percent of the animal which is edible) consumables are utilized by edible insects (Table 4) (Hoekstra and Mekonnen 2012; van Huis et al. 2013).

4.2 Greenhouse Gas Emission

Particularly, greenhouse gases (GHGs) are emitted to the atmosphere predominantly CO_2, nitrous oxide (NO_x), and methane (CH_4), from burning of fossil fuels and several agricultural and industrial processes. However, GHGs are also produced by the decomposition of organic matters. Recently, Dobermann et al. (2017) studied comparatively CO_2 emission from the dead parts of beef, pork, and insects. In the study, it is concluded that edible insects emit negligible or very little CO_2 emission. Overall, organic waste is probably the best option for insect rearing and for a better and comprehensive understanding the specific sources need to be deeply investigated for a particular insect species.

5 Future Prospects and Conclusion

Edible insects have essential and balance of nutrients and good levels of protein contents and fatty residues which are suitable for everyone's diet. They contain high-quality proteins, many vitamins, and amino acids. In addition, some edible insects were found to have medicinal properties. The use of insects as feed for humans, animals, and fishes makes them enough popular because of their feeding performance as well as socioeconomic benefits. Furthermore, due to the growing demand for animal resources as foods and declining availability of agricultural parameters like land, feed, and water, there is an urgent need to find alternative protein sources with low environmental risks. It is also worthwhile that the global fish reserves are dwindling, which are the sources of fishmeal. The use of insects in animal feed is a potential path to improve the sustainability of animal diets and meet the growing global demand for livestock products. It therefore remains a big question: "how insects can be made to reaching one's diet easily and their universal sale." To overcome the difficulties, additional supports should be given to popularize insect feeding throughout the society and more researches should be made on the needs and future opportunities to them so that entomophagy can be utilized in modern and sustainable food production system.

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Insect Pheromones and Its Applications in Management of Pest Population

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Abstract Insects are the members of largest phylum Arthropoda which comprise of numerous unique species. Numbers of species of insects pose threat to the modern agricultural system as pest and destroy crops and food products at different stages. To protect the crops from these pest species, modern agricultural practices involve a parallel evolving market of pesticides which are highly toxic and environmentally unsustainable. Broad-spectrum pesticides kill beneficial insect species along with the damaging pest species. Traces of these pesticide residues in soil and food produce have been reported to alter the soil macro- and mesofauna causing reduction of nutrient mobilization through mineralization processes. Moreover, increased use of chemical pesticide has been reported to cause gradual decrease in the food production recently. Extensive research in this field has given birth to the concept of integrated pest management based on the principle that pest population needs to be managed below their economic injury level and not to be eliminated. The most important requirement in this aspect is to understand the behavior of insect pest species and to identify the hormones that regulate the behaviors of these insects at different stages of their life cycle. At present, a huge number of pheromones have been identified in various insect species and their mechanism of action in modulating insect behaviors is being studied extensively. Though implications of insect pheromone in managing pest population are in its infancy, the potentialities of the pheromones in pest management have already been demonstrated by a number of researcher across the world in various agricultural systems. The present chapter attempts to delineate the details of insect pheromones and further elaborate the present status of their implications in management of pest population in different agricultural systems.

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1 Introduction

To meet the demands of rapidly expanding population, modern agriculture is facing many challenges. Significant attention and attempts are being taken across the globe toward the development of innovative tools and strategies for management of ever evolving pesticide-resistant pest. Several researches have demonstrated that killing or eliminating pest species leads to reduction of crop productivity and make the agricultural ecosystem highly unstable. Therefore, key factors like environmental conservation, resistance management, food safety, and economic viability are now placed in the center place while formulating policies and practices of the pest management to be used in commercial agricultural sectors. Thus, a combinatorial approach involving biological, physical, chemical, cultural, and conventional pest management systems taken all together gives rise to the concept of integrated pest management which has become one of the important practices in this twenty-first century. In this context, modulation of insect behaviors through factors like pheromone can become a boon for controlling pest population without eliminating any insect species.

Use of tools that promote behavioral manipulation such as pheromones has already been reported to complement the current pest management systems. Successful implications of pheromone in pest population in agricultural system can lead to tremendous decrease in the application of broad-spectrum insecticides. There will be no hazards to nontarget and beneficial insects because pheromones have higher degree of specificity to target insects. Several pest management approaches nowadays use semiochemicals like insect pheromones against a wide group of insects worldwide. This chapter gives an insight into the insect pheromones and their potential usage in the field of integrated pest control with special mention to chemical basis, types, mode, and mechanism of action and possible use of pheromones in pest control in present and future prospective.

2 Pest, Pesticides, and Their Effect on Crop Productivity

Pest (*Gk. Pestis*, meaning *to annoy*) can be any organism that causes irritation. A pest is an animal or plant present at a place where it is not desired and is disadvantageous to human interests including crops, livestock, and forestry. It includes fungi, bacteria, viruses, insects, nematodes, and rodents which transmit disease, destroy crops, store products, and directly or indirectly affect human health and welfare.

Pesticides are a group of chemicals that are used to control, repel, or destroy pests of any sort. Pesticides are a line of attack to make sure that the farmers get the desired crop yield without any concern of the harmful effects on the consumer and environment. Broad-spectrum pesticides kill pests over a range and are nonspecific whereas specific pests are targeted by narrow-spectrum pesticides. Pesticides are classified in six different types on the basis of the target organism like insecticides, herbicides, rodenticides, bactericides, fungicides, larvicides. Again they are classified as organophosphate, organochlorine, and carbamate on the basis of their chemical composition and toxicity. Direct and indirect exposure to pesticide makes people vulnerable to various health risks. Farmers and their family members get occupationally exposed to chemical pesticides on almost regular basis and have been reported to develop numerous pesticide-induced toxicity in their bodies. Pesticides enter into the body through various routes such as dermal, oral, and nasal. Moreover, concerns over the pesticide application can be attributed to following factors:

- Wind carries the pesticide.
- The produce can hold the pesticide if not cleaned properly.
- Pesticide can remain in both produce and animals.
- It can be transported to river and water bodies as runoff.

Pest species keep on evolving themselves through random genetic recombination and mutation induced by the pesticide itself as well as the other environmental factors. Improper and unregulated uses of pesticides in several agricultural systems have recently resulted in generation of a number of resistant pest species. These resistant pest species grow and reproduce in a very rapid rate and impart serious damage to crops across the globe. To control such resistant pest population, new generation pesticide with high specificity and toxicity to pest are being developed regularly. Very often implication of combination of different types of pesticides showing synergistic negative effect on pest species in various proportions is required to control the increasing pest population. Pesticide industry and production and uses of high yield variety of seeds in several cropping systems have been observed to grow hand in hand because most of the high yield seed variety are observed to have reduced immunity toward attack of pest species. Accumulation of pesticide residues in soil has been observed to directly reduce the nutrient mobilization in soil by hampering the process of mineralization (Moorman 1989). The pesticide residues also biomagnify through food chain and ultimately reach to humans causing serious health problems including incidence of cancer and other neurodegenerative diseases. Uses of chemical pesticides to eliminate the pest species open doors for numerous other serious problems in plants as well as environmental compartments making them highly unsustainable. There is urgent necessity for development of pesticide and pest control strategies which are environmentally sustainable

2.1 Effect of Pesticides

Several reports emphasize the harmful effects of pesticide application. Agricultural runoff very often causes contamination of aquatic system such as river, pond, and sometimes groundwater. Many broad-spectrum pesticides have been reported to effect nontarget micro- and macrofauna such as beneficial insects, fish, birds, and nontarget plants in agricultural system causing loss of ecological services rendered by these species in maintaining soil health and crop productivity. As most of the pesticide used in modern agricultural system for pest control are neurotoxic in nature, occupational exposure to pesticides during their formulation, production, transportation, mixing, and spraying in agricultural farm is of major concern. It is noteworthy to mention that a higher risk of hazard is noticed during manufacture and formulation of pesticides. The workers in such cases are frequently handling toxic chemicals during the production including toxic solvents, inert carriers, raw materials, and, finally, the pesticides. As a common contaminant in water, air, soil, and on nontarget organisms, pesticide and their residues harm plants and animals ranging from beneficial soil microorganisms, nontarget plants, fish, insects, birds, and other wildlife (Hurley et al. 1998).

An interesting case regarding the decline in the bald eagle population in the United States was reported by the US National Academy of Sciences which is due to exposure to DDE, a metabolite of pesticide DDT that causes the thinning of eggshell (Liroff 2000). Some of the pesticides mimick or antagonize the natural hormone in the human body system and disrupt the hormonal signaling. The long-term, low-dose exposure of endocrine disruptors is coupled with adverse effects on human health through reproductive abnormalities, hormone disruption, cancer, immune suppression, and diminished intelligence (Brouwer et al. 1999; Crisp et al. 1998; Hurley et al. 1998). Water contamination by pesticide residues is quite common as the pesticides from treated plants and soil can easily reach surface water through runoff. The US Geological Survey (USGS) based on a comprehensive set of studies done on major river basins across the country in the early to mid-1990s demonstrated the presence of several pesticides in more than 90% of water samples and fish populations from all streams (Kole et al. 2001). Similarly, pesticide-associated groundwater pollution is now a major problem of the world. A study performed by USGS shows that pesticides from every major chemical class can be found in groundwater. At least 21 transformation products and 143 different pesticides could be seen in groundwater. Again, in more than 43 states, groundwater is detected with pesticides over the past two decades (Waskom 1994).

Dr. Elaine Ingham, a renowned soil scientist, said that "If we lose both bacteria and fungi, then the soil degrades." Soil contamination with pesticide causes decline in such beneficial soil microorganisms like bacteria and fungi. Excessive use of pesticides and chemicals would therefore degrade soil quality. Though the indiscriminate use of pesticides would work for only a few years, it will kill the beneficial soil organisms that hold onto the nutrients (Savonen 1997). For an instance, to fix the atmospheric nitrogen to nitrates plants depend on a variety of soil microorganisms. But the use of herbicides triclopyr kills soil bacteria that carry out the conversion of ammonia into nitrite, thereby inhibiting nitrogen fixation (Pell et al. 1998). Similar to bacteria, beneficial fungi are also affected by pesticides. Several species of mycorrhizal fungi and its spores are exterminated by triclopyr (Chakravarty and Sidhu 1987) and oxadiazon (Moorman 1989) application. Again, plants exposed to glyphosate produces poor quality of seed (Locke et al. 1995). Application of such pesticides makes certain plants susceptible to various diseases posing threat to endangered plant species (Brammall and Higgins 1988).

3 Chemical Basis of Insect Pheromones

The term pheromone is derived from Greek words "pherein" which means to carry and "horman" meaning to excite or stimulate. Insect pheromones are a class of semiochemicals generally produced by specialized exocrine glands associated with the cuticle. Pheromones are low molecular weight volatile organic molecules that elicit behavioral response within the individuals of same species and can be used to communicate between members of the same or the opposite sex (Phillips 1997). Insect pheromones are secreted in extremely low amount, i.e., nanograms to micrograms per unit of time. Moreover, the amount of pheromones secreted varies with the insect species. Pheromone release depends on certain factors like circadian rhythm, temperature, presence of food sources, and age of the insects. Some of the examples of insect pheromones are farnesene, grandisol, blattellaquinone, acetosyringone, dihydroactinidiolide, ethyl oleate, nonacosane, farnesol, syringaldehyde, and vaccenyl acetate. The basis of communication by these pheromones causing behavioral response involves its release from the body of the first individual (emitter), its propagation through the air or water, and, finally, its detection by the receiver present in another individual (receiver). Pheromones in insects are basically received by antennae present on the head. The signals can be very persistent that stays in a certain place in active condition for many days. In some cases, pheromones are very much effective in attracting mates that are far away. Pheromones that are long-lasting help in marking the territory or food sources. Pheromones that get degraded rapidly provides short-lived signals that are helpful in providing an immediate message, such as signaling reproductive readiness for a brief period or a short-term alarming of danger. Structural diversity of pheromones are essential to perform diverse types of functions with specificity. However, pheromones basically range over a chemical having small hydrophobic molecules to hydrophilic peptides (Fig. 1).

4 Types of Insect Pheromones

Based on the type of interaction among the emitters and receivers, insect pheromones are categorized into several types as discussed in the section below.

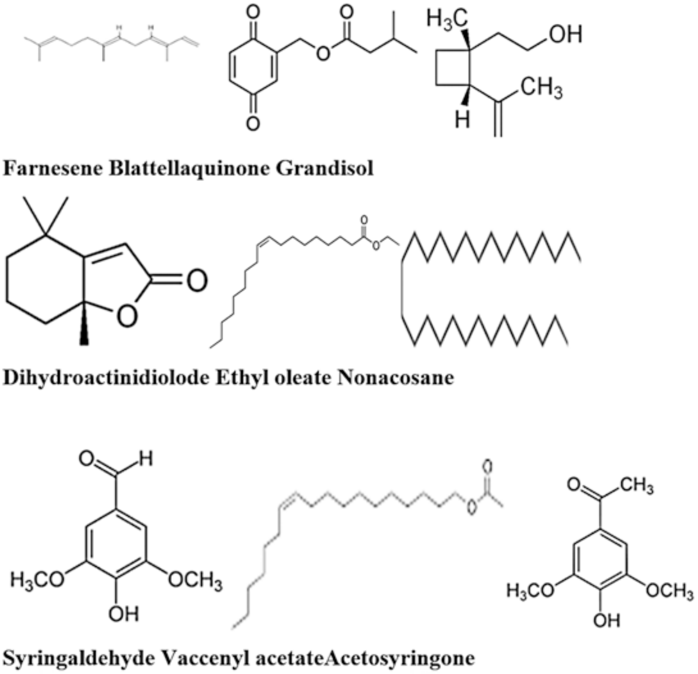

Fig. 1 Chemical structures of some common insect pheromone

4.1 Sex Pheromones

The sex pheromones are basically released in order to attract potential mates that are far away (e.g., moths). Sex pheromones though released in low concentrations are effectively detected by the sensitive chemoreceptive sensilla in insects' antenna (Regnier and Law 1968). The factors like time of day, availability of host plants, and weather govern the release of sex pheromone (Law and Regnier 1971).

4.2 Alarm Pheromones

Alarm pheromones are released as a responsive act against attack by natural enemies (e.g., aphids). Moreover, such pheromones promote dispersal and avoidance behavior in conspecific insects. Moreover, it has also been seen that alarm pheromones cause aggressive response in some social insects like bees and leaf-cutting ants (Ginzel 2010).

4.3 Aggregation Pheromones

Aggregation pheromones are responsible for delivering signals that promote intraspecific group formation and mating at a food source (Tinzaara et al. 2002). Some species of bark beetles have been reported to release pheromones that facilitate recruitment of other sex individuals leading to their aggregation in feeding site (e.g., Scolytidae: Coleoptera) (Blomquist et al. 2010).

4.4 Anti-aggregation Pheromones

As a contrary to the aggregation pheromone, these compounds cause the dispersal and disaggregation of individuals. Such dispersal works best under resource-limited environment to encourage the maintenance of optimum spacing.

4.5 Oviposition-Deterring or Epideictic Pheromones

Epideictic pheromones facilitate certain female insects that avoid hosts that are already used by conspecifics for egg deposition. Such responses reduce the intraspecific competition between the individuals (Stelinski et al. 2007). During the ovipositor dragging process after egg laying, a kind of compound called oviposition-deterring fruit-marking pheromones is deposited by females of several fruit fly species (e.g., Diptera: Tephritidae), e.g., Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Prokopy et al. 1978).

4.6 Trail Pheromones

Such pheromones are primarily secreted by social insects (e.g., ants and termites). In order to guide other members of their colony, social insects use these compounds for trailing or to mark feeding or nest sites (Table 1).

5 Mechanism of Pheromone Action

The animals intend to pass signal release pheromones through their exocrine glands into the surrounding air as cloud of liquid vapors that carries information about the emitter animals (Bossert and Wilson 1963). Many factors govern the effective range of the pheromone action. The range of transmissions of pheromone signal depends

Table 1 List of the insect pheromones and their chemical nature

Name of the pheromone	Chemical nature and molecular formula	Insect species	Types
Grandisol	$C_{10}H_{18}O$ A monoterpene containing a cyclobutane ring, an alcohol group, an alkene group, and two chiral centers	Cotton boll weevil	Aggregation pheromone (Merck Index, 11th Edition, 4442)
Farnesene	$C_{15}H_{24}$	Termites Food attractants for the apple tree pest, the codling moth	Alarm pheromone (Šobotník et al. 2008)
Blattellaquinone	$C_{12}H_{14}O_4$	German cockroach (*Blattella germanica*)	Sex pheromone (Nojima 2005)
Acetosyringone	$C_{10}H_{12}O_4$ Phenolic natural product and is a chemical compound related to acetophenone and 2,6-dimethoxyphenol	Male leaf-footed bug (*Leptoglossus phyllopus)*	Used in communication system (Aldrich et al. 1976, 1979)
Dihydro-actinidiolide	$C_{11}H_{16}O_2$ Volatile terpene	Red fire ant	Pheromone for queen recognition (Yao et al. 1998)
Ethyl oleate	$C_{20}H_{38}O_2$ Fatty acid ester formed by condensation of oleic acid and ethanol. It is a colorless to light yellow liquid	Honeybees	Primer pheromone (Leoncini et al. 2004)
Nonacosane	$C_{29}H_{60}$ A straight chain hydrocarbon	*Orgyia leucostigma* Female *Anopheles stephensi*	
Syringaldehyde	$C_9H_{10}O_4$ A colorless solid soluble in alcohol and polar organic solvents	*Scolytus multistriatus*	Oviposition-deterring or epideictic pheromones (Meyer and Norris 1967)
Vaccenyl acetate	$C_{20}H_{38}O_2$ A volatile chemical compound which is the acetate esters of vaccenyl alcohol	*Drosophila* *longhorn beetle*	
Isoamyl acetate	$C_7H_{14}O_2$ An organic compound that is the ester formed from isoamyl alcohol and acetic acid	Honeybee	Alarm pheromone (Boch et al. 1962)
Periplanone- B	$C_{15}H_{20}O_3$	Female American cockroach (*Periplaneta Americana*)	Sexual attractant (Okada et al. 1990)
Benzyl acetate	$C_9H_{10}O_2$ Organic ester formed by condensation of benzyl alcohol and acetic acid	Orchid bees	Intraspecific pheromones and also sex pheromone (Schiestl and Roubik 2004)
Bombykol	$C_{16}H_{30}O$	Female silkworm Moth of wild silk	Sex pheromone (Kuwahara 1984)

on its rate of diffusion, the volatility of the compound, olfactory efficiency of the receiver, wind currents, and its stability in air. Primarily two mechanisms are broadly accepted on how the pheromones exert its affect on central nervous system. The first category of pheromones displays immediate response whereas the second category of pheromones shows delayed behavioral response. Interestingly long-range chemical communications that are documented thoroughly are the sex pheromones indulged in finding mates. For instance, chemical compounds released by female moths send indication of their availability to male moths over long distances for mating purpose (Rau and Rau 1929 and Butenandt 1963). In another notable case, it is reported that pheromones secreted from female emperor moths could attract male moths present about 11 km away (Butenandt 1963). Usually, the sex pheromone signals secreted by females are perceived by the antenna of males. If the antennae are removed or coated, all the sexual behavioral responses by males are eliminated (Butenandt 1963). Moreover, to enhance the effectiveness of the signaling process, the exocrine glands of various social insects are generally fortified with a variety of chemical called as primers and releasers. These primers and releasers including the pheromones collectively regulate the social behavior of insects.

The insects species having a social organization use alarm pheromones to warn about the approaching danger to other members in the colony. Upon the receipt of alarm signal almost all insects behave in a similar manner. First of all, the insects orient osmotactically from low to high pheromone concentration source and behave hysterically. In some cases, they attack the pheromone source. This frantic behavioral response can be seen in ants by keeping a crushed worker ant near the entrance of their nest. Moreover, social insects appear to perform recruitment by chemical communication. Such recruitment is widespread in ants, termites, and bees. Recruiting pheromones constitutes mainly trail-marking substances which are most widely studied in insects. The insects that use trail pheromones use that signal as navigational aids which helps other members of the colony to orient into a particular direction to a distant location. The distance may vary in different insects, from hundreds of meters in bees to kilometers in terrestrial insects. As discussed earlier, the distance travelled by pheromone signal and perception by receiver insect depends on the volatility of the compound, its stability in air, its rate of diffusion, wind currents, and olfactory efficiency of the receiver. Two scientists, namely, Bossert and Wilson (1963), demonstrated the relation between the diffusion behavior of pheromones and they put forward a mathematical equation to predict the concentration of pheromone at a particular distance from a point source as a function of time. The concentration may be calculated by using the equation as below:

$$U(r,t) = (Q/2D\pi r)\,erfc\left\{\mathrm{r}/(4\,\mathrm{D}\,\mathrm{t})^{1/2}\right\}$$

where the $U(r,t)$ is the concentration in molecules/cm^3, Q represents the rate of emission of vapor from the source measured in molecules/sec, $\boldsymbol{D}$ is the diffusion coefficient of the evaporating substance in air in cm^2/sec, $\boldsymbol{r}$ is the distance from the emission source in cm, t represents the time from the beginning of emission in seconds, and erfc is the complementary error function. When alarm pheromone is secreted by any worker ant in a group, the alarm usually spreads outward in a wavy manner from the source and the signal molecules reach to the resting ant workers through its diffusion in the air. In fact, the pheromone molecules released by the worker ants are often found to diffuse outward at a faster rate than the alarm proliferation. Interestingly, the worker ants do not evoke the alarm behavior till there is enough concentration of pheromone accumulated on their antennae needed for the signal response. This concentration of pheromones in molecules/cm^3 at the chemoreceptors is regarded as the behavioral threshold concentration (K). For the estimation of threshold concentrations (K), when a group of insects are placed near glass capillary tube with liquid pheromone, the responding insects in effect become a measuring device. In this device setup the distance ***(r)*** from the source of signal to the receiver insect and the time *(t)* from the advent of the pheromone signals to the elicitation of response can be directly applied into the Bossert and Wilson's equation of diffusion behavior. Moreover, the emission rate (Q) of the odorant or signaling pheromones in molecules/sec can be calculated by observing the decrease in the volume of liquid pheromone in the capillary per unit time with the help of an ocular micrometer. Again, the diffusion coefficient ***(D)*** can be found out either experimentally or from standard tables. To find out the behavioral threshold concentrations ***(K)***, these values of the emission rate (Q), distance ***(r)***, the time ***(t)***, *and* diffusion coefficient ***(D)*** can be applied in the diffusion equation.

6 Pheromones and Their Possible Implications in Pest Control

To control the insect pests by pheromone requires three important items: (1) pheromone molecules, (2) trap for pests, and (3) a support for the trap in the field. Sex pheromones play an important role in controlling the pests in fields. Furthermore, sex pheromones are employed in three ways in pest management.

6.1 Detection and Monitoring

One of the important applications of pheromones is to detect and determine the temporal distribution of insects. Mostly, males act as the responder to female pheromones. Trap bait is used for this purpose which is designed to mimic the ratio of chemical components and the emission rate of calling females. Different designs of trap baits are being tested every year (Heath et al. 2006; Kim et al. 2011; Leskey et al. 2012). Moreover, the type of trap design essentially regulates the effectiveness of the trap under use for monitoring insect populations. According to the behavior of the target insects, the trap design and size are selected. The insecticide use and other pesticide control strategies can be developed by using the information available from the trap catches. For instance, trap catches are helpful in specifying failure pheromone effect on the disruption of mating. They can also indicate when to reapply a pheromone. In this regard, the most important thing to undertake is the vigilant monitoring and meticulous data interpretation. It is noteworthy to mention that such traps attracting males would be highly beneficial in destroying males, thereby controlling their population control. The advantages of using pheromones for monitoring pests include specificity, high sensitivity, ease of use, and lower costs (Laurent and Frérot 2007; Wall 1990). The data collected from monitoring traps can be utilized in an IPM program that includes components such as species detection, early warning, timing of control treatments, population trends, and dispersion of target pests (Nealis et al. 2010; Wall 1990). The design, color, and placement (including vertical height) may influence the monitoring efficiency of pheromone-baited traps. For example, in monitoring adults of jasmine moth, *Palpita unionalis* Hubner (Lepidoptera: Pyralidae), funnel traps were more efficient compared to adhesive traps and a higher number of males were caught along the edge than in the interior of the groves (Athanassiou et al. 2004). Pheromones are used in pest management programs in number of cases especially for detection, monitoring, and deciding the timing of pesticide spray programs, e.g., the codling moth (*Cydia pomonella*) in pear and apple orchards in Australia (Williams 1989), the *Heliothis* spp. in the USA (Lopez et al. 1990), *Spodoptera litura* in India (Ranga Rao et al. 1991), apple leaf roller (*Bonagota cranaodes*) in Brazil (Kovaleski et al. 1998), *Leucinodes orbonalis* in Bangladesh (Alam et al. 2003; Uddin et al. 2008; Mazumder and Khalequzzaman 2010), sesiid moth (*Macroscelesia japona*) in orchards of Ibaraki Prefecture in Japan (Islam et al. 2007), and nettle moth (*Parasa lepida*) of family Limacodidae in orchards in Gifu Prefecture in Japan (Islam 2009). Pheromones can be combined with attractive plant-derived kairomones to increase the efficiency of monitoring traps (Knight et al. 2005). Furthermore, traps with pheromone/kairomone blends are attractive to both males and females and may improve the predictive correlations between adult capture and variables such as egg density and timing of egg hatch (Knight et al. 2005).

6.2 Mass Trapping

The use of sex pheromones in baited traps is greatly helpful in capturing male moths leading to the prevention of mating and declination in their population. Use of pheromone have been proved to be an efficient and economical approach in mass trapping and controlling pest population, e.g., cotton weevil (*Anthonomus grandis*) baited through aggregation pheromone (Cork et al. 2003), Japanese strain of *Phyllocnistis citrella* trapped by the lure containing Z_7, $Z_{11\text{-}16}$: Ald (Vang et al. 2008), and nettle moth, *P. lepida*, mass trapping through lure bait $Z_{7,9\text{-}10}$: OH (Islam 2009). Another interesting example is brinjal (*Solanum melongena* L.), an economically important crop in South and Southeast Asia. More than 50% fruit losses are commonly reported in these regions due to the fruit borer, *Leucinodes orbonalis*, as its larvae bore the brinjal shoot and fruits (Cork et al. 2005). Blends comprising between 1 and 10% of *E*11-16: OH and *E*11-16: Ac caught even more male *L. orbonalis* than *E*11-16: Ac alone in a study done in the fields in India. Up to 500 μg (*E*)-11-hexadecenyl acetate when baited attracted and trapped more *L. orbonalis* male moths than six virgin females (Zhu et al. 2006). Again, a positive correlation is established between the rate of release pheromone and trap catches. A highest dose of 3000 μg was tested to effectively catch male moths at a significant rate than lower doses of pheromones (Cork et al. 2001).

6.3 Mating Disruption

During mate location, sex pheromones are commonly used as long-range cues to orient insect species toward potential mates. Synthetic blends of sex pheromones can be used to permeate the environment and disrupt the orientation of males to females, thereby inhibiting the mating process (Byers 2007; Witzgall et al. 2008). The mating disruption can be achieved by the use of sex pheromones. In such setup, high concentrations of sex pheromone traps are kept at fixed intervals all the way through the desired field. As there is high concentration of sex pheromone, the area becomes saturated where the males become unable to find females which thereby prevents mating and controls pest population. Specifically, it involves the deployment of multiple synthetic pheromone point sources that act to divert, arrest, and possibly deactivate males seeking mating partners (Miller et al. 2010; Peng et al. 2012). In contrast to traditional insecticide-dependent pest management programs, mating disruption does not affect nontarget organisms and is environmentally sound and approved for organic and biorational production systems. Furthermore, it does not affect the efficacy of biological control agents making it well suited for sustainable IPM programs (Kirsch 1988; Vacas et al. 2012). Factors such as pheromone application rate (trap density), optimum dispenser design, and dispenser height are important for the proper and cost-effective implementation of mating disruption technique (Alfaro et al. 2009; Epstein et al. 2006, 2011). For example, mating disruption was more effective against codling moth when pheromone dispenser density was high (Epstein et al. 2006). Mating disruption can also be combined with conventional pest management programs or an IPM program to reduce insecticide applications, particularly when the pest density is high (Atanassov et al. 2002). Cost effectiveness, uniformity of application, and ease of use are some of the factors that can be considered when several mating disruption dispensers and formulations are available for a single pest (Piñero and Prokopy 2003; Trimble 2007; Dorn and Piñero 2009).

6.4 Pheromone Traps

Pheromone traps can be made at very low cost at home. Moreover, the traps are also procured which are commercially available. This kind of pheromone-baited lure effectively brings male moths into trap. In such design of trap proper design is highly essential as the killing of insects will be achieved when they enter the trap.

The behavior of the target insect dictates the type of trap to be used in the field. Funnel traps, winged traps, and delta traps are reported to be the most effective traps in controlling the pest population. These traps work at very low densities and highly sensitive in attracting the insects. Such traps are frequently used for sample and monitor and determine the first appearance of exotic pests in a particular area. However, in an isolated area where pest density is very low, mass trapping works best (El-Sayed et al. 2006).

7 Conclusion

Application of pheromone in pest management is a rapidly evolving noninvasive and ecofriendly method and is going to be one of the most preferred modalities for pest control in the future. Application of pheromone in maintaining the insect pest population below economic injury level in integrated pest management provides a sustainable and environmentally friendly replacement to the broad-spectrum insecticides, either as monitoring or management tools of critical IPM programs (Zijlstra et al. 2011). However, there is necessity of isolation and characterization of more number of pheromones from pest species so that pheromones with high specificity to a particular pest species can be developed. Presently research and innovations are required to address problems of areas related to the use of multiple pheromones to monitor several pests simultaneously. Use of pheromones for behavioral manipulation of natural enemies, understanding the mechanisms underlying mating disruption and other pheromone-based control approaches, and the use of multiple pheromones for controlling several pests simultaneously in a mating disruption scenario necessitate further research to optimize the processes. The ultimate challenge will be to increase the adoption of pheromone-based pest management technologies by making them cost-effective, but without sacrificing efficacy.

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Non-protein Chemical Compounds from Lepidopteran Insect Cocoons

M. Sayed Iqbal Ahamad, Kari Neetha, and Shyam Kumar Vootla

Abstract Cocoons are the specialized naturally engineered structures constructed by lepidopteran insect silkworms. They are natural protein composites of fibroin and sericin as a nonwoven biological structural material. This complex structure is presumed to be optimized through evolution over millions of years. It provides the prime protection for the silkworm pupae from biotic and abiotic factors and facilitates the thermal insulation to the inactive pupa stage of the insects, during metamorphosis. In present chapter chemical compounds other than silk proteins in commercially valuable lepidopteran insect cocoons which are responsible for sustained protection of the insect pupae inside the cocoon which is predicted to be the key factors to achieve the unique structural and chemical barriers to protect the pupa within the cocoons are described. Biophysical analysis of the various ingredients in the cocoons was performed. The role of host plants in the biosynthesis or the sequestration of the phytochemicals or the active compounds was evaluated qualitatively to understand the interaction of the host plants and non- protein chemical constituents from lepidopteran insect cocoons specifically the domesticated and wild silkworms.

Keywords Bioactive compounds · FT-IR · GC-MS · Lepidopteran insects · Silkworm cocoons · Sequestration

1 Introduction

Insects constitute the most diverse group of living organisms on the world. They are cosmopolitan in nature found in every terrestrial and aquatic habitat. Insects are considered as the most species-rich taxonomic group on Earth. Nearly 9, 50, 000 insect

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species have been reported and many millions are yet to be discovered (Groombridge 1992). Insect species are estimated to account for more than half of all species on Earth, and beetles alone represent a quarter of all described species (Southwood et al. 1979; Stork 1988). The members of family Saturniidae are one of the largest and most spectacular of the lepidopteran insects and estimated 1300–1500 different species distributed worldwide (Grimaldi and Engel 2005). They occupy all types of ecosystems and perform many important ecological functions as pollinators, seed predators, decomposers and parasitoids (Sodhi et al. 2010). Insects have significant economic role in silk industry to beekeeping and act as the pollinators for horticultural and agricultural produce. In many regions, insects form an important component of the human diet. Some insects have great value in Chinese medicine (Lokeshwari and Shantibala 2010).

Silks produced by domesticated silkworm *Bombyx mori* are the most comprehensively investigated due to its availability; silk is used in textiles and historical medical value as a suture material. This structural variety and the versatility of these biopolymers and systems have important implications for the design, development and manufacturing of new and artificial assemblies for tissue engineering those that are critical to biomedical and biotechnological applications which would lead to industrial production. The silk proteins of non-mulberry silkworms are biochemically and immunochemically distinct from the mulberry silkworm *Bombyx mori* (Nayak et al. 2012).

The tropical Indian tasar silkworm *Antheraea mylitta* ($n = 31$) is a natural fauna of tropical India. It exists in various forms of nearly 44 ecological ecoraces in different geographical niches of India depending on food plants and environmental conditions (Renuka and Shamitha 2013). The wild *Antheraea mylitta* is a sericigenous insect in India distributed naturally in the forest fields of Andhra Pradesh, Bihar, Madhya Pradesh, Maharashtra, Orissa and Telangana. It is a polyphagous insect feeding on a number of food plants primarily on *Terminalia arjuna* and *Terminalia tomentosa* and *Shorea robusta* and secondarily on *Ziziphus*, *Tectona*, *Bauhinia*, *Lagerstroemia* etc. (Renuka and Shamitha 2015). The range of distribution is between 12–31° N latitude and 72–96° E longitude. *Antheraea mylitta* species is endemic and distributed in different geographical regions of India in the form of different ecological races. It leads to phenotypic diversity traits like fecundity, voltinism, cocoon weight and the preference of host plants (Mahendran et al. 2006; Sinha 2011).

In India commercially valuable lepidopteran insects tasar silkworms are largely found in dry tropical forests, as well as in moist deciduous forest areas of red loamy and black clayey regions within maximum temperature range of 30–34°C and a minimum of 18–21°C, the annual precipitation ranging from 925 to 939 mm in dry deciduous and 1000–1275 mm in moist tropical deciduous forest areas (Suryanarayana and Srivastava 2005). These silkworms are reported to be univoltine, bivoltine and trivoltines; the voltinism is mainly regulated by environmental factors such as temperature, relative humidity, day length and rainfall (Kar et al. 2000). Humidity also plays an important role in growth of the larvae, triggering the moth emergence and preventing pupal desiccation.

In addition to the silk fibres, insect extracts have been extensively used in folk medicines from thousands of years (Hardy and Scheibel 2010). In Chinese traditional medicines insects and insect-based products are used in the treatment of throat and ear infections, tuberculosis, influenza, cancer and many other diseases and ailments (Costa-Neto 2005; Rajkhowa and Deka 2016). Naturally insects and their products are highly evolved entities in the course of evolution, because insects face various challenges in their life cycle due to microbe-infested niches they occupied, and their success, vast numbers and diversity indicate the presence of very effective immune systems producing powerful antimicrobial, cytotoxic and medicinally valuable chemical factors (Figueirêdo et al. 2015).

The chemical defence strategies of insects evolved including odorous repellents to avoid or to kill or inactivate the defending individual predatory organisms including microorganisms. It is a common defence mechanism among the innumerable adaptations that have been evolved in response to selection by natural enemies (Zhang et al. 2011). Even though insects and their products are quite neglected for modern biomedical research, they have provided valuable products like honey and silk which are utilized in folklore medicine from thousands of years (Ratcliffe et al. 2011). By considering this, we selected commercially exploited lepidopteran insects of domesticated and wild silkworms to screen the active non-protein chemical components from their cocoons.

The domesticated mulberry silkworms are monophagous insects and feed on exclusively mulberry leaves, whereas non-mulberry silkworms are polyphageous in nature and feed on diverse types of leaves. The feeding habit and the growing conditions of the silkworms directly influence the chemical composition of the cocoons. The phytochemicals from the host plants of silkworms might be sequestered to cocoons (Bharani et al. 2002). Host plant specificity of silkworms achieved by coevolution of plants and mono- or oligophagous herbivorous insect leads to the accumulation of host plant-derived chemicals in the cocoons. The allelochemicals or phytochemicals produced by plants play a vital role in the life cycle of plant-feeding insects. These chemicals affect the behaviour, survival, growth and fecundity of insects. Similar observation was reported in tasar cocoons for biological functions of secondary metabolites like tannins, terpenoids, saponins, flavonoids and phytosterols sequestered into the larvae to cocoons (Bindu et al. 2006). The secondary metabolites consumed by the silkworms from the host plants are sequestered with silk proteins and play a significant role in cocoon formation (Kumar and Mandal 2017).

2 Classification of Insects

Insects are the most species-rich taxonomic group on Earth. Nearly 9, 50, 000 insect species have been identified and many millions still await for discovery (Groombridge 1992). Insect species are estimated to account for more than half of all species on Earth, and beetles alone currently represent a quarter of all described species (Southwood 1978; Stork 1988). The recent estimation of 10, 13,825 named insect species compiled in the Catalogue of Life (64) is based on numerous separate catalogues for different taxa.

The insect order Lepidoptera consists of 4 suborders, 15,578 genera and 1, 57, 424 species (van Nieukerken et al. 2011). The commercially exploited silkworms are broadly segregated into domesticated and wild in nature. The domesticated silkworms belong to the family Bombycidae, which is mulberry silkworm *Bombyx mori*. It is exclusively domesticated and reared under controlled rearing conditions. But wild silkworms belong to the family Saturniidae and are polyphagous in nature and feed on various types of leaves. The wild non-mulberry saturniid variety of silkworms is of three types: tasar, muga and eri. The Indian tropical tasar *Antheraea mylitta* feeds on the leaves of *Terminalia arjuna*, *Terminalia tomentosa* and *Shorea robusta*. The muga silk is produced by *Antheraea assama* which feeds on *Machilus bombycina*. Eri silk is produced by *Samia ricini* whose primary host plant is *Ricinus* species (Kundu et al. 2008).

All serpiginous insects belong to phylum Arthropoda, class Insecta, order Lepidoptera, and superfamily Bombycoidea and families of Bombycidae and Saturniidae are the only two families whose members produce natural silk. There are several species of silkworm that are used in commercial silk production. Wild silkworms tropical tasar *Antheraea mylitta* and muga *Antheraea assama* are indigenous to India (Chakraborty et al. 2015).

3 Insect Cocoons

Insects spin a silk web as cocoon to facilitate protection and ensuring proper inner environment inside the cocoon during the metamorphosis. The structure and composition of insect cocoon shows hierarchical composites of silk fibres and sericin proteins and other non-protein chemical components which confer a number of unique properties to the cocoon (Chen et al. 2012a). The architecture of cocoons and the chemical compounds present in the cocoons play a crucial role to provide protection of the pupae during metamorphosis. Such cocoons are equipped with a variety of chemical compounds which include antibacterial, antifungal, insecticidal and insect repellent etc. The biomimetic potential of the cocoons, the relationships between their structure and function, which have been evolved over millions of years of evolution (Blossman-Myer and Burggren 2010).

The insect cocoon features of great interest in the properties of flexibility, damage resistance and light in weight. The cocoons of every species have their own individual features. But they all have a multilayer structure with silk fibres connecting layers than aligned in the individual layers. The interlayer bonding is much weaker than the intralayer bonding. The porosity of the cocoon layers decreases from inner to outer layers (Chen et al. 2012b). In addition to this, cocoon structures are optimized for combinations of diverse biological functions across the species range against various environmental challenges and threats. Some cocoons also possess extra calcium oxalate crystals on the cocoon surface like *Antheraea pernyi*. The natural interaction between their structure and function, which have been standardized over millions of years of evolution. All cocoons seems to be multifunctional and includes camouflage (Danks 2004), thermoregulation (Lyon and Cartar 1996) and humidity control (Blossman-Myer and Burggren 2010). However, the main function of all cocoons is to be the mechanical protection of the enclosed pupae from predators (Scarbrough et al. 1972; Waldbauer 1967).

The wild tasar silkworms grown on forest trees for their commercial value are subject to harsh environmental conditions in comparison with domesticated *Bombyx mori* silkworms due to pupal diapause in harsh weather for 3 months. Hence the non-protein active compounds from the wild cocoons and their biophysical characterization and their evolution in the cocoons of wild *Antheraea mylitta* silkworms will increase the commercial and biological applications in the field of entomology. In addition to the cocoon proteins other non-protein chemical compounds are studied and compared for domesticated and wild silkworms and their cocoons show slight different combination of physical, morphological, structural and chemical properties adapted to cope environmental conditions.

4 Protein Components of Insect Cocoons

The cocoons of the lepidopteran insects are composed of two major types of proteins, viz. fibroins and sericins. Silk is composed of two main proteins, the hydrophilic sericin and crystalline fibroin, with distinct properties and functions which dictate the structure of the cocoon (Kaplan et al. 1994). The fibroin proteins are the core proteins which constitute over 70% of the cocoon and are hydrophobic glycoproteins secreted from the posterior silk gland (PSG). The sericins secreted by the middle silk gland (MSG), which are gluelike proteins, constitute 20–30% of the cocoon and are hot water-soluble glycoproteins that hold the fibroin fibres together to form the naturally stable fibroin-sericin composite cocoon structure (Kundu et al. 2008).The strong adhesive property of sericin is attributed to the hydrogen bonding potential of the vast number of hydroxyl amino acids present in it (Anghileri et al. 2007). The built and strength of different cocoons like tasar cocoons and lustrous golden yellow colour of muga silks and mulberry silks renders its difference in the sericin protein and other non-protein components of the cocoons.

5 Non-protein Chemical Compounds of Lepidopteran Insect Cocoons

The cocoons of wild lepidopteran insect *Antheraea mylitta* are the largest and toughest morphology with a peduncle, the typical feature of Saturniidae silkworms. The average weight of the cocoons is 1–1.5 g. The cocoons along with silk proteins are even covered with surface impurities such as the inorganic salt of calcium oxalate. The tropical tasar silkworm *Antheraea mylitta* is an endemic wild silkworm of central parts of Indian forests (Fig. 1). Unlike domestic silkworm cocoons which are

Fig 1 Wild *Antheraea mylitta* silkworms

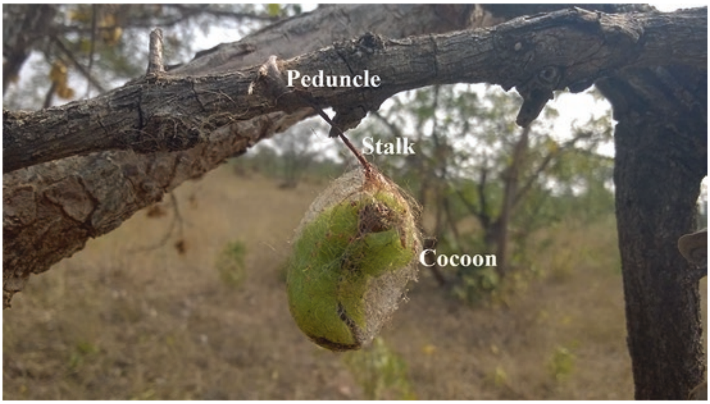


Fig. 2 *Antheraea mylitta* cocoon construction in wild conditions

processed and handled for commercial silk production under supervision, *Antheraea mylitta* cocoons are spun on trees in wild and the cocoon structure is peculiar with the peduncle and with hard built (Fig. 2) (Kar et al. 2005). Current research on silkworm is focused only on textile applications. The chemical properties of the cocoons and constitution of bioactive non-protein chemical compounds in the cocoons and their functions need to be explored. The screening of chemical compounds of the cocoons, which are presumed to be the chemical protective entities of the wild lepidopteran cocoons and their long-term utilization for biomedical applications, needs more exploration.

As an insect model silkworms have been well studied for entomological research and silkworm cocoons have been used for textile applications from thousands of years (Guo et al. 2016). The gluelike sericin envelops the core fibroin fibres with successive adhesive layers and ensures the cohesion of the cocoon by gluing the silk threads together. In lepidopteran insect cocoon fibroin 70%, sericin 25% and non-sericin components 5%, respectively. The non-protein components in the cocoons include carbohydrate, salt wax and other chemicals (Wang et al. 2012). Silkworms are host-specific insects, feeding plants specificity of the insects is due

Table 1 Qualitative tests for active compounds from mulberry and non-mulberry cocoons

Active compounds	A (Mulberry)	B (Tasar)	C (Eri)	D (Muga)
Alkaloids	+	+	+	-
Saponins	-	+	-	-
Steroids	-	+	-	-
Phenols	+	+	+	+
Flavonoids	+	+	+	-
Terpenoids	-	-	-	-
Tannins	-	-	-	-
Fatty acids	+	+	+	+
Carboxylic acids	+	+	+	+
Volatile oils	+	+	+	-
Fixed oils	+	+	+	+
Aldehydes	+	-	+	-

Fig 3 Silkworm cocoons of (**a**). Mulberry, (**b**). Tasar, (**c**). Eri, (**d**). Muga

to the co-evolution of plants and mono- or polyphagous herbivorous insects. The host plant-derived chemicals or allelochemicals play a significant role in the life of plant-feeding insects. These allelochemicals affect the survival, growth, fecundity and population dynamics of insects. But to the non-adapted insect pests, the same allelochemicals function as deterrents or poisons (Bindu et al. 2006).

In present research the qualitative analysis of phytochemicals was tested for alkaloids, saponins, steroids, phenols, flavonoids, terpenoids, tannins, fatty acids, carboxylic acids, volatile oils, fixed oils and aldehydes in the methanolic extracts of lepidopteran insect of mulberry and non-mulberry silkworm cocoons by using standard methods and compiled in Table 1 (Roy et al. 2011). The alkaloids and volatile oils are positive in mulberry, tasar and eri except muga cocoons (Fig. 3). The terpenoid and tannin tests are negative in all the cocoons. The tests for phenols, fatty acids, carboxylic acids and fixed oils are positive in all the four cocoons. The aldehyde test is positive in mulberry and eri cocoons, whereas saponins have shown a positive test in tasar cocoons. In the cocoons of mulberry silkworms three flavonoid 5-glucosides from the sericin layer and many other flavonoids were identified in yellow-green cocoon of the Sasammayu silkworms. The flavonoids from silkworm cocoons are known effective for free radical scavenging, antioxidation, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Wang et al. 2012).

The active compounds traced in the cocoons are presumed to be sequestered from their host plants. The host plant *Morus nigra* (Moraceae) leaf-fed mulberry silkworm cocoons were selected for the study; hence we observed identical phytochemicals except alkaloids in mulberry cocoons as compared to their host plant. Due to these bioactive compounds mulberry plants are utilized for medicinal applications (Wadood et al. 2013; Jiang and Nie 2015; Natić et al. 2015). The primary host plant for tasar silkworm is *Terminalia arjuna* (Combretaceae); it has been reported for its antimicrobial properties and to treat cardiovascular disease (Kaur et al. 2014; Smritikana and Pranoy 2017). In this study similar phytochemical compounds of *Terminalia arjuna* leaves observed in tasar cocoons except steroids were not detected in the tasar host plant (Desai and Chanda 2014). The extract of *Ricinus communis* (Euphorbiaceae), the primary host plant for eri silkworms, qualitatively showed the same phytochemical constituents observed in eri cocoons except alkaloids. But saponins and steroids which are not observed in cocoons were also reported in the host plant. The allelochemical sequestrations from host plant to the cocoons in muga cocoons evaluated from the major primary host plant for muga silkworms is *Persea bombycina* (Lauraceae), the phytochemicals of phenols, fatty acids, carboxylic acids and fixed oils are only observed in the muga cocoons (Neog et al. 2011a, b). The alkaloids, saponins, steroids, flavonoids, terpenoids, tannins, volatile oils and aldehydes are not observed in the cocoon extract. These observations highlight not only the phytochemical sequestrations from host plant to cocoons and biosynthesis of the active compounds by the silkworm larvae during spinning of the cocoons. The mechanism or metabolic pathway for phytochemical sequestration or the biosynthesis of the bioactive compounds is observed.

The chemical constituents of insect cocoons and their role in defence strategies in nature are neglected in the field in entomology research. Along with the mechanical design and architectural features of the cocoons, chemical constituents present in the cocoons also play an active role to protect inactive pupae which are entrapped in the cocoon during metamorphosis. Survival of the insects in wild environmental conditions is determined by the chemical weaponry, comprising repellents or deterrent odours that reduce the possibility to defending predator to be killed or loses its potential. It is a natural defence mechanism among the several adaptations that have evolved in response to selection by natural enemies (Zhang et al. 2011). Insect repellents are substances that act locally or at a distance, deterring an arthropod from flying to landing on or biting human or animal skin. Many synthetic broad-spectrum chemicals have been industrialized as repellents; they are the most effective but more persistent on skin and may cause environmental and human health risks (Nerio et al. 2010). But the essential oils and other complex mixtures of volatile compounds derived from plants have been characterized for repellent properties on various haematophagous arthropods. Some of the plant-based repellents have emerged as better insect repellents than synthetics. Even though insect repellent potential is directly depending on the nature of essential oil, it possesses short lasting from a few minutes to as long as 2 h depending on their volatility (Anuar and

Yusof 2016). Therefore, there has been an increase in search efforts for natural, long-lasting, less volatile and eco-friendly repellents from various sources.

We investigated the insect repellent alkaloids and other volatile oils from wild *Antheraea mylitta* cocoons. The tropical tasar silkworm, *Antheraea mylitta*, is an important commercially valuable insect for Asian non-mulberry silk industry and produces tasar silk. After domesticated *Bombyx mori* silkworm, tasar silkworm is the second largest silk-producing, silk-spinning insect (Akai 2000). It is polyphagous and feeds on a variety of food plants such as *Terminalia arjuna*, *Terminalia tomentosa* and *Shorea robusta* and the secondary food plants *Terminalia bellirica*, *Terminalia chebula*, *Terminalia paniculata*, *Terminalia catappa*, etc.; hence we presume the allelochemicals sequestered from their host plants.

Biophysical analysis of *Antheraea mylitta* methanolic cocoon extract by FT-IR and FT-Raman spectroscopic studies are synchronized to identify functional groups, molecular vibrations and bonding patterns of the active compounds and confirmed the compounds by GC-MS as fatty acids, alkane hydrocarbons, esters, terpene alcohols, aldehydes, aromatic carboxylic acids and steroidal compounds. Further antimicrobial potential of tasar cocoon extract on bacterial strains of *Escherichia coli*, *Staphylococcus aureus* (Fig. 4) and antifungal potential on *Candida albicans* and *Aspergillus flavus* (Figs. 5, 6, 7 and 8). The identified antimicrobial compounds of 1,3,5-triazine-2,4-diamine, nonadecanoic acid, oleic acid, cyclopropanepentanoic acid, 2-undecyl-, methyl ester, bis(2-ethylhexyl) maleate, decanal, 1,2-benzenedicarboxylic acid, cholestane, ethyl iso-allocholate and cytotoxicity inducing trichloroacetic acid hexadecyl ester compiled in Table 2 and chemical structures shown Fig. 9, which are may be the responsible components to inhibit the selected microorganisms in antimicrobial assay.

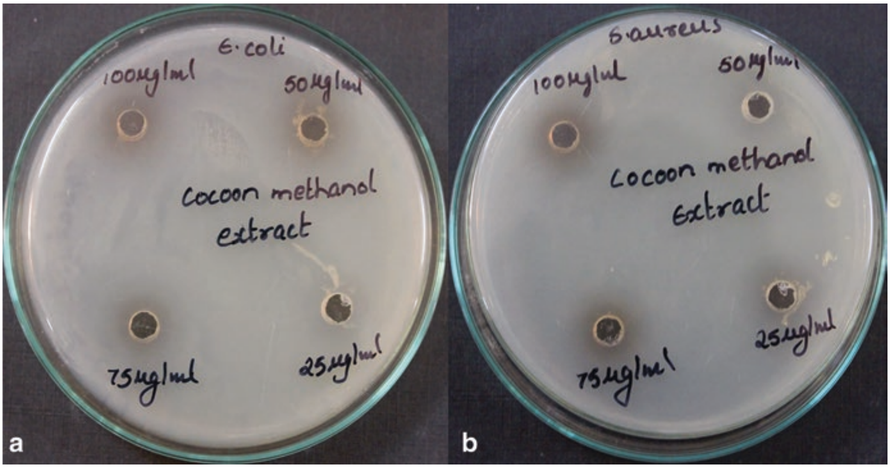


Fig. 4 Antibacterial activity of *Antheraea mylitta* cocoon extract on (**a**) *Escherichia coli* and (**b**) *Staphylococcus aureus*

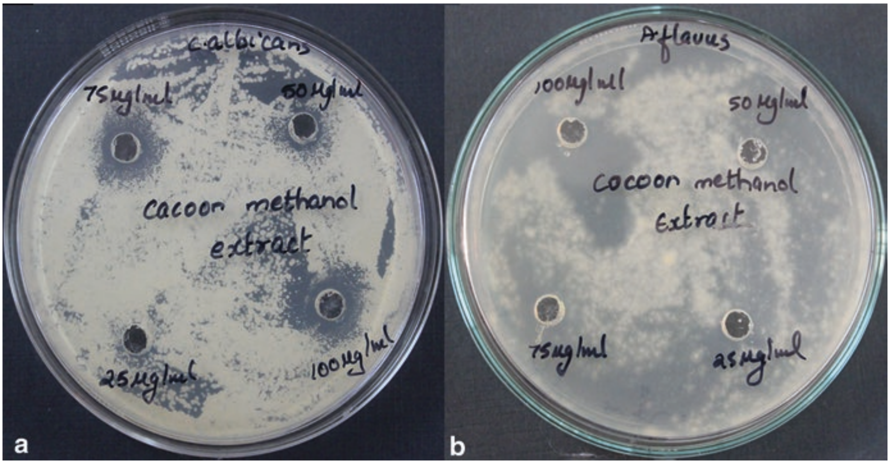


Fig. 5 Antifungal activity of *Antheraea mylitta* cocoon extract on (**a**) *Candida albicans* and (**b**) *Aspergillus flavus*

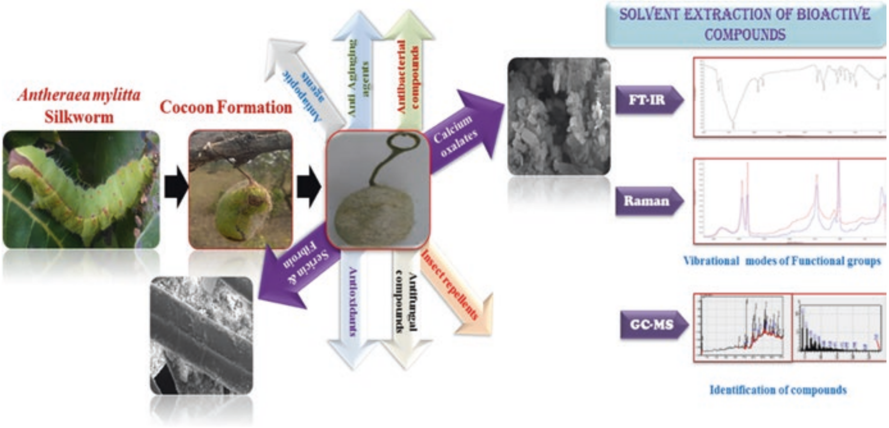


Fig. 6 Graphical representation of tasar cocoon components and their identification

The antimicrobial action of essential oils traced in tasar cocoons may inhibit the gram-positive bacteria by damaging the cell walls and cytoplasmic membrane of bacteria which leads to the leakage of the cytoplasmic contents and consequently its inactivation. A steroid cholestane screened in present study contains imidazole moiety. Some other gram-positive bacteria which are sensitive to imidazole and whose cell membranes are rich in unsaturated fatty acids cause rearrangement of the microbial membrane components which results in the loss of cell viability and finally lysis of the cells occurs (Hashim et al. 2016). For antimicrobial potential of the cocoon extract, along with fatty acids other bioactive compounds traced in cocoon by their individual or synergistic action are responsible for microbial inhibitory activity in natural environment.

Fig. 7 Biotic and abiotic factors affecting cocoon components

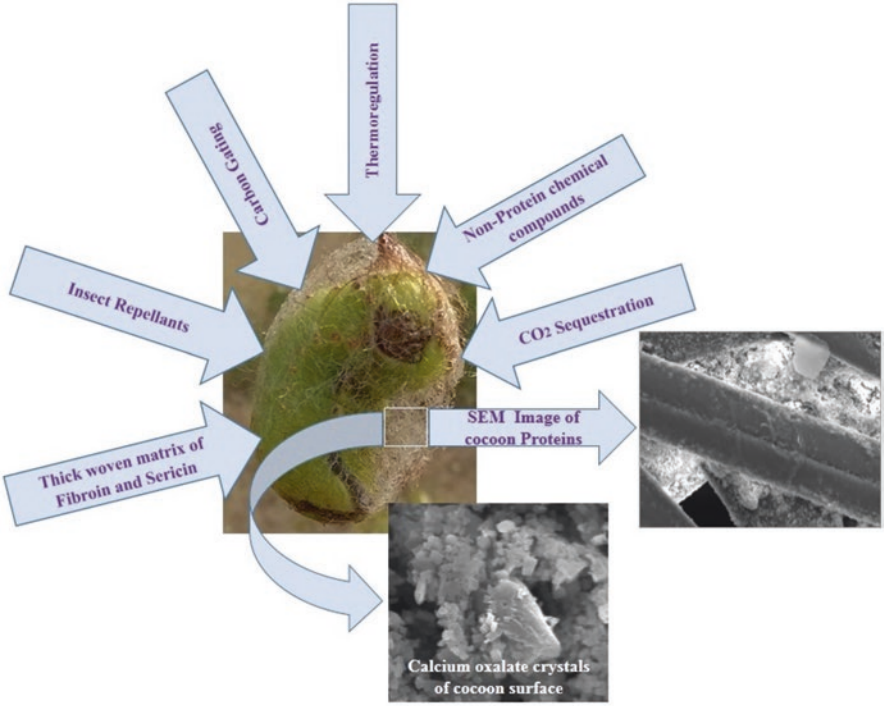


Fig. 8 Functions and components of lepidopteran insect cocoon

Table 2 Non-protein chemical compounds from *Antheraea mylitta* cocoons

Name of the compound	Structure	Molecular weight	Chemical nature
1,3,5-Triazine-2,4-diamine	$C_3H_5N_5$	111.105	Nitrogen containing heterocyclic compound
Nonadecanoic acid	$C_{19}H_{38}O_2$	298.503	Free saturated fatty acid
Heptacosanoic acid, methyl ester	$C_{28}H_{56}O_2$	424.743	Saturated fatty acid methyl ester
Oleic acid	$C_{18}H_{34}O_2$	282.461	n-3 polyunsaturated fatty acid
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424	Saturated fatty acid
2-Tridecyne	$C_{13}H_{24}$	180.329	Alkane hydrocarbon
Cyclopropanepentanoic acid, 2-undecyl-, methyl ester	$C_{20}H_{38}O_2$	310.514	Ester
2-Methyl-Z,Z-3,13 octadecadienol	$C_{19}H_{36}O$	280.488	Terpene alcohol
Z,Z-8,10-Hexadecadien-1-ol	$C_{16}H_{30}O$	238.409	Terpene alcohol
Octadecanoic acid	$C_{18}H_{36}O_2$	284.477	Saturated fatty acid
Bis-(2-ethylhexyl) maleate	$C_{20}H_{36}O_4$	340.497	Fatty acid ester
12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280.488	Terpene alcohol
Decanal	$C_{10}H_{20}O$	156.265	Aldehyde
Beta carotene	$C_{40}H_{56}$	536.872	Carotenoid
1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	166.130	Aromatic dicarboxylic acid
Cholestane	$C_{27}H_{48}$	372.670	Steroid
(4Z)-5,9-Dimethyl-4,8-decadienal	$C_{12}H_{20}O$	180.287	Aldehyde
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.624	Steroid
Trichloroacetic acid hexadecyl ester	$C_{18}H_{33}Cl_3O_2$	387.812	Ester of chloroacetic acid
Undecanal	$C_{11}H_{22}O$	170.291	Aldehyde

The screened chemical constituents might be involved in the protection of *Antheraea mylitta* cocoons in wild environment by their individual or synergistic action. We presume that the non-protein chemical composition of wild silkworm cocoons other than silk sericin and fibroin proteins is responsible for biologically active cocoons for their sustainability, integrity and stability for long duration in wild harsh environmental conditions. By this study the strategic developments for the economic, commercial extraction of non-protein bioactive compounds during degumming process of cocoons of the silkworms can be explored for medicinal importance.

The tasar silkworm cocoons possess chemical composition which is strong enough to face threats during their life cycle. The clear mechanism of the cocoon alkaloid synthesis or sequestration from host plants to the cocoons and their role in silkworms needs to be elucidated. The insect-based chemical products including antimicrobial agents, their synthesis strategies and the mechanism of action may play a crucial role in the development of new drugs in the field of biomedical research.

Mulberry silk has been produced for at least 5000 years. In additional to textile applications, in Chinese medicine, silk has been used for a variety of human disor-

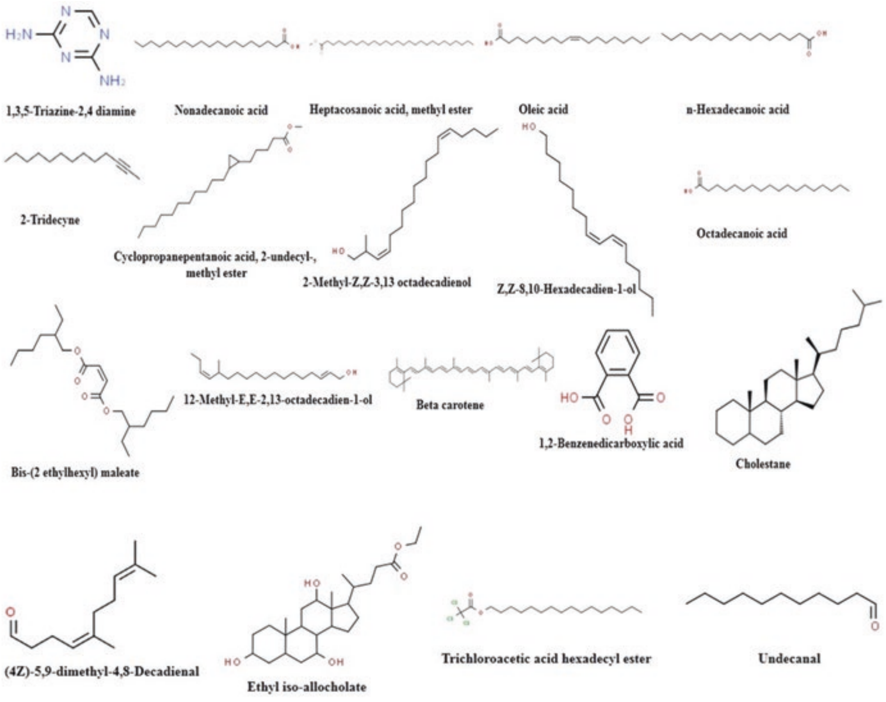


Fig. 9 Chemical structure of non-protein compounds from *Antheraea mylitta* cocoons

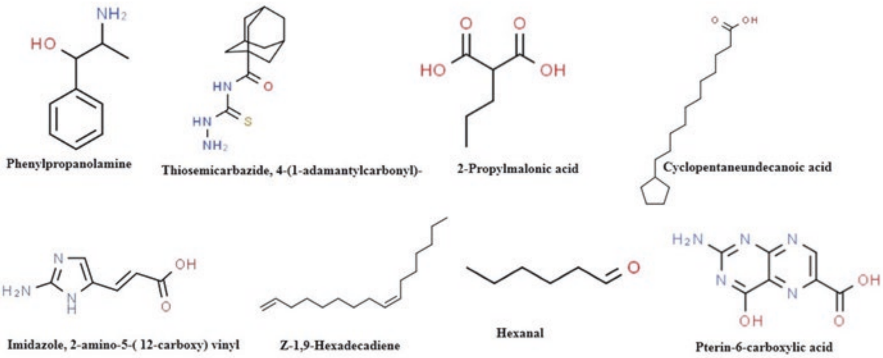
ders including the relief of spasms and flatulence. Interestingly, silkworm larvae have also been prescribed for treating impotence only for, subsequently, a vasodilator compound enhancing no production to be extracted from *Bombyx mori* larvae and to be a candidate for the therapeutic treatment of vascular impotency (Ratcliffe et al. 2014).

The methanolic extract of *Bombyx mori* cocoons identified eight non-protein compounds of cyclopentaneundecanoic acid (20.62%), pterin-6-carboxylic acid (17.98%), Z-1, 9-hexadecadiene (25.68%) and hexanal (9.09%). The antimicrobial activity of these compounds was reported (Kavitha et al. 2009; Parkouda et al. 2011; Qin et al. 1997; Dandekar et al. 2015). The minimum peak area percentage is observed for imidazole, 2-amino-5-(12-carboxy)vinyl (4.14%), an antimicrobial heterocyclic compound. The identified compounds and their bioactivity are presented in Table 3 and chemical structures compiled in Fig. 10.

The cocoons of the mulberry silkworm *Bombyx mori* feeding on *Morus alba* leaves contain flavonoids (Hayashiya et al. 1959). These flavonoids were identified according to their mass, NMR and UV-Vis spectral properties as derivatives of kaempferol and quercetin (aglycones and mono-triglycosides) (Tamura et al. 2002; Hirayama et al. 2008). Even quercetin with amino acid (proline) substituents has been found in the cocoons of *Bombyx mori*. Some flavonol glycosides found in the mulberry silkworm cocoons were not reported in mulberry leaves implying that the

Table 3 Non-protein chemical compounds from mulberry silkworm cocoons

Name of the compound	Structure	Molecular weight	Chemical nature
Phenylpropanolamine	$C_9H_{13}NO$	151	Amino derivative
Thiosemicarbazide, 4-(1-adamantylcarbonyl)	$C_{12}H_{19}N_3OS$	253	Urea derivative
2-Propylmalonic acid	$C_6H_{10}O_4$	146	Fatty acid derivative
Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254	Fatty acid derivative
Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207	Carboxylic acid derivative
Imidazole, 2-amino-5-(12-carboxy) vinyl	$C_6H_7N_3O_2$	153	Heterocyclic compound
Z-1,9-Hexadecadiene	$C_{16}H_{30}$	222	Alkyne
Hexanal	$C_6H_{12}O$	100	Aldehyde



Phenylpropanolamine
Thiosemicarbazide, 4-(1-adamantylcarbonyl)-
2-Propylmalonic acid
Cyclopentaneundecanoic acid
Imidazole, 2-amino-5-(12-carboxy) vinyl
Z-1,9-Hexadecadiene
Hexanal
Pterin-6-carboxylic acid

Fig. 10 Chemical structure of non-protein compounds from *Bombyx mori* cocoons

larvae had probably modified these compounds from dietary flavonols (Tamura et al. 2002; Hirayama et al. 2013). Flavonoids can possibly shield cocoons against UV-B radiation (Daimon et al. 2010) or increase their antioxidant capacity (Kurioka and Yamazaki 2002). The cocoon extracts of *Bombyx mori* have shown antimicrobial activity that is probably due to antimicrobial proteins (Pandiarajan et al. 2011), but the possible role of flavonoids in this aspect seems to be unknown.

Ethanolic extract of *Bombyx mori* cocoons reported *n*-pentanyl-3,4-(dioxymethylene)benzene, *n*-pentanyl-2,3-dioxymethylene-4-hydroxybenzene, *n*-decanyl-3,4-dioxymethylene-4-hydroxybenzene, dihydrocitronellyl glucoside and dihydrocitronelloyl glucuronosidic ester which are the five new phytoconstituents that have been isolated from the silk cocoons of *Bombyx mori* which are considered as medicinally significant compounds (Kaskoos and Naquvi 2012).

The cocoons of the *Myrascia* species of lepidopteran insects contained sacs filled with oily terpenoids (Common and Bellas 1977). These sacs originate from the larval phase, as the larvae sequester oils from their host plant. The oily sacs are possibly aimed at deterring predators of the cocoons. The silkworm *Attacus atlas* is a lepidopteran insect that produces silk with aesthetic value. These moths have maximum wing span to reach 300 mm which is the largest wing of all moths. This is native of China and Southeast Asia in tropical rainforest habitats at altitudes around 1500 m. It feeds on a variety of plants like *Erythrina variegata* and *Cananga odorata* species. They often move from one plant to another during their development (Chen et al. 2014). The variations in diet and climate resulted in diversity and properties of the cocoons and fibres produced by silk within the same type. The microstructure of the cocoon investigation by scanning electron microscopy (SEM) and X-ray spectroscopy (EDS) revealed the surface of the cocoon reveal that by feeding the larvae of *Attacus atlas* with *Erythrina variegate* resulted in cocoon with chlorine (Cl) around 2.96 mass%. Chlorine is not found in the cocoon that was feeding with *Cananga odorata*, but the elemental calcium (Ca) is present around 14.37 wt%. This calcium gives good biocompatibility since calcium is essential for cell physiology. In addition to these other elements of carbon (C), oxygen (O) and potassium (K) are also reported (Tjokorda et al. 2017).

The methanolic extraction of eri cocoons revealed eight chemical compounds from respective GC-MS studies. The concentration of nonadecanoic acid (44.43%), 9-octadecenal (39.92%) and acetic acid (4.78%) is predicted more because of the maximum of peak area percentage. Nonadecanoic acid is reported as insect pheromone and antimicrobial, 9-octadecenal is used as a flavouring agent and acetic acid is an antimicrobial agent. Another major constituent of the extract 3-pyridinecarboxaldehyde (3.37%) is reported for its insecticidal and antifungal properties (Agoramoorthy et al. 2007; Al-Wathnani et al. 2012; Jasim et al. 2015). Other minor compounds traced by GC-MS and their bioactivity are mentioned in Table 4 and chemical structures compiled in Fig. 11.

The GC-MS analysis of muga cocoon extract revealed six prominent chromatogram peaks. The major compounds with more peak area percentage revealed are benzoxazol, 2,3-dihydro-2-thioxo-3-diallylaminomethyl- (50.83%), cyclopentaneundecanoic acid (31.57%), benzeneethanamine, 2,5-difluoro-beta,3,4-trihydroxy-N-methyl- (11.96%) and phenylephrine (3.07%). The cyclopentaneundecanoic acid is extracted from *Nocardia* species and confirmed its antimicrobial properties (Kavitha et al. 2009) and phenylephrine is used as nasal decongestant, cardiotonic and vasoconstrictor agent (Meltzer et al. 2015). The GC-MS analysis and the bioactivity of the compounds is presented in Table 5 and chemical structures compiled in Fig. 12.

Table 4 Non-protein chemical compounds from eri cocoon extract

Name of the compound	Structure	Molecular weight	Chemical nature
2-Methylaminomethyl-1,3-dioxolane	$C_5H_{11}NO_2$	117	Aldehyde
3-Nitropropionic acid	$C_3H_5NO_4$	119	Fatty acid derivative
3-Pyridinecarboxaldehyde	C_6H_5NO	107	Aldehyde
Propanedionic acid	$C_3H_4O_4$	104	Carboxylic acid
Acetic acid	$C_2H_4O_2$	60	Carboxylic acid
Butylurea	$C_5H_{12}N_2O$	116	
Nonadecanoic acid	$C_{19}H_{38}O_2$	298	Fatty acid
9-Octadecenal	$C_{18}H_{34}O$	266	Aldehyde

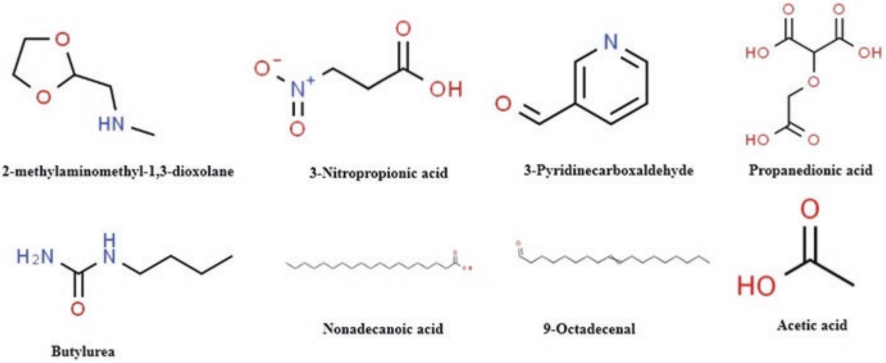


Fig. 11 Chemical structure of non-protein compounds from eri silkworm cocoons

Table 5 Non-protein chemical compounds from of muga cocoon extract

Name of the compound	Structure	Molecular weight	Chemical nature
4-Fluorohistamine	$C_5H_8FN_3$	129	
Phenylephrine	$C_9H_{13}NO_2$	167	Phenethylamine
Thiophene-3-ol	C_4H_4OS	100	Heterocyclic
Benzeneethanamine, 2,5-difluoro-beta, 3,4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	
Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254	Fatty acid derivative
Benzoxazol, 2,3-dihydro-2-thioxo-3-diallylaminomethyl-	$C_{14}H_{16}N_2OS$	260	–

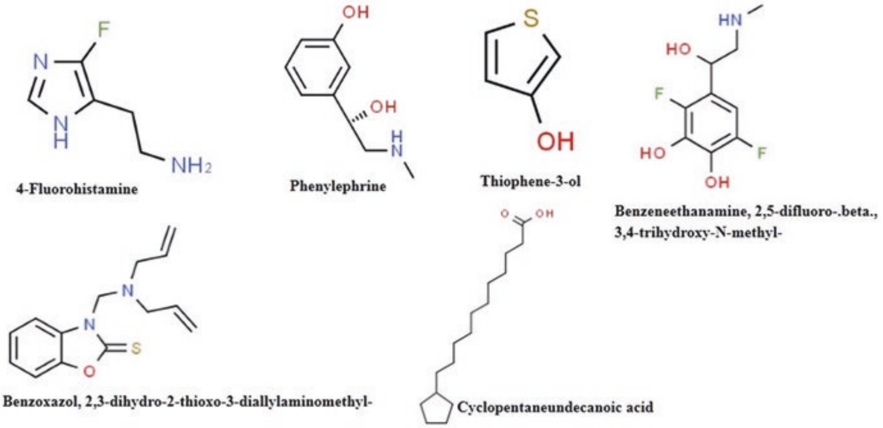


Fig. 12 Chemical structure of non-protein compounds from muga silkworm cocoons

In nature insects and their products are highly evolved entities in the course of evolution, because insects face various challenges in their life cycle due to microbe-infested niches they occupy, and their success, vast numbers and diversity indicate the presence of very effective immune systems producing powerful antimicrobial, cytotoxic and medicinally valuable factors (Figueirêdo et al. 2015). Insect extracts have been used extensively in folk medicines from thousands of years (Hardy and Scheibel 2010). The chemical defence strategies of insects evolved including odorous repellents to avoid or to kill or inactivate the defending individual predatory organisms including microorganisms. It is a common defence mechanism among the innumerable adaptations that have been evolved in response to selection by natural enemies (Zhang et al. 2011).

6 Conclusion

In this chapter we reviewed the non-protein chemical compounds from the cocoons of commercially important lepidopteran insects. The research on the lepidopteran insect cocoons is exclusively focused on the protein components of the cocoons but the non-protein compounds of the cocoons are highly neglected. We identified various classes of non-protein chemical compounds in different economically important insects and their applications for the protection of the insects. The chemical defence, the sequestration of the allelochemicals from host plants to the cocoons and their function and mechanism of action need extensive research in the field of entomology.

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