Mohamed Fawzy Ramadan *Editor*

Cardamom (*Elettaria cardamomum*): Production, Processing and Properties

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Editor
Mohamed Fawzy Ramadan
Professor of Food Chemistry and Biochemistry
Department of Clinical Nutrition
Faculty of Applied Medical Sciences
Umm Al-Qura University
Makkah, Saudi Arabia

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Dedicated to my M.Sc. supervisor and scientific mentor, Mahmoud Z. Sitohy, Emeritus Professor of Agricultural Biochemistry at Zagazig University, Egypt. He is the Director of the Technology, Innovation and Commercialization Office of Zagazig University and a Member of Agriculture and Food Council, Ministry of Scientific Research. He was awarded Zagazig University Appreciation Award in Basic Sciences (2010), the State Award for Excellence in Agricultural Sciences (2014), and the State Medal of Science and Arts of the first class (2017).

Preface

Conventional medicine extensively uses medical plants, spices, herbs, and plant-based products to fight diseases. Cardamom (*Elettaria cardamomum*) is highly prized for its nutritional, medical, and nutraceutical uses. Agronomic, genetic, and climatic factors impact the cultivation and yield of cardamom. Cardamom contains a variety of bioactive phytoconstituents that have health benefits, including phenolics, fatty acids, vitamins, tocols, and sterols. The biological traits of cardamom (i.e., analgesic, anticancer, and antidiabetic effects) are due to these bioactive phytoconstituents. Medical uses for cardamom fruits or capsules include treating cataracts, cardiac, digestive, and kidney diseases, asthma, tooth and gum infections, diarrhea, and nausea. The cardamom cultivation, botanical and ethnobotanical traits, medicinal applications, composition, food and non-food uses, and pharmacological potentials are covered and highlighted in this book.

Cardamom (Elettaria cardamomum): Production, Processing, and Properties focuses on the botany, cultivation, horticultural practices, ethnobotany, post-harvest, phytochemistry, extraction methods, biochemistry, functionality, nutritional value, health-enhancing traits, ethnomedical uses, and processing of cardamom to create a multidisciplinary forum for discussion. The book also covers cardamom's uses in nutrition, food, cosmetics, and pharmaceutical. The outcomes explore their proper uses in developing nutraceuticals, pharmaceuticals, functional food, and drug.

Organized into three sections, *Cardamom (Elettaria cardamomum): Production, Processing, and Properties* addresses:

Part I. Cardamom: Cultivation, Species, and Cultivars
Part II: Cardamom: Chemistry, Functionality, and Health-Promoting Properties
Part III: Cardamom: Technology, Processing, and Applications

Intending to provide a comprehensive contribution to the scientific community involved in food sciences, horticulture, clinical nutrition, health, and pharmacology, this book comprehensively reviews the aspects that led to the recent advances in cardamom (*Elettaria cardamomum*) biochemistry, production, and functionality. The editor hopes the book will be a rich source for researchers, students, and developers in related disciplines.

The editor sincerely thanks all authors for their excellent contributions and their cooperation. The Springer Nature staff's help and support, especially *Daniel Falatko* and *Sofia Valsendur*, was essential for completing my task and is highly appreciated.

Makkah, Saudi Arabia Mohamed Fawzy Ramadan
30 March 2023

Description

Cardamom [*Elettaria cardamomum* (L.) Maton.] is known as the "*Queen of Spices*," due to its distinctive flavor. Cardamom capsules are known for their fruitful health-enhancing qualities and are utilized as a spice and flavoring in the food products. The capsules of dried fruits, mostly from the *Elettaria, Amomum*, and *Aframomum* genera of the Zingiberaceae family, make up cardamoms. The diverse uses of *E. cardamomum* capsules have a number of positive health consequences pertinent to contemporary pharmaceutical viewpoints. Cardamom essential oil (CEO) has a number of pharmacological impacts, while CEO is deemed GRAS by the FDA. The applications of *E. cardamom* have been revealed by in vitro and in vivo medical studies. Several bioactive constituents have been isolated from *E. cardamomum*. Product derived from cardamom capsules contains monoterpenes (i.e., 1,8-cineole, α-pinene, α-terpineol, linalool, linalyl acetate, and nerolidol), which have medicinal potentials. *E. cardamom* flavonoids, anthocyanins, terpenoids, alkaloids, and other phenolics have been used to control pulmonary, kidney, cardiovascular, and lung-associated disorders.

Recent research focuses on studying phytochemicals, bioactive compounds, and therapeutic traits, investigating the mode of action and toxicological impacts of medical plant extracts and bioactive phytochemicals. Cardamom is of significant importance due to its widespread food and medical uses. Although cardamom products are already commercially available in the international market, it is hard to find a comprehensive book on the production, processing, chemistry, and properties of *E. cardamomum*.

Cardamom (Elettaria cardamomum): Production, Processing, and Properties is a valuable scientific work for novel food, horticultural, pharmaceutical, and nutraceutical developers, as well as the R&D researchers in the fields that use spices, herbs, and medical plants.

Key Features

- Production, processing, chemistry, and functional traits of cardamom
- Cardamom phytochemicals and its health-promoting effects
- Food, non-food, and technological applications of cardamom

Readership

- Food biochemistry, clinical nutrition, phytotherapy, pharmacology, biochemistry, and horticulture students and researchers.
- Developers of nutraceuticals, functional food, and pharmaceuticals, as well as R&D researchers in the fields, apply spices, herbs, and aromatic plants.

Mohamed Fawzy Ramadan
Makkah, Saudi Arabia
30 March 2023

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About the Editor

Mohamed Fawzy Ramadan is a Professor of Food Chemistry at the Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia. Prof. Ramadan obtained his Ph.D. (Dr. rer. nat.) in Food Chemistry from the Berlin University of Technology (Germany, 2004). Prof. Ramadan continued his post-doctoral research at ranked universities such as University of Helsinki (Finland), Max-Rubner Institute (Germany), Berlin University of Technology (Germany), and the University of Maryland (USA). In 2012, he was appointed Visiting Professor (100% teaching) in the School of Biomedicine, Far Eastern Federal University in Vladivostok, Russian Federation.Prof. Ramadan published over 350 research papers and reviews in international peer-reviewed journals. He edited and published tens of books and book chapters (Scopus *h*-index is 45 and more than 7500 citations). He was an invited speaker at several international conferences. Since 2003, Prof. Ramadan is a reviewer and editor in several highly-cited international journals, such as the *Journal of Medicinal Food* and *Journal of Advanced Research*. He is currently the editor-in-chief of *Journal of Umm Al-Qura University for Medical Science*.Prof. Ramadan received several national and international prizes, including Abdul Hamid Shoman Prize for Arab Researcher in Agricultural Sciences (2006), the Egyptian State Prize for Encouragement in Agricultural Sciences (2009), European Young Lipid Scientist Award

(2009), AU-TWAS Young Scientist National Awards (Egypt) in Basic Sciences, Technology and Innovation (2012), TWAS-ARO Young Arab Scientist (YAS) Prize in Scientific and Technological Achievement (2013), and Atta-ur-Rahman Prize in Chemistry (2014).

Chapter 1
Introduction to Cardamom (*Elettaria cardamomum*): Production, Processing, and Properties

Mohamed Fawzy Ramadan

1.1 United Nations Sustainable Development Goals and Health-Enhancing Plants

A peaceful and sustainable world is the goal of the United Nations Sustainable Development Goals (SDGs; https://sustainabledevelopment.un.org). The third SDG, "*Good Health and Well-Being*," strives to improve a healthy existence by utilizing health-improving plants and environmentally friendly methods in the food business (Ramadan, 2021, 2022, 2023).

Developing new functional plant-based products that might improve human health is possible. Current innovations will impact future dietary habits (McClements, 2019). Herbs, spices, and medical plants have been utilized to create novel nutraceuticals and medications. Almost 80% of people worldwide use traditional medicine, according to the World Health Organization (WHO). Besides, WHO emphasized the value of researching medicinal plants for their potential to improve healthcare (i.e., safety, efficacy, quality assurance, quality control, dosage, clinical trials, toxicity, drug interaction, and therapeutic uses). Due to advancements in clinical nutrition, there is much interest in aromatic plants as phytoconstituent-rich sources for nutraceuticals, healthy food, and medications. The role of plant-based active ingredients and oils in promoting health has expanded (Ramadan, 2021, 2022, 2023; Ramadan et al., 2022).

M. F. Ramadan (✉)
Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia
e-mail: mfhassanien@uqu.edu.sa

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1.2 Cardamom (*Elettaria cardamomum*): Production, Processing, and Properties

Consuming spices, medicinal plants, and herbs rich in health-enhancing phytoconstituents might expand the consumers' life span. The abundant bioactivity of phytoconstituents in medicinal plants, herbs, and spices makes them natural active compounds (Gantait et al., 2022; Gidwani et al., 2022; Ramadan, 2021, 2022, 2023; Ramadan et al., 2022).

Due to its distinctive flavor and perfume, cardamom [*Elettaria cardamomum* (L.) Maton., family: Zingiberaceae] is known as the "*Queen of Spices*" around the world. The third most costly spice, after saffron and vanilla, is cardamom. Traditional medical uses for small cardamom [*E. cardamomum* (L.) Maton.] fruits or capsules include treating asthma, tooth and gum infections, cataracts, diarrhea, nausea, and digestive, cardiac, and kidney diseases. Moreover, *E. cardamomum* capsules are known for their fruitful health-enhancing qualities and are utilized as a spice and flavoring in meals (Prabhakaran Nair, 2006; Sangeeth & Suseela Bhai, 2016; Ashokkumar et al., 2020; Tanveer et al., 2020; Nimisha et al., 2021; Surendran et al., 2021; Singletary, 2022; Ramadan et al., 2022; Abdel-Rasoul et al., 2023; Abdel-Hameed et al., 2023; Mandal et al., 2023).

Little cardamom, green cardamom, and genuine cardamom are all names for *Elettaria cardamomum*, which is cultivated in Tanzania, India, Nepal, Indonesia, Guatemala, Sri Lanka, and India. The capsules of dried fruits, mostly from the *Elettaria, Amomum*, and *Aframomum* genera of the Zingiberaceae family, make up cardamoms. The most important of these is *Elettaria cardamomum* (L.) Maton. Bengal, Nepal, and Southeast Asian countries are the native habitats of the false cardamom, giant cardamom, or black cardamom, all members of the related genus *Amomum*. Southeast Africa is home to the African cardamom, or *Aframomum danielli*, as it is scientifically termed. Because of high pricing, only Asian nations engage in international trading of small and large cardamom. Medicinal and culinary uses for cardamom (*E. cardamomum* (L.) Maton.) capsules include treating asthma, dental and gum infections, kidney and digestive problems, nausea, cataracts, and cardiac issues (Prabhakaran Nair, 2006; Sangeeth & Suseela Bhai, 2016; Ashokkumar et al., 2020; Tanveer et al., 2020; Nimisha et al., 2021; Surendran et al., 2021; Singletary, 2022; Ramadan et al., 2022; Abdel-Rasoul et al., 2023; Abdel-Hameed et al., 2023; Mandal et al., 2023).

Cardamom's bioactive components, which include proteins, minerals, carbohydrates, lipids, flavonoids, essential oils, terpenoids, and carotenoids, have been found through phytochemical investigations. Also, the diverse uses of *E. cardamomum* capsules have a number of positive health consequences pertinent to contemporary pharmaceutical viewpoints. They also come into play in scents. Cardamom essential oil (CEO) has a number of pharmacological impacts, including anticancer, antibacterial, insecticidal, antidiabetic, and antioxidant traits. The CEO is deemed GRAS by the FDA. The historic use of *E. cardamom* has been supported by contemporary *in vitro* and *in vivo* pharmacological research. Tens of bioactive constituents

have been isolated from *E. cardamomum*. Cardamom capsules' distinctive aroma and their value as a medicinal, innovative food, and nutraceutical are influenced by CEO and other bioactive substances. Depending on the type of plant and processing method, CEO content ranges from 6% to 14%. There was also evidence of chemotype variation based on geographic origin. Product derived from capsules contains monoterpene components that have medicinal effects, including 1,8-cineole, α-pinene, α-terpineol, linalool, linalyl acetate, and nerolidol. They also include the ester of α-terpinyl acetate. Depending on the method of extraction, the primary phytochemicals were α-terpinyl acetate (*ca*. 42%), eucalyptol (*ca*. 27%), linalyl acetate (*ca*. 8%), and linalool (*ca*. 7%). Flavonoids, anthocyanins, terpenoids, alkaloids, and other cardamom phenolics have been used to control pulmonary, kidney, cardiovascular, and lung-associated disorders. CEO showed high MIC values against Campylobacter species and reduced *Bacillus subtilis* spore. CEO also disrupted the quorum sensing of *C. violaceum*. In models of rabbit intestinal perfusion and carrageenan-induced rat paw edema, CEO's anti-inflammatory and antispasmodic properties were demonstrated. When CEO is kept in a normal atmosphere, its flavor quickly deteriorates. The components of its phytochemicals might also alter as a result of the change in scent or flavor (Prabhakaran Nair, 2006; Sangeeth & Suseela Bhai, 2016; Ashokkumar et al., 2020; Tanveer et al., 2020; Nimisha et al., 2021; Surendran et al., 2021; Singletary, 2022; Ramadan et al., 2022; Abdel-Rasoul et al., 2023; Abdel-Hameed et al., 2023; Mandal et al., 2023).

1.3 Cardamom (*Elettaria cardamomum*) Market

The popularity of healthy cuisines is linked to the increased demand for cardamom (*Elettaria cardamomum*) worldwide. Cardamom (*Elettaria cardamomum*) has an outstanding international market because of the increase in the world population with its consumption requirements. FAOSTAT (https://www.fao.org/faostat) reported that, in 2021, the global area harvested by nutmeg, mace, and cardamom is 469,631 ha. The world yield of nutmeg, mace, and cardamom is 3129 kg/ha, and the world production of nutmeg, mace, and cardamom is 146,952.34 tonnes.

The ginger family Zingiberaceae includes the tropical plant known as cardamom (or cardamon). Various plants belonging to the genera *Elettaria* (small cardamom) and *Amomum* (big cardamom) are referred to as spices. The world's two largest producers of cardamom are Guatemala and India. India produces both varieties of cardamom, while Guatemala only produces small cardamom. Nepal, Sri Lanka, Indonesia, Tanzania, Bhutan, Laos, and Malaysia are additional producing nations. *Aframomum corrorima*, often known as Ethiopian or false cardamom, is a plant that grows in Ethiopia. Its seeds are dark brown or black (https://www.cbi.eu/market-information/spices-herbs/cardamom, Accessed on 20 March 2023). Only saffron and vanilla are more expensive than cardamom as a global spice. It is primarily utilized in Middle Eastern and Indian cuisines and health items.

The European market potential for cardamom is reported (https://www.cbi.eu/market-information/spices-herbs/cardamom, Accessed on 20 March 2023). The demand for cardamom in Europe is rising. For exporters of cardamom from developing countries, Germany, the United Kingdom, the Netherlands, Sweden, France, and Finland could be regarded as the most attractive European markets. These countries combine high import rates, established markets for spices and herbs, and cardamom use for expanding ethnic markets or conventional recipes. Sustainable spice trends influence the European market, and organic certification is a rapidly expanding market segment. The high-end market is also influenced by the interest in single origin (https://www.cbi.eu/market-information/spices-herbs/cardamom, Accessed on 20 March 2023).

1.4 Cardamom (*Elettaria cardamomum*) in the International Scientific Literature

Coriandrum sativum highly attracts international research. As a result, hundreds of contributions were published on cardamom (*Elettaria cardamomum*). For example, a search with the keyword "*Cardamom* and *Elettaria cardamomum*" in PubMed (March 2023) resulted in 231 published contributions to *Elettaria cardamomum* production, cultivation, and the bioactivity of phyto-extracts, essential oil, fatty acids, active compounds, and industrial uses.

This section gives a current analysis of the cardamom (*Elettaria cardamomum*) in the literature. On 20 March 2023, searches were conducted using the Scopus database to retrieve cardamom (*Elettaria cardamomum*) publications. The search string: "cardamom" OR "*Elettaria cardamomum*" used to retrieve bibliometric information from the online Scopus database (https://www.scopus.com/home.uri, accessed on 20 March 2023) and bibliographic data, i.e., publication count, publication year, countries of origin, document type, and journals, were recorded. A careful search of "cardamom" or "*Elettaria cardamomum*" in Scopus (www.scopus.com) exhibited that the number of documents published is exceptionally high (*approx.* 630 till March 2023). Of the published contributions, *ca.* 560 were research contributions, 23 review contributions, 20 book chapters, and 12 conference articles. The contributions count on *Elettaria cardamomum* from 2001 to 2021 are presented in Fig. 1.1. The contributions annually published on *Elettaria cardamomum* have increased from 3 articles in 2001 to 44 in 2021. Between 2001 and 2021, Fig. 1.2 represents the distribution of document types on *Elettaria cardamomum*, which includes research contributions (497), book chapters (19), review articles (17), and conference papers (9). The contributions are related to the subject fields (Fig. 1.3) of Agricultural and Biological Sciences (298 contributions), Biochemistry, Genetics, and Molecular Biology (106 contributions), Pharmacology, Toxicology, and Pharmaceutics (83 contributions), Environmental Science (67 contributions),

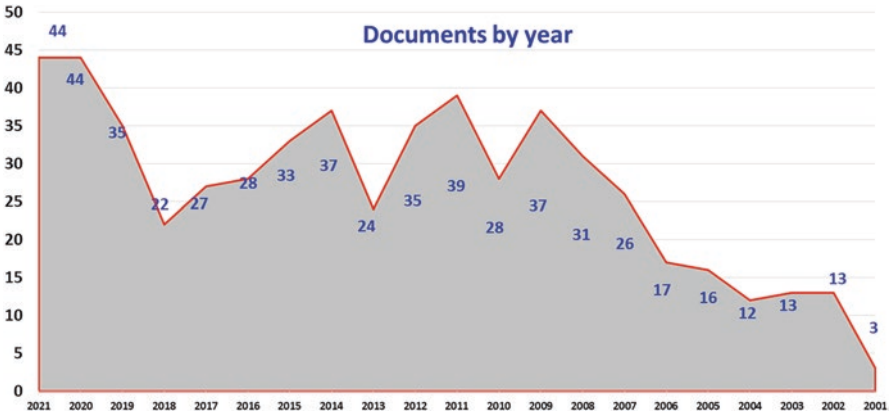

Fig. 1.1 Scholarly output on cardamom from 2001 to 2021. (www.scopus.com)

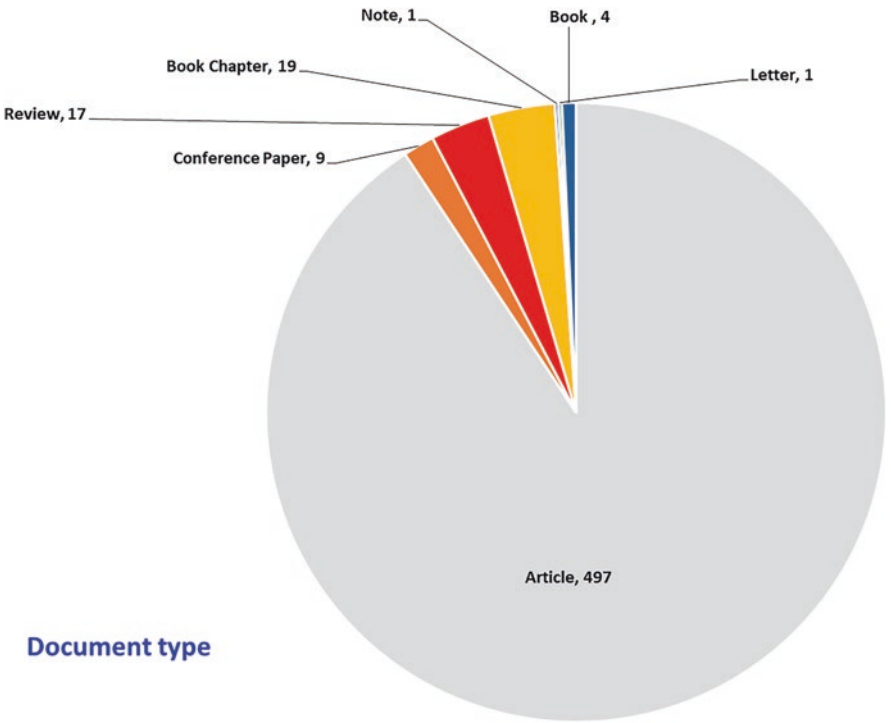

Fig. 1.2 Distribution by types of document on cardamom from 2001 to 2021. (www.scopus.com)

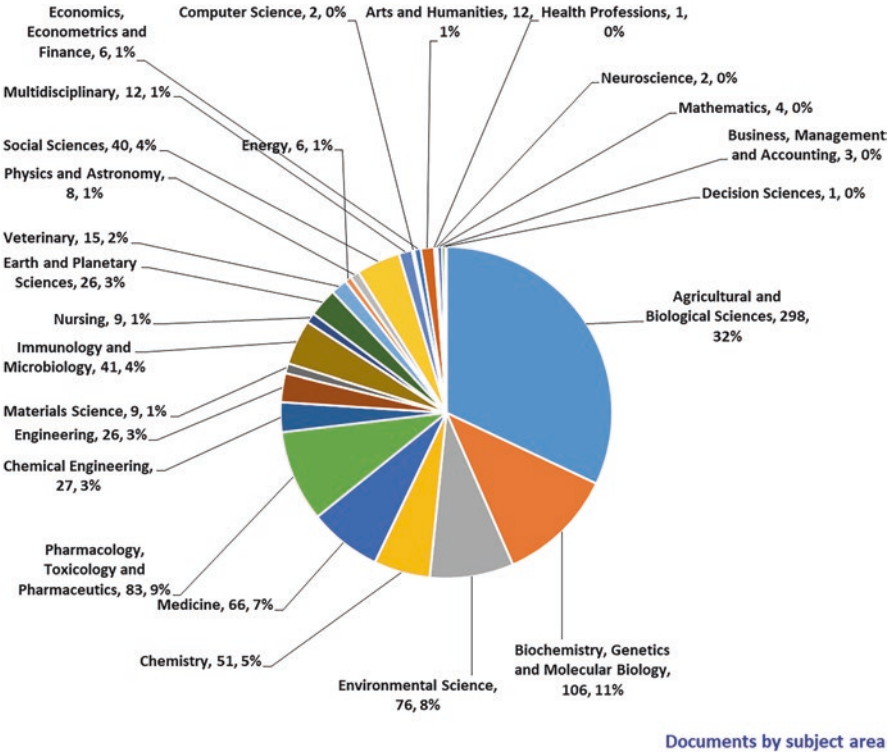

Fig. 1.3 Distribution by subject area of documents on cardamom from 2001 to 2021. (www.scopus.com)

Medicine (66 contributions), Chemistry (51 contributions), Immunology and Microbiology (41 contributions), and Social Sciences (40 contributions).

Scientists from India (286), the USA (47), Iran (30), the UK (24), Japan (18), and Saudi Arabia (18) emerged as major authors (Fig. 1.4). Scientific journals with the highest numbers of contributions were Journal of Food Science And Technology (12), Current Science (8), Asian Agri History (6), International Journal of Pharma And BioScience (6), Zootaxa (6), Ecology Environment And Conservation (5), European Journal of Plant Pathology (5), Journal of Essential Oil Bearing Plants (5), Journal of Ethnopharmacology (5), Journal Of Food Science (5), Phytotherapy Research (5), Agroforestry Systems (4), Journal of Plantation Crops (4), Natural Product Communications (4), Raffles Bulletin of Zoology (4), Tropical Ecology (4), Annals Of Botany (4), and Applied Biochemistry And Microbiology (3).

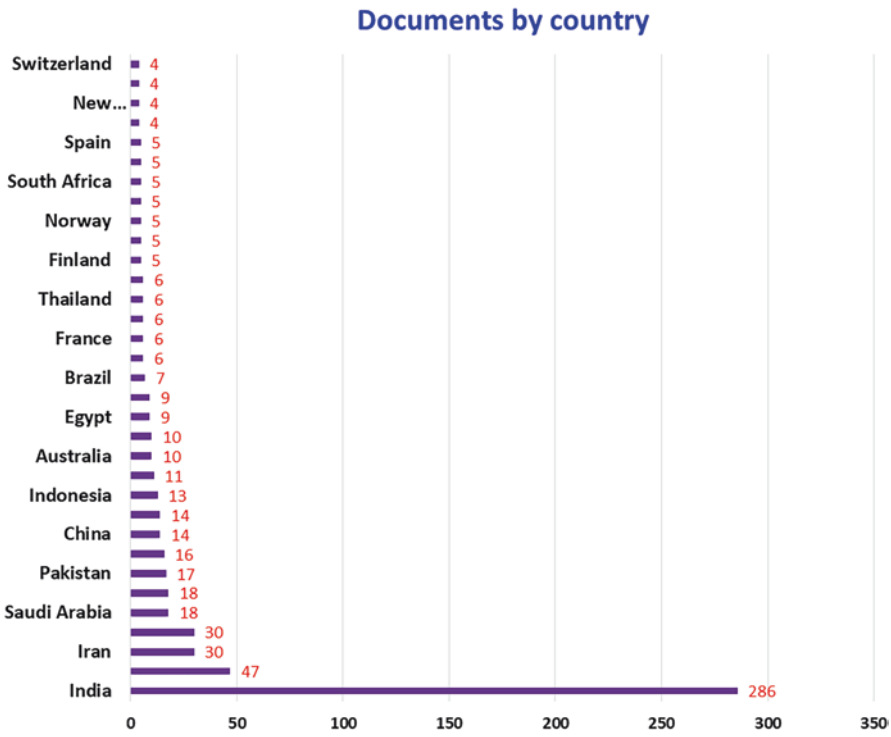

Fig. 1.4 Distribution by country of documents on cardamom from 2001 to 2021. (www.scopus.com)

1.5 Aims and Features of the Book

Cardamom (Elettaria cardamomum): Production, Processing, and Properties focuses on the botany, cultivation, horticultural practices, ethnobotany, post-harvest, phytochemistry, extraction methods, biochemistry, functionality, nutritional value, health-enhancing traits, ethnomedical uses, and processing of cardamom with the goal of creating a multidisciplinary forum for discussion. The distribution of cardamom in the botanical world, its phytochemical components, food uses, and biological traits are all covered in the book. The book also covers cardamom's possible uses in nutrition, food, cosmetics, and pharmaceutical items.

The tentative manuscript submission has various developments in nutrition, food sciences, chemistry, and horticultural researches. Organized into three sections, *Cardamom (Elettaria cardamomum): Production, Processing, and Properties* includes comprehensive chapters under main sections, namely

Part I. Cardamom: Cultivation, species, and cultivars
Part II. Cardamom: Chemistry, functionality and health-promoting properties
Part III. Cardamom: Technology, processing, and applications

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Part I
Cardamom: Cultivation, Species, and Cultivars

Chapter 2
Cardamom (*Elettaria cardamomum*): Production and Postharvest Treatments

Walid Nosir and Mohamed Abdelkader

2.1 Introduction

Cardamom is one of the oldest spices in the world. Cardamom refers to herbs belonging to two genera within the ginger family: *Elettaria* (little cardamom) and *Amomum* (large cardamom). Both kinds have a small seedpod with a triangular cross-section, a spindle-like structure, a papery shell, and small black seeds. Elettaria pods are light green in hue, whereas Amomum pods are bigger and dark brown.

Elletaria cardamomum is widely cultivated in mountainous regions of South India, Sri Lanka, Papua New Guinea, Tanzania, and Guatemala. It is approximately 7 mm in diameter, green, and smells slightly sweeter than its larger relative. *Amomum subulatum*, often known as Nepal cardamom, is a spice farm in the sub-Himalayan Indian states of Sikkim and West Bengal. It is usually 20–50 mm long and black/brown in appearance.

Cardamom is mainly utilized in the Middle East, where gahwa, a cardamom-coffee beverage, is widespread. In India, it is prominently featured in curries, pickles, custards, and spice blends such as garam masala, and it is also consumed as a nut and used as an aromatic and essential oil in perfumes. Cardamom is available in organic and conventional varieties and has a gentle, ginger-like sweetness. Moreover, Cardamoms are used as cooking spices, flavorings in food and drink, and medications. One of the most expensive spices, cardamom seeds, can be used whole or ground to make curry powder, pickles, sausages, cakes, and confections (Rajathi et al., 2017).

Cardamom (*Elettaria cardamomum*) is Bhutan's most valuable spice crop. Approximately 17,000 farmers in Bhutan are involved in its cultivation, earning

W. Nosir (✉) · M. Abdelkader
Department of Horticulture, Faculty of Agriculture, Zagazig University, Zagazig, Egypt
e-mail: w.nosir@agritech.college

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between $500 and $1200 per farmer. In addition, Bhutan earned $13 million in foreign exchange in 2014.

It is planted at lower altitudes, obtaining between 1700 and 2500 millimeters of precipitation annually, and prefers hill shade and moist conditions. In areas with high annual precipitation (2500–4000 millimeters), farmers cultivate the crop. The plant grows slowly throughout the dry season and can tolerate temperatures as low as 1 °C. The plant is extremely frost-sensitive.

A cardamom farmer stated, "Low-intensity snowfall is not always detrimental to production capacity, but prolonged exposure to sunlight will dry out the leaves." In Bhutan, leaf drying in huge cardamom is an urgent issue. The practice of shade management ought to make cardamom cultivation environmentally pleasant, long-lasting, and economically viable without harming the ecology (Kumar et al., 2014). The Cardamom Hill Reserves (CHR) climate has a relatively high variability of daily cycles (surface air temperature and relative humidity) compared to a low variability of yearly cycles, which made it easier for small and severe pests and diseases to occur and spread throughout the season (Murugan et al., 2022).

Multiple sources indicate that Bhutan's environment is ideally suited for its cultivation and ample opportunities for its expansion in terms of acreage and volume. In Gelephu and Tsirang, few government farms and private nurseries produce saplings from seeds. They are mainly cultivating Bharlange for production. The majority of farmers propagate seedlings asexually. This has exacerbated the cardamom industry in Bhutan due to the spread of illness. Disease incidence has exacerbated the big cardamom industry in Bhutan by limiting productive life, becoming a source of disease transmission, and consequently dampening the enthusiasm of those involved in the large cardamom value chain. We must discourage these behaviors.

The availability of high-quality, ailment-free saplings is essential to developing this business. Therefore, we must distribute two extension pamphlets to farmers while selling huge cardamom saplings. This pamphlet should provide all pertinent information about cardamom so that farmers can choose the optimal variety for their environment and other planting specifics. In addition, the government should provide them with all of Bhutan's plantation production facilities.

This chapter will overview the numerous propagation procedures, their pros and cons, equipment, supplies, and labor requirements. In addition, it will assist farmers in establishing nurseries and disease-free, high-quality plants.

2.2 Global Production

Before yields drop, cardamom plants are commercially productive for four to six years. The spaced pods along the panicle contain brown or black seeds so small that it takes four pods to fill a quarter-teaspoon, making it one of the most expensive spices in the world. In 2006, the global production of small and large cardamom was roughly 70,000 metric tons, with Guatemala and India accounting for 45% and 21%, respectively, of total production.

Guatemala solely cultivates little cardamom, although India cultivates both varieties. Guatemala and India traditionally dominated cardamom production, although Indonesia has emerged as a major player since 2003. Indonesia was the third largest producer in 2006, accounting for 18% of overall output. In 2006, Indonesia was the leading producer of large cardamom, accounting for 45% of total output. Nepal (23%), India (15%), and China (14%) accounted for the majority of the remaining production.

2.3 Markets

Europe, the Middle East, South Asia, and South East Asia are the primary markets for cardamom. From 2006 to 2008, the global demand for cardamom increased gradually from 31,448 to 37,710 metric tons. Following the 2008 global financial crisis, imports plummeted to 25,566 MT before recovering marginally to 26,944 MT in 2009. Saudi Arabia is the top importer of cardamom in the world. In Saudi Arabia, coffee consumption looks to be a significant demand driver for cardamom. In Arabia, pre-ground cardamom coffee typically contains five to ten grams of ground spice per 250 grams of coffee in retail stores. However, enormous quantities of cardamom may be utilized on exceptional occasions or to honor a guest with a generous gesture. During the three months between Ramadan (August 2011) and the Hajj (November 2011), the Muslim pilgrimage to Mecca, consumption tends to surge as one to two million Muslims enter the nation.

The majority of Saudi Arabia's imports originate in Guatemala. In 2006, Guatemala supplied 94% of Saudi Arabia's cardamom imports by volume; in 2010, this ratio dropped to 78%. India accounts for a lesser proportion of Saudi Arabia's imports, yet this proportion is growing over time. In 2006, Saudi Arabia imported 5% of its cardamom from India; by 2010, this proportion had climbed to 18%. From 2006 to 2010, Saudi Arabian import quantities ranged between 7188 and 10,300 metric tons, while import values increased from $43.7 million to $154.1 million.

India is the largest consumption market for cardamom, although Saudi Arabia is the top importer. India, unlike Saudi Arabia, produces a large amount of cardamom for internal consumption. India consumed roughly 18,100 MT of cardamom in 2009, considering international trade flows (imports and exports) and domestic output.

European Community. From 2006 to 2010, EU cardamom imports declined from 1666 to 1593 metric tons. In 2010, the leading EU importers were Germany (445 MTs), the Netherlands (431 MTs), and the United Kingdom (414 MTs). Historically, the United Kingdom has been the greatest importer since cardamom is in high demand among the country's sizable Asian population. It has also become a common ingredient in baking and dessert dishes on the European continent.

Like Saudi Arabia, the European Union buys most of its cardamom from Guatemala. In 2010, the European Union imported 1368 metric tons from Guatemala and just 225 metric tons from the rest of the world, including 159 metric tons from

India. Although EU import volumes remained relatively consistent during the period, import values soared from US$5.4 million in 2006 to US$27.7 million in 2010 due mainly to price hikes.

United States of America. The United States remains a small cardamom importer. From 2006 to 2010, the United States imports rose from 512 MT to 660 MT. Guatemala is the principal provider. In 2010, the United States bought 458 MT from Guatemala and 202 MT from everywhere else, including 177 MT from India. Like the EU, US imports rose from 512 MT to 660 MT. Guatemala is the principal provider. In 2010, the United States bought 458 MT from Guatemala and 202 MT from everywhere else, including 177 MT from India. Similar to the EU, the United States experienced significant increases in import values from 2006 to 2010, from $2 million to $13.4 million.

2.4 Suppliers

Guatemala is the world's top provider of cardamom, producing roughly 23,000 metric tons each year. Most of Guatemala's exports go to the Middle East, while only a small amount is sent to the European Union. As the market leader, Guatemalan cardamom output significantly impacts global prices. Global prices increase when Guatemalan spices'quality and/or quantity decline. In 2007, Guatemalan cardamom exports reached an all-time high of $137,2 million. In 2009, exports topped this amount, hitting $172,3 million. Regarding trade, five to six corporations are responsible for 80% of exports.

Guatemalan cardamom is *Elettaria cardamomum*, a species endemic to the Malabar coast of India. It is usually grown at an altitude of 250–1500 m with annual precipitation of 1000–3500 mm and temperatures between 10 and 35 degrees Celsius (50 and 86 degrees Fahrenheit). In contrast to India, where cardamom is grown under a forest canopy, it is grown in the open without shade and at higher altitudes where temperatures are cooler in Guatemala. Regionally, the Department of Alta Verapaz is responsible for almost 70% of Guatemala's output. India is the second-largest producer of cardamom in the world and, in 1970, accounted for around 56% of the global export market.

Guatemala surpassed India in production during the 1979–1980 season. However, India's market share has decreased due to high domestic prices, high production costs, and low yields. (Center for Agricultural Policy with Prosperity Initiative, 2009). Cardamom cultivation is labor-intensive and accounts for 60%–70% of production costs.

From 2003 to 2009, India's total cardamom production (big and little cardamom) exceeded 15,000 metric tons per year. The only exception happened in 2007–08 when total production reached 14,390 MT. Due to its high domestic demand, India imports more cardamom than it exports. India imported 4554 MTs in 2003 and 5846 MTs in 2009, whereas it exported 1714 MTs in 2003 and 3025 MTs in 2009.

The disparity between imports and exports becomes more obvious during years of poor domestic output. During the 2007–2008 low production season, India's net imports (i.e., imports minus exports) of cardamom were 7816 MT to supplement production.

2.5 Seasonality

Due to ideal climatic conditions, Guatemalan farmers harvest year-round, most occurring between September and March. This affords the nation significant benefits during the off-season. The harvest season in India extends from September to February, with the most productive months being October and November, immediately following the wet season. As a result, India's farmers typically rely on rain-fed produce during the summer monsoon season. Nepal, China, and Vietnam have a shorter harvest season from September to December.

Additionally, Gebreazgaabher (2016) noted that mature unripe capsules dried on a wire mesh bed for 05 days scored the highest oleoresin and essential oil content of dried seeds in small cardamom cultivated in Western Ethiopia. Similar mature unripe capsules dried on a wire mesh bed for five days yielded the most dried husk essential oil. On the other hand, mature unripe and mature ripe capsules that were smoke-dried for 15 days produced the lowest amounts of oleoresin and essential oil, respectively. Also, mature unripe capsules dried using smoke for 15 days produced dried husk with the lowest essential oil content.

2.6 Cardamom Propagation Methods

The reproduction of large cardamom should comprise three techniques: sexual, asexual, and micro-propagation (Fig. 2.1).

2.6.1 Sexual Method

High-yielding seeds sexually propagate large cardamom. This technology allows for the mass manufacture of seedlings. It avoids transmitting viral infections via seeds; therefore, the seedlings produced are free of viral diseases such as "Chirkey" and "Foorkey"; nonetheless, the nursery must be protected from new infections from surrounding plantations. In order to improve seed germination, Seid et al. (2019) found that soaking seeds in 80% alcohol for 30 min was the most successful method (78.1%), followed by soaking seeds in 25% acetic acid for 10 min (69.5%) at six weeks after sowing. The tap water for 12 h and the 5% sulfuric acid for 10 min

Fig. 2.1 Morphological characters or cardamom plants, leaves, flowers, capsules, and seeds

treatments resulted in significantly greater mean germination values than the control. While soaking seeds in tap water for 24 h revealed poorer mean germination values than the control treatment performance, soaking seeds in 10% sulfuric acid for 5 min, 25% nitric acid for 10 min, and 50% nitric acid for 15 minutes did not.

Advantages

- Seedlings have a longer lifespan than suckers removed from plantations.
- Seedlings generated using this technique are free of viral infections.
- Expensive in comparison to vegetative propagation.
- Varieties may not be true to type because they may be hybrids.
- Pollinated by a wild or uncultivated variety.
- This method may facilitate the transfer of fungal infections.
- Seedlings generated using this method will be more expensive than asexual propagation.

The following processes are required for sexual reproduction

2.6.1.1 Primary Nursery

Location Determination for Primary Nursery

(a) Choose a spot with appropriate sunshine, lighting and irrigation water.
(b) The location must be free of snow and frost; south-facing nurseries receive adequate light and heat.
(c) The Site must be at least 500 m from the main plantation to prevent pest and disease transmission.
(d) There should be no standing water. There should be no trees nearby, as they would impede sunlight illumination.
(e) A loamy soil that is rich in organic materials is desirable.
(f) Road access will facilitate input and output shipment and minimize expenses.

2.6.1.2 Choice Selection

(a) Large cardamom is cross-pollinated extensively. Thus, more care must be taken when selecting the shrub. The presence of wild cousins of cardamom might degrade the quality of the seeds. It will lack true varietal characteristics.
(b) Select cultivars based on their yield potential, pest and disease resistance, and altitude.
(c) Varieties with disease resistance include Zongu-Golse, Seremna, Bharlange, etc.

2.6.1.3 Plant Selection

(a) Select disease-free plants that perform well and are true to type.
(b) The chosen variety should have a proven track record of strong production potential over the past few years.
(c) Maintain high-performing plants as mother stock, as stated in the section of the varietal identification manual devoted to variety maintenance.

2.6.1.4 Capsule Collection

(a) Gather capsules from a high-yielding, well-kept plantation.
(b) Select only mature, large, and disease-free capsules.
(c) Extract it from healthy, productive-appearing spikes.
(d) Allow the bundle of capsules to warm for two to three days, so they are simple to separate.
(e) Select well-matured, larger-sized capsules from the base and middle of the spikes.
(f) Sort capsules according to size and weight. Utilize only large and heavy capsules.

Selection of Seeds

(a) Select capsules from productive, healthy bushes that are true to type.
(b) The capsules in the tip region are tiny and should not be used for propagation.
(c) Select mature capsules that contain black seeds. Separate the light seeds from the heavy seeds. Use only substantial seeds.

2.6.1.5 Plant Extraction

(a) Cardamom seeds retain their sticky mucilage coating. Therefore, removing the mucilage with sand and ash will be required.
(b) Repeat the procedure until no mucilage remains on the seeds.
(c) Rubbing the seeds with jute bags and sand is another method for extracting the seeds.
(d) Rinse the extracted seeds with clean water and allow them to air-dry for three to four days. The dried seed can be planted immediately or kept. It should be kept in a cool, dry location.
(e) The germination rate of seeds collected by this method ranges between 30% and 50%.
(f) Procedures for seed extraction according to the Cardamom Research Center, Ilam, Nepal, 2015
(g) After extraction from the capsule, the cardamom seed is mixed with one kilogram of sand and a half kilogram of ash, and vigorous leg rubbing is performed. This process must be carried out five times.
(h) The seeds are then stored in muslin fabric. Next, the pit is dug to a depth of one and a half feet, of which a half foot is filled with sand.
(i) Place the seed packet on the sand.
(j) The pack is once again coated in half-foot-level sand.
(k) Practicing this method between the latter week of January and the first week of February will yield favorable results. After one and a half months, the seed is extracted from the pit for seeding in nurseries.
(l) Germination will commence one month after planting.
(m) The germination rate was approximately 65%.
(n) The non-germinating seeds will decompose in the soil.

2.6.1.6 Acidic Removal of Mucilage

(a) According to the Indian Cardamom Research Institute in Gangtok, Sikkim, cardamom seeds are submerged for 10 min in 25% nitric acid (25 mL nitric acid and 75 mL water).
(b) The seeds are washed thoroughly under running water to remove the acid.
(c) Following this seed treatment, germination begins four to five months after sowing. Germination is dependent on the nursery's temperature and humidity.

2.6.1.7 Sterilization of Nursery Soil

(a) Soil sterilization can reduce insect infestation by covering nursery beds with clear plastic for at least a month before planting.
(b) Additionally, manure can be sterilized using the same method.
(c) The optimal time to sterilize soil is before September.

2.6.1.8 Application of Manure in a Nursery

(a) Adequate manure should be added to nursery beds that have been properly prepared.
(b) Comply with soil laboratory suggestions.
(c) Two square meters of clay soil require 20–25 kg of well-rotted compost, one kg of ash, and 10–15 kg of sand. These ingredients should be thoroughly combined.
(d) The seedbeds must be devoid of clods and weeds, as well as fine-grained.
(e) Black plastic mulching is also utilized for rapid germination.
(f) Application deadline: prior to September.

2.6.1.9 Preparation of the Crib

1. A one-meter-wide and -long nursery bed must be prepared according to the site and practicality.
2. The seedbeds must be elevated 25–30 cm above the ground.
3. There should be a minimum distance of one meter between two nursery beds.

2.6.1.10 Seed Planting

(a) Seeds can be sown immediately after extraction. b. The optimal time to sow seeds is between the last week of September and the middle of February.
(b) One gram of treated/extracted large cardamom seeds per square meter of nursery space is suggested.
(c) The seeds are spread at a depth of 2 cm with a row-to-row gap of 2 cm.
(d) The seeds are coated with a thin layer of fine-grained dirt from above.

2.6.1.11 Mulching

Mulching retains soil moisture and decreases weed pressure in the nursery by preventing evaporation.

(a) After sowing the seeds, straw or plastic sheets should be used to mulch the beds.
(b) If the straw is used as mulch, the thickness should be between 3 and 4 cm.

2.6.1.12 Germination

(a) The time required for large cardamom seed germination depends on the technique of seed treatment employed. However, it takes three to twelve months.
(b) It is reported that 30–65% of huge cardamom seeds germinate.
(c) As soon as germination begins, the mulch should be removed to enable photosynthesis in the seedlings.

2.6.1.13 Management of Shade

(a) Shade is required to protect seedlings from direct sunlight, hail, heavy rain, and snow. 50% shade should be given using shade nets or bamboo roofs. Additionally, it will protect them from insect attacks and infections.
(b) Provide distinct shade nets to different varieties of giant cardamom to preserve the integrity of the types in the same nursery and facilitate identification during transplanting.
(c) Irrigation is performed every three to four days thrice, depending on soil moisture content and rainfall.
(d) An overhead sprinkler or drip irrigation system can be installed in the nursery for labor-saving watering efficiency. Do not permit flooding at any cost.

2.6.1.14 Additional Advice for Secondary Nursery

Maintain the seedling in the secondary nursery for 10–12 months with proper care.

- Each seedling should attain 45–60 cm in height with 5–10 tillers after 10–12 months.
- The old mother plant of the primary nursery may be eliminated while the young offspring are preserved.
- Count one tiller and one healthy vegetative bud as one seedling at the time of sale.

2.6.1.15 The Packaging of Seedlings

- Seedlings are packaged in jute bags lined with sphagnum moss soaked in water.
- Seventy to one hundred seedlings can be packed in each bundle. In nurseries, 100 saplings per pack are typically utilized.
- It should be transportable with ease.

2.7 Asexual Method

Asexual propagation takes stem sections or root tissue from the parent or donor plant, treats this tissue with plant growth regulators, and encourages adventitious root or shoot production in a controlled/open environment. Large cardamom reproduces asexually through its suckers and rhizome buds (tissue culture).

Advantages of the Asexual Method of Reproduction

- They are easier and less expensive than other approaches for establishing orchards.
- It begins bearing fruit in the second year after planting.
- Superior, high-yielding mother bush-origin saplings will produce superior results.
- It is possible to create many saplings with characteristics identical to the mother plant (desired varieties).
- A three-year reduction in orchard establishment time compared to sexual approaches.

2.8 Negative Aspects of Asexual Reproduction

- Diseases will be transmitted to freshly planted orchards if the mother plant is infected.
- It will exponentially spread the disease.
- Challenging to transport.
- It is not recommended to take saplings from older mother stocks that are fewer than 15 years old, as the loss of vigor can render new orchards sensitive to pests and weaker overall.
- Demand a substantial application of manure.
- Select only healthy, high-performing bushes.
- The cleanliness of the mother plant is crucial for asexual replication.
- It is predicted that the incidence of cardamom fungal and viral illnesses will be high during asexual propagation. Before and after usage, the knife and cutting device must be sterilized with hot water. Utilize equipment when they have cooled.

Preservation of parent stock or a variety of repository

The Repository is the best quality mother stock of Large Cardamom. The storehouse of varieties is guarded with great care within a net. The planting components (suckers, rhizomes, roots, and one plant) are extracted from this mother stock.

To obtain high-quality, disease-free planting material for purposes of propagation.

Facilities necessary for the scientific repository

1. Tunnel with a 50% shading net
2. Irrigation systems (drip or sprinkler irrigation)
3. Appropriate application of manure based on soil test results
4. The optimal location for the repository will be 700 m above sea level
5. Frequent disease test

How do we create the repository from the ground up?

- Select disease-free, high-yielding, and true-to-type varieties from the field • Test for illnesses and genetic abnormalities in the laboratory

Maintain the chosen varieties in the repository.

- Maintain high-yielding, type-true mother stocks of many kinds.
- They must continue to get intensive care and management.
- Each variety should be kept in its cabin.
- They must be primarily confined beneath agro-shade netting.
- Spray fungicides such as blitox 50 at a rate of 2 mL/L water every 15 days.
- Manure should be applied at 10 kg per bush twice or thrice yearly.
- Shower irrigation should be performed as needed.
- Removal of dead, older leaves must be done.
- A restricted number of individuals should be permitted inside, but only after donning an apron.
- These bushes' plant parts should be used for propagation.

2.9 Propagation Through Suckers

Large cardamom is most easily propagated through direct separation from its mother plant on the plantation. Therefore, it is commonly utilized by farmers since it is less expensive than other propagation techniques. However, this approach has a considerable risk of transmitting viral and fungal diseases.

Establishment of a rootstock propagation nursery

Selection of plant materials for establishing a nursery

- Select a high-yielding, disease-free plantation of the desired variety.
- The plantation should have a three-year track record of high yields (depending on the variety).

- For planting in the sucker multiplication nursery, a healthy, matured tiller with two immature tillers or vegetative buds exhibiting the desirable features of the selected variety should be chosen.

2.10 Location of a Sucker Propagation Nursery

- The distance between the nursery and the main plantation should be at least 500 m to prevent pest and disease transmission in the nursery.
- The nursery should be kept in the shade of a forest or the shade pandals with 50% shade utilizing agro shade nets.

2.11 The Construction of Trenches

- Trenches of 30 cm in width and 30 cm in depth are dug across the slope of the field at length appropriate for the terrain. Maintain a 30 cm separation between trenches.
- The trenches should be filled with well-composted cattle dung or compost and properly mixed with the topsoil.

2.12 Introduction of Suckers

- Plant the suckers with a mature tiller and two immature tillers or buds 30 cm apart in the trenches.
- The optimal season for planting is between May and June.
- Following planting, the plant base is mulched with dried forest leaves; • the annual multiplication rate is approximately 1:8.

Managing and maintaining the nursery

- Consistently monitor the sucker multiplication nursery and remove any old, off-type, or unhealthy plants.
- Mulch the nursery with dry grasses and leaves.
- Weeding is performed intermittently based on weed pressure.
- Irrigation should be performed throughout the dry season, often between November and March, depending on the moisture content of the soil.
- Irrigate the bush prior to uprooting and selling saplings.
- Trim a third of the apical and root portions to reduce transpiration and facilitate transport.
- Limit the length of the pseudo stem to one foot to minimize bending during handling.

2.13 Packaging of Plants for Shipment

- Count one plant with one bud, rhizome, and roots when packing saplings.
- At the time of sowing, farmers can separate them from the entire bundle.
- Treat them with a copper-oxychloride solution at a concentration of 2 cc per liter of water.
- The uprooted plants should be placed in a jute bag with sphagnum moss.

Apply water on the bundle intermittently.

The covering of the sapling with jute bags and sphagnum moss will help to conserve water.

2.14 Tissue Culture

Tissue culture is the *in vitro* cultivation of plant or animal cells, tissue, or organs on a nutritional medium under aseptic conditions, typically in a glass container. Sometimes, tissue culture is referred to as sterile or in vitro. This method allows it to retain living cells outside an organism's body for an extended duration.

Plant tissue culture is a collection of procedures to maintain and develop plant cells, tissues, or organs in sterile circumstances on a nutrient-rich culture medium with a specified chemical composition.

2.14.1 Importance of plant tissue culture in large cardamom

The propagation process enables the year-round generation of many disease-free, high-quality plantlets. Plant tissue culture has numerous applications, including mass propagation of plants through micro-propagation or *in vitro* culture; generation of high-quality planting material; production of phytochemicals and high-value pharmaceutical cosmetics and food additives; and generation of phytochemicals (CSIR 2016). In addition, the apical growing portion or meristematic tissue is regarded as virus-free. Its rapid proliferation in aseptic laboratory conditions is practiced. However, it must be screened for numerous illnesses prior to cultivation.

Thus, this technology can be utilized in large-scale cardamom propagation to generate disease-free seedlings with the appropriate traits rapidly. Therefore, this method can be utilized in large-scale cardamom propagation to generate many disease-free seedlings with the appropriate characters.

2.14.1.1 Instruments and Nutrition Culture Medium Preparation

Apparatus Necessary

1. Pliers
2. Scissors
3. Blade holders
4. Disposable but sterile surgical instruments
5. Petri dishes with two paper filter tips
6. Pipette 1000 mL
7. 50 mL test tube
8. 500 mL beakers
9. One thousand-milliliter container with crew caps
10. Three 350 mL early mire flasks containing blades or pointed carpels.
11. A package that contains disposable pipettes.
12. Pipettes

These devices require a thorough cleaning. After thoroughly cleaning, the items are wrapped in newspaper and taken to an autoclave.

All culture vessels must be washed before use in tissue culture. Then, they are sterilized. These are sterilized for one hour in an autoclave.

Glass and plastic containers used for culture must be thoroughly cleaned and autoclaved for one hour. They are then immersed for 16 h in chromic acid.

Then, glass and plastic utensils are rinsed with water to eliminate chromic acids, soaked in detergent water, and cleaned using the hard deluge method. The glass and plastic items are after that submerged in clean water. The detergents are subsequently removed by rinsing.

Finally, the glass and plastic utensils are rinsed under running water, placed on clean trays, and dried in an oven between 60 and 80 °C. The dried glassware can now be utilized.

2.14.1.2 Decontamination of Culture Media

The next crucial step is creating the culture media used in the micro-propagation procedure. A medium for plant tissue culture will contain various macro and micro-elements, including vitamins, amino acids, and, most significantly, sucrose, which acts as a supply of carbon, the plant's primary source of glucose. Next, the salts must be carefully weighed and dissolved in deionized water following the supplied relational data. Precautions must be made to prevent the precipitation of salts, as this reduces their availability to plants. This is because the salt with the highest solubility is dissolved first.

Generally, plants can absorb nutrients with a pH range of 5.6–5.8. Once the medium has reached the correct pH, it is ready to be poured into a 250 mL early mire flask, 50 mL test tube, 100 mL flask, or 20 mL flask. A semi-solid media

requires preparation. The procedure calls for 0.75 to 0.8 grams of agar-agar. This quantity must be regulated to supply the culture with optimal hydration.

Pouring medium, 100 mL of medium is poured carefully into each 250 mL early mire flask and plugged with cotton plugs made of nonabsorbent cotton wool coated with muslin cloth and fastened at the top to enable easy gripping of the plug during inoculation. The next step is autoclaving the culture media to sterilize it. An autoclave is a device used to sterilize equipment and supplies by exposing them to saturated steam under high pressure. After preparing the media and preparing the various equipment for use, they are autoclaved for 20 min at high temperature, i.e., 120 °C, and pressure of 15 Pascals.

These parameters are used to sterilize devices and media without altering their chemical composition, such as sucrose. As explants, meristematic tissue from such a location can be harvested. Different types of explants, such as leaves, petals, buds, ovaries, seeds, anthers, and nodule segments, can be employed for plant tissue culture because each plant cell can produce a new individual. However, care must be taken to obtain these explants from actively growing, disease-free plants. Having some meristematic regions with high cell activity is advantageous. Immediately upon collection, explants are placed in clean water to prevent the entry of air bubbles, bacteria, and pollutants from the cut or exposed portions and to prevent browning due to phenolic oxidation. These explants are subsequently transported to the lab for surface sterilization. Explants are chopped with scissors into smaller pieces and then placed in a Petri dish containing clean water.

Several hair brushes are then used to clean the surface of the explant with mild detergents, such as twin 20, as a wetting agent. After washing the surface, the explants are picked up and placed in a plastic container with a light fungicide or antibiotic solution. The explants are then swollen for a few minutes and rinsed with sterile deionized water multiple times.

Explants are soaked in sterile, deionized water and placed in an inoculation chamber.

2.14.1.3 Inoculation of Plant Tissues

Now, our explants are prepared for the subsequent inoculation procedure. Before entering the inoculation chamber through the double doors, it is essential to don a clean cotton lab coat and take an air shower. Inoculation involves the transfer of plant specimens into media under aseptic circumstances.

2.14.1.4 Making Ready the Laminar Hood

The laminar hood is specialized in inoculation equipment. By closing the laminar hood's shutter, ultraviolet light can destroy all bacteria. Turn on the UV light for approximately 40 min. It is highly hazardous to the eyes and skin of humans. Therefore, the shutter is covered with a black sheet and will not open until the UV

light has been turned off. After 30 min, turn off the UV light and turn on the chamber light. After opening the shutter, activate the airflow. Prior to operating the laminar hood, it is necessary to wear a clean and sterile face mask and head covering. In addition, the panel of the laminar facing us is equipped with an efficient particle air filter.

The explants are treated with the mild mercuric chlorides solution for a few minutes after being rinsed with 70% ethanol for a few seconds. A basic rule of thumb is to employ a mild solution for a longer duration instead of a powerful solution for a shorter length. Inoculation of plant cuttings. After being treated with the sterilizing agent, the solution is decanted into a waste beaker.

The explants are then rinsed multiple times with sterile de-ionized water to eliminate traces of mercuric chloride. All the apparatus is then dipped in the 70% ethanol, flamed with the spirit lamp's light and allowed to cool. After the explants have cooled, they are gently plucked with forceps and placed in Petri dishes lined with filter paper to absorb any extra water.

Using a surgical blade, the surface of the explants exposed to mercuric chloride is removed. Next, each nodule segment is carefully inserted into the medium and enclosed in the test tube, and care is given to prevent the explant from 20 from touching the lip of the flask, which is again flamed, and then the cap is replaced over the mouth of the flask to seal it snugly. Finally, the surface of the test tube is labeled with the name of the plant, the medium, and the date of inoculation.

2.14.1.5 Incubation for Development

The cultures are now prepared for incubation in the culture lab under optimal circumstances of 16 h of light followed by 8 h of darkness. The temperature is between 25.5 and 27.0 degrees Celsius, and the relative humidity is 40%, and it is monitored promptly. After a few days, between 5 and 15 days, all cultures with bacterial or fungal contamination are eliminated, and the healthy cultures are permitted to continue growing. After a period, many shoots from a single sample are immersed in a single flask. Once the shoots have reached a height of approximately 3–4 cm, they are rooted. Generally, auxins encourage roots in each shoot by adding them to the culture medium. Once the shoots have established roots, they must be toughened for acclimatization in the open air.

Acclimatization and Repotting of Seedlings

Tissue-cultured raised plantlets (TCPs) are now transported to a greenhouse or outdoor environment. As a result, they are exposed to many shocks, including temperature, humidity, nutrition, carbon dioxide, and airflow. The humidity levels in the greenhouse and fields are significantly low. Higher light intensities and septic surroundings are stressful to the TCPs because they have beyond their in vitro comfort zone.

After the plantlets have rooted and reached 3–4 inches, transfer them to a different potting mixture, including garden soil, sand, and well-21 decomposed farmyard

manures in a ratio of 1:1:1. Other mediums, such as soil rite, vermiculite, perlite, and coco pit, may also be utilized to prepare the mixture. After 45–60 days, these acclimatized seedlings can be transferred to the outdoor environment. These plants are now effectively prepared for extreme environments with a very low mortality rate.

2.14.1.6 Protocol for the Culture of Large Cardamom Tissue

In vitro micro-propagation is one of the most effective alternative replication techniques for quick clonal mass propagation of healthy, high-yielding plants with minimal illness. MS supplemented with 1.0 mg/L BAP and 1.0 mg/L IBA is the optimal medium for root and shoot induction. This methodology is effective for large-scale cardamom production and multiplication.

Consolidation of Tissue-Cultured Plants

(a) Due to the fragility of newly emerging plants, they are put at an ambient temperature outside the incubation chamber for 24–30 h.
(b) Once these plantlets have reached maturity at room temperature, they should be transferred to a glasshouse or green net house where the insects (Aphid) cannot enter.
(c) The culture medium shall consist of sand, vermin-compost, and forest soil in a ratio of 2:0.5:1.
(d) The formalin 1% solution is then utilized to sterilize this medium. (39% Formaldehyde is commercially available).
(e) The individual who will care for the seedlings must wear sterilized aprons.
(f) There should be lime water at the entrance to the shade house, net house, or glass house.
(g) The individual caring for the seedlings must not smoke.
(h) For insect control in organic sapling development, 3–5 mL of Nibicidin or Marglobe per liter of water is utilized. It is a pesticide containing neem.
(i) For organic agriculture, a month of solarization in white plastic will eliminate infections.
(j) The seedlings are planted in benches separated by 20 × 10 cm.
(k) The media should be fertilized with 200 kg of nitrogen, 200 kg of phosphorus, and 100 kg of potash per hectare.
(l) A 0.5 kg of micronutrient mixture should also be added per hectare. The plantlets and media should be sprayed with a 0.02% bavistin solution regularly.
(m) They are covered with white plastic for four to six days to increase humidity, but excessive heat will kill the seedlings. Consequently, care should be taken.
(n) The water used to irrigate plantlets should be treated with an ultraviolet rays filter to sterilize the water.
(o) The insects can be eliminated by using electric-netted bats to kill them.

2.15 The Sale of Seedlings for Planting

The seedlings are wrapped in sphagnum moss for transit to the planting field and secured in jute bags. It should have eight to nine leaves and a one-foot height. In Sikkim, Pseudomonas fluorescens is frequently employed as a bioagent for disease control in big cardamom. For large-scale cardamom farming, the Indian Cardamom Research Institute, Regional Station, Spices Board, Tadong, Gangtok, and Sikkim developed the mass multiplication technique and field application schedules.

The bio-agents suppress pathogenic fungi by their properties such as rapid growth, rapid multiplication, antagonism to disease-causing organisms, hyperparasitism, competition with other microorganisms, etc., suppress pathogenic fungi, promote plant growth, and protect large cardamom plants from numerous soil-borne fungal diseases. Then, these plants are transported to the fields for planting.

2.16 Nursery Pest and Disease Management

2.16.1 Insects

White grub, thrips, aphids, and sand stem borer are some of the most prevalent insect pests of a big cardamom nursery, but stem borer insects are not significant in a nursery environment.

Biology of Thrips

The mature female lays eggs on the surface of the leaf or fruit. Before hatching, the egg develops blisters. Then, the banana-shaped, white eggs are placed individually into plant tissue. Early-stage larvae are pale and have red eyes. After feeding, larvae become yellowish-colored. On average, mature larvae are around 1 mm in length. A metamorphosis follows two larval instars into the pre-pupal stage: pale yellow with red eyes and small wing pads.

Pupae are slightly larger than larvae, with longer wing pads and larger eyes. It begins as yellow and gradually darkens as it ages. In the pupal stage, the antennae are bent backward over the head. Prepupal and pupal stages do not consume food.

Adult:

- As the greenhouse thrips evolves into an adult, its head and thorax darken to black, and its abdomen changes from yellow to yellow-red to brown to black.
- Cool temperatures impede the color changes.
- The legs stay a pale yellow, and the antenna has eight segments.

The greenhouse thrips are parthenogenic, reproducing without mating, and males are rarely observed.

- It is a poor flier and almost always resides in the shaded portions of the plant.

Damage Symptoms

- This pest feeds largely on attractive plant foliage.
- Initially, it attacks the bottom surface, then as feeding continues and the population grows, it migrates to the higher surface.

The leaves become discolored and deform between the lateral veins; severely injured leaves turn yellow.

Aphid Thrips-Biology

Eggs are kidney-shaped and placed singly in the delicate portions of the leaf sheath, known as racemes.

Nymph: Nymphs are little, slender, fragile, and straw-colored.

Adult Aphids are a tiny, dark greyish brown, between 1.25 and 1.5 mm long, and have fringed wings.

Symptoms of damage

- Panicles become stunted.
- Flowers and immature capsules fall off, limiting the number of capsules produced.
- Infestation causes the creation of corky encrustations on the capsule, resulting in its deformed and shriveled state.
- Such pods lack their characteristic perfume, and their seeds are underdeveloped.

Natural Enemies of the Aphid

Parasitoids: *Aphidius colemani*, *Aphelinus* spp.

Lacewing, ladybird beetle, spider, and syrphid larvae are predators.

Stem Borer

Due to the development of huge cardamom stems in the natural environment, the infestation is a severe problem in the fields. In the nursery, the stem's development is not as advanced. It is advised to rough them up in the event of an infestation. The net prohibits entry from the outside.

White Grub

Adult beetles emerge between March and April and deposit eggs in the soil. The eggs are delicate, ellipsoid, and off-white, measuring approximately 1 mm along their longest axis.

Grubs: When mature, the larvae are typically around 38 mm long, pale or cream-colored, and fatty. The newly hatched grubs appear between June and August and continue to mature through October and November.

During this time, feeding grubs are often found in the top 6 inches of soil; however, they may move deeper if the soil is extremely dry. The larvae consume soil organic materials and plant roots. The adults are typical chafer beetles, primarily brown and 19–20 mm long.

Damage Symptoms
Affected plants exhibit foliage yellowing, leaf burning, defoliation, and death. In addition, a root system inspection will reveal that the roots have been gnawed off, leaving behind calloused stumps. H. disparilis also feed on plants at the soil level, producing stem damage followed by plant death.

Natural Predators of the White Grub
Nematode: *Heterorabditis* nematode.

- White grub can be managed by applying 1 g of Metarhizium per square meter.
- Damping off seedlings can be controlled by applying 0.5 mL/L of a mild carbendazim solution.
- The most damaging insects to seedlings should be eliminated or eradicated (Chloropiriphus 2 gram).
- Neem-based organic pesticides such as nimbicidine and marglobe can be administered to seedlings at a concentration of 3 mL/L of water.
- In the case of viral illnesses, the affected plant must be mutilated and discarded.

Lepidoptera-Biology
Egg: 300–800 eggs are placed on the underside of shade tree leaves.

The egg period ranges from 13 to 20 days.

Larvae are hairy and have a dark-gray body and a light-brown head. Larva traverses ten instars in 5 months.

Pupa: Pupate is buried in the dirt at 2–5 cm; the pupa is encased; the pupal duration is 7–8 months.

The adult is a big, ochre-colored moth measuring 70–80 mm with post-medial lines on the wings.

Damage Symptoms
- The caterpillars collect on the trunks of shade trees and then descend to the cardamom plants
- They devour the leaves of the cardamom plants, quickly defoliating them.

Figure 10: Adult Fig. 11: Cardamom caterpillar DISEASES

Common illnesses include Damping off, Chirkey and Foorkey, Blight, Wilt, and Leaf streak.

Chirkey Disease Manifestations
- The symptom is most noticeable on young, developing leaves, where distinct pale green to yellow longitudinal stripes run parallel.

A mosaic-like pattern of pale streaks can be observed on the delicate leaves.

- The growth, flowering, and yield of infected plants fall gradually, and they ultimately perish. Fig. 12: Symptom of Chirkey

Virus and Transmission

- The insect vector transmits the pathogen.
- It can also spread by the planting of infected suckers.
- This disease is transmitted via the movement of infected suckers from one location to another.
- The illness can also be transmitted mechanically by harvesting knives.

Foorkey Disease Manifestations

- Numerous little tillers grow at the base of infected plants, which become stunted and produce no harvest.
- The size of the leaves decreases; they become faintly curled and pale green in hue. Occasionally, slightly enlarged leaves resembling betel leaves are also observed.
- The inflorescence is stunted and produces no flowers or fruits.

Virus and Transmission

- The illness is caused by a virus and is spread mechanically through sap by insect vectors such as the black banana aphid, Pentalonia nigronervosa, and Micromyzus kalimpongensis.

Regulatory Measures

Due to Foorkey being a viral disease, infected plants cannot be treated entirely; nevertheless, losses can be avoided by implementing the following management practices:

- Monitor the plantation often, especially during the wet season, and carefully identify unhealthy plants.

Foorkey Illness

as soon as symptoms show, remove and destroy diseased plants (uproot and destroy).

- Infected plants could be uprooted en masse and burned at the village/community level to eradicate the disease. Utilize seedlings from approved nurseries. Never harvest plant material from an infected plantation or plants that appear healthy from badly contaminated areas.
- Sucker propagation is only recommended through certified multiplication nurseries.
- Establish nurseries around 500 m apart from the primary plantation to prevent aphid invasion
- Preserve clean clumps by removing old tillers with loosened leaf sheaths to prevent aphid colonization.
- The use of clean field equipment is strongly advised.

Reducing Disease Symptoms

Seedlings are contaminated with black spots around the soil and the base of the seedling.

Causal Organisms
Numerous types of fungi, including *Rhizoctonia solani, Fusarium* sp., *Pthium* sp., *Phytopthora* sp., etc.

Control Measures
- Special care must be taken in cardamom nurseries to eradicate this illness.
- Blitox 50 at 2 mL/L of water can save seedlings

After irrigation, excess water must be drained. Then, remove the infected seedlings by hand.

Visit and inspect the nursery regularly to assess any damage.

Grades and Standards Cardamom is rated based on color, clipping (i.e., pods with the tops removed), size, bleached or unbleached status, the amount of foreign matter present, and product provenance. If applicable, grading is performed in line with a pertinent national standard, such as those utilized by Indian producers. In addition, ISO standard 882-1 gives general recommendations for cardamom1 grading, handling, and packaging.

In 1969, the American Spice Trade Association (ASTA) adopted the original Cleanliness Specifications for spices, seeds, and herbs in the US market. These have been changed several times, most recently in 2007. ASTA Cleanliness Specifications are intended to meet or surpass FDA Defect Action Levels (DAL). The DAL refers to Title 21, Part 110.110 of the Code of Federal Regulations, which permits the Food and Drug Administration (FDA) to define maximum levels of natural or unavoidable faults in foods for human consumption that pose no health risk.

Common Grades Generally, the weight in grams per liter and color are the defining factors for quality. In addition to color (green or yellow) and drying process, the proportion of open fruit pods (also known as "open pods") impacts quality (mechanical or sun).

The definitions listed below pertain to popular Indian Grades:

Bold is a typical export grade in which 90% or more of the cardamom pods have a diameter of at least 6.5 mm. The product has a mature green hue and a gram-per-liter weight of 415 grams.

Super Bold is a high-quality cultivar in which all pods must have a diameter of at least 8 mm. The product has a mature green hue and weighs more than 450 grams per liter.

Extra Bold is a popular export grade in which all pods have a diameter of at least 7 mm. The product has a mature green hue and a gram-per-liter weight of 435 grams.

The cardamom in bulk has not been graded. As such, it contains mature and immature capsules of all sizes, along with black, yellow, and/or split cardamom.

Small is a grade with pods ranging in diameter from 5.5 mm to 6.5 mm. There are around 385 grams per liter.

Open/Splits cardamom is of lower quality, as more than 60% of the pods are "open" (seeds exposed), and the pods may be greenish or pale yellow. All ripe pods will have a diameter of at least 6.5 mm.

Seeds are the dark brown/black seeds of cardamom pods (i.e., husk entirely removed). Typically, the weight per liter is between 550 and 600 grams.

Fruits are often overripe pods with a faint yellow hue. More than 425 grams per liter are present per liter.

The following descriptions pertain to typical Guatemalan Grades:

- Jumbo Green is an extra-large variety of green cardamom pods.
- Imperial Best Green and Fancy Green Extra are two varieties of green pods.
- Fancy Green are green pods of medium size.
- The Imperial Mixed Green pods are big and pale green.
- Mixed Green are pods of various hues.
- Mixed Green Split are medium large open green pods.
- Yellow Mixed pods are medium to big and closed
- MYQ, or Mixed Yellow Quality, is a medium-sized, light-brown ground cardamom.

Seeds are cardamom without its husk.

Packaging

Due to its high value, cardamom is typically packaged in double-layered bags (42 kg to 50 kg) and rarely delivered in boxes without bags. Polybags are increasingly used to line single-ply cloth bags. As color is a significant determinant of pricing, black polybag liners are employed to shield the greener, higher-quality grades from light.

Premium grades from Guatemala are packaged in protective bags and transported in 5 kg cartons, with eight of these comprising a master carton. In addition, cardamom husks are occasionally transported in compacted bales weighing up to 300 kg or lose bags.

Harvesting. When fruits are fully matured yet still green, they are harvested. It is essential for quality that the white seeds within the green pods have become brown or black. Due to the uneven nature of fruit ripening, individual fruits are harvested at the optimal stage of ripeness. Fruits are also harvested with care to prevent bruising and damage. In India, two forms of harvesting are utilized: light and hard. In light harvesting, only fully mature pods are collected, but in hard harvesting, semi-mature pods are also removed.

Postharvest Handling The postharvest procedures include washing, curing (drying), cleaning, polishing, sorting, grading, and packing. Occasionally, pods are treated with 2% washing soda (sodium carbonate) for 10 min after harvesting to stabilize chlorophyll and impart a more vibrant green hue. Drying cardamom capsules as soon as possible after harvest is essential to minimize flavor loss. It is also essential that the drying process be as brief as possible to prevent mold growth on the capsules and preserve their vibrant green color.

There are numerous methods for reducing the fruit's moisture content from approximately 75% at harvest to 13% for safe storage. Sun-drying, solar-drying, wood-fired drying, electric/gas drying, and humidity-controlled drying are

examples of drying methods. In sun-drying, cardamom pods are spread out on a concrete floor to dry using the sun's natural heat, while in solar drying, they are placed in a particular dryer away from direct sunlight. The traditional drying process in India is wood-fired drying, which often takes a substantial amount of firewood. The smoke from the fire can impart an unpleasant smoked flavor to the pods and cause some of the cardamom to burn. Therefore, cardamom pods dried using this technique are not of the greatest grade.

Globally, the cardamom import industry has not yet recovered from the global economic crisis of 2008. In 2008, global imports totaled 37,700 MT, while in 2010, they decreased to 26,900 MT. Based on these numbers, the cardamom export market has space for expansion. Since 2008, global imports have been expanding, but at a relatively moderate rate. Although much will depend on the global economy, this import trend should continue and possibly reach 30,000 MT per year. The consumption of cardamom in India and Saudi Arabia corresponds closely with changes in income. Early in 2011, sources said Middle Eastern purchasers withdrew from the market due to political upheaval in the region; however, they have since returned and progressively resumed supplying the market.

According to recent pricing patterns, prices should remain constant and at their current level. This results from a good crop in Guatemala in 2011 and a relatively stable economic climate in Saudi Arabia. However, a disappointing harvest in India in 2011 due to fungal disease outbreaks could increase prices shortly, as India would need to import more to meet domestic demand. Besides economic factors, climatic circumstances must also be considered. Because the cardamom plant requires a continuous period of rain alternated with periods of good sunlight, production suffered. In 2010, La Nia delivered Guatemala above-average rainfall, boosting agricultural output.

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Chapter 3
Cardamom Botany, Cultivars, and Genetic Diversity

Martha Ikechukwu, Alfred Maroyi, and Ahmad Cheikhyoussef

Abbreviations

DNA	Deoxyribonucleic acid
GD	Genetic diversity
ISSR	Inter simple sequence repeat
ITS	Internal transcribed spacer
POWO	Plants of the World Online
RNA	Ribonucleic acid

3.1 Introduction

Elettaria cardamomum (L.) Maton is a perennial herbaceous monocotyledon belonging to the family Zingiberaceae. The genus name "*Elettaria*" is derived from the word "elettari", a vernacular name for this species in Malabar in India (Nair, 2020). The species name "*cardamomum*" comes from the Latinization of the Greek word "kardamomom", which describes an Indian specie (Nair, 2020). The genus *Elettaria* Maton has been recorded from South India to West Malesia, represented

M. Ikechukwu
Sam Nujoma Marine and Coastal Resources Research Centre, Sam Nujoma Campus University of Namibia, Henties Bay, Namibia

A. Maroyi
Department of Botany, University of Fort Hare, Alice, South Africa

A. Cheikhyoussef (✉)
Science and Technology Division, Multidisciplinary Research Service, Centre for Research Services, University of Namibia, Windhoek, Namibia
e-mail: acheikhyoussef@unam.na

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by *E. cardamomum* and *Elettaria ensal* (Gaertn.) Abeyw. (POWO, 2023). *Elettaria cardamomum* (L.) Maton is the most important species known as small cardamom, green Cardamom, true cardamom, ceylon cardamom, and malabar cardamom (Opara & Chohan, 2021).

Other species previously included in the *Elettaria* genus include *E. brachycalyx* S. Sakai & Nagam., *E. kapitensis* S. Sakai & Nagam., *E. linearicrista* S. Sakai & Nagam., *E. longipilosa* S. Sakai & Nagam., *E. longituba* (Ridl.) Holttum, *E. multiflora* (Ridl.) R.M.Sm., *E. rubida* R.M.Sm., *E. stoloniflora* (K. Schum.) S. Sakai & Nagam and *E. surculosa* (K. Schum.) B.L. Burtt & R.M.Sm. (Burtt & Smith, 1983; Smith, 1986; Sakai & Nagamasu, 2000) have been reclassified into a new genus, *Sulettaria* A.D. Poulsen & Mathisen, based on distributional, morphological and molecular data such as plastid matK, ndhF and trnL-F, and nuclear ITS and At103 sequences (Poulsen et al., 2018). Guatemala is the largest producer of cardamom in the world, grown commercially. Other producing countries include Cambodia, El Salvador, Laos, Sri Lanka, Papua New Guinea, Vietnam, and Tanzania (Govindarajan et al., 1982; Cyriac et al., 2016). This chapter summarizes the latest literature on the cardamom botanical information, cultivars varieties and genetic and genomic research, highlighting the latest techniques applied to this important crop to improve the product and its quality attributes.

3.2 Cardamom Botany

Elettaria cardamomum is commonly known as cardamom, green, small or true cardamom and naturally occurs in southwest India (POWO, 2023). The common name cardamom is also widely used for several species belonging to the *Aframomum* K. Schumann and *Amomum* Roxb. genera. Such species include *Aframomum corrorima* (A. Braun) P.C.M.Jansen (Ethiopia), *A. daniellii (Hook. f.)* K. Schumann (Cameroon) and *A. melegueta* (Roscoe) K. Schumann (West Africa), *Amomum acre* Valeton (South-East Asia), *A. aromaticum* Roxb. (Eastern Himalayas), *A. compactum* Soland. ex Maton (South-East Asia), *A. krervanh* Pierre ex Gagnepain (South-East Asia), *A. ochreum* Ridley (South-East Asia), *A. subulatum* Roxb. (Eastern Himalayas), *A. testaceum* Ridley (South-East Asia), *A. uliginosum* J.G. König ex Retz. (South-East Asia), *A. xanthioides* Wallich ex Baker (South-East Asia) and *A. xanthophlebium* Baker (South-East Asia) (Wardini & Thomas, 1999. However, the study focuses on *E. cardamomum,* a non-contentious scientific name (Wardini & Thomas, 1999; Ashokkumar et al., 2020) which has been in use since 1811 (POWO, 2023). *Elettaria cardamomum* has been introduced in Australia, Bangladesh, Cambodia, Costa Rica, Guatemala, Laos, Nusa Tenggara Island, Papua New Guinea, Réunion, São Tomé and Príncipe, Seychelles, Tanzania, Thailand, Trinidad-Tobago and Vietnam (Randall, 2017; POWO, 2023).

Elettaria cardamomum is a robust perennial herb, growing up to 5 m in height from a thick and branched rhizome. Several erect and leafy shoots with leaf sheaths arise from the rhizomes with numerous decumbent flowering shoots. The leafy

shoots have a limited life span, and the first year is mainly for vegetative growth, the second year for reproductive growth, that is, developing flowers and fruits, and the third year for senescence (Anandaraj & Sudharshan, 2011). New buds are formed from the base of the old shoots in the first and second years in a clump of old shoots (Anandaraj & Sudharshan, 2011). The species has distichous leaves, alternate, lanceolate in shape, with an acuminate apex, dark green and glabrous above, light green and glabrous or pubescent beneath. The petioles are sheathing at the base and forming a pseudostem with other sheaths. The inflorescence of *E. cardamomum* is long, erect, a prostrate panicle, arising from the rhizome and leaning against the soil when ripe. The inflorescence has axillary and tubular bracteole, lanceolate and alternate bracts. The bisexual and zygomorphic flowers are borne on long racemose panicles from the rhizome. The most conspicuous part of the flower is the whitish lip or labellum located at the tip of the corolla tube. The calyx is green in color and shortly three-toothed and persistent. The corolla tube is as long as the calyx tube, with narrow spreading pale green lobes.

The flowers have violet nectar guides leading to the corolla tube. Studies on the pollination biology of *E. cardamomum* (Belavadi et al., 1997; Sinu & Shivanna, 2007) showed that social bees such as *Apis dorsata*, *A. cerana* and *Trigona iridipennis* are the pollinators of the species. The single fertile stamen bears bi-lobed anthers which are sessile with small and capitate stigma. The pistil is trilocular, and each locule bears over 20 ovules with axile placentation. The inferior ovary consists of three iunited carpels and a slender style with a small capitate stigma on the top to the anther along the crest. The style is filiform and terminates with a laterally compressed, cup-shaped stigma slightly above the anther. The stigmatic cup's inner surface is receptive and lined with a viscous exudate. *E. cardamomum* has globose or subcylindrical fruits (Fig. 3.1), which are pale green to yellow and brown when dry with aromatic and dark brown seeds with a thin mucilaginous aril.

E. cardamomum has been recorded in evergreen montane forests and among boulders on loam soil to quartz gravel at altitudes ranging from 600 m to 1500 m above sea level (Wardini & Thomas, 1999). The Western Ghat coast in Kerala, India, is the center of origin and diversity of *E. cardamomum* (Nair, 2020).

Fig. 3.1 Small cardamom, *Eletaria cardamomum* pods and seeds

Eight species from *Elettaria* sp. of Sarawak have been reported by Sakai & Nagamasu (2000). Four reported species were newly described as *E. linearicrista*, *E. longipilosa*, *E. brachycalyx* and *E. kapitensis*. In addition, *E. surculosa* and *E. stolonifera* species, previously included in *E. multiflora*, are considered independent species (Sakai & Nagamasu 2000). They further examined the recent collections and emphasized that anther dehiscence patterns, the form of the labellum and anther crest are important characters for the taxonomy of *Elettaria* sp. As plant domestication yields different morphological and physiological traits, it is usually known as the domestication syndrome, typically used later to distinguish between domesticated crops and their wild ancestors (Kuriakose et al., 2009). It was reported that the domestication of cardamom in India led to an increase in branches number and the number of inflorescences per clump, as well as significantly increased the total number of flowers per clump. Consequently, the number of flowers and flowering duration were remarkably higher/longer in cultivated varieties than in wild cardamom, and these features are essential to increase cultivars' fruit yield significantly (Kuriakose et al., 2009).

3.3 Cardamom Cultivars

Elettaria cardamomum is variable, and several cultivars, such as laxiflora, malabar, mysore, mysorensis and vazhukka (Wardini & Thomas, 1999; Madhusoodanan et al., 2002; Anandaraj & Sudharshan, 2011; Parthasarathy & Prasath, 2012; Alam et al., 2019) have been described based on differences in plant structure, leaf, flower and fruit characteristics (Table 3.1).

3.3.1 Laxiflora Cultivar

The plant specimens of the Laxiflora cultivar are comparatively less robust and shorter than those of the Mysorensis cultivar (Nair, 2020). The leaves of the plant specimens of the Laxiflora cultivar are glabrous and characterized by short petioles. The plant specimens of the Laxiflora cultivar have flexuous and lax decumbent panicles. The flowers of the plant specimens of the Laxiflora cultivar are produced in 4–40 short lax racemes (Nair, 2020). The capsules of the plant specimens of the Laxiflora cultivar are variable ranging from oblong to oblong fusiform.

3.3.2 Malabar Cultivar

The Malabar cultivar is characterized by a prostrate panicle, which is usually 60 cm to 90 cm long, and the plant specimens are medium-sized, growing to a height ranging from 2 m to 3 m on maturity. The leaves are usually 30–45 cm long; their upper

Table 3.1 Characteristics of *E. cardamomum* cultivars

	Cultivar					
Character	Laxiflora	Malabar	Mysore	Mysorensis	Vazhukka	References
Adaptability	–	Lower altitudes at 600–900 m asl	Higher altitudes at 900–1200 m asl	–	Wide range	Wardini and Thomas (1999); Nair (2020), and Mathew et al. (2022)
Capsules	–	Round to oblong	Bold and elongated	–	Round to oblong	Nair (2020), and Paul (2022)
Capsule color at maturity	–	Pale, golden or yellow	Green	–	Green	Nair (2020), and Paul (2022)
Chromosome numbers	–	2n = 48	2n = 50	–	–	Chandrasekhar and Sampathkumar (1986)
Fruits	–	Small, globose, oblong, rounded or ovoid and lightly ribbed	Fusiform, 3-angled and ribbed	–	–	Wardini and Thomas (1999) and Nair (2020), and Mathew et al. (2022)
Leaf petiole	–	Short	Long	–	Long	Wardini and Thomas (1999)
Panicles	–	Prostrate	Erect	Semi-erect	Semi-erect	Wardini and Thomas (1999); Nair (2020), and Mathew et al. (2022)
Petiole size	Short	Short	Long	–	Long	Wardini & Thomas (1999) and Nair (2020)
Plant stature	–	Medium-sized, usually less than 3 m in height	Robust, about 3 m to 4 m tall	Robust, about 3 m to 5 m tall	Robust, about 3 m to 5 m tall	Wardini and Thomas (1999); Nair (2020), and Mathew et al. (2022)
Productivity	–	Less productive	More productive	–	More productive	Nair (2020), and Paul (2022)

side may be pubescent or glabrous. The fruits of the Malabar cultivar plant specimens are small, globose, oblong, rounded or ovoid and lightly ribbed. The Malabar cultivar has been recorded at an altitude ranging from 600 m to 1200 m above sea level. The Malabar cultivar is less susceptible to shoot borer infestation of thrips (*Taeniothrips cardamomi*), a common cardamom pest, and the cultivar can thrive in areas characterized by low rainfall and unpredictable weather conditions (Nair, 2020) but susceptible to katte or marble or mosaic disease caused by cardamom mosaic virus (Wardini & Thomas, 1999).

3.3.3 Mysore Cultivar

Erect panicles characterize the Mysore cultivar; the plant specimens are robust and grow up to 3 m to 4 m in height. The leaves of Mysore cultivar plant specimens are lanceolate or oblong and glabrous on both the lower and upper sides. The capsules of the cultivar are ovoid in shape, bold, and dark green. The fruits of Mysore cultivar plant specimens are fusiform, 3-angled and ribbed. The Mysore cultivar has been recorded at an altitude ranging from 900 m to 1200 m above sea level and thrives in areas characterized by high and well-distributed rainfall patterns (Nair, 2020). The Mysore cultivar plant specimens are resistant to katte, marble, or mosaic disease caused by the cardamom mosaic virus (Wardini & Thomas, 1999).

3.3.4 Mysorensis Cultivar

The plant specimens of the Mysorensis cultivar are robust, tall, and possess either glabrous or pubescent leaves. Flexous panicles characterize the plant specimens of the Mysorensis cultivar. The flowers of the plant specimens of the Mysorensis cultivar are produced in short racemes, and the capsules are bold and distinctly three-angled (Nair, 2020).

3.3.5 Vazhukka Cultivar

The Vazhukka cultivar is a natural hybrid between Malabar and Mysore cultivars (Nair, 2020). Therefore, this cultivar exhibits characteristics that are intermediate between both of these two cultivars. The plant specimens of the Vazhukka cultivar are robust with semi-erect or flexuous panicles. The leaves of the Vazhukka cultivar are deep green, oblong or lanceolate or ovate. The capsules of the Vazhukka cultivar are bold, globose or ovoid (Nair, 2020). The Vazhukka cultivar plant specimens have been recorded at a wide range of altitudes ranging from 600 m to 1200 m above sea level (Nair, 2020).

Raissa et al. (2020) indicated the availability of a wide range of germplasm pool cardamom in Indonesia, with no variety of commercial fruit production produced in Indonesian cardamom a few years ago. Since in-depth knowledge is required for the features of different accessions of Cardamom to be used in breeding, it is essential to collect suitable accessions from the cardamom gene pool (Krishna et al., 2020).

3.4 Genetic Diversity of Cardamom

Like any other organism, plants change over generations by developing special inheritable features through evolution. The changes are sponsored by genetic variations that can arise from either mutations or genetic recombination. Genetic variation is the allelic differences of genes in DNA or RNA arrangements in the genetic pool of a population (Begna, 2021). Genetic variations alter gene activity or protein function; as a result, different traits are introduced into the plant. The introduced traits may manifest in height, flower color, fruit size and other phenotypic expressions (Govindaraj et al., 2015). As the plants reproduce, the introduced traits become increasingly common in the new population, making the descendant population different from the ancestral one. The genetic variation within a population or species is referred to as genetic diversity.

Genetic diversity is the broadest term consisting of all the variations between the different genetic materials concerning the genetic makeup of crop species (Begna, 2021). The frequency and diversity of alleles among individuals within a particular population or species drive it. Genetic diversity in plant species such as *Elettaria cardamomum* allows them to adapt to various environmental conditions, such as fluctuating climate and soil conditions. A high genetic diversity in any given species indicates many individuals with a wide variety of traits, and a low genetic diversity translates into a genetically homogenous species. Genetic diversity is the basis for the development of elite varieties of a crop, such as *E. cardamomum,* with desirable characteristics. *E. cardamomum* has high genetic diversity, from which better-performing genotypes for traits of interest can be selected (Anjali et al., 2016). Diversity in plant genetic resources such as those of *E. cardamomum* provides an opportunity for plant breeders to develop new and improved cultivars with desirable characteristics for farmers (yield potential and large seed, etc.) and breeders (pest and disease resistance) (Preethy et al., 2022).

Genetic diversity in crop species such as *E. cardamomum* is caused by mutation, evolution, domestication, plant breeding and migration (Begna, 2021). The mutation is the sudden heritable changes in genetic diversity that occasionally occur through an aberration of genetic materials like DNA, RNA, and protein within the cells. It is the source of genetic variation and increases genetic diversity in species. Evolution occurs when generations of plants undergo a gradual change. As a result, a new crop species different from its earliest and primitive descendants arise. Evolution leads to the transformation of genetic diversity through gradual processes, resulting in new crop species. Domestication is the transformation of wild progenitors to cultivated species through a continuous selection of crop plants with desirable traits to satisfy human needs.

Adopting high-yielding varieties of *E. cardamomum* with resistance to biotic and abiotic stresses, big seed and fruit size, and early matured crop plants results in genetic alteration of morphological and agronomical characters (Govindaraj et al., 2015). During the plant breeding process, the genetic composition of crop plants is changed by using genetic diversity to produce preferred phenotypic traits. Genetic

diversity is introduced by crossing genetic materials of diverse origins to develop superior genotypes in yield and resistance to diseases and pests, among many different traits. During migration, gene flow occurs through seed and pollen dispersal. Gene flow leads to the introduction of new alleles, increases variability within the species, and possibly results in new combinations of traits.

Genetic diversity could be assessed using phenotypic variations in quantitative and qualitative traits. Quantitative variations are based on morphological markers (i.e., markers related to variation in shape, size, color and the surface of various plant parts) (Jose et al., 2022). On the other hand, qualitative variations are either based on biochemical or molecular markers. Biochemical markers are multimolecular forms of enzymes coded by various genes but have the same functions. Molecular markers are specific fragments of DNA found at specific locations of the genome that could be identified within the whole genome. They are used to 'flag' the position of a particular gene or the inheritance of a particular character. Molecular markers are phenotypically neutral (Yaman, 2022). Based on these variations, three main varieties of *E. cardamomum* are recognized: Mysore, Malabar and Vazhukka. The most distinguishing quantitative traits in *E. cardamomum* are plant height and length of the flower stalk, while qualitative traits include capsule and panicle shapes, capsule color, and leaf texture (Babu et al., 2012). Anisha et al. (2020) studied the genetic diversity in *E. cardamomum* using inter-simple sequence repeat (ISSR) markers and reported 13 released varieties of cardamom, including selections and hybrids from different sources in the Southern parts of India.

The genetic diversity (GD) of *Amomum subulatum* Roxb. has been reported by Chaudhary et al. (2016), of which 16 samples were analyzed using 25 random primers, which led to the generation of 169 different loci with a high polymorphic value of 90.9%. Two GD indexes of Nei (0.295) and Shannon Information (0.448) suggested high GD for the studied large cardamom accessions with 4 major clusters under the Cardamom Dendrogram have been identified for the cultivars from the Eastern hills of Nepal. Raissa et al. (2020) indicated the availability of a wide range of germplasm pool cardamom in Indonesia, with no variety of commercial fruit production produced in Indonesian cardamom a few years ago. Since in-depth knowledge is required for the features of different accessions of cardamom to be used in breeding, it is essential to collect suitable accessions from the cardamom gene pool (Krishna et al., 2020).

3.4.1 Genetic Diversity and E. cardamomum *Crop Breeding*

From the very beginning of agriculture, natural genetic diversity has been exploited within crop species to meet subsistence food needs. Though unconscious of spontaneous mutations and recombination, humans have always selected superior crops for breeding (Wolter et al., 2019). Seeds from the best-performing plants were retained after harvest and sowed in the next season, continuously improving characteristics favourable for local production (Voss-Fels et al., 2019). These traditional

breeding approaches have been highly successful in delivering elite crop varieties with high yields and other enhanced traits, and even today, a practice that underpins the current crop improvement (Wolter et al., 2019). The formulation of Mendel's Laws of Heredity, however, added a twist to the classical breeding approaches through targeted crossing between parental genotypes, and it increased selection efficiency using marker-assisted selection and genomic selection (Wolter et al., 2019).

Genetic diversity has tremendous significance for developing superior cultivars in crop improvement. Crossing different genetic materials can produce crops of superior performance and desirable hybrids (Begna, 2021). This is evident in the *Vazhukka* variety (a hybrid between *Mysore* and *Malabar* varieties) of *E. cardamon,* as depicted in Table 3.1. Since all the varieties of *E. cardamomum* are interfertile, the observed variations are probably due to natural crossing (Parthasarathy & Prasath, 2012). Cyriac et al. (2016) developed microsatellite markers in small cardamom (*Elettaria cardamomum* Maton) using the selective hybridization enrichment method. These markers are informative and can be further utilized for generating reliable molecular data to assist in small cardamom improvement.

Nadiya et al. (2016) performed transcriptome sequencing and de novo transcriptome assembly of a wild and five cultivar genotypes of cardamom, *E. cardamomum* (L.) Maton using Ion Proton RNA sequencing technology. They further generated functional annotations that could provide valuable insights into differences between wild progenitor and cultivated cardamom and may, enrich the plant transcriptome database, and provide new insights into cardamom genomics (Nadiya et al. 2017).

3.4.2 Erosion of E. Cardamomum *Genetic Diversity*

The success of crop improvement lies in efficiently identifying and incorporating genetic diversity from various plant genetic sources, including currently cultivated cultivars, newly developed cultivars, landraces, wild and near relatives of cultivated cultivars, and germplasm collections with elite and/or mutant plants (Swarup et al., 2021). Modern crop varieties, especially, are being developed primarily for high-yielding potential under well-endowed production conditions (Govindaraj et al., 2015). This is especially true for *E. cardamon w*hich has seen a threefold increase in production in the past 20 years (Joseph et al., 2022). The continuous increase in production coupled with consistently superior quality of the crop results from the extensive coverage of single genetic cultivars. With the advent of new biotechnological tools and techniques, this process of genetic manipulation is being accelerated, and it shortened the breeding cycles. As a result, it can be carried out more precisely (neglecting environmental effects) and fast-track manner than the classical breeding techniques (Govindaraj et al., 2015).

Prolonged inbreeding, however, can lead to genetically homogenous populations, which may result in linkage drag and the transfer of deleterious genetic material genetically linked to the desirable trait (recessive identical alleles)

(Wolter et al., 2019). Even in a natural population, severe reductions in population size, the so-called genetic bottleneck, leads to loss of genetic diversity and increased susceptibility to infectious pests and diseases, increasing the chances of extinction of an individual crop in question. Given the limited geographic distribution of *E. cardamon*, the above factors may lead to genetic erosion. In order to avert genetic erosion, there is a need to preserve the wild cardamom populations, as they may have genetic variability and possess alleles that can be used for future crop improvement programs (Anjali et al., 2016).

Genome editing is the newest breeding tool used to introduce genetic variations with precision and accuracy, compared with other breeding methods that rely on random mutation. Multiple traits are introduced into an array of germplasm to generate the final crop product (Shylaja & Nair, 2022). Regardless of the source of genetic variation (e.g., native germplasm, biotechnology, or genome editing), the resulting cultivar has a unique genetic makeup and the addition of novel phenotypic traits.

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Chapter 4
Plant Morphological Traits of *Elettaria cardamomum*

Mohammad Rafiq Khan and Shamaila Aslam

4.1 Introduction

Spices are extensively used for flavor, color, aroma and preservation and are obtained from different parts of the plants: bark, buds, flowers, fruits, leaves, stems, roots, seeds, and stigmas. The term spice is distinguished from the term herb in that the latter is usually derived from the leaves of a plant and used in cooking, but any other part of the plant, often dried, is called a spice. Some examples are cloves buds; cinnamon-dried bark; ginger roots; peppercorns berries, cumin aromatic seed; saffron flower's stigma and cardamom pods of a perennial plant (Rajathi et al., 2017). Cardamom is one of the most expensive spices prepared from the seeds of different plants classified in the genus *Elettaria*. The capsules of small *Elettaria cardamomum* commonly encountered in commercial markets are shown as a zigzag heap (Fig. 4.1).

Apart from being a famous flavoring agent on the food tables of the world, it claims many applications as a medicine to cure some diseases. Cardamom, for example, may help lower blood pressure due to its antioxidant and diuretic traits by promoting urination that removes extra water from the living body, e.g., around the heart or in other body organs and tissues. Cardamom powder could increase the activity of certain enzymes that help in fighting cancer. The spice may also enhance the ability of natural killer cells to attack tumors. Cardamom tea has been observed to cause a slimming effect on the human body without any harmful effects. The

M. R. Khan (✉)
Department of Environmental Science and Policy, Lahore School of Economics, Lahore, Pakistan
e-mail: drrafiq@lahoreschool.edu.pk

S. Aslam
Director of Clinical Research, Northwest Houston Clinical Research PLLC, Tomball, TX, USA

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Fig. 4.1 Capsules of small *Elettaria cardamomum*

major constituents of its essential oil are 1,8-cineole, α-terpinyl acetate, sabinene, and β-linalool. As narrated above, these could be used in food, aroma, and pharmaceutical applications (Ashokkumar et al., 2019, 2020, 2022). Due to its extensive uses highlighted above, it is called "*The Queen of Spices*" in India. Intensive research is currently underway to extend the spectrum of its applications.

4.2 Nomenclature and Classification

Cardamom, sometimes called cardamom or cardamom (Wikipedia, Encyclopedia Britannica), is a spice made from the seeds of several plants in the genera *Elettaria* and *Amomum* in the family Zingiberaceae. Both genera are native to the Indian Subcontinent and Indonesia (Wikipedia). Their small seed pods recognize them: triangular cross-sect and spindle-shaped, with a thin, paper-like outer shell and small, black seeds. *Elettaria* pods are light green and smaller, while *Amomum* pods are larger and dark brown.

The binomial nomenclature and botanical classification of cardamom are:

Kingdom	Unranked (Angiosperms) Plantae
Family	Zingiberaceae
Genus:	*Elettaria* and *Amomum*
Species:	*Elettaria cardamomum*/*cardamom*

4.3 Occurrence

Elettaria genus naturally occurs in India, Nepal, Bhutan, Pakistan, Nepal and Indonesia (Dictionary. Com). According to a Review by Rajathi et al. (2017), cardamom originated from the coastal area of India. It is grown in Guatemala, Tanzania, Sri Lanka, El Salvador, Vietnam, Laos and Cambodia. India is the leading exporter of dried cardamom. Cardamom is known as the "*Queen of Spices*" that grows from a thick rootstalk up to around 6–10 feet in height and is indigenously grown in the evergreen forests of the Western Ghats in South India. Different types of *Elettaria* are encountered that are distinguished based on the size and color of their pods. Based on the size of the pod, it is called small cardamom (Choti Elachi in India and Pakistan), while a similar species *Amomum* is called big cardamom (Moti Elachi) based on pod size. Based on the color of the pod former is called green cardamom, while the latter is called dark brown cardamom (KPT International Online).

Both species used for cardamom are native throughout tropical and subtropical Asia. The oldest references to cardamom are found in Sumer and the Ayurvedic literature of India (Baser, 2002). *Elettaria cardamomum* has gained the status of an export commodity and earner of foreign exchange due to its outstanding quality. According to a report from Export Development Board (EDB) Sri Lanka, due to the unique flavor of green cardamom cultivated in Sri Lanka, the green cardamom produced in Sri Lanka is known as Ceylon Cardamom that is available in the export market in the form of whole pods, powder and essential oil. The country meets about 0.1% of the global demand for cardamom, which amounts to 4000–5000 tons annually. Australia, Canada and Estonia, and India, major producers and exporters, are the primary buyers of Ceylon Cardamom, available in two grades. It is mainly cultivated in Kandy, Matale, Kegalle, Nuwara Eliya, Ratnapura and a part of Galle. Three types of cardamom are found in Sri Lanka and categorized based on the inflorescence shape as Malabar, Mysore and Vazhukka.

Cardamom consumption has drastically increased throughout the world during the last two decades. However, cardamom is mainly consumed in Middle Eastern countries, India, Pakistan, European countries, the USA, and Japan. Middle Eastern countries, such as Saudi Arabia and the United Arab Emirates, and South-East Asian countries, such as India, account for over 60% of global consumption.

4.4 General Structure and Morphology of Plant, Roots, Stem, Branches, Leaves and Seeds

4.4.1 General Structure of Plant

The general structure of the plant and the composition of its different parts are described below. The morphological structure of *E. cardamom* is exhibited in Fig. 4.2, while that of cardamom in Fig. 4.3.

Fig. 4.2 *Elettaria cardamomum* plants

Fig. 4.3 *Elettaria cardamomum* capsules

The leafy off-shoots of the cardamom plant grow up to 1.5–6 m (5–20 feet) from the roots and are equipped with the tendency to branch. The flowering off-shoots are approximately 1 m/3 feet long and may be upright or sprawling in different directions. Every shoot bears many flowers about 5 cm/2 inches in diameter, with greenish petals and a purple-veined white lip. The whole fruit is 0.8–1.5 cm long and is a green three-sided oval capsule containing 15–20 dark, reddish brown to brownish black, hard, angular seeds. The essential oil of cardamom occurs in large parenchymal cells under the epidermis of the seed coat. The essential oil content of the seed is from 2% to 10%. Cineole and α-terpinyl acetate are principal components (Encyclopedia Britannica, Baser, 2002).

According to another report from India, cardamom is a perennial plant with a tall pseudo stem formed by the encircling of leaf sheaths wrapped one over the other. Depending on the variety, a normal fully grown plant may reach 2–4 m in height. The genus name comes from *Elettaria*, the vernacular name for this plant in Malabar, India.

4.4.1.1 Appropriate Agronomic/Climatic Conditions (MFPI)

Annual Rainfall: 1500–4000 mm
Temperature: 10–35 °C
Altitude: 600–1200 m above the Mean Sea Level
Season: December to June
pH: 5.5 to 6.5
Humidity: 75%

4.4.1.2 Essential Oil

The essential oil of cardamom capsules is responsible for the characteristic aroma of cardamom. The essential oil yield and chemical constituents of 22 different accessions of cardamom have been evaluated and reported (Ashokkumar et al., 2020). The essential oil yield was from 4.5% to 9.5%, indicating substantial variations. The GC/MS analysis results discovered 24 constituents that constituted 98.1%–100% of total essential oil. The main fractions were "oxygenated monoterpenes (40.7%–66.7%), monoterpene hydrocarbons (23.1%–58.6%), and sesquiterpenes (0.1%–2.0%). Among the monoterpenoids, the predominant constituents were α-terpinyl acetate (29.9%–61.3%) followed by 1,8-cineole (15.2%–49.4%), α-terpineol (0.83%–13.2%), β-linalool (0.44%–11.0%), and sabinene (1.9%–4.9%). Two sesquiterpene constituents, cardinen, nerolidol, and *p*-cresol (a phenol derivative), were identified. The compositional data were subjected to euclidean-distance-based similarity analysis, which showed two major clusters. The major constituents of cardamom essential oil (*CEO*) are 1,8-cineole, α-terpinyl acetate, sabinene, and β-linalool that could be used in food, aroma, and pharmaceutical applications. The researchers (Ashokkumar et al., 2020) have reported the minimum and maximum range, retention time, and retention index of essential oil compounds in 24 cardamom accessions (Table 4.1).

4.4.1.3 Sowing and Cultivation

The cardamom seeds are obtained from standard seed suppliers, sown and initially cultivated to prepare the seedlings in primary and secondary nurseries. The seedlings are then planted in pits of appropriate size dug in land fields, subsequently filled with compost and suitable soil. Cardamom is usually grown as a rainfall crop, but occasional sprinkling with water is also done for its better growth and enhanced yield. The compost mix of 25 t/ha, 75 kg N, 75 kg P and 150 kg K/ha (MFPI).

Table 4.1 Minimum and maximum range, retention time, and retention index of essential oil compounds in 24 cardamom accessions

No.	Compound	RTa	Rib	RIc	Area %		
					Minimum	Maximum	Mean
1.	α-Thujene	8.3	924	930	0.1	1.0	0.2
2.	α-Pinene	8.6	948	943	0.6	1.5	1.0
3.	Sabinene	9.6	969	975	1.9	4.9	3.5
4.	β-Pinene	9.8	974	979	0.2	0.5	0.3
5.	β-Myrcene	10.0	988	990	0.9	1.9	1.4
6.	3-Carene	10.6	1008	1011	0.1	0.7	0.1
7.	α-Terpinolene	10.7	1022	1022	0.1	1.9	0.3
8.	Limonene	11.1	1024	1029	0.9	9.4	2.3
9.	1,8-Cineole	11.2	1026	1031	15.2	49.4	34.5
10.	β-Cymene	11.3	1042	1030	0.1	0.7	0.2
11.	γ-Terpinene	11.9	1054	1059	0.2	1.4	0.4
12.	β-Linalool	13.0	1082	1087	0.4	11.0	2.0
13.	Terpinen-4-ol	15.2	1137	1137	0.4	3.2	1.8
14.	α-Terpineol	15.6	1143	1140	0.8	13.2	3.4
15.	β-Terpineol	15.7	1158	1159	0.3	2.7	0.8
16.	β-Citral	15.8	1174	1174	0.1	0.5	0.2
17.	Nerol 1	6.8	1228	1229	0.2	1.1	0.7
18.	Linalyl acetate	16.9	1231	1231	0.2	4.4	1.2
19.	α-Citral	17.3	1264	1267	0.1	0.6	0.2
20.	α-Terpinyl acetate	19.4	1333	1300	29.9	61.3 43.5	
21.	Geranyl acetate	19.9	1379	1381	0.1	2.3	0.9
22.	p-Cresol	22.9	1382	1385	0.2	9.0	1.0
23.	γ-Cadinene	23.3	1513	1513	0.3	0.4	0.1
24.	Nerolidol	24.2	1564	1563	0.1	2.0	0.7

Source: Ashokkumar et al. (2020)

aRT, Retention time; bRI, Retention index (experimental) on Rxi®-5 Sil MS column; cRI, Retention index in literature; Monoterpene hydrocarbons, 1–8 and 11; Oxygenated aRT, Retention time; bRI, Retention index (experimental) on Rxi®-5 Sil MS column; cRI, Retention index in literature; Monoterpene hydrocarbons, 1–8 and 11; Oxygenated

4.4.1.4 Growth

The cardamom plants start bearing two to three years after planting. Panicles appear from the base of the plants; it is generally starting to appear in January. The flowering starts in August. The peak stage of flowering is May to June. The time for fruit to mature is about 120 days after flowering. Fruits are small trilocular capsules containing 15–20 seeds. On maturity, seeds turn dark brown to black.

4.4.1.5 Harvesting

The harvesting starts in August–September and extends into February–March. Matured cardamom capsules are picked up by hand. The yield ranges from 200 to 250 kg per hectare.

4.4.1.6 Genome

Anjali et al. (2016) conducted the variation in *Elettaria cardamomum* Maton (cardamom) based on genome size, cytological studies and molecular marker data. The relative 2C genome size and the number of base pairs of cardamom were determined through flow cytometric analysis using propidium iodide staining. The nuclear DNA content was estimated in various species sections representing individuals from wild and cultivar genotypes as the internal reference standard. Chromosome number from the growing root tip was examined following standard protocols. The chromosome number was found to be 2n = 48. Among the thirty cardamom accessions studied using ISSR markers showed a very prominent level of genetic diversity. Thus the analysis revealed the existence of genetic variability within the studied cardamom accessions. The plant specimens also differed significantly in their genome size.

According to a recent study by Nadukeri et al. (2020), cardamom is a cross-pollinated crop propagated through both vegetative and seed methods; thus, variability is present among its genotypes. Their investigation from the ANOVA statistical analysis showed that all growth characters exhibited significant differences at 1 and 5 percent levels except for the trait of leaf breadth. The results indicate that the "Phenotypic coefficient of variation was slightly higher than the genotypic coefficient of variation for all the growth characters. The highest GCV (23.5%) and PCV (24.3%) were recorded for the trait number of leaves per tiller. High heritability (99.0%) and genetic advance over a mean (48.2%) were noticed for the trait number of vegetative buds per clump."

4.5 Morphology of Stem

Though there are a few studies on the chemical characteristics of its leaf oil from other phytogeographical regions, the presence of oil in the stem and the chemical composition of essential oil from stems have not been reported in detail. However, a study was undertaken to characterize the chemical composition and antioxidant activity of leaf and stem essential oil of E. cardamom. The essential oil extracted by hydro-distillation from the leaf and stem revealed the presence of "43 and 37 compounds, representing 92.7% and 92.1% of total oil, respectively, by GC/MS analysis. The major constituents of the leaf essential oil were 1,8-cineole (20.6%), camphene (18.0%), camphor (10.0%) and tricyclene (7.36%), whereas α-terpinyl

acetate (19.7%), 1,8-cineole (10.3%), caryophyllene oxide (7.13%) and β-eudesmol (4.85%) were rich in the stem essential oil. Oxygenated monoterpenes were the major terpenic fraction in the leaf and stem essential oil of *E. cardamomum*. The free radical scavenging ability assessed by DPPH· (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay showed that leaf oil had better activities as compared to stem oil. The findings demonstrated that *E. cardamomum* growing wild in Eastern India could be considered an important bioresource and natural antioxidant."

4.6 Morphology of Branches and Leaves

The structure of the branches and leaves of cardamom is shown in Fig. 4.4, and of leaves in Fig. 4.5.

Cardamom is a clumping plant with between 10 and 20 leafy shoots arising from the rhizome. There are several additional flowering shoots. The leaves are lanceolate and dark green. Cardamom is a pungent, aromatic, herbaceous perennial grown for its glossy textured foliage and edible use (Plant Finder). Cardamom is grown for

Fig. 4.4 Structure and morphology of branches and leaves

Fig. 4.5 Structure and morphology of leaves

its fruits used as a spice. Cardamom is a clumping plant with between 10 and 20 leafy shoots arising from the rhizome. The shoots are pseudo stems composed of overlapping leaf sheaths.

Cardamom leaves, like their pods and seeds, stand among the oldest and most expensive spices in their history on earth due to their unique flavor and aroma. In addition, the seeds and leaves have been used in cooking to prepare various delicious dishes. Cardamom is a ginger family member originating from India, Pakistan, and Nepal. The cardamom leaves bear the typical flavor of cinnamon and salt and have a freshening taste.

Cardamom leaves have also been used to make some excellent drinks. An important example is their fresh-tasting infusion. The leaves are used in sauces or stocks also. The products derived from cardamom leaves also combine very well with fish. Their warming brings out the best of its flavor.

Cardamom Leaves are available annually and can easily be stored for up to seven days at a temperature of 2–7 °C. Cardamom leaves are produced in a socially responsible culture and meet hygienically designed kitchen standards. The product is ready for use because it is clean and is grown hygienically.

The spectrum of the products from cardamom leaves is very broad: "Blanching, Blending, Blitzing/Mashing, Bruising, Canning, Cold infusion, Confiture, Deep frying, Drying, Extracting, Fermenting, Freezing, Gvery broad, Grilling, Hot infusion, Macerate, Marinating, Oil, Pickling, Smoke, Sous vide, Steaming, Syrup Gin, Rum, Vodka, Chocolate, Coffee, Prawns, Lobster, Crab, Crustaceans others, Cod, Monkfish, Fish (saltwater), Banana, Berries, Blueberry, Cherry, Coconut, Kalamansi, Lime, Mandarin, Passion fruit, Plum, Granatapfel, Deer, Rabbit, Game, Mint, Anise, Beef, Goat, Lamb, Offals, Porc, Veal, Duck, Black pepper, Cinnamon, Vanilla, Beetroot, Ginger, Rhubarb, Couscous, Liquorice, Olive oil, Ponzu, Rice, Soy sauce, Sugar, Yuzu"

"Gin is a colorless distilled beverage widely consumed worldwide, produced by several methods, but always with juniper as the predominant taste. The volatile content of gin is made up of terpenoid compounds, mainly from juniper berries, but also from other botanicals such as coriander seeds, angelica root or citrus peel. Although some authors have used various methods to develop a vocabulary to describe the sensory characteristics of gin, no uniform vocabulary has been widely adopted. The most usual attributes defined by the tasters were juniper and coriander, but spice, liquorice, aniseed, floral and fruity attributes were also reported."

Sensory evaluation and consumer research of beverages of alcoholic beverages have been conducted (Woodward, 2012).

The images of some related recipes are given below.

Chocolate, ginger ice cream & olive crunch, lemon cress and cardamom leaves (**Jan Hartwig & Thomas Barosch**)

Pig's chin, cardamom leaves, bell pepper and celeriac (**Marcel Fischer**)

Steamed cardamom leaves with scallops

Spicy shakerato (**Lisette Dawtrey**)

4.7 Structure and Composition of Seeds

The structure and composition of the seeds are exhibited in Fig. 4.6.

The basic physical properties of cardamom seeds, such as length, width, thickness, geometric mean diameter, thousand seed mass, and sphericity, have been reported as dependent on their moisture content, according to a report from India (Gebreselassie, 2012). These were studied at moisture content 9.90%, 13.5%, 18.4%, and 23.2% on a wet basis and are reported "to increase from 17.01 to 17.30 mm, 5.68 to 6.57 mm, 5.02 to 5.35 mm, 7.86 to 8.47 mm, 120.8 to 165.6 g, and 0.46 to 0.49, respectively, with the increase in the moisture content from 9.9% to 23.2% n weight basis. As the moisture content increased from 9.9% to 23.2%, the bulk density, true density, and porosity decreased from 408.2 to 358.9 kg/m^3, 926.5 to787.1 kg/m^3, and 55.9% to 54.4%, respectively. In addition, the angle of repose increased from 72.16° at 9.9% to 73.80° at 23.2% moisture content. In contrast, the static coefficient of friction increased with the increase in moisture content from 9.9% to 23.2% on three different surfaces. The highest static coefficient of friction was recorded when cardamom seed against plywood (0.47–0.56), and the lowest static coefficient of friction against mild steel (0.41–0.50). The static coefficient of friction between cardamom seeds and galvanized iron surface increased from 0.44–0.53 within the studied moisture content range."

Cardamom seeds are widely used to make many products, including dishes and medicines. The major use of cardamom seeds is in preparing cardamom tea, a herbal tea made from the seed pods of the cardamom plant, including both its types: Green cardamom and Black cardamom. Green cardamom seeds come from

Fig. 4.6 Structure of cardamom seeds

the pods of the plant *Elettaria cardamomum. The* lack of cardamom comes from the plant *Amomum cardamomum*. *The former claims* an intensely strong flavor that is both spicy and slightly sweet. Black cardamom tea has a smoky flavor contrasted by a refreshing, slightly minty aroma. The seeds can be poured directly into hot water or cardamom powder. Cardamom seeds are picked by hand and may be used even in the raw state to make tea or after harvest in October. They are then either crushed or packaged as desired to make tea. The cardamom seeds can also be packed as tea bags and floated into the market for sale as culinary spices, as shown in the images below.

Benefits of Cardamom Tea

Cardamom tea has long been used in South Asia to treat various ailments. Like Indian masala chai tea, cardamom tea is an Ayurvedic and traditional medicine staple. Today, research shows that cardamom may have health benefits when consumed

regularly. Thus, let us know how to brew cardamom tea. About 2 cups of water are heated in a stovetop pot to prepare cardamom tea. The water is brought to a boil, removed from the stove, and 4 cardamom pods are added. Tea leaves are added to this s if they are used in planting. The contents of the pot top are steeped for 5 min before adding milk or sugar. A long reading is requisite to know the complete spectrum of tea health benefits of cardamom tea. Its major medical benefits are outlined below

1. Cardamom tea may aid weight loss and prevent severe obesity disease. Cardamom tea may help accelerate weight loss by streamlining the body's digestive processes. Thus it helps prevent fat buildup while helping the liver dispose of waste products more quickly. Ground cardamom helps prevent obesity. In a study on animals published in Lipids in Health and Disease (Rehman et al., 2017), the research workers found that cardamom improved glucose intolerance and prevented the deposit of abdominal fat. Cardamom was also shown to affect the liver by ameliorating fibrosis positively. Another study published in the Journal of Diabetes and Metabolic Disorders examined the effects of cardamom on pre-diabetic women. The researchers observed that cardamom consumption increased insulin sensitivity and decreased bad LDL cholesterol.
2. Cardamom tea is good for oral health. It helps in protecting dental health by inhibiting bacterial growth (Chen et al. 2020). The bacterial growth on the surface of the teeth causes dental caries, a commonly encountered disease due to the breaking of tooth enamel by acids. In addition, the bacterial fermentation of carbohydrates produces acids. Drinking cardamom tea can help neutralize the acids produced by bacteria and prevent plaque buildup, cavities, and dental caries. In addition, the anti-bacterial properties of cardamom also effectively treat halitosis, commonly known as bad breath. Bad breath is caused when bacterial buildup in the mouth begins and tends to feed on food particles. Cardamom helps eliminate bacteria to keep the breath fresh all day.
3. It may help in quitting smoking. Cardamom tea may be beneficial for those who are trying to quit smoking. Research published in Addictive Behaviors examined the potential of cardamom gum to aid nicotine withdrawal. The results showed that vanilla and baked apple cardamom gums effectively reduced nicotine withdrawal symptoms, including dysphoria, anxiety, and tension (Cohen, 2010).
4. It boosts the immune system. Like many herbal teas, cardamom tea may help treat and prevent the common cold and flu. It contains antioxidants and vitamins that fight off viruses, fungi, and bacteria. A study published in Ethnobotanical Leaflets found that cardamom may effectively prevent viruses such as streptococcus, which causes sore throat. In addition, researchers found cardamom effective against staph infections and fungal infections, including candida (Majdalawieh & Carr, 2009).
5. It protects heart health. Cardamom contains high levels of potassium that are good for heart health (Rastogi, 2017). Potassium is a vasodilator, decreasing inflammation and pressure on arteries and blood vessels. This means that drink-

ing cardamom tea regularly may help lower high blood pressure. As a result, it could help improve blood circulation and lower your risk of heart attack and blood clots.

6. It acts as a digestive aid. Cardamom tea has long been used as a digestive aid to soothe stomach ailments, including gas and bloating. It was used in Turkey and Arabic societies to treat intestinal worms. Crushed cardamom seeds have anti-inflammatory properties that soothe irritated stomach muscles. This helps to prevent the contractions that cause stomach pains. Cardamom is a natural carminative, which means it relieves gas. Drinking cardamom tea during or after meals can help streamline digestion and prevent gas. Some research also shows that cardamom tea may be beneficial in the treatment of irritable bowel syndrome, although results have been inconclusive (Streit, 2018).

Like ginger tea, cardamom tea can help treat nausea. Sip this hot tea before you board a boat or plane if you suffer from motion sickness. Drinking cardamom tea may also help ease morning sickness, but consult a physician before drinking cardamom tea if you are pregnant.

Side Effects of Cardamom Tea

Cardamom tea has not been shown to have any serious side effects when consumed in moderation. This herbal tea may interact with certain medications, so it is a good idea to talk to your doctor before drinking cardamom tea if you have a health condition. Research shows cardamom may interact with blood thinning medications and some antidepressants, so limit or avoid use if you take these medications.

Cardamom tea may cause allergic reactions in specific individuals. Therefore, stop using it immediately if you experience a runny nose, itchy throat, or difficulty breathing when drinking cardamom tea. Likewise, do not drink cardamom tea if you are allergic to either of the cardamom plants.

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Chapter 5
Viral Diseases of *Elettaria cardamomum*

Sangeetha Elangovan and Tennyson Jebasingh

5.1 Introduction

Elettaria cardamomum Maton (Small Cardamom) is an herbaceous, perennial, rhizomatous and shade-loving monocotyledon plant that belongs to the *Zingiberaceae* family. *Zingiberaceae,* with 56 genera with 1300 species, is the largest family in the order Zingiberales (Kress et al., 2002). This family contains flowering plants, most of which are aromatic herbs. Cardamom is among them; the dried capsules or fruits have the greatest economic value. Cardamom is the third most expensive spice in the world. Due to its aroma and health benefits, it is considered the 'Queen of spices'. It is the third most expensive spice globally, with a worldwide turnover of over 10 billion USD (Khan et al., 2020). Cardamom is cultivated in Guatemala, India, Sri Lanka, Indonesia, and Nepal. In India, cardamom is cultivated in the Western Ghats of southern India. India, the 'land of spices,' once occupied a premier position in the global cardamom market, but now Guatemala is the largest producer of cardamom with an annual yield of 23,000 metric tons (Sasikumar et al., 2012). The widespread incidence of diseases adversely affects the cardamom industry, substantially reducing total production and productivity. Bacterial, fungal and viral diseases are common in the cardamom plant, but viral diseases are of significant concern.

The viruses like cardamom mosaic virus (CdMV), Nilgiri necrosis virus, infectious variegation virus, cardamom vein clearing virus (CdVCV) (Venugopal, 1995) and banana bract mosaic virus (BBrMV) (Siljo et al., 2012) affect the cardamom plantation. Recently, cardamom transcriptome datasets have reported two more viruses-Cardamom virus X and cardamom polerovirus (Sidharthan et al., 2021). In

S. Elangovan · T. Jebasingh (✉)
Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India
e-mail: jebasingh.biological@mkuniversity.org

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order to control the viral disease, the phenotypical and genotypical characterization of a virus is needed. The common visible symptoms of viral diseases include mosaic, chlorosis, necrosis, leaf wilt, ring spots, etc. However, the mixed infection of *Katte* and *Kokke kandu* disease was observed on cardamom plantations (Bhat et al., 2020). The mixed infection of two or more viruses may infect a plant and have a synergistic effect (Syller, 2012). Therefore, the discrimination of one viral disease becomes cumbersome in these cases. Thus, the visual diagnosis attributed to the virus diagnosis is untenable (Biju et al., 2010), and to characterize the virus, the genome and proteome of the virus are essential. The complete genome of cardamom-infecting viruses is available for CdMV, BBrMV, and CdVCV (Elangovan et al., 2019; Bhat et al., 2018, 2020), and for other viruses, minimal information is available. The protein-protein interaction (PPI) studies from these viruses are also very limited. This chapter discusses the symptoms of the cardamom viral disease, transmission vector, host range, genome of a virus, detection methods and PPI.

5.1.1 Cardamom Mosaic Virus

Cardamom mosaic disease, a viral disease of small cardamom, is locally known as *'katte*,' a disorder known as marble disease (Uppal et al., 1945; Varma & Capoor, 1953). The prevalence of the disease is observed in the Western Ghats of Southern India. The disease was also observed in Guatemala and Sri Lanka (Mayne, 1951; Gonsalves et al., 1986). The major production constraint encountered by the cardamom industry is due to the cardamom mosaic disease (Varmudi, 2000).

The disease is caused by the cardamom mosaic virus (CdMV). CdMV belongs to the Macluravirus genus of the *Potyviridae* family. The CdMV was initially considered as a member of Potyvirus, and later by using 3'terminal sequencing, it was proposed as a Macluravirus member (Jacob & Usha, 2001). *Potyviridae* is the largest plant-infecting virus family, with approximately 200 members. Most of the economically essential viruses belong to this family. The Maculravirus genus is the second largest in the Potyviridae family.

5.1.2 Host

The host range of CdMV is narrow; small cardamom is the only reported natural host of CdMV. However, by mechanical inoculation, several other hosts from the *Zingiberaceae* family like *Amomum connecarpum*, *A. involucraltum*, *A. microstephanum*, *A. muricatum*, *A. pterocarpum*, *A. subulatum*, *Alpinia neutans*, *A. mutica*, *Curcuma neilgherrensis*, *Hedychium flavescens*, *Zingiber cernuum* and *Maranta arundinacea* of *Marantacea* family were reported (Rao & Naidu, 1973; Viswanath & Siddaramaiah, 1974).

5.1.3 Transmission

The viral disease is transmitted primarily through the infected rhizome and by the aphid in a non-persistent manner (Uppal et al., 1945; Naidu et al., 1985). The aphid *Pentalonia nigronervosa f. caladii* was reported first. However, later it was found that 12 other aphids, namely *Aphis craccivora Koch., A. gossypii Glover, A. nerii, A. rumicis L., Brachycaudus helichrysi L., Greenidia artocarpi W., Macrosiphum pisi Kalt., M. rosaeformis Das, M. sonchi L., Schizaphis cyperi van der Goot, S. gramimum Rondm., Pentalonia mgronervosa f. typic a* and *P. nigronervosa f. caladii* van der Goot (Varma & Capoor, 1958; Rao & Naidu, 1973; Ghosh et al., 2017).

5.1.4 Symptoms

The symptoms of the disease are chlorosis of young leaves, which later develop into pale or dark green stripes and yellow mosaic patches parallel to the veins (Venugopal, 1995). Symptom variation is found among different isolates due to continuous cultivation. Based on this, the CdMV isolates from Karnataka, Kerala and Tamil Nadu were further classified into 6 distinct subgroups (Jacob et al., 2003) (Fig. 5.1).

5.1.5 Structure

The flexuous filamentous particles were observed under an electron microscope with 800 nm in diameter and 11–12 nm in width. It also showed that the central cavity of 4–6 nm in width is surrounded by coat proteins (CP) (Jacob & Usha, 2002).

5.1.6 Genome

CdMV contains single-strand RNA as a genome. The monopartite genome contains a viral genome linked at 5′UTR and poly A tail at 3′ end. Like Potyvirus, the Macluravirus genus also encodes a large ORF and a short overlapping ORF. The

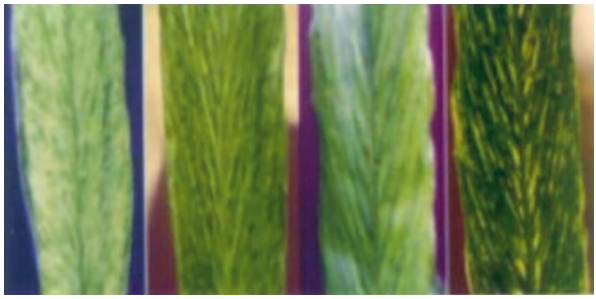

Fig. 5.1 Symptom variation in CdMV-infected cardamom leaves. (Jacob et al., 2003)

large ORF encodes a polyprotein which is then cleaved into nine proteins HC-Pro, P3, 7K, CI, 9K, VPg, NIa, NIb and CP by the action of HC-Pro (through *cis* protease activity) and NIa Protease (through *cis* and *trans* protease activity). A short overlapping ORF produced due to translational slippage in the P3 cistron encodes P3N-PIPO (Chung et al., 2008). Compared with Potyvirus, the P1 coding region and the 5′ terminal sequences of HC-Pro are lacking in CdMV (Elangovan et al., 2019) and in the entire members of Macluravirus.

In 2001, Jacob and Usha, based on the 3′ end sequence of CdMV possessing partial nuclear inclusion b (NIb), complete coat protein (CP), and 3′ untranslated region (3′ UTR) classified CdMV under the *Macluravirus* genus. Later, the same group cloned the remaining upstream sequences of CdMV till the very 5′ end of the CI coding sequence (Jebasingh et al., 2008; Jebasingh et al., 2011; Jebasingh et al., 2013). Finally, the complete genome sequence of CdMV was annotated from the transcriptome sequence of CdMV infected cardamom through Next generation sequencing (NGS) and then cloned (Elangovan et al., 2019).

5.1.7 Characterization of CdMV Protein and Interaction with Host

The CdMV genome information paves the way to study the CdMV protein. The recombinant expression of CdMV CP in the heterologous system *E. coli* showed the development of an inclusion body in the cytoplasm. The aggregation of CdMV CP formed as a filamentous aggregation of 100–150 nm in length (Jacob & Usha, 2002). It has also been shown that CdMV CP could be used as a high-density epitope display system to develop a vaccine for HIV and Leptospirosis (Subha et al., 2013; Vikram et al., 2016).

The other CdMV proteins like NIb, NIa and VPg were expressed in *E. coli* (Jebasingh et al., 2008, 2011, 2013). The expression of NIb protein with hexahistidine tag in *Escherichia coli* led to insoluble aggregates. Out of all the approaches (making truncated versions to reduce the size of protein; replacing an amino acid residue likely to be involved in hydrophobic intermolecular interactions with a hydrophilic one; expressing the protein along with chaperones; expression in Origami cells for proper disulphide bond formation, in *E. coli* as a fusion with maltose-binding protein (MBP) and *Nicotiana tabacum*) to obtain the RdRp in a soluble form, only expression in *E. coli* as a fusion with MBP and its expression in *N. tabacum* were successful (Jebasingh et al., 2008). CdMV VPg forms as aggregated proteins in *E. coli*. The conformational variations between native, denatured and refolded CdMV VPg were observed with fluorescence spectroscopy and CD. Native and refolded CdMV VPg showed unordered secondary structure in the CD spectrum (Jebasingh et al., 2011). The NIa protease of CdMV forms inclusion bodies in *E. coli* solubilized with 8 M urea, refolded and purified by Nickel-Nitrilotriacetic acid affinity chromatography (Jebasingh et al., 2013).

Recently, the interaction of CdMV VPg with the cardamom histones and tomato SGS3 was studied (Palani et al., 2020, Palani thesis, 2018 unpublished data). The ER motif of the MYST family HAT is meant for the acetylation of histones (H3, H4) in the nucleosome (Adachi et al., 2002). ER motifs are predicted in NIa proteases of some Macluraviruses *viz*. ArLV, CdMV and CYNMV. In CdMV, the NIa interaction with the H3 and H4 is assessed using yeast two-hybrid (Y2H), which shows no interaction (Palani et al., 2020). Nevertheless, the assay identified the interaction of CdMV VPg (responsible for the nuclear shuttling of NIa protease) with both H3 and H4. This interaction may impact the host defense mechanism (Palani et al., 2020). The CdMV VPg also showed strong interaction with tomato SGS3 in Y2H has provided a clue regarding the role of CdMV VPg in suppressing the host RNA silencing defense mechanism (Palani thesis, 2018 unpublished data).

5.1.8 Virus Detection

CdMV spread is controlled only by uprooting the infected plants. Therefore, the detection of CdMV is essential in order to avoid the spread of the disease. The CdMV detection using ELISA with the Indian CdMV isolate showed a positive reaction against the antiserum of Guatemalan CdMV (Saigopal et al., 1992; Jacob & Usha, 2001). The RT-PCR method to detect the core region of CP was reported by Biju et al. (2010). Compared to the ELISA and conventional RT-PCR, the SYBR green-based RT-qPCR was reported as a highly sensitive method for CdMV detection (Siljo et al., 2014). Though the methods are reliable, the earlier diagnosis of CdMV on-site is developed, which is highly sensitive and less laborious (Elangovan thesis, 2022 unpublished data).

5.1.9 CdMV Resistance in Cardamom

Prolonged infection of CdMV helps the plants to develop natural resistance. To date, the Malabar variety of small cardamom is resistant to CdMV. In addition, the natural *katte* escape (NKE), IISR-Vijetha and Appangala 2, a hybrid of NKE, were found as resistant cultivars (Venugopal, 1999, 2002; Prasath et al., 2010; Kumar et al., 2017).

In addition, attempts were made to develop CdMV-resistant cardamom by pathogen-derived resistance. The diversity among the CP of various isolates might be utilized for CdMV resistance transgenically (Jacob et al., 2003). Jebasingh et al. (2017) developed the transgenic cardamom with the NIb gene, which might be a stepping stone in developing CdMV resistant variety.

5.2 Banana Bract Mosaic Virus

The Banana bract mosaic virus belongs to the Potyvirus genus of the *Potyviridae* family. The virus was first reported in banana plants in 1979. The occurrence of banana bract mosaic virus (BBrMV) in small cardamom was reported by Siljo et al. (2012). During the survey on CdMV in small cardamom, a new viral disease with a chlorotic streak on veins was observed.

5.2.1 Host

Bananas (*Musa* spp), flowering ginger (*Alpinia purpurata*) and small cardamom are the natural hosts for this virus.

5.2.2 Transmission

In bananas, the virus is transmitted through the aphids *viz Pentalonia nigronervosa, Aphis gossypii, Aphis craccivora and Rhopalosiphum maidis* in a non-persistent manner (Magnaye & Espino, 1990; Munez, 1992; Thomas & Magnaye, 1996; Selvarajan, 2006). The existence and breeding of *Pentalonia nigronervosa* in both bananas and cardamom might play a role in the horizontal transfer of the disease (Venugopal, 2002; Siljo et al., 2012). BBrMV is also transmitted through bits and suckers. Transmission through the soil was not reported (Thomas & Magnaye, 1996) (Fig. 5.2).

5.2.3 Symptoms

Spindle-shaped intravenous yellow or light green streaks appeared continuously or discontinuously along the veins and midrib. These streaks later combine and give veins a yellow or light green hue. In the pseudostem and petioles, there is a discontinuous mottling in the form of spindles. In severe cases, a plant that is afflicted has suppressed tillering. The condition is given the term “chlorotic streak” because of its distinctive feature, the development of intravenous chlorotic streak (Siljo et al., 2012).

5.2.4 Structure

BBrMV has flexuous filamentous particles with 700–850 nm in length and 12–15 nm in width (Ha et al., 2008; Bhat et al., 2018).

Fig. 5.2 Spindle-shaped streaks in veins and pseudostem. (Siljo et al., 2012)

5.2.5 Genome

BBrMV cardamom isolate was isolated from the Green gold variety of cardamom, and the complete genome was sequenced. The genome shared 96.7% identity with the genome of the banana isolate (Bhat et al., 2018). The virus consists of a monopartite genome that encodes a large ORF and a short overlapping ORF. The large ORF encodes a polyprotein which is then cleaved into 10 mature proteins, namely, P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa, NIb, CP and the short overlapping ORF encodes P3N-PIPO (Chung et al., 2008; Bhat et al., 2018).

5.2.6 Proteins of BBrMV

The P1 and NIa proteins of BBrMV were expressed in a heterologous system and characterized as serine protease (Patil et al., 2021). BBrMV CP is expressed and purified as a soluble protein (Dilip et al., 2020). The viral protein-host interaction was reported from the BBrMV (banana isolate) VPg, which interacts with the eukaryotic initiation factor 4E (Anuradha et al., 2021).

5.2.7 Detection

The detection methods of BBrMV include ELISA and RT-PCR. The detection of BBrMV in cardamom is by SYBR-green-based RT-qPCR, reverse transcription loop-mediated isothermal amplification (Siljo et al., 2012, 2014; Siljo & Bhat, 2014). BBrMV CP is a potent antigen for serological detection (Dilip et al., 2020). The on-site diagnostic kit developed with the CP helps to detect the BBrMV infection in the field sample (Selvarajan et al., 2020).

5.3 Cardamom Vein-Clearing Virus

The cardamom vein-clearing virus (CdVCV) causes vein-clearing disease, locally known as *kokke kandu*. The virus sequence was obtained through small RNA and transcriptome sequencing (Bhat et al., 2020). CdVCV belongs to the *Nucleorhabdovirus* genus of the *Rhabdoviridae* family (Bhat et al., 2020). The mixed infection of *Katte* and *Kokke kandu* disease was also observed in cardamom plantations (Bhat et al., 2020).

5.3.1 Disease Transmission and Symptoms

The disease is transmitted from infected to healthy plants through the aphid *Pentalonia caladii*. The symptoms include vein chlorosis, rosetting, loosening of leaf sheath and leaf shredding. It also forms hook-like tillers where freshly developing leaves become entangled in the older leaves. Other symptoms like leaf sheath mottling, fruit cracking and seed sterility caused by light green streaks with shallow furrows on the capsules (Venugopal, 2002).

5.3.2 Genome

The viral members of Nucleorhabdovirus contain a monopartite genome (Jackson et al., 2005). Due to the virus replication site being the nucleus (in contrast to the Cytorhabdovirus, which replicates in the cytoplasm), this virus genus was named Nucleorhabdovirus. They are enveloped viruses and have bacilliform structures. The size of the virions is about 45–100 nm in diameter and 130–300 nm in length (Bhat et al., 2020).

The CdVCV consists of a negative ss RNA genome with a 13.3 Kb size. The monopartite genome contains six ORF which encode nucleocapsid (N), phosphoprotein (P), movement protein (P3), matrix protein (M), glycoprotein (G),

Fig. 5.3 Cardamom leaves show vein chlorosis symptoms and emerging leaves entangled in the older leaves. (Bhat et al., 2020)

RNA-dependent RNA Polymerase (L) (Bhat et al., 2020). The nucleorhabdovirus G and M proteins are the structural components of the virion envelope, P3 is a movement protein, and the N, P, and L proteins are involved in the replication process by interacting with the genomic RNA and forming ribonucleoprotein core (Dietzgen et al., 2017). In addition, all six protein contains importin α dependent nuclear localization signals (NLS) in which the P, P3 and G proteins can localize to cytoplasm also (Goodin et al., 2001; Dietzgen et al., 2015; Wu et al., 2018; Bhat et al., 2020) (Fig. 5.3).

5.4 Nilgiri Necrosis Virus

The necrosis disease in cardamom is caused by the Nilgiri necrosis virus (NNV). Nilgiri necrosis disease got its name since it was initially identified in a severe form in Nilgris, Tamil Nadu. The disease incidence was also found in Kerala and Karnataka (Venugopal, 1995). Every cultivar of cardamom is prone to illness (Sridhar, 1988).

5.4.1 Transmission

The disease is transmitted through the infected rhizome and not through seed, soil or insect (Sridhar, 1988; Venugopal, 1995). In order to find the aphid for transmission, the cardamom aphid *Kanakarajiella cardamomi* was fed with the NNV-infected cardamom and the disease's transmission to healthy plants was studied.

The study proved that the *K. cardamomi* is not an NNV-transmitting vector (Selvakumaran et al., 1997), and the other studies also proved that the NNV is not transmitted through the aphid (*P. nigronervosa f. caladi*), thrips (*Sciothrips cardamomi*) and whitefly (*Dialeurodes cardamom*) (Nair, 2020).

5.4.2 Symptoms

The symptoms on immature leaves appear broken or continuous pale to yellowish streaks extending from the midrib to the leaf margins. These streaks turn reddish brown in the later stages of illness. Along these stripes, leaf shredding is frequently seen. Margin distortion results in a smaller leaf size. In the early stages of infection, plants only generate a small number of panicles and capsules, but as the infection progresses, tillers become severely stunted and are unable to produce panicles and capsules (Sridhar et al., 1991) (Fig. 5.4).

5.4.3 Virus Structure

The genome information of the Nilgiri necrosis virus was not reported so far. However, the preliminary study on the leaf dip electron microscopy indicated the presence of the flexuous rod-shaped virus particles. The particle with 570–700 nm × 10–12 nm in length and width, respectively. The virus belongs to the Carlavirus genus (Naidu & Thomas, 1994).

Fig. 5.4 Nilgiri necrosis virus-infected plants showing yellow streaks in cardamom leaves. (Source: Small cardamom: Diseases and Symptoms, Vikaspedia)

5.5 Infectious Variegation Virus

The uncharacterized virus found in Kerala and Karnataka cardamom plantations is tentatively named infectious variegation virus. The disease incidence is 15% (Venugopal, 1995).

5.5.1 Symptoms and Transmission

The infected leaf showed distinctive light green and dark green lamina-based radiating stripes that range from narrow to large. Other typical symptoms include stunting, tiller distortion, and leaf distortion. Within a year of infection, the infected plants become stunted with numerous tillers and lose their productivity. The aphid *P. nigronervosa f. caladii* was found to transmit the disease in just 2% of cases. The disease is virtually eradicated due to rouging in all plantations (Venugopal, 1995).

5.6 Conclusion

The small cardamom plant is infected with different viral diseases; sometimes, mixed viral infections are also observed. The diseases are identified through visible symptoms and several detection methods. The visual diagnosis fails in case of mixed infection, where the discrimination of one viral disease becomes cumbersome. Thus, the serological detection methods with the viral-specific antibody may help the detection process. In order to control the viral diseases of cardamom, genome characterization and PPI among the viral proteins and host proteins are needed. Nevertheless, these studies are limited to cardamom viruses. Complete studies on these viruses may help to control the disease and thus to increase cardamom production.

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Chapter 6
Cardamom Wild Genotypes

Lydia Ndinelao Horn, Fimanekeni Ndaitavela Shivute, and Selma Ndapewa Nghituwamhata

6.1 Introduction

Cardamom (*Elettaria cardamomum* Maton) is a cross-pollinated herbaceous, perennial monocotyledonous plant from the Zingiberaceae family and the order Scitamineae (Nadukeri et al., 2020). Cardamom is believed to be one of the largest families of monocotyledons, comprising about 52 genera and 1500 species (Nadiya et al., 2015). The herb belongs to three genera in the Zingiberaceae family: *Elettaria*, *Amomum* and *Aframomum* (Sabulal & Baby, 2021). Among the three genera, Cardamom (*Elettaria cardamomum* Maton), also known as small cardamom (Ashokkumar et al., 2020), has been one of the most popular and important spice crops since ancient times, with its dried capsules widely used in herbal medicines, spices, flavoring agents, and cosmetics (Korikanthimath et al., 2001; Nadiya et al., 2015; Anjali et al., 2016). Small cardamom originates from India's moist habitats of the evergreen forest of the Western Ghats in the Southern part of the country (Prasannan & Jose, 2021; Nadiya et al., 2015). The large cardamom, also known as false or black cardamom, belongs to the *Amomum* and *Aframomum* family, and it is native to Nepal, Sikkim, Bengal and Southeast Asian countries. In contrast, the African cardamom (*Aframomum danielli* (Hook.f.) K. Schum) is native to Africa and commonly found in south-eastern parts covering Tanzania, Cameroon, Madagascar, and Guinea (Ashokkumar et al., 2020). Internationally the three

L. N. Horn (✉) · F. N. Shivute
Zero-Emission Research Initiative (ZERI) Multidisciplinary Research (MR), Centre for the Research Services University of Namibia, Windhoek, Namibia
e-mail: lhorn@unam.na

S. N. Nghituwamhata
Regional Universities Forum for Capacity Building in Agriculture (RUFORUM), Kampala, Uganda

M. F. Ramadan (ed.), *Cardamom (Elettaria cardamomum): Production, Processing and Properties*, https://doi.org/10.1007/978-3-031-35426-7_6

cardamom groups are classified as green cardamom, black cardamom, and Madagascar cardamom (Heryanto & Syukur, 2021).

The crop is mainly propagated by seeds and grows well under 40–50% shade cover, which hinders its production (Nadukeri et al., 2020; Mathew et al., 2022). As crop production continues under shade in the native habitat, demands for developing varieties adaptable to various climatic conditions and reducing crop vulnerability by promoting genetic diversity are increasing (Ashokkumar et al., 2020). Additionally, the cultivated cardamom has a narrow genetic base, which also affects its production, and thus a need to broaden its genetic base by developing new varieties with wide adaptability (Mathew et al., 2022). This chapter provides information on using wild relatives of cardamom to increase its genetic base for developing and improving potential commercial varieties.

6.2 Importance and Uses of Cardamom

Cardamom is favored for its medicinal and aromatic benefits (Sarac, 2021; Malhotra et al., 2021), smell, flavor, and nutritional quality (Nair, 2020), as well as income and employment for local households (Kettel et al., 2020; Jamir, 2021). It is known as the *Queen of spices* due to its popularity and importance, making it the third most expensive spice in the world after saffron and vanilla (Nadiya et al., 2015). Cardamom capsules contain a distinctive aroma, and nutraceutical and pharmaceutical substances are primarily attributed to their essential oils and other high-value antioxidant and gastroprotective bioactive metabolites (Hamzaa & Osman, 2012). Study by Ashokkumar et al. (2020) revealed from the chemo profiling of GC-MS analysis that∼98% of the essential oils were represented by eleven bioactive phytochemicals, while the remainder were trace levels.

6.3 Agronomic Practices and Production of Cardamom

Cardamom is a cross-pollinated crop mostly propagated through seeds and suckers with a large diversity of wild and cultivated features in its natural population (Bhat & Goudar, 2020). The small cardamom is widely cultivated in India, Guatemala, Sri Lanka, Nepal, Indonesia, Costa Rica, Mexico, and Tanzania (Ashokkumar et al., 2020). However, its production is widely affected by climate-related indicators (Maharjan et al., 2019), thus a need for urgent intervention to curb the effects of climate change on the production of this economically important crop. One of the major problems in cardamom production is insect pests such as thrips (*Sciothrips cardamomi*) that attack its capsules, changing its texture and appearance. The increase in pest damage led to the uncontrolled use of pesticides in the growing area, which wiped out the natural enemies of pests (Murugan et al., 2017; Ashokkumar et al., 2020). Possible interventions should focus on integrated

management concerning agronomic practices, plant protection measures, the development of new varieties, and post-harvest handling approaches (Sharma & Katoch, 2019).

6.4 Genetic Diversity and Variation Among Cardamom Germplasm

The basic chromosome number for cardamoms is X = 12 and 2n = 48, which is a balanced tetraploid in nature (Heryanto & Syukur, 2021). Genetic diversity refers to the variations among the alleles of a gene, and it may be examined at the nucleotide level within the DNA sequence (Singh et al., 2021). The genetic makeup of any organism's genotype describes its complete set of genes. Padmini et al. (2000) revealed variations in economically significant features within and across tiny cardamom cultivars. In the search for productive genotypes, there is a need to collect and evaluate germplasms to identify the level of variation in the cardamom germplasm (Nadukeri et al., 2020). Diversity in both the wild and cultivated forms of cardamom has been reported in several studies that linked a large genetic variability of cardamom to India, the center of origin. Understanding the genetic variability among genotypes in a breeding program helps efficiently utilize the genotypes (Prasannan & Jose, 2021). A study on the analysis of intraspecific variation in cardamom using genome size, cytological studies, and molecular marker data revealed the existence of genetic variability among cardamom accessions and a significant difference in their genome size (Anjali et al., 2016). A diversity study on promising and released genotypes developed through selection by various research institutions in India has revealed closeness among genotypes by forming clustered together (Gaur et al., 2012). A separate study analyzed the genotypes of cardamom and found that no duplicates were observed among germplasm collections and that they were distinct from each other as a reflection of diversity (Nirmal et al., 2012). Furthermore, Kumar et al., (2018) have reported three different morphotypes in cardamom. These include Malabar, Vazhukka, and Mysore. The Vashukka and Mysore types are believed to be stronger than the Malabar morphotypes.

The differences in traits of economic importance differ from panicle type, non-branched to branched raceme, female sterility, and cleistogamy (Madhusoodanan et al., 1994; Padmini et al., 2000; Kumar et al., 2018). Cardamom germplasm exhibits rich genetic diversity that could be utilized for innumerable agronomic traits such as yield attributes, quality, drought tolerance, insect pest, and disease-tolerant attributing characters (Zakir, 2019). Genetically resistant varieties have been the major component in integrated disease management (IDM) among various breeding programs (Kumar et al., 2018). The above information shows that there is high diversity among the wild relatives of cardamom that need to be exploited to benefit this crop's further advanced development.

6.5 Wild Genotypes and Their Contribution to Cultivar Development

Cardamom is prone to pests and diseases that result in total production reductions and high yield loss. However, the wild cardamom genotypes are rich in some novel alleles that could positively contribute to the genetic improvement of the cultivars (Nadiya et al., 2015). The genetic materials from the wild relatives can be crucial in developing new breeds and improving the existing varieties to generate superior cultivars with desirable characteristics, including tolerance to pests and diseases (Nadiya et al., 2019). Various research activities have been reported on the genetic variation analysis of wild cardamom accessions using various biotechnological methods. For example, microsatellite markers were successfully developed using the selective hybridization enrichment method to characterize Cardamom with 140 microsatellite repeats identified from 270 clones (Cyriac et al., 2016). A different study evaluated different cardamom accessions and found high diversity among the germplasm. Some were identified as related to the wild relatives on phylogenetic analysis using a dendrogram that revealed a complex distribution pattern of how diverse the accessions under study were (Nadiya et al., 2015). A diversity study on thirty accessions by Anjali et al. (2016) using C53 (feral from Bonacaud) revealed a high level of genetic diversity ($h = 0.38$, $I = 0.54$, $P = 80.77\%$) and the analyzed plant specimens showed a significant difference among their genomes. A study on intraspecific variations reported by Anjali et al. (2016) revealed genetic variability within wild cardamom accessions.

Some prominent steps in defining descriptors for cultivars and breeding and introducing new alleles from unutilized germplasm accessions include morphological characteristics and marker-assisted research (Ranjanan et al., 2021; Gurung, 2020; Jacob et al., 2020). On the other hand, genetic diversity studies on cardamom using inter-simple sequence repeat (ISSR) markers were reported to have found a high level of genetic redundancy (Anisha et al., 2020). The ISSR markers were also found to have differentiated the small cardamom genotypes based on yield (Prasannan & Jose, 2021), a milestone achievement in cardamom improvement programs. In the study of Cardamom as a spice and medicine, miRNA profiling of cultivar and wild cardamom genotypes concluded that wild genotypes constitute stronger drought tolerance and have better floral development than cultivated ones (Nadiya et al., 2019). Various research studies suggest that the wild relatives of Cardamom are still the base of genetic materials that can be utilized to develop valuable and diverse germplasm. Hybridization with the wild relatives could therefore be the best option for developing better varieties and broadening the genetic base of cultivated cardamom than selection.

6.6 Genetic Characterization of Cardamom Germplasms

Genetic resource characterization identifies or classifies plant accessions (de Vicente et al., 2005). Characterization in the context of gene banks and germplasm management describes traits often highly heritable, visible to the naked eye, and equally expressed across all habitats. At the same time, genetics is the process of identifying variation brought on by variations in DNA sequences, particular genes, or modifying influences (de Vicente et al., 2005). Genetic characterization describes heritable traits according to the Mendelian principle, including particular DNA sequences (Miglani, 2000). Conservation of genetic resources involves acquiring germplasm, preserving it, and assessing beneficial features to take advantage of the knowledge produced by applying molecular marker technologies (de Vicente et al., 2005). Two fundamental strategies for protecting genetic resources are *in-situ* and *ex-situ* conservation. When a specie is allowed to continue in its ecosystem as part of a natural or carefully managed ecological continuity, it is said to be *in situ.* At the same time, *ex-situ* involves conservation in botanic gardens and keeping seeds or vegetation in genebanks (Khanna & Neeta, 2012). The main objective of genetic resource conservation is to maintain widespread genetic variation within each species with a known or potential value to ensure that it is accessible for use to both current and future generations (Khanna & Neeta, 2012).

6.7 Conservation of Cardamom

Genetic resource conservation is the best way to conserve the biodiversity of various species, including plants, and it is done through *in vitro* and cryopreservation techniques, especially when conventional storage methods are ineffective or scarce (Agrawal et al., 2021). The selection of cardamom for better agronomic traits started around 210 years ago (Anjali et al., 2016). However, the conservation of cardamom genetic resources only started in the early 1960s, and some of the collected germplasm accessions are conserved in ex-situ and *in vitro* germplasm (Muchie, 2021; Mathew et al., 2022), while some remain in clonal repositories in the field for protection (Zakir, 2019; Malik et al., 2022). Approximately 600 variants of cardamom are preserved under in-situ germplasm conservation at the National Active Germplasm Site at ICAR-IISR Regional Station, Appangala, India (Anjali et al., 2016).

Depletion of forest area, the extensive distraction of forest tree growths as well as the changes in the agro-ecological conditions have collectively led to a sharp decline in the population of many species, including cardamom, a worldwide concern that is making the conservation of genetic diversity an important aspect of nature (Saji et al., 2019; Gupta et al., 2019). Therefore, the priorities of breeding programs worldwide should focus on the conservation and utilization of the genetic resources of cardamom.

6.8 Importance of Genetic Characterization and Conservation

Characterization of any germplasm is the first step toward proper utilization of any germplasm as it gives a comprehension of the variability in yield that informs the utilization of the genotypes in breeding programs (Prasannan & Jose, 2021). Therefore, proper utilization of germplasm material depends on the appropriate and adequate characterization of the available variability after its collection and conservation. Furthermore, crop breeders require a diverse breeding population to select genetically diverse breeding stock. Therefore, genetic identification is the first important stage in any breeding operation, and molecular markers are valuable tools for identifying and characterizing a variety of genotypes (Ramakrishnan et al., 2019).

Understanding the genetic composition of accessions and variations in different characters enables the conservation of germplasm, which involves gathering and managing germplasms through gene identification to increase the value of genetic resources (De Vicente et al., 2005; Nadukeri et al., 2020). De Vicente et al. (2005) have outlined some of the uses of genetic characterization in the conservation of crop germplasm as follows:

1. Genetic characterization offers trustworthy data for determining the level of genetic variation, the composition of samples and populations, and the magnitude of variations in traits and diversity between populations, found in various regions.
2. Molecular characterization helps determine the reproductive success of individuals, species' breeding patterns, and the presence of gene flow.
3. Molecular data enables the interpretation of phylogeny and provide fundamental information for understanding taxonomy, domestication and evolution.
4. Molecular information is used to determine the need for decreasing the size of germplasm collections
5. Molecular technologies provide cost-effective and comprehensive genotypic profiles of accessions in germplasm identification.
6. Molecular data serve as the benchmarks for tracking changes in the genetic structure of the accession caused by natural selection or human intervention.
7. Genetic characterization helps identify useful genes in germplasm to maximize conservation efforts.

6.9 Conclusion

The importance of cardamom in the spice, medicine and cosmetic industry is widely known. However, cardamom production in its native habitat has increased over the years, posing a threat to its narrow genetic base. Furthermore, the depletion of forests and other factors such as drought and pests also threatens cardamom production

as a shade crop. Therefore, there is an increasing demand for developing new varieties adaptable to various climatic conditions to increase and broaden their genetic diversity. To achieve this, there is a need to dig deeper into the understanding of the crop by utilizing the wild relatives of the crop commonly found in India, which is the center of origin for the crop. Genetic characterization and conservation of cardamom germplasm is a major step that will lead to adequately identifying genotypes in breeding programs. Several genetic diversity studies in India revealed genetic variability within the wild relative accessions of cardamom, providing a breakthrough to the breeding programs.

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Part II
Cardamom: Chemistry, Functionality and Health-Promoting Properties

Chapter 7
Composition and Functional Properties of Cardamom Seeds

Chin Xuan Tan, Seok Shin Tan, and Seok Tyug Tan

Abbreviations

Aβ42	amyloid beta peptide
GC-MS	Gas chromatography-mass spectrometry
HOSC	Hydroxyl radical scavenging capacity,
MRSA	Methicillin-resistant *Staphylococcus aureus*
ORAC	Oxygen radical absorbance capacity,
PTSD	Post-traumatic stress disorder
RDSC	Relative DPPḤ radical scavenging capacity
SCO_2 seed extract	supercritical carbon dioxide cardamom seed extract

7.1 Introduction

Cardamom (*Elettaria cardamomum*), also known as '*Queen of Spice*', true cardamom, or green cardamom, is a plant species belonging to the Zingiberaceae family. As one of the world's highly prized and most expensive spices (Chempakam & Sindhu, 2008), it is widely cultivated in the moist, well-drained soil of the shady area. The ovoid, tri-lobular cardamom capsule (fruit) changes from green to golden yellow upon ripening.

C. X. Tan (✉)
Department of Allied Health Sciences, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti Bandar Barat 31900, Kampar, Perak, Malaysia
e-mail: tanchinxuan@ymail.com; tancx@utar.edu.my

S. S. Tan · S. T. Tan
Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway 47500, Selangor, Malaysia

M. F. Ramadan (ed.), *Cardamom (Elettaria cardamomum): Production, Processing and Properties*, https://doi.org/10.1007/978-3-031-35426-7_7

Table 7.1 Physical measurements of cardamom

Region	Weight of capsules (g)	Husk (%)	Seed (%)
Karnataka			
Coorg	23–25	26.0–27.0	73.0–74.0
Mudigere	23–24	25.5–28.0	72.0–74.5
Kerala			
Alleppey green	23	27.7	72.3
Wayanad	20–22	28.0–38.0	62.0–72.0
Tamil Nadu			
Nelliampathy	12–18	26.0–31.0	74.0
Yercaud	23–26	27.0	73.0

Source: Chempakam and Sindhu (2008)

Since the fourth century BC, the cardamom capsule has been used by Indian Ayurveda, Roman, and Greek doctors in treating health conditions such as constipation, asthma, bronchitis, cold, cough, carminative, and gum infection (Ashokkumar et al., 2020). The seed, covered with a white mucilaginous coat and embedded inside the cardamom fruit, has been studied for its functionality in food, pharmaceutical, and cosmetic applications. This chapter aims to review the physical and chemical composition of cardamom seeds. Besides, the functional traits of cardamom seeds are also compiled and summarized.

7.2 Physical Measurements of Cardamom

India is one of the main producing countries of cardamom. The cardamom capsule is ready to be harvested after about 120 days of flowering. Ripe cardamom contains 15–20 seeds per capsule. The plants are widely cultivated in altitudes of 900–1400 m above the mean sea level of south India, which are Karnataka, Kerala, and Tamil Nadu (Ashokkumar et al., 2020). The physical measurements of cardamoms harvested from different regions of south India are shown in Table 7.1. The seeds contributed most of the cardamom capsule (62–74.5%), followed by the husk (25.5–38.0%).

The proportional distribution of seed husks of different varieties of cardamom seeds, Mysore, Malabar, Guatemala, and Vazhukka, were summarized in Table 7.2. The proportion of seed was greater than the husk, corresponding to the results reported in Table 7.1.

7.3 Macronutrients and Micronutrients of Cardamom Seed

The proximate composition is important for product development and quality control of foods. Irrespective of the varieties, cardamom seed was rich in carbohydrates (42.1–49.0%), protein (11.4–13.5%), and crude fiber (8.50–13.1%) (Table 7.3).

Table 7.2 Proportional distribution of different varieties of cardamon

Variety	Husk (%)	Seed (%)
Mysore	38	62
Malabar	24	76
Vazhukka	30	70
Guatemala	34	66

Source: Padmakumari Amma et al. (2010)

Table 7.3 Composition of cardamom seed

Parameter	Mysore	Malabar	Vazhukka	Guatemala
Carbohydrates	42.42	49.05	42.95	42.14
Protein	13.53	12.72	11.40	13.06
Moisture	10.33	9.00	11.00	10.33
Oil	7.90	8.79	7.9	8.60
Ash	8.07	6.97	8.16	8.52
Crude fiber	13.16	8.50	11.70	12.09

Source: Padmakumari Amma et al. (2010)

More than 70% of the proteins ingested by humans are sourced from storage proteins of plant seeds and legumes (Krishnan & Coe, 2001). The proteins content of cardamom seed was comparable with bitter melon seed (11.8%) but lower than Kalahari melon (24.5%), kenaf (21.8%), pumpkin (26.5%), and roselle (14.9%) seeds (Nyam et al., 2009).

Meanwhile, the ash content of cardamom seed (6.97–8.52%) was greater than bitter melon (3.6%), Kalahari melon (2.6%), kenaf (5.9%), pumpkin (4.6%), and roselle (5.1%) seeds (Nyam et al., 2009). This indicates that the cardamom seed is an excellent source of minerals. Potassium (843.2 mg/100 g), phosphorus (151.2 mg/100 g), calcium (109.8 mg/100 g), and magnesium (102.7 mg/100 g) were the main mineral constituents of cardamom seed (Abera et al., 2019). Other minerals found in the seed were selenium (37.14 mg/100 g), manganese (29.32 mg/100 g), iron (11.66 mg/100 g), zinc (1.57 mg/100 g), and boron (0.41 mg/100 g). Limited information on the vitamin composition of cardamom seed has been reported. However, two lipid-soluble vitamins were detected in the cardamom seed oil. The total carotenoids and tocopherols of cardamom seed oil were 0.05 μmol/kg and 38.4 μmol/kg (Parry et al., 2006). Individual tocopherols isolated from the cardamom seed oil were α- (10.4 μmol/kg), γ- (4.3 μmol/kg), and δ- (4.3 μmol/kg) tocopherols. The amounts of carotenoids and tocopherols of cardamom seed oil were the lowest compared to other seed oils (onion, parsley, pumpkin, mullein, and milk thistle) investigated under the same experimental conditions (Parry et al., 2006).

7.4 Fatty Acids Composition

Table 7.4 shows the fatty acids profiles of oils in the cardamom seed and its defatted seed flour. Oleic (49.2%), palmitic (26.4%), and linoleic (15.2%) acids were the predominant fatty acids in cold-pressed cardamom seed oil. Compared with other seed oils, it was found that the total monounsaturated fatty acids of cardamom (51.3%) were lower than parsley (81.0–81.1%) but greater than onion (25.4–26.6%), mullein (16.4%), pumpkin (36.7%), and milk thistle (25.2%) (Parry et al., 2006; Ramadan et al., 2022).

Extraction of oil from the plant seed generates seed meal as a by-product. Depending on the oil extraction parameters, this seed meal may still contain a low amount of oil. Mechanical extractions such as cold-pressing and extrusion expelling often result in lower oil recovery than organic solvent extractions. As evidenced by Parry et al. (2008), the oil yields of cold-pressed seed meals when subjected to Soxhlet extraction were in the order of parsley (17.6%) > pumpkin (12.3%) > milk thistle (7.5%) > cardamom (0.7%). The proportional distribution of the individual fatty acids in the oil isolated from the raw cardamom seed is distinctly different from the oil isolated from the cardamom seed meal. Palmitic (47.6%), oleic (40.6%), and stearic (6.6%) acids were the predominant fatty acids in the oil extracted from cardamom seed meal. More than 55% of the oil isolated from the cardamom seed meal was composed of saturated fatty acids (Table 7.4). This is in contrast to the oil isolated from raw cardamom seeds, where saturated fatty acids constituted 30.8% of the total fatty acids. The difference might be attributable to high temperatures and extraction durations in the Soxhlet extraction. Generally, mild extracting conditions used in oil extraction could better preserve the polyunsaturated fatty acids (Tan et al., 2018).

Table 7.4 Fatty acids composition

	Oil	
Fatty acid (g/100 g)	Raw seed[a]	Seed meal[b]
C14:0	1.5	1.0
C16:0	26.4	47.6
C18:0	2.3	6.6
C20:0	0.4	1.0
C16:1	1.6	1.5
C18:1	49.2	40.6
C20:1	0.5	0.9
C18:2	15.2	0.7
C18:3	2.7	ND
Total saturated	30.8	56.2
Total monounsaturated	51.3	43.1
Total polyunsaturated	17.9	0.7

Sources: [a] Parry et al. (2006); The oil was obtained by cold-pressing
[b]Parry et al. (2008); The oil was obtained by Soxhlet extraction
ND Not detected

7.5 Phytochemicals Content of Cardamom Seed

Phytochemicals are compounds derived from plants that may provide benefits to human health. A solvent or a mixture of solvents is required to extract these phytochemicals from the plants (Abubakar & Haque, 2020). Screening is a preliminary step to detect the existence of phytochemicals in the extract. Table 7.5 shows the phytochemical screening of cardamom seed extracts. Compounds such as alkaloids, saponins, and quinones were not detected. Due to the variation in the finding results, the presence of tannins, which are astringent water-soluble polyphenols, in cardamom seed extracts remains inconclusive. Such conflicting results could be explained by the use of different experimental protocols in the screening of tannins. Literature data indicate the presence of flavonoids and terpenoids in the seed extracts; however, information on the types and quantity of these compounds in the extract is scarce.

Polyphenols are phytochemicals with antioxidant properties. The polyphenol compounds detected in the methanolic cardamom seed extract include the derivatives of caffeic acid, gallic acid, *p*-coumaric acid, ellagic acid, hydroxycinnamic acid, catechin, stilbene, quercetin rutinoside, quercetin-3-coumarylo-galactoside, quercetin 3-oglucuronide, and sinapic acid (Joanna Brodowska et al., 2014). Three polyphenols (quercetin, rutin, and kaempferol) were isolated from the methanolic cardamom seed extract using thin-layer chromatography (Masoumi-Ardakani et al., 2017).

7.6 Microbiological Content of Cardamom Seed

Cardamom seed is exposed to various environmental contaminants during post-harvest processing. In addition, one of the main usages of cardamom seed is a raw ingredient in preparing desserts, curries, meat dishes, and beverages. Hence, it is worth investigating the microbiological quality of this seed.

Table 7.5 Phytochemical screening of cardamom seed extracts

Parameter	Methanolic extract[a]	Methanolic extract[b]	Aqueous extract[c]
Alkaloids	×	×	ND
Flavonoids	√	√	√
Saponins	×	×	ND
Tannins	×	√	√
Quinones	×	ND	×
Terpenoids	√	ND	√

Sources: [a]Bano et al. (2016)
[b]Masoumi-Ardakani et al. (2017)
[c]Cárdenas Garza et al. (2021)
ND Not determined
√: Present, ×: Absent

Table 7.6 Total mesophilic, fungal, and *Enterobacteriaceae* counts

Analysis	CFU/g	
	Raw seed	Ozone treatment
Total mesophilic bacteria count	10^5	10^3
Total fungal count	10^2	<10
Total *Enterobacteriaceae* count	10^2	<10

Source: Joanna Brodowska et al. (2014). Not detected at the level 10 CFU/g

Table 7.6 shows the total count of mesophilic bacteria, fungal, and *Enterobacteriaceae* of cardamom seed. Total mesophilic bacteria count, also known as total plate count, is used to estimate the bacterial population of a food sample. Good quality spices contain a total mesophilic bacteria count of $<10^4$ (International Commission on Microbiological Specifications for Foods, 2005). The level of mesophilic bacteria count in cardamom seed (10^5 CFU/g) was the same as the cardamom spices (10^4–10^9 CFU/g) sold in the retail spice outlets of India (Banerjee & Sarkar, 2003), indicating the occurrence of bacterial contamination. Four bacteria (*Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus megaterium*, and *Bacillus pumilus*) were isolated from the cardamom seed (Joanna Brodowska et al., 2014). Among these, the number of *Aeromonas hydrophila* (10^5 CFU/g) and *Bacillus cereus* (10^4 CFU/g) were the highest. Meanwhile, the fungal and *Enterobacteriaceae* counts of the seed were within acceptable levels (International Commission on Microbiological Specifications for Foods, 2005).

The use of contaminated cardamon seed in ready-to-eat food may lead to food spoilage and causes foodborne outbreaks. The high number of mesophilic bacteria in cardamom seeds may attribute to the poor sanitation of post-harvest processing. The effect of ozone treatment on the microorganism content of cardamom seed was investigated by Joanna Brodowska et al. (2014). After being treated with ozone (concentration: 160–165 g/m^3, pressure: 0.5 atm, and flow rate: 0.1 L/min) three times, the total mesophilic bacteria count of the seed reduced to the level that was safe to be consumed by human (Table 7.6). In addition, a quantification study indicated that the number of *Aeromonas hydrophila* and *Bacillus cereus* were reduced to 10^2 CFU/g, and the *Bacillus megaterium* and *Bacillus pumilus* were not detected in the ozone-treated seed. Moreover, the total fungal and *Enterobacteriaceae* counts were below the detection limit (<10 CFU/g). These results suggest ozone treatment could be feasible to ensure the cardamom seed is microbiologically clean and safe for direct consumption.

7.7 Volatile Organic Composition of Cardamom Seed

Gas chromatography-mass spectrometry (GC-MS) is an analytical instrument used to identify the organic compounds of a food sample. The volatile composition of three different varieties of cardamom seed was reported by Debabhuti et al. (2021).

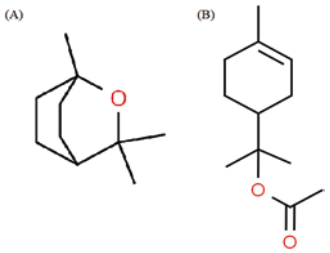

Fig. 7.1 Chemical structures of (**a**) 1,8-cineole and (**b**) α-terpinyl acetate

Six volatiles were detected when the powdered seed sample was subjected to a headspace GC-MS analysis. The average concentrations of these volatiles were in the order of 1,8-cineole (73.3%) > α-terpinyl acetate (16.06%) > α-pinene (6.49%) > β-pinene (2.66%) > γ-terpinene (1.32%) > terpinen-4-ol (0.12%).

Both 1,8-cineole and α-terpinyl acetate are the main volatiles in the cardamom seed extract but do not show the same trend. The concentration of α-terpinyl acetate (30.6–41.2%) was greater than 1,8-cineole (22.0–28.1%) (Paul et al., 2020; Yassin et al., 2022). The difference might be due to the experimental protocol used. Commonly, an extract requires to be dissolved in a solvent (e.g., hexane, methanol, and ethyl acetate) and centrifuged to obtain a supernatant. The supernatant is then injected into a GC-MS for volatile analysis. Meanwhile, the volatile constituents are usually investigated for the seed sample by placing it into a vial and sealed. The vial is heated for a particular duration, and the vapor released is then introduced to a GC-MS for volatile analysis. Irrespective of the experimental protocols, 1,8-cineole and α-terpinyl acetate are the main volatiles in the cardamom seed.

Figure 7.1 shows the chemical structures of both compounds. 1,8-cineole, or eucalyptol, is a colorless monoterpene with a camphor-like odor and spicy, cooling taste. This compound was well-documented in alleviating oxidative stress and inflammatory responses *via* regulating the nuclear factor-kappa B and nuclear factor erythroid-2-related factor 2 pathways (Cai et al., 2021). On the other hand, α-terpinyl acetate is a monoterpene ester with herbaceous floral and lavender odor. In addition, this compound demonstrated a high antimicrobial effect against dermatophytes and fungi (Vaičiulytė et al., 2021).

7.8 Functional Properties

7.8.1 Food Preparations and Skin-Penetration Enhancing Properties

Coffee contains caffeine and natural acid compounds such as chlorogenic and organic acids. Brewing coffee releases these acid compounds, and people with acid reflux, irritable bowel syndrome, and gastric ulcers might not be suitable to take coffee regularly. Coffee could brew together with cardamom seed to reduce the acid

in coffee and neutralize the effects of caffeine (Douillard, 2012). Besides, chewing the seed can sweeten the breath (Chempakam & Sindhu, 2008). Therefore, the seed has been used a natural breath freshener for centuries.

Cardamom oleoresin is a resinous product containing the volatile and non-volatile components of the seed. The aroma of cardamom oleoresin is stronger than the raw seed but weaker than the essential oil. Cardamom essential oil has been described as warm, sweet, spicy, citrusy, and lightly camphorated (Chempakam & Sindhu, 2008). These aromas are associated with a variety of terpenoids in the oil. It has been reported that the exposure of cardamom oleoresin and essential oil to acid, oxygen, light, or heat will greatly influence these terpenoids and might lead to an undesirable petroleum-like aroma (Chempakam & Sindhu, 2008). This limits the usage of cardamom oleoresin in food preparation that requires high temperatures and acidic conditions. One way to overcome this shortcoming is through microencapsulation. According to Krishnan et al. (2005), microencapsulation of the cardamom oleoresin with gum arabic could entrap the aroma for 6 weeks. This allows the application of cardamom oleoresin in beverages at lower ranges of pH (pH < 4) and the manufacture of pastry products that uses high oven temperature (> 150 °C).

On the other hand, cardamom essential oil could interact with the skin's stratum corneum and increase the active compounds' diffusion capacity by the lipid intercellular pathway (Sengottuvelu, 2011). As evidenced by Huang et al. (1995), incorporating 1% cardamom essential oil into 2% of indomethacin, piroxicam, and diclofenac enhanced the absorption of these drugs. The skin-penetration-enhancing properties of cardamom essential oil are mainly due to cyclic monoterpenes (Sengottuvelu, 2011).

7.8.2 Antioxidant Properties

Antioxidants are natural or synthetic compounds that can scavenge and suppress free radicals formation in the human body. In the food industry, antioxidants are used to prevent or delay the oxidation of food products. The antioxidant activity could be monitored by an array of assays with various mechanisms, including reducing power, hydrogen atom transfer, metal chelation, and single electron transfer, among others.

Several assays have been utilized to measure the antioxidant potential of defatted cardamom seed flour (Table 7.7) and cardamom seed oil (Table 7.8). The oxygen radical absorbance capacity (ORAC) value of defatted cardamom seed flour was the lowest compared to the defatted milk thistle, mullein, and parsley (98.2–1130.7 μmol TE/g) flours. On the contrary, the ORAC value of cardamom oil was greater than milk thistle, onion, and mullein seed oils but lower than parsley seed oil. It was reported that the peroxyl radical could interact with unsaturated fatty acids in membranes to induce lipid peroxidative damage. Both studies done by Parry et al. (2006, 2008) hypothesize that the lipophilic antioxidants of cardamom seed correspond to the scavenging potential of peroxyl radicals.

Table 7.7 Antioxidant capacity of defatted seed flour

	μmol TE/g seed flour		
Seed flour	ORAC	HOSC	RDSC
Cardamom	35.3	22.6	19.5
Parsley	390.0	311.5	18.1
Mullein	98.2–127.3	74.3–75.3	21.2–24.0
Milk thistle	1130.7	893.0	61.1

Source: Parry et al. (2008)
μmol TE/g Trolox equivalents in micromoles per gram, *ORAC* Oxygen radical absorbance capacity, *HOSC* Hydroxyl radical scavenging capacity, *RDSC* Relative DPPH radical scavenging capacity

Table 7.8 Antioxidant capacity of seed oils

Seed oil	ORAC (μmol TE/g oil)	RDSC (%)
Cardamom	941.5	58.2
Parsley	537.0–1097.5	9.2–13.4
Mullein	26.9	ND
Milk thistle	125.2	67.3
Onion	4.6–17.5	22.7–24.2

Source: Parry et al. (2006)
μmol TE/g Trolox equivalents in micromoles per gram, *ORAC* Oxygen radical absorbance capacity, *RDSC* Relative DPPH radical scavenging capacity, *ND* Not determined

Hydroxyl radical is a potent mutagen that attacks DNA to form different base and ribose derivatives. It is one of the most reactive oxygen species in the biological system. Compared with other defatted seed flours (74.3–311.5 μmol TE/g), cardamom seed flour's hydroxyl radical scavenging capacity (HOSC) value was the lowest. This indicates that defatted cardamom seed flour exhibits a low antioxidant effect toward hydroxyl radicals.

The effect of antioxidants on DPPḤ is linked to their hydrogen-donating ability (Tan et al., 2022). The relative DPPḤ radical scavenging capacity (RDSC) value of defatted cardamom seed flour was greater than defatted parsley seed flour but lower than defatted mullein and milk thistle seed flours. Meanwhile, the DPPḤ radical scavenging ability of cardamom seed oil was stronger than onion and parsley seed oils but lower than milk thistle seed oil. These suggest cardamom seed exhibits a moderate scavenging effect towards free DPPḤ radical, compared with other defatted seed flour and seed oil investigated under the same experimental conditions.

7.8.3 Pharmacological Properties

Cardamom seed extract has proven to have numerous benefits to human health. These benefits are summarized below:

7.8.3.1 Antimicrobial

The antimicrobial potentials of cardamom seed extracts obtained from different solvents, namely, ethanol, hexane, ethyl acetate, and water, were investigated by Yassin et al. (2022). Among these four extracts, ethyl acetate seed extract exhibited the greatest antibacterial proficiency against *Salmonella typhimurium,* whereas the ethanol seed extract exhibited the greatest antibacterial proficiency against *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Escherichia coli*. Meanwhile, aqueous seed extract showed no antimicrobial action against *Pseudomonas aeruginosa*. These microorganisms are the common causative agents of food poisoning. Therefore, Yassin et al. (2022) concluded that ethanol seed extract, rich in α-terpinyl acetate and 1,8-cineole, could be utilized as a natural preservative effective in inhibiting microbial growth in food products.

7.8.3.2 Anti-Anxiety

Post-traumatic stress disorder (PTSD) is an anxiety disorder characterized by numbing, hyperarousal, and recurring flashbacks and nightmares. Results of the elevated plus-maze rotarod open-field tests indicated that the anxiety-like behaviors in the PTSD rats were significantly improved after receiving the treatment of 400 mg/kg of methanolic seed extract (Masoumi-Ardakani et al., 2017). Furthermore, a previous study indicated that quercetin influenced corticotrophin-releasing factor-induced anxiety through $GABA_A$ receptors (Bhutada et al., 2010). Hence, the anxiolytic effects of cardamom seed extract could be attributable to the presence of quercetin.

7.8.3.3 Anti-Alzheimer Activity

Alzheimer's is a neurodegenerative disorder characterized by an insidious onset with slow impairments in cognitive functions. Monoterpenoids such as 1,8-cineole and α-terpinyl acetate have been proposed as potential modulators of Alzheimer's disease (Habtemariam, 2018). Under optimized conditions (Pressure: 200 bar, Extraction time: 90 min, and temperature: 50 °C) of the supercritical carbon dioxide extraction, cardamom seed extract enriched in 1,8-cineole (SCO_2 seed extract) was obtained (Paul et al., 2020). Owing to strong anti-oxidative activity, SCO_2 seed extract and pure commercial 1, 8-cineole extract prevented the ferrous and ascorbate-induced hydroxyl radical formation. Meanwhile, SCO_2 seed extract was more potent than commercial 1, 8-cineole extract in preventing the oligomerization of amyloid beta peptide (Aβ42), a main amyloid plaque found in the brain of patients diagnosed with Alzheimer's disease. This may be due to the synergistic effects of other monoterpenoids in the SCO_2 seed extract in inhibiting Aβ42 aggregation. In another study, ethanol cardamom seed extract and α-terpinyl acetate were evaluated for their

anti-Alzheimer potentials (Chowdhury & Kumar, 2020). Results indicated the inhibitions of acetylcholinesterase and butyrylcholinesterase enzymes of ethanolic cardamom seed extract were better than α-terpinyl acetate, possibly due to the presence of other monoterpenoids or phytochemicals in the seed extract.

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Chapter 8
Composition and Functional Properties of Cardamom Essential Oil

Mustafa Kiralan, Onur Ketenoglu, and Sündüz Sezer Kiralan

8.1 Introduction

In recent years, the demand for natural products has received increasing attention. Plant essential oils are important sources of natural products due to their various biological properties, including antibacterial, antifungal, antiviral, antimycotoxigenic, and antioxidant activities (Tariq et al., 2019, Mutlu-Ingok et al., 2020). Essential oils are obtained from different parts of plants, such as leaves, stems, flowers, seeds, roots, fruit rinds, resins, or barks, by numerous techniques, including modern techniques such as supercritical fluid extraction, subcritical extraction liquid, solvent-free microwave extraction, and conventional techniques such as hydro-distillation, steam distillation, hydro diffusion, and solvent extraction (Aziz et al., 2018, Hanif et al., 2019, Baptista-Silva et al., 2020). Furthermore, essential oils are secondary metabolites of plants which are complex mixtures of volatile compounds belonging to various chemical classes: alcohols, ethers or oxides, aldehydes, ketones, esters, amines, amides, phenols, heterocycles, and mainly the terpenes (Dhifi et al., 2016). Due to their chemical composition, essential oils have been used in many fields, like cosmetics, foods, and medicine (Hanif et al., 2019). Besides, essential oils are generally considered safer than synthetic additives, so they are preferred as potential alternatives to synthetic ones in various fields, especially food applications (Angane et al., 2022).

M. Kiralan (✉) · S. S. Kiralan
Faculty of Engineering, Department of Food Engineering, Balikesir University, Balikesir, Turkey

O. Ketenoglu
Faculty of Agriculture, Department of Food Engineering, Eskisehir Osmangazi University, Eskisehir, Turkey

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Cardamom, [*Elettaria cardamomum* (L.) M] is a member of the family Zingiberaceae and is a valuable spice due to its unique, pleasant aroma and various health benefits. Guatemala and India are major producers of small cardamom (Reyes et al., 2006, Mathew et al., 2022). The seeds (fruits, capsules) are used in various foods as a spice and flavoring agent (Ashokkumar et al., 2020). The proximate composition of seeds presented the following composition: carbohydrates (68.2%), water (19.2%), protein (10.6%), oil (2.4%), and ash (5.3%) (Sontakke et al., 2018). Besides, the essential oil is a valuable constituent in the chemical composition of cardamom seeds yielding up to 8% essential oil on a dry basis, according to the variety, plant parts, and extraction methods used (Ashokkumar et al., 2020). Oxygenated monoterpenes are the major class of essential oils (63.0%), with 1,8-cineole attaining 55.4% of the essential oil composition (Noumi et al., 2018).

Cardamom essential oil has been a valuable ingredient in various foods, perfumery, and traditional applications (Ashokkumar et al., 2021). Besides, numerous studies showed that cardamom essential oil exhibited various biological activities, including antioxidant (Misharina et al., 2009), antibacterial (Cui et al., 2020), anti-inflammatory (Alam et al., 2021a), anti-hypercholesterolemic (Nagashree et al., 2017), insecticidal (Goudarzvand Chegini & Abbasipour, 2017), and other miscellaneous activities (Ashokkumar et al., 2020). This chapter examines studies on cardamom essential oil's chemical composition and functional properties.

8.2 Cardamom Essential Oil Extraction

The yield of essential oil differs depending on many factors, such as variety, geographical location, climatic conditions, and extraction methods. One of the influential factors in the oil yield from cardamom seeds is the extraction method. The oil yield of cardamom extracted by various methods is summarized in Table 8.1.

Several methods, such as hydro-distillation, steam distillation, Soxhlet extraction, supercritical extraction, etc., are available for essential oils extraction (Morsy, 2015). Hydro-distillation is the most common method for the extraction of essential oil. Hydro-distillation and steam distillation have traditionally been used to extract cardamom essential oil. However, due to some disadvantages, such as the risk of decomposition of thermally sensitive compounds and unfavoured changes like hydrolytic reactions on essential oil compounds, new technological methods have been developed in essential oil extraction instead of traditional methods (Lucchesi et al., 2007).

Table 8.1 The yields of essential oil from cardamom seeds

Extraction method	Oil yield (%)	Reference
Hydro-distillation	4.5–9.6	Ashokkumar et al. (2021)
Enzyme assisted hydro-distillation	2.5	Chandran et al. (2012)
Solvent-free microwave extraction	2.70	Lucchesi et al. (2007)
Instant controlled pressure drop	4.43	Teresa-Martínez et al. (2022)
Hydro-distillation	2.52	

Enzyme treatments before essential oil extraction improve oil yield. Chandran et al. (2012) used a mixture of enzymes, namely, lumicellulase (cellulase, β-glucanase, pectinase, and xylanase) on seeds as a pre-treatment before extraction, and the researchers reported that the oil yield increased up to 2.5% from 1.9%. Baby and Ranganathan (2016) used individual enzymes, including Celluclast 1.5 L, Pectinex Ultra SP-L, Protease, and Viscozyme L, to extract cardamom essential oil. The pre-treatment of cardamom seeds with these individual enzymes enhanced the oil yield, as the pre-treatment with Viscozyme L gave the highest release of essential oil (7.83%) compared to untreated samples (6.73%) and that of other enzyme treatments (7.23–7.68%).

Morsy (2015) used the ultrasound technique to pre-treatment hydro-distillation essential oils. This new extraction technique improved the extractibility of cardamom essential oil and shortened extraction time in the hydro-distillation process. The extraction time in combined ultrasonic-assisted extraction and hydro-distillation was lower (≤1 h) than the traditional hydro-distillation process (6 h).

Teresa-Martínez et al. (2022) used a new technique, instantly controlled pressure drop before hydro-distillation in essential oil extraction. This technique increased essential oil yield up to 4.43% compared to solely hydro-distillation (2.52%).

Another green method, solar energy-based extraction, was used to extract cardamom essential oils, and the oil yield obtained by solar energy-based extraction (4.3%) showed similar values compared to traditional hydro-distillation (4.1%). Similarly, extraction time in solar energy-based extraction was close to the hydro-distillation method. However, solar energy-based extraction was 23–34% greener than traditional hydro-distillation (Al-Hilphy et al., 2022).

8.3 Cardamom Essential Oil and Its Composition

The chemical composition of cardamom essential oil varies depending on the geographical origin and the extraction methods used (Table 8.2). Especially novel extraction methods exhibit some advantages in the essential oil components compared to classical extraction methods. 1,8-cineole, α-terpinyl acetate, linalool, linalyl acetate, and α-terpineol (Fig. 8.1) are major aroma compounds that constitute the cardamom essential oil. These compounds give the characteristic aroma profile of cardamom essential oil.

Lucchesi et al. (2007) used a new extraction technique, solvent-free microwave, to examine the effects of extraction conditions on the essential oil composition. Compared with hydro-distillation, the higher level of oxygenated compounds and lower level of monoterpene hydrocarbons were identified in the cardamom essential oil extracted by solvent-free microwave extraction**.** In the essential oil composition, oxygenated compounds are more valuable constituents than monoterpene hydrocarbons in their contribution to the essential oil's fragrance.

Combining ultrasonic-assisted extraction and hydro-distillation provided more gentle conditions in extracting essential oils, preventing sensitive and thermally

Table 8.2 Essential oil composition of cardamom

Origin	Method	Constituents	Percentage (%)	References
India		α-Terpinyl acetate 1,8-Cineole α-Terpineol Nerol Linalool	39.28–47.92 15.88–27.34 2.25–7.37 Nd–6.81 Nd–9.38	Leela et al. (2008)
Unspecified	Solvent-free microwave extraction	1,8-cineole α-Terpinyl acetate Linalool Linalyl acetate α-Terpineol Terpin-4-ol	35.11–55.03 19.40–29.22 7.47–9.72 4.36–7.62 3.03–6.04 2.30–4.15	Lucchesi et al. (2007)
Unspecified	Hydro-distillation	1,8-cineole α-Terpinyl acetate Linalool Linalyl acetate α-Terpineol Terpin-4-ol	26.23 45.45 5.29 3.63 3.88 2.60	Lucchesi et al. (2007)
Guatemala	Hydro-distillation	α-Terpinyl acetate 1,8-Cineole Linalool α-Terpineol	36.50 33.93 5.10 4.62	Morsy (2015)
Guatemala	Ultrasonic-assisted extraction and hydrodistillation combination	α-Terpinyl acetate 1,8-Cineole Linalool α-Terpineol	22.94–40.56 26.59–39.34 5.47–7.60 3.58–4.96	Morsy (2015)
Iran	Hydro-distillation	α-Terpinyl acetate 1,8-Cineole Linalyl acetate Sabinene	36.61 30.42 5.79 4.85	Goudarzvand Chegini and Abbasipour (2017)
India	Hydro-distillation	α-Terpinyl acetate 1,8-Cineole α-Terpineol Linalool Sabinene	29.9–61.3 15.2–49.4 0.83–13.2 0.44–11.0 1.9–4.9	Ashokkumar et al. (2021)
Guatemala	Hydro-distillation	α-Terpinyl acetate 1,8-Cineole α-Terpineol	42.65 33.78 2.98	Ivanović et al. (2021)
China	Hydro-distillation	1,8-Cineole α-Terpinyl acetate Linalool α-Terpineol Sabinene	43.47 21.56 10.26 6.98 2.11	Tarfaoui et al. (2022)

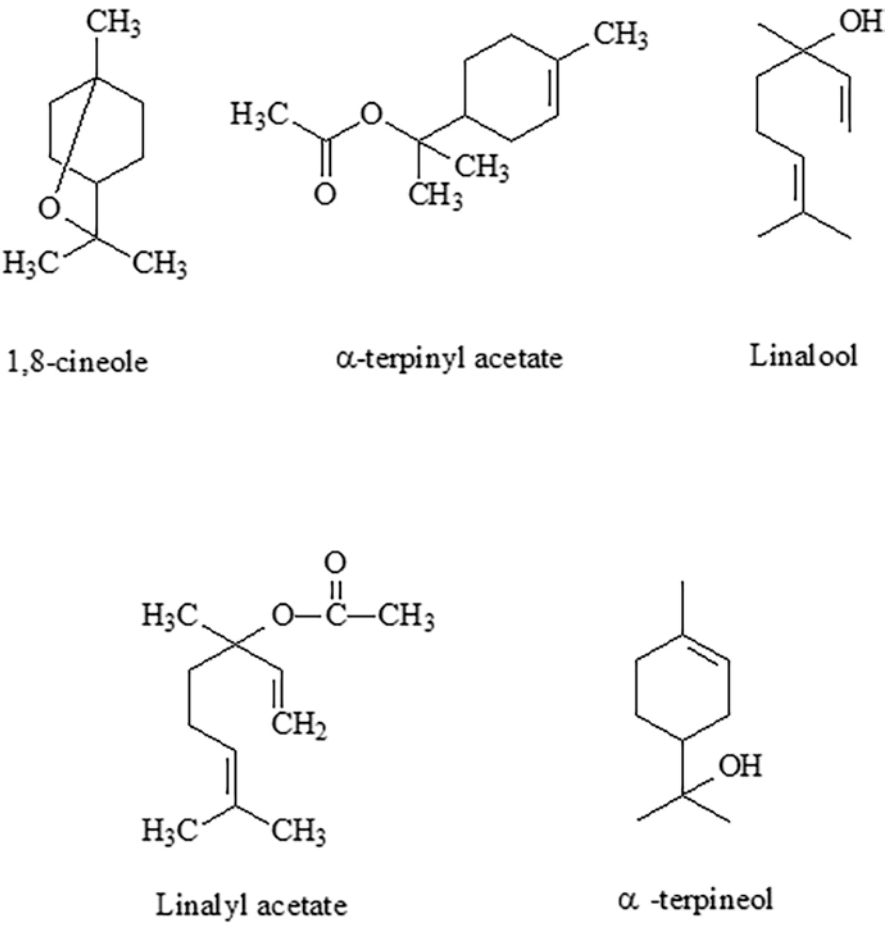

Fig. 8.1 Chemical structure of major compounds in cardamom essential oil

unstable compounds such as trans sabinene hydrate, 1-octanol, 4-thujanol and farnesol; these compounds could not be identified in cardamon essential oil obtained by hydro-distillation. Besides, this combined technique enhanced monoterpenes hydrocarbon concentration compared to the classical hydro-distillation (Morsy, 2015).

The chemical composition of cardamom essential oil was also affected by the enzyme (lumicellulase-a mixture of cellulase, β-glucanase, pectinase, and xylanase) assisted extraction. α-terpenyl acetate increased from 38.9 (control) to 48.6% by enzyme-assisted extraction. In addition, 1,8-cineole content decreased (20.8%) with enzymatic pre-treatment compared to the control (32.8%) (Chandran et al., 2012).

8.4 Functional Properties of Cardamom Essential Oil

The cardamom essential oil has various functional properties, summarized in Tables 8.3, 8.4 and 8.5.

8.4.1 Antioxidant Activity

Cardamom fruits are reported to be rich in antioxidant compounds with a level of 1.8 mmol/100 g. Cardamom essential oil inhibited 2-hexenal in the model assay (65.0% inhibition). In DPPḤ ((2, 2-diphenyl-1-picrylhydrazyl) test, the IC_{50} value of essential oil was lower with the value of 1.0 g/L compared with other essential oils (coriander, black pepper and white pepper) (Misharina, 2016). Handayani et al. (2019) demonstrated that the IC_{50} value of cardamom oil is 29.5 µg/mL, and also

Table 8.3 Antioxidant activities of cardamom essential oil

Assay	Result	Reference
DPPḤ	The IC_{50} value for leaf oil is 46.13 µg/mL IC_{50} value for stem oil is 81.32 µg/mL The inhibition rate of leaf oil was higher than stem oil	Jena et al. (2021)
ABTS	The IC_{50} value for leaf oil is 20.99 µg/mL IC_{50} value for stem oil is 61.5 µg/mL The inhibition rate of leaf oil was higher than stem oil	Jena et al. (2021)
DPPḤ	The IC_{50} value for cardamom oil is 29.53 µg/mL The antioxidant activity index (AAI) of essential oil is 1.33 Based on AAI, this oil is categorized as a potent antioxidant	Handayani et al. (2019)
DPPḤ	The inhibition percentage of essential oil is 37.41%. Moderate antioxidant activity was observed among various spice oils (cinnamon, cumin, wisteria, coriander)	Hassan et al. (2020)
Reducing power (RP)	At a 2.5 mg/mL concentration, the cardamom essential oil showed an RP of 2.23 related to BHA and a lower reducing power activity among 26 commercial essential oils	Wang et al. (2017)
β-Carotene bleaching (BCB)	At a 2 mg/mL concentration, the BCB activity level of cardamom essential oil was 11.2% The BCB activity of cardamom essential oil was lower than those of other samples	Wang et al. (2017)
1,1-diphenyl-2-picrylhydrazyl free radical scavenging (DFRS)	At a concentration of 1 mg/mL, the DFRS ability of cardamom essential oil was 11.29%. The DFRS activity of cardamom essential oil was lower than those of other samples	Wang et al. (2017)

Table 8.4 Antibacterial and antifungal activity of cardamom essential oil

Activity	Target microorganism	Control	Result	Reference
Antibacterial	*Staphylococcus aureus, Bacillus cereus, Escherichia coli, salmonella typhi*	Amikacin and gentamycin	The inhibition zone ranged from 66 to 88 mm at 3000 ppm The best inhibitory effect was observed against gram-positive bacteria, including *Staphylococcus aureus, Bacillus cereus*	Singh et al. (2008)
Antibacterial	*Aerococcus viridans, Bacillus cereus, B. subtilis, Enterococcus faecalis, Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus, S. epidermidis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabils, Salmonella typhimirium, Shigella flexenerii, Vibrio alginolyticus, V. parahaemolyticus* ATCC 43996, *V. vulnificus, Serratia marcescens Escherichia coli, Shewanella putrefaciens, Vibrio cholerae, Vibrio parahaemolyticus* ATCC 17802	Ampicillin	The ihibition zone ranged from 6.00 to 41.3 mm, with Best inhibitory effect @ 0.048 mg/ml against *Micrococcus luteus* The inhibition zone ranged from 7.00 to 20.6 mm, with Best inhibitory effect @ 0.097 mg/ml against *Vibrio parahaemolyticus*	Noumi et al. (2018)
Antifungal	*Candida tropicalis, C. parapsilosis, C. krusei, C. guilliermondi Candida glabrata, C. albicans, saccharomyces cerevisiae*	Amphotericin B	The inhibition zone ranged from 14.3 to 21.6 mm, with Best inhibitory effect @ 0.048 mg/ml against *Candida parapsilosis* The inhibition zone ranged from 15.3 to 18.6 mm, with best inhibitory effect @ 0.097 mg/ml against *Saccharomyces cerevisiae*	Noumi et al. (2018)

(continued)

Table 8.4 (continued)

Activity	Target microorganism	Control	Result	Reference
Antibacterial	*Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus mutans, Escherichia coli, Bacillus cereus, salmonella enteric, Pseudomonas aeruginosa, Candida albicans*	Bacterial cells without green cardamom essential oil	Disk diffusion assay showed that *C. albicans* and *S. mutans* were the most sensitive microorganisms The value of MIC for essential oil against tested strains were 10 mg/mL against *S. typhimurium, S. aureus* and 5 mg/mL against *S. mutans, C. albicans*	Asghar et al. (2017)
Antibacterial	*Staphylococcus aureus, S. epidermidis, Escherichia coli, Klebsiella pneumoniae, Proteus Mirabibilis, Acinetobacter baumannii, pseudomonas aeruginosa, Candida albicans, C. tropicalis*	Amoxicillin and ampicillin	The highest sensitivity, 20 mm, was recorded For *Staphylococcus aureus* with cardamom oil, followed by *S. epidermidis* with a 14 mm inhibition zone. The value of MIC essential oil against *Staphylococcus aureus* and *S. epidermidis* were 2 μL/mL	Tarfaoui et al. (2022)

MIC Minimum Inhibitory Concentration (mg/mL)

this oil is classified as a potent antioxidant based on the antioxidant activity index (AAI = 1.33).

Hassan et al. (2020) evaluated the antioxidant activity using the DPPḤ method among the five samples (cardamom, cinnamon, cumin, wisteria, and coriander essential oils). Moderate antioxidant activity was observed in cardamom oil, with an inhibition percentage of 37.4% in the studied essential oils.

In a study by Wang et al. (2017), the researchers evaluated the antioxidant activity of 26 commercially available essential oils by using various assays, including reducing power (RP), β-carotene bleaching (BCB) activity, Trolox equivalent antioxidant capacity (TEAC), and 1,1-diphenyl-2-picrylhydrazyl free radical scavenging (DFRS) ability. The cardamom essential oil had the lowest RP, BCB, and DFRS activities among the 26 commercial essential oils. However, the cardamom essential oil showed negligible TEAC results.

Jena et al. (2021) evaluated the free radical scavenging ability of essential oils from leaf and stem parts of cardamom using DPPḤ and ABTS ((2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assays. These assays showed that the leaf oil had better activity than stem oil. For example, in the DPPḤ assay, IC_{50} values of the leaf and stem essential oil were 46.1 and 81.3 μg/mL, respectively. Similarly, IC_{50} values of the leaf and stem essential oil were recorded as 20.9 and 61.5 μg/mL in ABTS assays.

Table 8.5 Other miscellaneous activities of cardamom oil

Activity	Result	Reference
Gastroprotective effect	Cardamom essential oil exhibited gastroprotective effects at 12–50 mg/kg doses in ethanol, aspirin, and ligation-induced pathologies	Jamal et al. (2006)
Anti-inflammatory and anti-infectious properties	Cardamom essential oil comprises a promising compound for combatting acute campylobacteriosis and, presumably, preventing post-infectious morbidities in rats	Heimesaat et al. (2021)
Cholesterol-lowering activity	The broilers fed the diets containing 50 mg/kg essential oil had lower LDL cholesterol than the control sample	Omidi et al. (2015)
Insecticidal activity	Against three insects (*Callosobruchus maculatus* Fabricius, *Tribolium castaneum* Herbst, and *Ephestia kuehniella*), essential oil exhibited strong insecticidal activity, especially against *E. kuehniella*	Abbasipour et al. (2011)
Insecticidal activity	Cardamom essential oil showed strong insecticidal effects on the tomato leaf miner *Tuta absoluta.* The effect of essential oil was stronger on adults and larvae than on their eggs	Goudarzvand Chegini and Abbasipour (2017)
Anticancer	Geraniol or cardamom essential oil (100 and 200 mg/kg/day for 26 weeks) inhibited oxidative stress and also improved antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione-S transferase in diethylnitrosamine (DENA)-induced oxidative stress in the kidney and brain of rats	Elguindy et al. (2018)

Tarfaoui et al. (2022) examined the antioxidant activity of cardamom essential oil using two antioxidant assays, including Ferric reducing power (FRP) and DPPḤ radical scavenging activity assays. According to the researchers, the cardamom essential oil showed weak activities in these assays. For example, in the DPPḤ test, the IC_{50} value of essential oil was 1.42 mg/mL, and the reducing power value of the essential oil was reported as 16.7 mg ascorbic acid equivalent/g.

Cardamom essential oil is also used to improve the oxidative stability of vegetable oils, and thus, it can be regarded as a natural antioxidant. Singh et al. (2008) added cardamom essential oil to mustard oil at 0.02% (v/v) and stored the samples at 60 °C for 28 days. Their findings revealed that the cardamom essential oil delayed oxidation of mustard oil under accelerated storage conditions compared to the control, while the synthetic antioxidants (BHA and BHT) had a stronger effect than the cardamom essential oil.

8.4.2 Antibacterial and Antifungal Activity

Cardamom essential oil also has excellent antimicrobial activity due to its volatile components. According to Singh et al. (2008), cardamom essential oil exhibited intense inhibition activity against *Staphylococcus aureus*, *Bacillus cereus*,

Escherichia coli and *Salmonella typhi* at 3000 ppm using the agar well diffusion method. In addition, the cardamom essential oil showed strong antifungal activity against *Penicillium purpurogenum, Fusarium graminearum, Aspergillus terreus,* and *Penicillium madriti* at 3000 ppm by the poison food method. Another antifungal assay, the inverted Petri dish method, demonstrated that cardamom oil exhibited moderate to strong antifungal activity against the tested fungal isolates (*P. purpurogenum, F. graminearum, A. terreus, P. madriti*) (Singh et al., 2008).

Noumi et al. (2018) reported that green cardamom essential oil showed high activity against seven yeast strains. The inhibition zone for essential oil ranged from 14.3 mm to 21.6 mm. Besides, the MIC and Minimum Fungicidal Concentration (MFC) levels ranged between 0.048–0.097 mg/mL and 6.25–12.5 mg/mL, respectively. In their study, cardamom essential oil exhibited antimicrobial activity against the twenty-five Gram-positive and Gram-negative bacteria, including those frequently associated with food contamination and human disorders. The inhibition zones were between 6–14.3 mm for cardamom oil.

Mutlu-Ingok et al. (2019) evaluated the individual and combined effects of cumin, cardamom, and dill weed essential oils on *Campylobacter jejuni, Campylobacter coli, Escherichia coli, Staphylococcus aureus*, and mixed cultures using the broth microdilution method. Cardamom essential oil showed satisfactory inhibition against Gram-negative (*C. coli, C. jejuni,* and *E. coli*) and Gram-positive (*S. aureus*) pathogenic microorganisms. Besides, mixed essential oils produced a higher inhibition effect than individual ones. The researchers noted that the high antimicrobial activity of cardamom essential oil might result from oxygenated monoterpenes, especially α-terpinyl acetate (43.4%) and 1,8-cineole (29.2%), as the major compounds of cardamom essential oil.

The essential oils of Guatemalan green cardamom and Indian green cardamom showed inhibition activities against *Pseudomonas aeruginosa* and *Escherichia coli*. The Minimum inhibitory concentrations (MICs) of Guatemalan cardamom oil and Indian cardamom oil against *P. aeruginosa* were 0.5 and 0.25 mg/mL, respectively. However, the MICs for these oils were 1 and 0.5 mg/mL against *E. coli*, respectively (Alam et al., 2021b).

Cardamom oil exhibited potent activity against *Staphylococcus aureus* and *S. epidermidis* with a high inhibition zone of 20 mm and 14 mm, respectively. Like bacteria, this essential oil exhibited potent activity against two yeast strains, including *Candida albicans* and *C. tropicalis*, with 13 mm and 12 mm, respectively, inhibition zones. Even low concentrations (2 μL/mL) of the essential oils inhibited the growth of the aforementioned microorganisms (Tarfaoui et al., 2022).

8.4.3 Other Miscellaneous Activities

Cardamom essential oil exhibited gastroprotective effects in rats at 12–50 mg/kg doses. In addition, the essential oil inhibited the gastric lesions induced by aspirin, ethanol, and pylorous ligature (Jamal et al., 2006).

Heimesaat et al. (2021) demonstrated that the treatment of cardamom essential oil lowered the intestinal pathogen burdens in *C. jejuni*-infected microbiota-depleted 1 L-10^{-}/$^{-}$ and improved clinical outcomes. Essential oil treatment also led to less distinct macroscopic and microscopic inflammatory sequelae in *C. jejuni*-infected 1 L-10^{-}/$^{-}$ mice and diminished pro-inflammatory immune responses upon *C. jejuni* infection.

Omidi et al. (2015) used cardamom essential oil as dietary supplementation with an inclusion level of 50 and 100 mg/kg in broiler feedings. The diets with cardamom essential oil decreased the low-density lipoprotein cholesterol compared to the control diet. In addition, the diet with cardamom essential oil at 50 mg/kg concentration positively affected blood cholesterol by decreasing the plasma cholesterol and low-density lipoprotein.

Cardamom essential oil exhibited insecticidal activity against the bruchid beetle, *Callosobruchus maculatus* Fabricius, the red flour beetle, *Tribolium castaneum* Herbst, and the flour moth, *Ephestia kuehniella* Zeller, which are important pests of stored various foods and causes severe damage to stored foods. The lethal time (LT_{50}) of essential oil on three insects decreased with increasing concentration (Abbasipour et al., 2011). In another study by Goudarzvand Chegini & Abbasipour (2017), cardamom essential oil showed insecticidal effects on the tomato leaf miner, *Tuta absoluta,* an important insect causing damage to the host plants, especially the Solanaceae family. The LC_{50} values for adults (7.88 and 1.55 µL/L) and larvae (1.88 µL/L) of this insect were lower compared to eggs (351.1 µL/L).

Elguindy et al. (2018) reported that cardamom essential oil exhibited an anticancer effect on diethylnitrosamine (DENA)-induced oxidative stress in the kidney and brain of rats. According to the researchers, cardamom essential oil or geraniol decreased the level of brain and kidney ornithine decarboxylase and the activity of brain acetylcholinesterase. Besides, cardamom essential oil or geraniol enhanced the antioxidant enzymes in the brain and kidneys of DENA-treated rats, including catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione-S transferase (Elguindy et al., 2018).

8.5 Conclusion

Cardamom is an essential plant with many health-promoting attributes, thus gaining worldwide attention. The essential oil of cardamom can be used in many application areas due to its varying composition in volatile compounds. Although hydrodistillation is the most common technique to extract cardamom essential oil, many other novel techniques have also been studied. Furthermore, the essential oil has many health benefits, including antimicrobial, antibacterial, anti-inflammatory, and anticancer effects, making this essential more important and valuable. Although cardamom is a lesser-known plant worldwide, it will surely gain its deserved importance when the number of research studies on this plant increases.

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Chapter 9
Composition and Functional Properties of Cardamom Fixed Oil

Mustafa Kiralan, Onur Ketenoglu, and Sündüz Sezer Kiralan

9.1 Introduction

Cardamom, [*Elettaria cardamomum* (L.) M], a member of Zingiberaceae family, has a significant value as a spice regarding its characteristic aroma and positive health-promoting traits. Guatemala and India are the two major producers of small cardamom (Reyes et al., 2006, Mathew et al., 2022). This plant is also known as "the Queen of spices" and "Heel khurd" (Nair, 2006, Jamal et al., 2006). Cardamom has been used worldwide for traditional pharmaceutical applications (Anwar et al., 2016). In addition, the flavor of cardamom seeds is identified as warm and slightly pungent, making it a beneficial flavoring agent in tea and food preparations (Chempakam & Sindhu, 2008).

The cardamom seeds have been reported to contain fixed oil at 1.32% (Daga et al., 2022) and 4% (Kasturi & Iyer, 1955). Despite the low oil content, seed oil contains valuable components in its composition. Cardamom oil is rich in monounsaturated fatty acids (44.8%), followed by saturated fatty acids (31.1%)(Daga et al., 2022). In addition, cardamom oil is characterized by oleic acid at 49.2% and palmitic acid at 26.4% (Parry et al., 2006). Besides, cardamom oil contains several minor compounds, such as tocopherols and sterols. Tocopherols are important compounds in edible oils that show strong antioxidant activity and also have various health benefits, including prevention of certain types of cancer, atherosclerosis, Parkinson's and Alzheimer's diseases, eyesight degeneration, diabetes, coronary heart disease, and other chronic ailments (Shahidi & De Camargo, 2016,

M. Kiralan (✉) · S. S. Kiralan
Faculty of Engineering, Department of Food Engineering, Balikesir University, Balikesir, Turkey

O. Ketenoglu
Faculty of Agriculture, Department of Food Engineering, Eskisehir Osmangazi University, Eskisehir, Turkey

M. F. Ramadan (ed.), *Cardamom (Elettaria cardamomum): Production, Processing and Properties*, https://doi.org/10.1007/978-3-031-35426-7_9

Almagro et al., 2021). The main tocopherol isomer is α-tocopherol with a 1.25 mg/100 g oil level, followed by γ-tocopherol (0.58 mg/100 g oil)(Ramadan et al., 2022). It has many health benefits, such as tocopherols in sterols, lowering low-density lipoprotein cholesterol and protecting against cardiovascular disease (Kopylov et al., 2021). Cardamom oil also contains different forms of sterols. β-sitosterol as the most abundant sterol, followed by sitostanol, campesterol, stigmasterol, Δ5-avenasterol, and citrostadienol (Ramadan et al., 2022). Besides, cardamom oil contains high amounts of total phenolics (3.9 mg gallic acid equivalents/g oil)(Ramadan et al., 2022).

Cardamom oil has some functional properties, such as antioxidant activity, oxidative stability, and antimicrobial activity. According to Parry et al. (2006), the ORAC antiradical test showed that cardamom oil had ORAC values over 100 μmol Trolox equivalents/g oil. In another antiradical assay, the DPPḢ test showed that cardamom oil had strong antiradical activity and quenched 58.2% of the radicals in the reaction mixtures in 10 min. In addition, the oxidative stability index (OSI) recorded for cardamom oil was over 63.5 h, comparable to the cold-pressed corn (66.0 h) and pumpkin (61.7 h) seed oils. Cardamom oil also exhibited antimicrobial activity against foodborne pathogens (*Salmonella enteritidis, Staphylococcus aureus, Listeria monocytogenes*, and *Escherichia coli*) and dermatophytic fungi (*Trichophyton mentagrophytes* and *Trichophyton rubrum*) (Ramadan et al., 2022).

9.2 Composition of Cardamom (*Elettaria cardamomum* L.) Fixed Oil

9.2.1 Fatty Acid and Acyl Lipids Profile

According to Ramadan et al. (2022), the neutral lipid (NL) was the greatest lipid class in cardamom oil and represented 96.6% of total lipids, followed by glycolipids (GL, 2%) and phospholipids (PL, 2%). The main subclass in NL is triglycerides (96.8% of total NL), followed by free fatty acids (1.18% of total NL), monoacylglycerols (1.10% of total NL), esterified sterols (0.78% of total NL) and diacylglycerol (0.06% of total NL). In GL class, the main subclass was esterified sterylglycosides (39.2% of total GL), followed by monogalactosyldiglycerides (22.1% of total GL), sterylglycosides (15.3% of total GL), cerebrosides (12.3% of total GL), sulphoquinovosyldiacylglycerol (5.52% of total GL), and digalactosyldiglycerides (5.22% of total GL). In PL class, phosphatidylcholine (49.7% of total PL) was the predominant compound, followed by phosphatidylinositol (31.2% of total PL), phosphatidylethanolamine (13.3% of total PL), and phosphatidylserine (5.62% of total PL).

The fatty acid composition of all lipid classes was also reported to be similar. Oleic acid ($C_{18:1}$) was detected as major fatty acid, ranging between 43.0% and 44.3%. The other most abundant fatty acid, palmitic acid ($C_{16:0}$), ranged from 21.1% to 21.8%. Linoleic acid ($C_{18:2}$) was the other predominant polyunsaturated fatty acid, between 17.5% and 17.9% (Ramadan et al., 2022).

Table 9.1 Fatty acid composition of cardamom oils (%)

		Ramadan et al. (2022)	Parry et al. (2006)	Daga et al. (2022)
Saturated fatty acids (SFA)	C14:0	2.40	1.5	0.73
	C16:0	21.6	26.4	28.5
	C18:0	6.30	2.3	1.90
	C20:0	1.21	0.4	–
	C22:0	0.45	–	–
Total SFA		31.96	30.8	31.1
Monounsaturated fatty acids (MUFA)	C16:1	2.53	1.6	1.85
	C18:1 (*cis* 9)	43.7	49.2	40.8
	C18:1 (*cis* 11)	–	–	2.16
	C20:1	0.79	0.5	–
Total MUFA		47.02	51.3	44.8
Polyunsaturated fatty acid (PUFA)	C18:2	17.6	15.2	17.6
	C18:3	3.42	2.7	6.39
Total PUFA		21.02	17.9	23.9

The major fatty acids in cardamom are oleic acid (C18:1), palmitic acid (C16:0), and linoleic acid (C18:2) (Table 9.1). Palmitic and stearic acids (as saturated fatty acid), oleic acid (monounsaturated fatty acid – MUFA) and linoleic acid (polyunsaturated fatty acid – PUFA) are the most common fatty acids.

Extraction conditions affect the fatty acid composition of cardamom oil. Hamdan et al. (2008) extracted ground seeds using supercritical carbon dioxide at 308–328 K and pressure of 10–30 MPa, sub-critical CO_2 at 298 K and 8–10 MPa, and sub-critical propane at 298 K and pressure of 2–5 MPa. Under extraction with CO_2 at 20 MPa and 308 K, oleic acid (C18:1) and linoleic acid (C18:2) decreased dramatically, while an increasing trend was observed in myristic acid (C14:0), margaric acid (C17:0) and unidentified fatty acid derivatives. The different parameters in sub-critical propane extraction did not change the fatty acid composition of cardamom oil.

9.3 Minor Bioactive Lipids

9.3.1 Tocopherols and Tocotrienols

Tocols (tocopherol and tocotrienols) are lipid-soluble antioxidants in, especially edible oils, the most important natural dietary sources of tocols. Tocols exhibit strong antioxidant activity towards lipid oxidation in foods and biological systems.

Table 9.2 Tocols in cardamom oil

Tocols	Ramadan et al. (2022) (mg/100 g)	Parry et al. (2006) (mg/kg)	Daga et al. (2022) (mg/100 g)
α-Tocopherol	1.25	10.4	30.4
β-Tocopherol	0.08	–	3.29
γ-Tocopherol	0.58	4.3	
δ-Tocopherol	0.28	1.6	4.01
α-Tocotrienol	0.05	–	–
β-Tocotrienol	0.03	–	–
γ-Tocotrienol	0.08	–	–
δ-Tocotrienol	0.03	–	–

As a result, tocols show various health benefits such as anticancer, antiobesity, antidiabetic, and cardioprotective (Durazzo et al., 2021).

Cardamom oil contains different patterns and amounts of tocols, predominantly α-tocopherol (Table 9.2). The other important isomers are γ- and δ-tocopherols. Daga et al. (2022) reported that α-tocopherol was highest in hexane-extracted cardamom oil with a 30.4 mg/100 g oil. However, cold-pressed cardamom oil contains lower α-tocopherol content (1.25 mg/100 g) than the hexane extracted (Ramadan et al., 2022).

9.3.2 Phytosterols

Phytosterols comprise a major portion of the unsaponifiable matter in vegetable oils. Phytosterols contribute to human health by preventing cardiovascular diseases, exhibiting antioxidant effects, and anti-inflammatory properties (Vilahur et al., 2019; Makhmudova et al., 2021; Poli et al., 2021). Cold-pressed cardamom oil contained high amounts of unsaponifiable matter (16.4 g/kg oil), of which phytosterols comprise a large part (222 mg/100 g oil). β-sitosterol was the predominant sterol (51.8% of total sterols) in cardamom oil, followed by sitostanol (12%). The other important sterols in cardamom oil are campesterol (8.2% of total sterols), stigmasterol (7.8% of total sterols), Δ5-avenasterol (6.6% of total sterols), and citrostadienol (4.4% of total sterols) (Ramadan et al., 2022). However, the content of β-sitosterol was higher in hexane-extracted cardamom oil (1736 mg/100 g, Daga et al., 2022) than in cold-pressed oil (115 mg/100 g, Ramadan et al., 2022).

9.3.3 Phenolics and Carotenoids

The total phenolic content of cold-pressed cardamom oil was 3.9 mg gallic acid equivalents/g oil and was reported to be higher than those of various cold-pressed oils such as cold-pressed boysenberry, blueberry, red raspberry, and marionberry

seed oils (1.5–2.0 mg GAE/g) (Parry et al., 2005). Daga et al. (2022) examined the individual phenolic compounds of cardamom oils, and their identified phenolics were hydroxybenzoic acid (0.50 mg/100 g oil), *p*-coumaric acid (0.24 mg/100 g oil) and kaempferol (0.03 mg/100 g oil) in oil samples.

The cold-pressed roasted pumpkin seed oil had the lowest total carotenoid concentration (0.05 μmol/kg) among cold-pressed oils, including onion, parsley, cardamom, mullein, pumpkin, and milk thistle oils. In addition, only zeaxanthin was identified as a carotenoid in cold-pressed cardamom oil (Parry et al., 2006).

Hamdan et al. (2008) evaluated carotenoids increased with pressure increase using supercritical fluid extraction with CO_2. The β-carotene content increased to 5.8 μg/g when the pressure was raised from 8 to 30 MPa. Likewise, an increase in the total amount of chlorophyll was observed with the increase in applied pressure (from 8 to 30 MPa). At 30 MPa, cardamom oil had higher values for the total chlorophyll content (4.53 μg/g).

9.4 Functionality of Cardamom Oil

9.4.1 Antioxidant Activity

Daga et al. (2022) used three antioxidant tests, including DPPḤ, ABTS, and FRAP assays, to determine the antioxidant activities of various cold-pressed oils. Cold-pressed cardamom oil exhibited moderate antioxidant activity among oils. DPPḤ, ABTS, and FRAP levels were 23.1 mg ascorbic acid equivalent/100 g oil, 897 mM Trolox equivalent/100 g oil, and 1923 mM Trolox equivalent/100 g oil, respectively.

Cold-pressed cardamom oil showed more radical scavenging activity than extra virgin olive oil against galvinoxyl and DPPḤ free radicals. After 60 min incubation, 32 percent of DPPḤ radicals were quenched by cardamom oil, while extra virgin olive oil was able to quench 9%. After 60 min incubation in an electron spin resonance assay, 33% of free radicals were quenched with cold-pressed cardamom oil, while 16% were quenched with extra virgin olive oil (Ramadan et al., 2022).

Due to its potent antioxidant capacity, cardamom oil is also used to increase the stability of vegetable oils with low oxidative stability. For example, during the Rancimat test at 120 °C, the induction period values of cardamom oil blends with sunflower oil were found to be higher than that of the blank oil [IP:532 min for sunflower oil and cardamom oil blend-8:2 (w/w); IP:405 min for sunflower oil and cardamom oil blend-9:1 (w/w); IP:180 min for sunflower oil) (Ramadan et al., 2022)].

9.4.2 Antimicrobial Activity

The antimicrobial activity of cold-pressed cardamom oil was studied. The oil was reported to have potent activity against a broad spectrum of food or human pathogens, including foodborne pathogens (*Trichophyton mantigrophytes*,

Trichophyton rubrum, Salmonella enteritidis, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*. The antimicrobial activity of cardamom oil was measured by recording the clear zone diameter (CZD, mm). The CZD value for *S. aureus, E. coli, S. enteritidis,* and *L. monocytogenes* are measured as 20, 18, 17, and 15 mm, respectively. As for fungi, The CZD values for *T. mentagrophytes* and *T. rubrum* were 32 and 30 mm, respectively. The minimum lethal concentration (MLC) of oils against microorganisms was evaluated. The MLC value ranged between 160 and 320 μg/mL for tested microorganisms (Ramadan et al., 2022).

9.5 Conclusion

Cardamom fixed oil is a significant product of the cardamom plant due to the diversity of such compounds as fatty acids, tocopherols, sterols, phenolics, and carotenoids. Oleic acid is the most abundant monounsaturated fatty acid of the fixed oil. In addition to the fatty acid composition, the aforementioned minor compounds give the plant significant health-promoting attributes, including antimicrobial and antioxidant activities. Since cardamom has increasing attention, further studies will help to reveal more functional properties of this important spice.

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Chapter 10
Health Aspects of True Cardamom (*Elettaria cardamomum*): Clinical Evidence and Proposed Mechanism

Tossaton Charoonratana

10.1 Introduction

Elettaria cardamomum (L.) Maton, or true cardamom, is in the Zingiberaceae family. True cardamom is native to India but is now extensively cultivated in Guatemala, Nepal, India, Indonesia, Sri Lanka, and Honduras. The world production is estimated to be 57,000–60,000 tons, while Guatemala shares around 65%–70% of world exports. The major importing countries are Saudi Arabia, United Arab Emirates, India, and Bangladesh (Joint FAO/WHO Codex Alimentarius Commission, 2021).

For centuries, humans have used true cardamom capsules for culinary purposes. The composition of cured true cardamom is carbohydrate 68.2%, protein 10.6%, and lipid 2.4%. It contains a significant amount of phosphorus (183 mg), magnesium (182 mg), potassium (124 mg), and sulfur (100 mg) per 100 g of cured capsules. The unique odor of this spice is from monoterpenoids which α-terpinyl acetate and α-terpineol provide a fragrance, and 1,8 cineole provides a harsh odor (Sontakke, 2018). Moreover, true cardamom contains a high amount of catechin (281.8 μg/g of dried cardamom) and other flavanols, such as epigallocatechin gallate (Ashokkumar, 2019). Oleic acid (49.2 g) and palmitic (26.4 g) are reported as the primary fatty acids in 100 g of cardamom oil (Parry, 2006). Flavonoids and terpenoids reported in true cardamom possessed various biological activities such as anti-inflammation, anti-oxidant, and also promoting endothelial function (Shafabakhsh, 2020; Kim, 2019).

Although true cardamom is commonly used as a seasoning and flavoring agent in food products, the Flavor and Extract Manufacturing Association (FEMA) estimates a low daily intake value of true cardamom (54.7 mg/day) (Burdock, 2009). Since it is

T. Charoonratana (✉)
Department of Pharmacognosy, College of Pharmacy, Rangsit University, Lak-hok, Muang Pathum Thani, Thailand

M. F. Ramadan (ed.), *Cardamom (Elettaria cardamomum): Production, Processing and Properties*, https://doi.org/10.1007/978-3-031-35426-7_10

difficult to draw the benefit of its secondary metabolites for health promotion when consumed as food, it is another option to use as a dietary supplement for a higher dose. Regarding safety, according to CFR title 21 section 182.10, true cardamom has been listed as GRAS (Generally Recognised As Safe) for human consumption as a food additive. However, human evidence is needed to evaluate its safety as a dietary supplement. Many clinical trials were performed, and no side effects were reported when consuming true cardamom at 3 g/day for 16 weeks (Singletary, 2022).

This chapter provided the potential use of true cardamom as a dietary supplement based on evidence in humans, such as randomized controlled trials and meta-analyses. Proposed mechanisms were also presented.

10.2 True Cardamom as a Dietary Supplement

The dietary supplement is the products used in dosage forms to enhance and improve the healthy function of the human body. However, it is not used as conventional food and is not intended to treat or prevent human diseases (Dietary Supplement Health and Education Act of 1994, 1994). True cardamom is linked to cardiovascular diseases and metabolic syndrome because of its secondary metabolites' biological activities and physiological effects. Additionally, true cardamom was used in Ayurvedic medicine for cardiac disorders (Gilani, 2008). This preliminary evidence supports its use as a dietary supplement called cardamom supplement (CS).

The World Health Organization (WHO) has identified five major risk factors for developing cardiovascular diseases; regularly unhealthy diet, not exercising regularly, smoking, harmful alcohol use, and diabetes. They cause effects on human body function, such as high blood pressure, high blood glucose, high hemoglobin A1C (HbA1C), and uncommon blood cholesterol, eventually resulting in cardiovascular diseases. Thus, the dietary supplement is one of the choices which consumers can take, and there are some pieces of evidence of CS which show the benefits of these parameters.

10.2.1 Effect on Blood Pressure

A meta-analysis was performed, and it was found that CS (powder 3 g/day) significantly decreased diastolic blood pressure (DBP) but did not affect systolic blood pressure (SBP) during 8-10 weeks (Izadi, 2022). CS is linked with a significant decrease in DBP from the forest plots. The analysis included three clinical trials with 244 participants and observed low heterogeneity. However, a study with substantial influence on the analysis showed high bias; more high-quality clinical trials are needed.

Although SBP did not decrease in people consuming CS, the subgroup analysis showed a positive effect in a clinical trial with people using CS for at least eight

weeks. The clinical trial by Zahedi et al. (2022) had high quality (Zahedi, 2022). However, the result was from 83 participants; more clinical trials with more participants are needed.

The mechanism of CS in lowering blood pressure is unclear. It was proposed that flavanols in true cardamom, which possessed vasorelaxant through increasing nitric oxide levels, had a role in the effect. Epigallocatechin gallate acts by activating the PI3K-Akt-NO/cGMP pathway and inhibits phosphodiesterase activity. It also modulates calcium channels and reduces reactive oxygen species. Moreover, it can increase miRNA-regulating aldosterone biosynthesis. Thus diuretic effect may associate with reduced blood pressure (Gilani, 2008; Maaliki, 2019).

10.2.2 Effect on Glycemic Indices

It was found that CS (powder 3 g/day) significantly decreased Hemoglobin A1C (HbA1C) and Homeostasis Model Assessment (HOMA-IR) index. However, it did not affect Fasting Blood Sugar (FBS), Quantitative Insulin Sensitivity Check Index (QUICKI), and insulin concentration during 8–12 weeks (Nameni, 2022).

From the forest plots, CS is linked with a significant decrease in HbA1C. The analysis included two clinical trials comprising 153 participants with low heterogeneity. However, neither study showed a low risk of bias; more high-quality clinical trials are required. It was proposed that flavanols in CS improved glucose homeostasis; epigallocatechin gallate was reported to increase glucagon-like peptide (GLP-1) secretion in vitro (Wen, 2022).

CS is linked with a significant decrease in HOMA-IR. The effect may be caused by catechin, which can activate peroxisome proliferator-activated receptor gamma (PPARg), subsequently increasing sensitivity to insulin. Three clinical trials with 247 participants were included in the HOMA-IR meta-analysis. All studies showed a low risk of bias, and low heterogeneity was observed (Shin, 2009).

From the analysis of four clinical trials with 320 participants, CS intake did not significantly alter FBS. The result was as same as the previous meta-analysis (Asbaghi, 2020). QUICKI and insulin concentration were not different between CS-received participants and control; high heterogeneity was observed; however, subgroup analysis was not presented. It is noted that four clinical trials with 330 participants are for insulin analysis, while two clinical trials with 153 participants are for QUICKI analysis.

10.2.3 Effect on Blood Lipid

Two meta-analyses were performed. It was found that CS (powder 3 g/day) did not significantly improve blood levels of triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL) during 8-12 weeks (Asbaghi, 2020; Shekarchizadeh-Esfahani, 2020).

From the forest plots, CS is linked with a significant decrease in triglyceride; however, a sensitivity analysis showed that removing a clinical trial from the meta-analysis made the overall effect of cardamom on triglyceride insignificant (Shekarchizadeh-Esfahani, 2020). The clinical trial by Aghasi et al. (2019) showed a strong influence on the analysis; however, it had a moderate risk of bias; thus, reliability should be a concerned. Although subgroup analyses showed that CS might reduce triglyceride after 12 weeks or in non-diabetic volunteers (Asbaghi, 2020), more clinical trials with higher populations and quality must be performed to conclude the effect of CS on triglyceride.

Although HDL did not increase in people consuming CS, the subgroup analysis showed that a clinical trial with people using CS for at least 12 weeks gained the benefit. In addition, the clinical trial by Daneshi-Maskooni et al. had a low risk of bias (Daneshi-Maskooni, 2019). However, the result was from 87 volunteers; more clinical trials with more volunteers are needed.

CS intake did not significantly alter total cholesterol and LDL; the subgroup analysis was performed based on clinical trial duration and diabetes status (Asbaghi, 2020). However, it is noted that only six clinical trials with 361 volunteers are represented.

10.3 Conclusion

CS powder 3 g/day positively affected diastolic blood pressure, hemoglobin A1C, and insulin sensitivity within 12 weeks. Flavanol, such as catechin and epigallocatechin gallate, were thought to involve in these effects. However, more extensive and longer-term trials are needed to consider the effects of CS on blood pressure, glycemic indices, and blood lipid.

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Chapter 11
Health-Promoting Effects of Cardamom (*Elettaria cardamomum*)

Ahmad Cheikhyoussef, Natascha Cheikhyoussef, Rajan Sharma, and Ahmed A. Hussein

Abbreviations

AD	Alzheimer's disease
BP	Blood pressure
CCB	Calcium channel blocking
CCl_4	Carbon tetrachloride
CFE	Cardamom fruit extract
CSE	Cardamom seed extract
DMBA	7,12-dimethylbenz[a]anthracene
EA	Estrogenic activity
IZD	Inhibition zone diameter
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
MTDL	Multi-targeted directed ligand
NMSC	Non-melanoma skin cancer
PTSD	post-traumatic stress disorder
TNF-α	tumor necrosis factor-α

A. Cheikhyoussef (✉)
Science and Technology Division, Multidisciplinary Research Service, Centre for Research Services, University of Namibia, Windhoek, Namibia
e-mail: acheikhyoussef@unam.na

N. Cheikhyoussef
Ministry of Higher Education, Technology and Innovation, Windhoek, Namibia

R. Sharma · A. A. Hussein
Chemistry Department, Cape Peninsula University of Technology, Cape Town, South Africa

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11.1 Introduction

Cardamom (*Elettaria cardamomum*) is an aromatic perennial plant cultivated in Burma, Guatemala, India, Malaysia, Pakistan, Papua New Guinea, Tanzania, and Sir Lanka. Cardamom's main consuming countries include Pakistan, Saudi Arabia, Denmark, Norway, Sweden, Russia, England, Japan, Iceland, the United States, and Germany. It is commonly known as green or true cardamom and has been used as a culinary ingredient in several traditional dishes (Ashokkumar et al., 2020b). Cardamom seeds and pods have a rich smell and are often added to desserts, hot and spicy plates, aromatic beverages, coffees and teas (Almeer et al., 2021). Cardamom is the third most expensive spice following saffron and vanilla. According to Watch Market (2022), the size of the cardamom global market is estimated to grow from USD 64580 million in 2021 to USD 74430 million by 2028.

Several phytochemical classes have been extracted from cardamom, including alkaloids, anthocyanins, fenchane-type monoterpenoids, fenchene, fenchone, fenchyl alcohol, flavonoids, flavones, labdane diterpenes, phenolics, coumarins, polyphenols, saponins, sterols, tannins and terpenoids (Al-Maliki, 2011; Gilani et al., 2008; Saeed et al., 2014; Asakawa et al., 2017; Liang et al., 2017; Noumi et al., 2018; Al-Yousef et al., 2021; Abdullah et al., 2022; Alkhalifah et al., 2022) with α-terpinyl acetate (34.9%) and 1,8-cineol (25.3%) (Fig. 11.1) in green cardamom (Asakawa et al., 2017; Paul & Bhattacharjee, 2018; Abdullah et al., 2021a; Ivanović et al., 2021; Cárdenas Garza et al., 2021), meanwhile, black cardamom samples

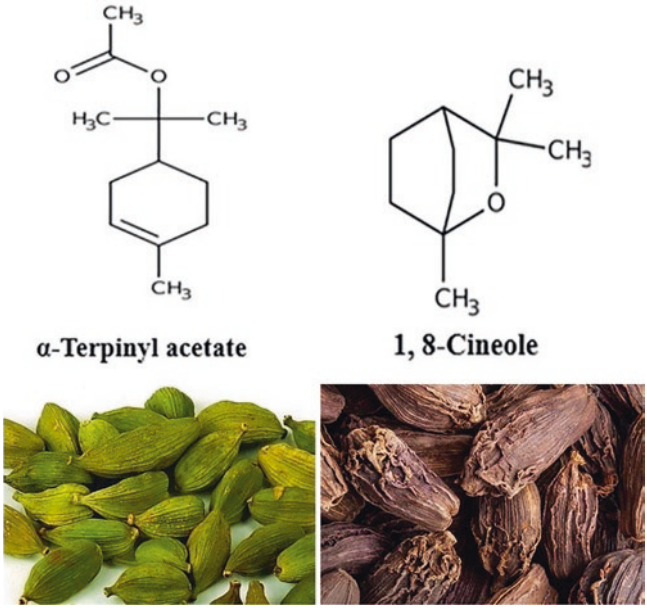


Fig. 11.1 Main bioactive compounds in Green and Black Cardamom

reported 1,8-cineol (44.2%) and α-terpinyl acetate (12.2%) being the main bioactive compounds as per the GC-MS analysis (Abdullah et al., 2021b).

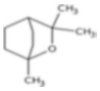
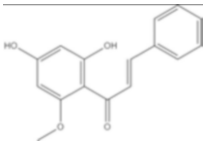
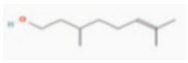
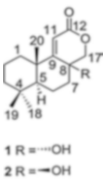
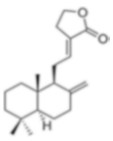
Other compounds of catechin (281 μg/g dry weight) and carotenoids (2.9 μg/g dry weight) (Ashokkumar et al., 2020a), cardamonin (Carvalho et al., 2012; Nawaz et al., 2020), limonene (Marongiu et al., 2004) were also reported. Other trace compounds include α-terpineol, citronellol, α-phellandrene, sabinene, myrceneborneol, camphor, γ-terpinene, p-cymene, terpinolene, myrcene, linalool, and α- and β-pinene, stigmasterol, geranyl acetate, eugenyl acetate, phytol, and β-sitostenone, borneol, nerol, linalyl acetate, neryl acetate, geraniol, farnesol, isosafrole, and nerolidol (Gopalakrishnan et al., 1990; Marongiu et al., 2004; Amma et al., 2015, Asakawa et al., 2017). In addition, polyphenolic compounds, such as gallic acid, tannic acid, caffeic acid and 4,5-dicaffeoyl quinic acid, were found in the ethanol extract of *E. cardamomum* (Moulai-Hacene et al. 2020). Meanwhile, Rahman et al. (2017) reported the presence of epicatechin, vanillin, *p*-coumaric acid, *trans*-ferulic acid, and ellagic acid in the ethanolic extract of cardamom, as well as Elguindy et al. (2016) reported the presence of gallic, tannic, caffeic and 4,5-dicaffeoyl quinic acid as per the HPLC analysis.

Several health benefits (Singletary, 2022) and biological properties of cardamom extracts from different plant parts, including seeds (Sengottuvelu, 2011), have been reported. These include anti-atherosclerotic (Winarsi et al., 2016), antibacterial and anti-biofilm (Abdullah et al., 2021a, b; Al-Maliki, 2011; Cui et al., 2020; Ghosh et al., 2015; Malti et al., 2007; Mahady et al., 2005; Souissi et al., 2020), anticancer (Ashokkumar et al., 2020a; Das et al., 2012), antidiabetic (Ahmed et al., 2017; Al-Yousef et al., 2021), anti-fungal (Sekine et al., 2007), anti-inflammatory (Arpitha et al., 2019, Cárdenas Garza et al., 2021; Izadi et al., 2022, Souissi et al., 2020), antioxidant (Amma et al., 2015; Alam et al., 2021; Ali et al., 2021; Arista et al., 2023; Ivanović et al., 2021; Saeed et al., 2014; Verma et al., 2009), antiviral (Sangeetha et al., 2022), and detoxifying properties (Farzin et al., 2022). Table 11.1 shows cardamom (*Elettaria cardamomum*) bioactive compounds, their biological activities, and most likely associated mechanisms. This chapter summarizes the latest literature on the traditional uses of cardamom and the present knowledge on the biological activities of cardamom plant parts and associated health-promoting effects.

11.2 Nutritional value

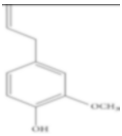
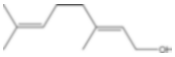
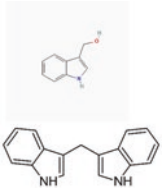
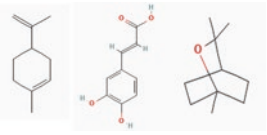
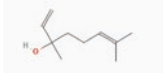
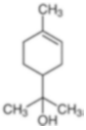
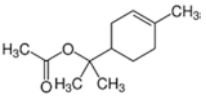
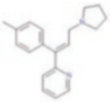
Cardamom is a rich source of dietary fiber, carbohydrates, proteins, minerals (copper, iron, manganese, sodium, and potassium), β-carotene (Zahirul Islam et al., 2020), and vitamins (C, A, niacin, pyridoxine, riboflavin, and thiamin) (Das et al., 2012; Abera et al., 2019; Sontakke et al., 2018). The proximate analysis of cardamom seeds on a dry weight basis resulted in 7.07% moisture, 1.39% fat, 11.4% protein, 8.24% crude fiber, 3.96% ash, and 83.1% carbohydrate (Ghosh et al., 2015). One hundred grams of cardamom contains approximately 1.21% and 175% of the daily value of manganese and iron, respectively. Furthermore, the low amount of sodium and cholesterol free property, with considerable potassium that aids in

Table 11.1 Cardamom (*Elettaria cardamomum*) bioactives and their reported biological activities

Compound name	Compound structure	Biological activity	Reference(s)
1,8-Cineol (eucalyptol)		Antibacterial (periodontal pathogens) Anti-inflammatory Antioxidant (lipoprotein metabolism) Gastroprotective activity: attenuate colonic damage in rats on acute TNBS-colitis	Souissi et al. (2020), Majdalawieh and Carr (2010), Cho (2012) and Santos et al. (2004)
α-Pinene		Spasmogenic, antispasmodic and sedative effects	Gilani et al. (2008)
Cardamonin		Hepatoprotective effect: reduces Acetaminophen-Induced Acute Liver Injury in mice via activating autophagy and nfe2l2 signaling. Anticancer agent: multiple cancer signaling pathways, it targets various signaling molecules, transcriptional factors, cytokines and enzymes, such as mTOR, NF-κB, Akt, STAT3, Wnt/β-catenin and COX-2. Inhibition of Oxazolone-Induced Atopic Dermatitis: Via NRF2 induction and the Th2 cytokine production inhibition	Xu et al. (2020), Nawaz et al. (2020) and Yoo et al. (2020)
Citronellol		Sedative effect: It sends signals directly to the olfactory system and trigger the brain to produce neurotransmitters, e.g., serotonin and dopamine. Also, it influences the neurophysiological brain activity, sympathetic and parasympathetic nervous system, and psychological and behavioral effects	Duke et al. (2002) and Cui et al. (2022)
Eletterins A and B	 1 R = ····OH 2 R = —OH	Anti-inflammatory effect: inhibit NO production induced by LPS on BV-2 microglia cells	Liang et al. (2017)
(E)-Labda-8(17),12(13)-dien-15, 16-olide		Anti-inflammatory effect: inhibit NO production induced by LPS on BV-2 microglia cells	Liang et al. (2017)

(continued)

Table 11.1 (continued)

Compound name	Compound structure	Biological activity	Reference(s)
Eugenol		Anti-cancer: In vivo tumor formation inhibition Immunomodulation Anti-inflammatory: reduces the inflammatory response and ameliorates the function of a specific organ, also it inhibits the liberation of inflammatory mediators from macrophages	Ghosh et al. (2005), Vishteh et al. (1986) and Barboza et al. (2018)
Geraniol		Antispasmodic and hypotensive	Duke et al. (2002) and Gilani et al. (2008)
9-Hexacosene		Anti-cancer: reduce edema size in mouse ears as induced by dimethylbenzene	Githinji et al. (2012) and Cárdenas Garza et al. (2021)
Indole-3-carbinol (I_3C) and diindolylmethane		Anti-cancer: chemopreventive properties: inhibit cancer-associated processes and regulate hormone activities in breast cancer. Antiviral activity: I_3C exhibits a significant antiviral activity (SARS-CoV-2 Omicron variant) with no toxicity effects	Acharya et al. (2010), Katz et al. (2018) and Centofanti et al. (2022)
Limonene, caffeic acid and cineole		Anti-cancer: block the activities of cyclooxygenase-2 and cytochrome P450 and downregulate several molecules' signal transductions	Bhagat and Chaturvedi (2016)
Linalool		Anti-inflammatory: reduce TNF-α and inhibits neutrophil activation	Abe et al. (2003)
α-Terpinol		Anti-inflammatory: inhibits pro-inflammatory mediator (IL-6) generation	Nogueira et al. (2014)
α-Terpenyl acetate		Anti-inflammatory: suppressing NO and COX pathways and decreasing the nitric oxide (NO)	Cárdenas Garza et al. (2021)
Triprolidine		Anti-COVID-19 has better docking and binding affinities to the various COVID-19 target proteins	Sangeetha et al. (2022)

physiology regulation, makes cardamom an excellent diet for the cardiovascular system (Ereifej et al., 2015; Vutakuri & Somara, 2018). Additionally, *E. cardamomum* possesses nutraceutical and functional food properties, and their regular consumption can potentially protect and assist humans in managing several chronic diseases (Ashokkumar et al., 2020b).

11.3 Traditional Uses

Several traditional uses have been reported for Cardamom in treating and managing asthma, diarrhea, gum infections, constipation, colic, diarrhea, dyspepsia, hypertension, epilepsy, kidney disorders, headache, congestive jaundice, inflammation of eyelids, immune-related disorders, nausea, snake and scorpion bites (Duke et al., 2002; Jamal et al., 2006; Majdalawieh & Carr, 2010; Daoudi et al., 2013; Ashokkumar et al., 2020b). In Ayurvedic medicine, cardamom, in combination with *Syzygium aromaticum,* was reported for antifertility treatment (Sethi et al., 1987) and food poisoning (Ashokkumar et al., 2020b). Ancient Egyptians used cardamom for breast-cancer anticipation as a prevention approach (Vutakuri & Somara, 2018). Other uses for cardamom have also been reported for gum, teeth, and throat infection, as well as against lung congestion, and pulmonary tuberculosis (Das et al., 2012), menstrual disorders (Bhatia et al., 2015). In Chinses medicine, bladder infection, children dysentery, constipation and stomachache (Kapoor, 1990; Duke et al., 2003).

Cardamom is commonly known as “Heel khurd” or “Choti ilaichi” and has been used in the Unani system of medicine to treat gastrointestinal disorders (Jamal et al., 2006). Cardamom seeds were reportedly used for appetite stimulation in people with anorexia, asthma, bronchitis, coughs, cold, and indigestion (Singh et al., 2008; Khan et al., 2011). Recently, Ashokkumar et al. (2020b) reviewed cardamom’s traditional uses and ethnopharmacology with detailed steps on preparation and administration. Two recent studies by Grover (2021) and Umakant (2022) reviewed the Ayurveda, pharmacological actions, and therapeutic significance of the queen of spices, *E. cardamomum* Maton (*choti elaichi*). Similarly, Kumar and Kumari (2021) reviewed cardamom’s traditional uses and biological activities.

11.4 Health Promoting Effects

11.4.1 Antimicrobial activities

Yassin et al. (2022) reported on the *in vitro* antimicrobial potency of *E. cardamomum* ethanol extract against multidrug-resistant of food poisoning bacterial strains of Methicillin-resistant *S. aureus* (ATCC 43300), *S. aureus* (ATCC 29213), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 9027), and *S. typhimurium* (ATCC 14023). The authors reported that the ethanol extract of *E. cardamomum* demonstrated the

highest antibacterial activity against the tested strains with inhibition zone diameter (IZD) ranging between 11.9 to 26.8 mm, as well as minimum inhibitory concentration (MIC) values of 0.25 and 0.50 mg/disc against *S. aureus* (IZDs = 16.8 mm) and *E. coli* (IZD = 12.3 mm) and minimum bactericidal concentrations (MBC) of 0.500 and 1.000 mg/disk respectively. Souissi et al. (2020) evaluated the antibacterial activities of cardamom fruit and seed extracts against periodontal infections causing pathogens of *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis*, and *Prevotella intermedia* with MIC of 0.5% [v/v], 0.25%, 0.062%, 0.125%, respectively and MBC of 1%, 0.25%, 0.062%, 0.25%, respectively. They further attributed the antibacterial activity to the presence of high amounts of 1,8-cineol (monoterpene) in cardamom fruit extract (CFE = 21.6%) and cardamom seed extract (CSE = 21.9%), which can disrupt the bacterial membrane of *P. gingivalis* and cell shrinking and damaged membrane of *Pseudomonas fluorescens* (de Sousa et al., 2012). Recently, copper oxide nanoparticles (CuO NPs) were synthesized using a cupric nitrate mixture, and the aqueous seeds extract of *E. cardamomum* and shown intense antimicrobial activity against pathogens (Venkatramanan et al., 2020). ZnONPs were also synthesized using an aqueous extract of green cardamom as a reducing agent in forming ZnONPs because cardamom extract is a rich source of flavonoids that holds great reducing properties (Pal et al., 2020). They reported antibacterial activities effective toward Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *P. aeruginosa*) strains. Other nanoparticles (gold) were designed whereby *E. cardamomum* seed extract was successfully synthesized and showed enhanced antibacterial activities against S. *aureus*, *E. coli* and *P. aeruginosa*. Furthermore, the resulting nanoparticles showed excellent cytotoxic activity as evaluated using HeLa cancer cell lines (Rajan et al., 2017).

Najafi et al. (2021) incorporated cardamom extract into sodium alginate (SA): polyvinyl alcohol (PVA) mat to design a wound dressing scaffold by electrospinning method and reported its antibacterial activities against *E. coli* and *S. aureus* with a significant reduction in the bacterial growth of 97% and 99%, respectively.

An earlier study by Malti et al. (2007) reported the antimicrobial activities of cardamom ethanolic extract at (300 μg/μL) with IZD ranging from 7 mm for *Pseudomonas aeruginosa* ATCC 27853 to 16 mm for *Bacillus cereus* ATCC 11778. However, the authors emphasized that the toxicity of cardamom dose (<0.3 mg/g) can induce inflammation in the brain, oxidative stress and cell necrosis in the heart. They further recommended that using *E. cardamomum* as a spice should not exceed 0.003 mg/g as no adverse effects were observed during their work at this amount.

11.4.2 Anti-cancer Activities

The cytotoxicity of *E. cardamomum* extracts against the MCF7 cell line was evaluated, and it was reported that the ethyl acetate extract exhibited the highest while the lowest was for aqueous extract with IC_{50} 114 and 187 μg/mL, respectively. Meanwhile, moderate toxicities with IC_{50} of 156 and 128 μg/mL for ethanol and

hexane extracts, respectively (Yassin et al., 2022). Almeer et al. (2021) reported the anticancer activities of green cardamom and, in combination with the anti-cancer drug; cyclophosphamide on Ehrlich solid tumors and provided evidence of its role as an apoptotic stimulator agent that can modulate the apoptotic-related genes and proteins significantly as well as increasing the glutathione levels and antioxidant enzymes, meanwhile decreasing the oxidative stress biomarkers. Furthermore, limonene and cineole from cardamom demonstrated promising effects against carcinogenesis (Acharya et al., 2010).

Saeed et al. (2014) confirmed that seeds and pods extracts of green cardamom have a strong antimutagenic potential against mutant strains *S. typhimurium* TA98 and *S. typhimurium* TA100. At the same time, none of the extracts showed mutagenicity. The results of this study support that the extracts from cardamom seeds and pods can be explored as potential chemotherapeutic agents against cancer and for the development of nutraceutical/functional food-based cardamom extracts. Gilani et al. (2008) reported that cardamom extract is safe even if the dose is high (10 g/kg), which supports the long-term safe use and the wide therapeutic and dietary attributes of the use of cardamom in diverse cultures worldwide. Das et al. (2012) examined cardamom efficacy against 7,12-dimethylbenz[a]anthracene (DMBA)-induced skin papilloma genesis in Swiss albino mice that closely resembles human Non-melanoma skin cancer (NMSC). They reported that oral administration of cardamom to DMBA-treated mice could up-regulate the phase II detoxification enzymes, such as glutathione-S-transferase and glutathione peroxidase, possibly through the activation of nuclear factor erythroid-2-related factor 2 transcription factor in carcinogen-treated (DMBA), and cardamom-treated 'DMBA+CARD' mice.

Additionally, in DMBA-treated mice, ingestion of cardamom led to the block of NF-κB activation and down-regulation of cyclo-oxygenase-2 expression. They concluded that cardamom has the potential to become one of the pivotal chemopreventive agents in preventing papilloma genesis on human skin. Sengupta et al. (2005) studied the effects of cardamom against azoxymethane (AOM) induced colonic aberrant crypt foci (ACF) in Swiss Albino mice. They further indicated that cardamom aqueous suspension has protective effects on ACF-induced colon carcinogenesis. The reduction ability for azoxymethane-induced colon carcinogenesis by cardamom extracts could be attributed to its anti-inflammatory, antiproliferative, and proapoptotic activities (Bhagat & Chaturvedi, 2016).

Farzin et al. (2022) reviewed the pharmacological properties of several medicinal plants in the prevention and treatment of breast cancer for the past 20 years. They indicated that regular consumption of *E. cardamomum* may aid in preventing breast cancer, mainly attributed to DNA repair, cell cycle, hormonal regulation, differentiation and apoptosis. In addition, cardamom extracts showed a chemopreventive effect on colorectal cancer (Bhattacharjee et al., 2007), increasing the detoxifying enzyme activity (GST) level. Furthermore, it decreases the lipid peroxidation levels in the treatment groups compared to the carcinogen control group. Furthermore, cardamom is an anti-myeloma drug with strong viability, whereby it inhibits the viability and proliferation of multiple myeloma (MM) cells (Zhihua et al., 2014). Possible mechanisms for the antitumor effects of green cardamom

extracts include modulation of several transcription signaling, growth factors, protein kinases and inflammatory cytokines (Sung et al., 2012).

11.4.3 Antioxidant Activities

Amma et al. (2010) studied four cardamom varieties: Mysore, Malabar, Vazhukka and Guatemala. The ethyl acetate extract of all varieties yielded the highest activity compared to other organic solvents, with the Malabar variety being identified as the best source of antioxidant compounds. Malabar cardamom has higher antioxidant activity regarding higher phenolic and flavonoid contents and reducing power. Cho (2012) indicated that 1,8-cineole (Table 11.1) possesses antioxidant capacity in lipoprotein metabolism and can reduce lipid accumulation in THP-1 liver cells of zebrafish.

Several studies indicated that phytochemicals from cardamom could boost the antioxidant defense system thanks to their rich phenolics content, including kaempferol, quercetin, luteolin, and pelargonidin, as well as phytosterols, and tocopherols (Abdullah et al., 2022; Ramadan et al., 2022). These active compounds provide protection to cells against adverse effects of oxidative processes via scavenging and inactivating the free radicals, e.g., DPPḤ assay against leaves extract IC_{50} = 594 µg/mL (Chowdhury & Islam, 2020), aqueous and methanol extracts (5 mg/L) of green cardamom seeds and pods DPPḤ scavenging of 46–91% and linoleic acid peroxidation inhibition of 34–83% (Saeed et al., 2014), as well as enhancing the enzymatic activities of glutathione and superoxide dismutase and reducing the malondialdehyde levels in the body (Abdullah et al., 2022). Furthermore, a recent study by Tarfaoui et al. (2022) reported that ethanolic extract of cardamom fruits exhibited DPPḤ (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging activity of IC_{50} = 0.423 mg/mL and ferric reducing power (FRP) of 95.03 FRP mg AAE/g.

11.4.4 Cardio-protection Effects

Goyal et al. (2015) reported on the protective effect of cardamom in isoproterenol (ISO)-induced myocardial infarction in rats and confirmed that cardamom extracts could significantly protect the myocardium and confers cardio-protective effects through free radical scavenging and antioxidant activities which led to cardiomyocytes preservation proven via reduced leakage of myocytes injury marker enzymes. In addition, other mechanisms were reported, including the attenuation of oxidative stress-mediated cardiovascular and heart damage or dysfunctions that could be attributed to cardamom phenolic acids, flavonoids, and sterols (Goyal et al., 2015).

Bibi et al. (2020) formulated *E. cardamomum* loaded phytosomes with an average vesicle size of 577 nm and a polydispersity index of 0.443. The developed phytosomes showed higher antioxidant and antimicrobial activities than its crude

extract, with an enhanced ACE inhibition activity (46%) than the crude extract (39%). Verma et al. (2009) reported that a green cardamom intake of 3 g/day can lower blood pressure in mildly hypertensive patients. Furthermore, it can increase visceral adiposity with decreased lean mass ensuring that decreased muscle mass can increase visceral adiposity (Zhang et al., 2015). In their recent review, Yahyazadeh et al. (2021) summarized the possible mechanisms for the hypotensive and cardio-protective effects of cardamom and its bioactive compounds, including calcium channel blocking and induction of NOS and endothelial NO, antioxidant properties and cholinergic effect. Izadi et al. (2022) reviewed the impact of green cardamom extracts on blood pressure and inflammatory markers among patients with metabolic syndrome and related disorders. Their findings indicated a significant decrease in diastolic blood pressure (WMD: −0.91 mmHg, 95%CI; −1.19, −0.62), high-sensitivity C-reactive protein (WMD: −1.21 mg/L, 95%CI; −2.18, −0.24), interleukin 6 levels (WMD: −2.41 ng/L, 95%CI; −4.35, −0.47) with no significant effect on systolic blood pressure.

11.4.5 Gastro-protection Effects

Vasudevan et al. (2000) reported that the aqueous extract of *E. cardamomum* can lead to gastric acid secretion increment in pentobarbitone anesthetized rats. Jamal et al. (2006) reported that cardamom extracts could inhibit the gastric lesions induced by aspirin and ethanol but have no effects on the ones induced by pylorous ligation. Gastrointestinal diseases have been treated with cardamom-based formula (Hamzaa & Osman, 2012), of which a mixture of coffee and cardamom was prepared to reduce the oxidative stress induced in rats via γ-irradiation. Earlier studies by Santos and Rao (2001) and Santos et al. (2004) reported the antioxidant and lipoxygenase inhibitory activities of 1,8-cineole. They showed that 1,8-cineole (50–200 mg/kg), via oral administration, led to significant attenuation of ethanol-induced gastric injury in a like nordihydroguaiaretic acid, a known lipoxygenase inhibitor indicating the gastroprotection against ethanol injury in the rat. They further provided evidence of its mechanism, which attenuates the colonic damage in rats with acute TNBS-colitis. It reduces myeloperoxidase activity and causes glutathione repletion, suggesting its potential as a dietary flavoring agent in preventing ulceration and gastrointestinal inflammation. A recent study by Qiblawi et al. (2020) reported that cardamom methanol extract concentrations ranging between 100–500 mg/kg, petroleum ether (12.5–150 mg/kg), and insoluble fractions (450 mg/kg) of the methanol extract significantly inhibited gastric lesions in rats induced via aspirin and ethanol which could be attributed to antioxidative, antiradical and anti-inflammatory activities of cardamom bioactive constituents. When combined with Arabic coffee, green cardamom extract showed increased total cholesterol and LDL concentration, but no effect on blood pressure was observed (Maha & Randa, 2013). They further indicated that an increased dose in such a combination (green cardamom in Arabic coffee) might increase cardiovascular risk.

11.4.6 Anti-dyslipidemia Effects

Yahyazadeh et al. (2021) indicated that active ingredients of cardamom could modify blood total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) in clinical trials (Fatemeh et al., 2017; Aghasi et al., 2019) and to control specific inflammatory and oxidative stress parameters in pre-diabetic people (Kazemi et al., 2017). *In vivo* studies as a pre-clinical step have indicated that cardamom powder (1 g/kg) via oral administration in a suspension form with 2% gum acacia (10 mL) for 12 days can decrease TC and TG levels and increase HDL levels in rats with dexamethasone-induced hepatomegaly, dyslipidemia, and fasting hyperglycemia (Nitasha Bhat et al., 2015). In another in-vivo study using Wistar hypercholesterolemia rats, cardamom powder (50 g/kg) for 8 weeks reported a restoration in lipid homeostasis and a significant reduction in atherogenicity index, showing the cardamom potential as a cardioprotective agent (Nagashree et al., 2017). Three mechanisms were associated with cardamom's anti-hyperlipidemic effects, including a) enhancing the rate of cholesterol degradative processes, b) increasing lipoprotein lipase activity, c) reducing lipid absorption in the intestine (Yahyazadeh et al., 2021). Shekarchizadeh-Esfahani et al. (2020) reviewed the impact of cardamom supplementation on lipid profile. They reviewed five eligible randomized controlled trials (RCTs) and showed that cardamom supplementation did not significantly change total cholesterol concentrations, low-density lipoprotein cholesterol, or high-density lipoprotein cholesterol. Meanwhile, they found a significant reduction in serum triglyceride levels.

11.4.7 Anti-inflammatory Activities

The anti-inflammatory activities of cardamom extracts of fruits and seeds were reported by Souissi et al. (2020) with a significant reduction in the secretion of IL-1b, TNF-a, and IL-8 by lipopolysaccharide-stimulated macrophages. An earlier study by Majdalawieh and Carr (2010) reported the anti-inflammatory activity of the cardamom extracts and related the activity to the presence of a high level of 1,8-cineole (eucalyptol), which is believed to attenuate LPS-induced inflammatory signaling pathways in lung alveolar macrophages as well as, aqueous cardamom extract enhances the cytotoxic activity of natural killer (NK) cells. A recent study by Shakeeb et al. (2022) reported that treatment for COVID-19 patients with a food supplement capsule as a natural anti-inflammatory (RECOVEREEZ FORTE™) resulted in an excellent anti-inflammatory action which is equivalent to steroids. They indicated that RECOVEREEZ FORTE ™ use for 5 days shows persistent anti-inflammatory action, recovery of COVID-19 symptoms and possible anti-viral action. Furthermore, when RECOVEREEZ FORTE™ consumed for 10 days, it may lead to the normalization of LDH. Nair (2020) indicated that a higher dose of cardamom reveals a higher anti-inflammatory effect on the skin than the effects of

the reference drug, indomethacin. Souissi et al. (2020) further provide evidence that anti-inflammatory activity may result from inhibiting the NF-kB signaling pathway, which could be used as a promising treatment and prevention of chronic inflammatory disorders, including periodontal diseases (Gupta et al., 2010). Cárdenas Garza et al. (2021) reported on the cardamom aqueous extract potential for their applications as natural anti-inflammatory adjuvants as they decrease the nitric oxide (NO) production in peritoneal macrophages.

11.4.8 Hepato-protective and Chemo-protective Effects

Alkhalifah et al. (2022) indicated that pretreatment with the cardamom extract (CE) prior to acetaminophen (APAP) administration could diminish serum levels of the hepatic function test and increase the nuclear factor erythroid 2-related factor 2 (Nrf2) nucleoprotein and HO-1 and NQO-1 pathway. In addition, the CE administration resulted in the down-regulation of malondialdehyde (MDA), inflammatory mediators of IL-1β, IL-6, TNF-α and NF-κB, and apoptotic markers of caspase 3, 9 and Bax. It amplified the enzymes of SOD, catalase, GSH-Px and GSH-R in the hepatic tissue of the studied samples. An earlier study by Shirwaikar et al. (1992) reported that Cardamom combined with other plants could reverse Carbon tetrachloride (CCl_4) induced–hepatotoxicity. Daneshi-Maskooni et al. (2017) reported on the effects of green cardamom on blood glucose indices, lipids, inflammatory factors, paraoxonase-1, sirtuin-1, and irisin in patients with nonalcoholic fatty liver disease and obesity. Elguindy et al. (2016) studied the effect of cardamom extract against diethylnitrosamine (DENA) at 200 mg/kg induced hepatocellular carcinoma (HCC). They reported a reduction in the levels of TNF, IL-1 and NF-κB, and a substantial increment in ornithine decarboxylase (ODC), hepatic malondialdehyde, and other liver injury markers, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT), as well as the enhancement of antioxidant enzymes activities following DENA treatment.

Another study by Qiblawi et al. (2015) reported that cardamom could reduce B(α)P-induced forestomach tumor incidence and yielded a significant enhancement for the hepatic activities (glutathione-S-transferases, superoxide dismutase, glutathione peroxidase, and catalase) in mice. Bhaswant et al. (2015) reported that green cardamom decreased plasma ALT and AST activity. However, it increased the plasma ALP activity.

11.4.9 Neuro-protective Effects

A recent study by Chowdhury and Kumar (2020) reported that α-terpinyl acetate is the main monoterpene component in *E. cardamomum* extract with acetylcholinesterase (AChE) inhibition. This compound can be used as a suitable lead compound

to develop a molecule that might have a multi-targeted directed ligand (MTDL) which has potential and disease amelioration effects in Alzheimer's disease (AD) (Abdul Manap et al., 2019). Another study by Gomaa et al. (2019) indicated that a terpenoid-rich *E. cardamomum* extract could prevent Alzheimer-like alterations induced in diabetic rats by inhibiting GSK3β activity, oxidative stress and pro-inflammatory cytokines in brainT2D rats. The beneficial effects of cardamom could be attributed to the anti-cholinesterase and antioxidant activity and the presence of endogenous antioxidants such as glutathione and superoxide dismutase (Kunwar et al., 2015) and showing a promising role as a natural memory booster in scopolamine-induced amnesia. Cardamom has also been observed to have the potential to alleviate AD-like changes through stimulation of attenuated insulin signal transmission in the brain, mitigation of related oxidative stress, and neuroinflammation (Gomaa et al., 2019). A1,8-cineole-rich extract (50 and 100 μM) of small cardamom seeds has been reported to be able to interfere with AD-related pathological events *via* impeding the production of reactive hydroxyl radicals, preventing the formation of Aβ42 deposits, and protecting cells from iron-induced death (Paul et al., 2020) or via GSK-3β down-regulation and Aβ production reduction by *in vivo* and *in vitro* BACE-1activity inhibition (An et al., 2022).

Bhaswant et al. (2015) studied the impact of green and black cardamom in a diet-induced rat model of human metabolic syndrome and reported that black cardamom is more effective in reversing the signs of metabolic syndrome than green cardamom. Furthermore, methanolic cardamom extract at 400 mg/kg was reported to reduce post-traumatic stress disorder (PTSD) like anxiety symptoms in Wistar rats (Masoumi-Ardakani et al., 2017). Abu-Taweel (2018) reported that when cardamom was consumed during pregnancy, offspring showed enhanced learning, memory, and behavior in mice offspring and could be helpful in Alzheimer's disease (AD). The same author added that cardamom administration leads to the enhancement of social activity, biochemical factors and development activities for Swiss-Webster mice offspring at different stages (Abu-Taweel, 2020).

11.4.10 Anti-diabetic Effects

In another clinical trial, Cheshmeh et al. (2021) presented that administration of green cardamom (3 g/day, 16 weeks) can control the expression of following diabetes and obesity genes; carnitine palmitoyltransferase 1A (CPT1A), fat mass and obesity-associated (FTO), leptin receptor (LEPR), lamin A/C, and peroxisome proliferator-activated receptor gamma (PPAR-γ) in women with polycystic ovary syndrome. Furthermore, *in vivo* study showed that cardamom powder at a dose of 1% of powder chow diet w/w for 8 weeks prevented obesity in high-fat diet-induced obese rats (Rahman et al., 2017). Yahyazadeh et al. (2021) summarized the anti-diabetic effects of cardamom and its bioactive compounds on the impact on glucose and insulin, whereby it decreases the glucose level and insulin resistance in the body, as well as increases the insulin level, pancreatic b-cells and glucose uptake

beside its remarkable antioxidant performance. Al-Yousef et al. (2021) studied the anti-obesity and antidiabetic activities of the aqueous extract of cardamom (1 mg/mL extract) in vitro and reported inhibition of pancreatic lipase of 62.2% with $IC_{50} = 288$ µg/mL, and α-amylase enzyme inhibition of 70.4% with $IC_{50} = 220$ µg/mL.

The effects of green cardamom supplementation on patients with type 2 diabetes mellitus (T2DM) have been reported by Zahedi et al. (2020, 2021, 2022). They indicated that cardamom intake (3 g/day) for 10 weeks functional properties of antioxidant, anti-inflammation, and blood pressure lowering properties might improve the endothelial function and lower the inflammatory biomarkers. Therefore, they hypothesized that cardamom supplementation could ameliorate endothelial dysfunction and mitigate inflammation in patients with T2DM. Furthermore, their results showed improvement of intercellular adhesion molecule-1 (ICAM), vascular cell adhesion molecule-1 (VCAM) and E-selectin serum levels and interleukin 6 (IL-6) reduction in the serum concentration with no effect on MMP9 and CD163 serum levels these patients were observed (Zahedi et al., 2021). In their latest findings, they reported a beneficial impact of green cardamom supplementation on systolic blood pressure (SBP), nitric oxide (NO) and high-sensitivity C-reactive protein (hs-CRP) levels in T2DM patients. They further recommended that improving blood pressure (BP) and inflammatory markers can help prevent complications and cardiovascular risk in T2DM patients (Zahedi et al., 2022).

11.4.11 Other Health-promoting Effects

Gilani et al. (2008) reported a combination of spasmogenic, spasmolytic, blood pressure (BP)-lowering, vasodilator, cardio-suppressant, diuretic and sedative activities for Cardamom crude extract using *in vitro* and *in vivo* techniques. These gut stimulatory and inhibitory effects were reported to be mediated via cholinomimetic and Ca^{++} antagonist mechanisms, respectively, as well as lowering the blood pressure (BP) through combined aspects of cholinergic and calcium channel blocking (CCB) pathways (Gilani et al., 2008). Estrogenic activity (EA) for cardamom methanolic extract (0.20 nM E2Eq mg^{-1} and anti-estrogenic activity of 0.02 µM ICI182780Eq mg^{-1} as well as androgenic activity of 0.30 nM R1881Eq mg^{-1} were reported by Real et al. (2015) using MCF-7 breast cancer cells using the in vitro receptor-specific bioassays. Based on their findings, they further concluded that methanol cardamom extract could interfere with the endocrine system via one or more hormonal receptors, and they further recommended future research work is needed to confirm cardamom's role as endocrine disrupters in humans (Real et al., 2015). Abdel-Rahman et al. (2017) reported the ameliorative effects of cardamom extracts on the cerebellum and midbrain of adult male albino rats. They showed that pre -and post-treatment with cardamom could ameliorate the hazardous effects of uranyl acetate dehydrate (UAD) intoxication. Paul et al. (2019) designed nanoliposomes to protect cardamom seed extract, enhancing *in vivo* therapeutic efficacies in restoring type 2 diabetes and hypercholesterolemia. They used soya

phosphatidylcholine and Tween 80 *via* probe-sonication. Furthermore, they reported that administration of the nanoliposomes (orally) in rats (550 mg/kg b.w.) could restore the rats' normal fasting blood glucose (FBG) levels and serum lipid profiles on day 35 through the specific regulations of related key enzymes.

Two clinical trials indicated the anti-obesity effects of cardamom. These include the administration of cardamom (3 g/day) for 3 months leads to the increment of sirtuin (SIRT1) from 1.2 to 1.3 ng/mL and reduces inflammation in overweight patients with non-alcoholic fatty liver disease (Daneshi-Maskooni et al., 2018). Naik and Ali (2018) studied the anticonvulsant activity of cardamom extract and reported an increment in the γ-aminobutyric acid (GABA) level. Another use for cardamom extract is for treating asthma in isolated tracheal tissues (Khan et al., 2011). They reported the possible mode of bronchodilator action, where crude cardamom extract affected the relaxation of both carbachol and K^+-induced contractions, such as verapamil, a Ca^{2+} antagonist (Khan et al., 2011). The antiviral activity of cardamom has been reported recently by Sangeetha et al. (2022). They found that docking and dynamics analyses of the target receptors with the lead phytocompound (Triprolidine) from *E. cardamomum* discovered possible inhibitory effects.

11.5 Conclusions

E. cardamomum extracts possess several bioactive compounds and demonstrate a potential for employing these extracts in the biofortification and formulation of safe and efficient food preservatives for several uses in aroma, cosmetics, food, pharmaceutical, and biomedical applications. The rich nutritional profile for cardamom as a source of dietary fiber, carbohydrates, proteins, and micronutrients of copper, iron, manganese, potassium, vitamins A, C, and B complex (B1–3 and B6) is responsible for its nutraceutical properties and as such finds uses as a functional ingredient in several functional food formulations and nutritional supplements. Although the literature has focused on the antimicrobial, anticancer and anti-inflammatory activities of cardamom extracts, the cardioprotective, gastroprotective, hepatoprotective and neuroprotective properties have increasingly reported a bearing on the development of the next generation of drugs.

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Chapter 12
Composition and Functional Properties of Cardamom Leaves

Reham Hassan Mekky, Mostafa M. Hegazy, Wael M. Afifi, Ahmad E. Mostafa, Hatem S. Abbass, and Muhamad Abd-Elraouf

Abbreviations

ABTS	2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
AgNPs	Silver nanoparticles
CD	Carbon dots
DPPH	2,2-diphenyl-1-picrylhydrazyl
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography coupled with mass spectrometry
TEAC	Trolox equivalent antioxidant capacity
XRD	X-ray diffraction

R. H. Mekky (✉)
Department of Pharmacognosy, Faculty of Pharmacy, Egyptian Russian University, Badr City, Cairo, Egypt
e-mail: reham-mekky@eru.edu.eg

M. M. Hegazy · A. E. Mostafa · M. Abd-Elraouf
Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt
e-mail: MostafaHegazy@azhar.edu.eg; Aemostafa@azhar.edu.eg; mhmraouf@azhar.edu.eg

W. M. Afifi · H. S. Abbass
Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

Department of Pharmacognosy, Faculty of Pharmacy, Sinai University, Ismailia, Egypt
e-mail: wael.afify@su.edu.eg; hsam8406@azhar.edu.eg

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12.1 Introduction

Cardamom is one of the world's ancient spices, native to India and Sri Lanka. Cardamom belonging to the family *Zingiberaceae*, known as the **"Queen of Spices"** is an important plantation crop in India (Chempakam & Sindhu, 2008). The name cardamom is used for species within three genera in the Ginger family (Zingiberaceae). One of these genera is *Amomum*, where the species are mainly found in Asia and Australia. Another, *Aframomum*, is distributed in Madagascar and other African countries, and *Elettaria* is distributed from India to West Malaysia. The genus *Elettaria* has seven species. The (small) cardamom of commerce belongs to the genus *Elettaria* and species *cardamomum* (Kaur et al., 2013). The cardamom plant is a 2–4 m tall herbaceous perennial with branched subterranean rhizomes from which several leafy shoots arise, forming a clump (Anandaraj & Sudharshan, 2011; Кханкан, 2019). Leafy shoots have a limited life span; the first year is mainly for vegetative growth, the second year for reproductive growth (flowers and fruits), and the third year is a senescence and death stage. New buds are formed from the base of the old shoots in the first and second year and, thus, in a clump of the old shoot (Anandaraj & Sudharshan, 2011). Young shoots and buds can be seen in varying numbers. The leaves are lanceolate in shape, and the lamina tapers into a sharp tip, 25–90 cm long and 5–15 cm wide. Leaves are dark green and shiny on the upper surface and pale green on the lower surface. The lower surface of the leaf could be smooth (glabrous) or pubescent (hairy), depending on the variety. Leaves are large, lanceolate to oblong-lanceolate, and glabrous on both surfaces (Madhusoodanan et al., 2002).

12.2 Ecology and Growing Conditions

Cardamoms are shade-loving plants; forest trees generally provide overhead shade. They grow well in places with well-distributed yearly rainfall and temperatures between 10° and 35 °C. Cardamom cultivation is mainly rain-fed and depends largely on the monsoon rains (Govindarajan et al., 1982). Cardamom grows wild in the shade in the forests of southern India as it does not tolerate direct sun. It thrives best in areas with uniform warm temperatures of 24–30 °C and means annual rainfall of 1500 mm distributed throughout the year on well-drained soil rich in organic matter. Shade is essential during the hot summer and rainy seasons, so shade is thinned. Cardamoms are traditionally cultivated under shade trees. Tall trees having well-spaced branching habits and small leaves are ideal shade trees for cardamom (Lim, 2013).

There are two main types of cardamom:

1. Small green cardamom (*Eletteria cardamomum*).
2. Large red/black cardamom (*Amomum subulatum* Roxb).

The most common type is the small green cardamom, while large cardamom is mainly grown in India, with some in Nepal and Bhutan. They both come from the Zingiberaceae family of plants (Ashokkumar et al., 2020). Since cardamom seeds

and pods are manually-collected and in high demand worldwide, they are considered the third most expensive spice in the world (Lim, 2013). In this sense, there is a high demand for the cardamom essential oil from other cardamom parts, as in the case of the leaves considered an agri-food residue. Agri-food residues are considered a source of bioactive metabolites and bioresources that continue to make a sustainable, economical model. In this context, several studies focused on the agri-food residues significance as source of bioactive metabolites and biofuels being a more sustainable alternative in many industries (Abouzed et al., 2018; Mekky et al., 2019, 2022; Gómez-Cruz et al., 2021; 2022).

12.3 Bioactive Metabolites Present in Cardamom Leaves

Several studies revealed cardamom leaves' phytochemical constituents, giving a new perspective on such under-valued agri-food residue. Tracing literature regarding the cardamom leaves, it was found that a total of 79 metabolites were detected (Table 12.1 and Fig. 12.1). They are classified into monoterpenes (Fig. 12.2), sesquiterpenes (Fig. 12.3), diterpenes, alcohols, aldehydes, ketones, acids, esters, other phenolics, and nitrogenous compounds (Fig. 12.4) employing GC-MS for the analysis of the extracts (Mahmud, 2008; Asakawa et al., 2017; Prabu et al., 2019; Sharma et al., 2020; Jena et al., 2021). It bears noting that monoterpenes, followed by sesquiterpenes, were the main observed classes in cardamom leaves, with 40 and 24 derivatives, respectively (Table 12.1, and Fig. 12.1). The demand for cardamom essential oil in the international market is increasing, while cardamom seeds and pods are the third most expensive spice in the world (Lim, 2013). Therefore, the chemical profiling of volatile compounds of different cardamom plant parts is explored in several research records to provide a more economical source of cardamom essential oil (Mahmud, 2008; Jena et al., 2021). In this context, (Mahmud, 2008) reported that the major components of cardamom leave essential oil was (4-terpineol, 1,8-cineol α-terpinolene, *p*-cymene, α -Terpinene, and α -Terpineol) whereas Sharma et al. (2020) accounted that the major components of cardamom leave essential oil was (terpinen-4-ol, eucalyptol, *p*-cymene, *trans*-phytol, and *cis*-sabinene). Moreover, Jena et al. (2021) studied the essential oils composition of cardamom leaves, where the major constituents of the essential leaf oil were (1,8-cineole, camphene, camphor, and tricyclene).

12.4 Biological Activity

12.4.1 Anti-scabies Activity

The *E. cardamomum* leaves essential oil was evaluated for its *in vitro* anti-scabies potential against *Sarcoptes scabiei* mites (Sharma et al., 2020). Based on the percent mean mortalities study, a 10% concentration of *E. cardamomum* essential oil killed all the mites within 60 min, similar to Permethrin®, whereas a 5% diluted solution took

Table 12.1 Bioactive metabolites reported in cardamom leaves

#	Compound	Molecular formula	Occurrence	Geographical Origin	Extraction procedure	Method of Analysis	Reference
Monoterpenes							
1	(E)-β-Ocimene	$C_{10}H_{16}$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
2	δ-3-Carene	$C_{10}H_{16}$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
3	α-Fenchene	$C_{10}H_{16}$	Dried leaves	Tokushima, Japan	extracted with diethyl ether for a week	GC-MS	Asakawa et al. (2017)
4	Fenchone	$C_{10}H_{16}O$	Dried leaves	Tokushima, Japan	extracted with diethyl ether for a week	GC-MS	Asakawa et al. (2017)
5	α-Pinene	$C_{10}H_{16}$	Dried leaves	Tokushima, Japan	Steam Distillation	GC-MS	Asakawa et al. (2017)
			Dried leaves	Tokushima, Japan	extracted with diethyl ether for a week	GC-MS	Asakawa et al. (2017)
			Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India	Hydrodistillation	GC-MS	Sharma et al. (2020)

6	*β*-Pinene	$C_{10}H_{16}$	Dried leaves	Tokushima, Japan	Steam Distillation	GC-MS	Asakawa et al. (2017)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
7	*p*-Cymene	$C_{10}H_{14}$	Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
8	Myrcene	$C_{10}H_{16}$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
9	*cis*-Sabinene	$C_{10}H_{16}$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
			Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
10	Limonene	$C_{10}H_{16}$	Dried leaves	Tokushima, Japan	Steam Distillation	GC-MS	(Asakawa et al. 2017)
11	*α*-Thujene	$C_{10}H_{16}$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
			Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)

(continued)

Table 12.1 (continued)

#	Compound	Molecular formula	Occurrence	Geographical Origin	Extraction procedure	Method of Analysis	Reference
12	Camphene	$C_{10}H_{16}$	Dried leaves	Tokushima, Japan	Steam Distillation	GC-MS	Asakawa et al. (2017)
			Dried leaves	Tokushima, Japan	extracted with diethyl ether for a week	GC-MS	Asakawa et al. (2017)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
13	Camphene hydrate	$C_{10}H_{18}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
14	Terpinolene	$C_{10}H_{16}$	Dried leaves	Tokushima, Japan	Steam Distillation	GC-MS	Asakawa et al. (2017)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
15	α-Terpiene	$C_{10}H_{16}$	Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
			Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
16	γ-Terpinene	$C_{10}H_{16}$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
			Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
17	δ-Terpineol	$C_{10}H_{18}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)

18	α-Terpineol	$C_{10}H_{18}O$	Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
19	α-Terpinyl acetate	$C_{12}H_{20}O_2$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
20	4-Terpineol	$C_{10}H_{18}O$	Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
21	1,8-Cineole	$C_{10}H_{18}O$	Dried leaves	Tokushima, Japan	Steam Distillation	GC-MS	Asakawa et al. (2017)
			Dried leaves	Tokushima, Japan	extracted with diethyl ether for a week	GC-MS	Asakawa et al. (2017)
			Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)

(continued)

Table 12.1 (continued)

#	Compound	Molecular formula	Occurrence	Geographical Origin	Extraction procedure	Method of Analysis	Reference
22	2 (10)-pinen-3-one, (±)-[pinocarvone]	$C_{10}H_{14}O$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
23	1-Cyclohexene-1-methanol, 4-(1-methyl phenyl)-(p-mentha-1,8-dien-7-ol) (Perillic alcohol)	$C_{10}H_{16}O$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
24	Linalool	$C_{10}H_{18}O$	Dried leaves	Tokushima, Japan	Steam Distillation	GC-MS	Asakawa et al. (2017)
			Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
25	2H-pyran-3-ol, 6-ethenyl tetrahydro-2,2,6-trimethyl- (Linalool oxide pyranoside)	$C_{10}H_{18}O_2$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
26	3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethylene-(α-compholenal)	$C_{10}H_{16}O$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
27	Ascaridole	$C_{10}H_{16}O_2$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
		$C_{10}H_{16}O_2$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
28	Bicyclo [3.1.1]hept-2-ene-2-methanal, 6,6-dimethyl-(Myrtenol) (Darwinol)	$C_{10}H_{16}O$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)

29	Bicyclo [3.1.1]hept-2-ene-2-corboxaldehyde, 6,6-dimethyl-(Myrtenal)	$C_{10}H_{14}O$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
30	Borneol	$C_{10}H_{18}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
31	Bornyl acetate	$C_{12}H_{20}O_2$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
32	Camphor	$C_{10}H_{16}O$	Dried leaves	Tokushima, Japan	Steam Distillation	GC-MS	Asakawa et al. (2017)
			Dried leaves	Tokushima, Japan	extracted with diethyl ether for a week	GC-MS	Asakawa et al. (2017)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
33	Carvenone	$C_{10}H_{16}O$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
34	*cis*-Menth-2-en-1-ol	$C_{10}H_{18}O$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
			Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
35	Geranial	$C_{10}H_{16}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)

(continued)

Table 12.1 (continued)

#	Compound	Molecular formula	Occurrence	Geographical Origin	Extraction procedure	Method of Analysis	Reference
36	Nerol	$C_{10}H_{18}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
37	*trans*-Piperitol	$C_{10}H_{18}O$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
38	*trans*-Sabinene hydrate	$C_{10}H_{18}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
39	Tricyclene	$C_{10}H_{16}$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
40	Tran-3 (10)-caren-2-ol	$C_{10}H_{16}O$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
Sesquiterpenes							
41	*β*-Caryophyllene	$C_{15}H_{24}$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
		$C_{15}H_{24}$	Dried leaves	Tokushima, Japan	extracted with diethyl ether for a week	GC-MS	Asakawa et al. (2017)
		$C_{15}H_{24}$	Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
42	Caryophyllene oxide	$C_{15}H_{24}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
43	Germacrene D	$C_{15}H_{24}$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)

44	Isoledene	$C_{15}H_{24}$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
45	δ-Cadinene	$C_{15}H_{24}$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
46	γ-Cadinene	$C_{15}H_{24}$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
			Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
47	β-Bisabolene	$C_{15}H_{24}$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
48	β-Elemene	$C_{15}H_{24}$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
49	α-Bergamotene	$C_{15}H_{24}$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
50	α-Gurjunene	$C_{15}H_{24}$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
51	(Z)-β-Farnesene	$C_{15}H_{24}$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
52	β-Farnesene	$C_{15}H_{24}$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
53	2E,6E-Farnesol	$C_{15}H_{26}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)

(continued)

Table 12.1 (continued)

#	Compound	Molecular formula	Occurrence	Geographical Origin	Extraction procedure	Method of Analysis	Reference
54	2Z,6E-Farnesol	$C_{15}H_{26}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
55	Z, Z-Farnesyl acetone	$C_{18}H_{30}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
56	(E)-Nerolidol	$C_{15}H_{26}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
57	Carotol	$C_{15}H_{26}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
58	Caryophylladienol II	$C_{15}H_{24}O$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
59	Cubenol	$C_{15}H_{26}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
60	Guaiyl acetate	$C_{17}H_{28}O_2$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
61	Humulene epoxide	$C_{15}H_{24}O$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
62	Humulene epoxide II	$C_{15}H_{24}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
63	*β*-Eudesmol	$C_{15}H_{26}O$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
64	Longifolenaldehyde	$C_{15}H_{24}O$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)

Diterpenes							
65	Phytol	$C_{20}H_{40}O$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
66	Retinal, 9-cis-	$C_{20}H_{28}O$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
Alcohols							
67	Heptan-2-ol	$C_7H_{16}O$	Dried leaves	Tokushima, Japan	Steam Distillation	GC-MS	Asakawa et al. (2017)
			Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
68	1-Heptatriacotanol	$C_{37}H_{76}O$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
Aldehydes and Ketones							
69	*trans*-2-Decenal	$C_{10}H_{18}O$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
70	Cryptone	$C_9H_{14}O$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
71	4-Phenyl-2-butanone	$C_{10}H_{12}O$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
Acids							
72	Aroma decanoic acid	$C_{10}H_{20}O_2$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
73	Hexadecanoic acid (Palmitic Acid)	$C_{16}H_{32}O_2$	Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
			Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)

(continued)

Table 12.1 (continued)

#	Compound	Molecular formula	Occurrence	Geographical Origin	Extraction procedure	Method of Analysis	Reference
Esters							
74	Methyl jasmonate	$C_{13}H_{20}O_3$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
75	Z-Methyl cinnamate	$C_{10}H_{10}O_2$	Dried leaves	Odisha, Eastern India	Hydrodistillation	GC-MS	Jena et al. (2021)
		$C_{10}H_{10}O_2$	Fresh leaves	Morni Hills, India.	Hydrodistillation	GC-MS	Sharma et al. (2020)
76	E-Methyl cinnamate	$C_{10}H_{10}O_2$	Dried leaves	Tokushima, Japan	Steam Distillation	GC-MS	Asakawa et al. (2017)
Other phenolics							
77	Apiole	$C_{12}H_{14}O_4$	Fresh leaves	Lahore, Punjab, Pakistan	Hydrodistillation	GC-MS	Mahmud (2008)
78	Asarone	$C_{12}H_{16}O_3$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)
Nitrogenous compounds							
79	Benzeneethanamine, α methyl- (Amphetamine)	$C_9H_{13}N$	Dried leaves	Kerala, South India	Extracted with ethanol	GC-MS	Prabu et al. (2019)

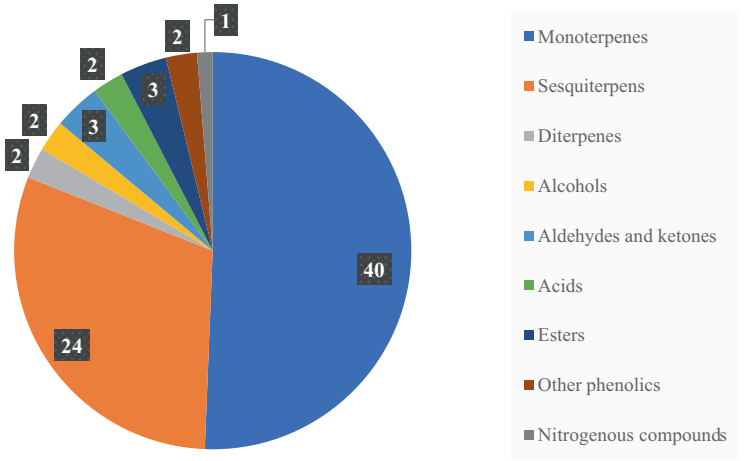

Fig. 12.1 Classification of different bioactive metabolites reported in cardamom leaves

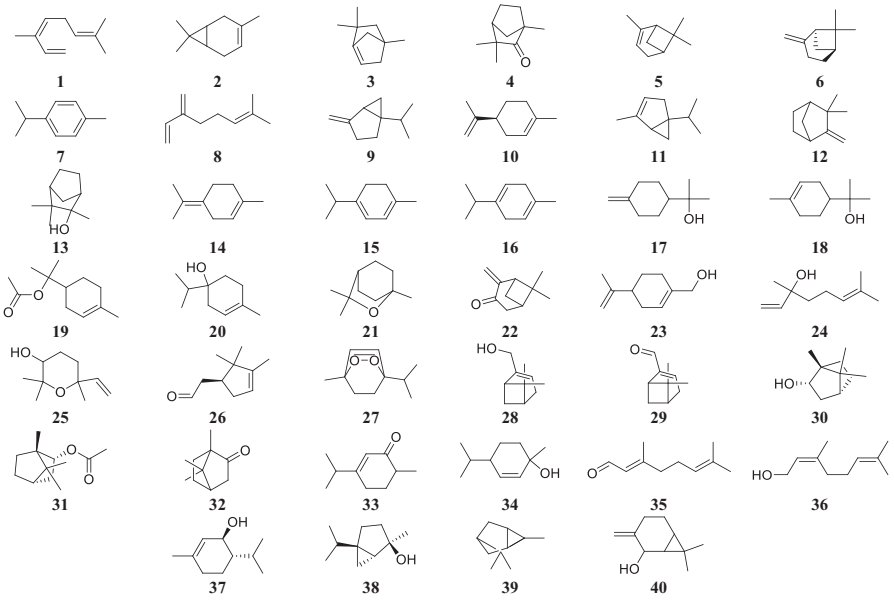

Fig. 12.2 Chemical structures of monoterpenes reported in cardamom leaves

80 min to kill all the mites. The natural components terpinen-4-ol, γ-terpinene, and eucalyptol with insecticidal activity may be responsible for the anti-scabies potential of the oil. Furthermore, eucalyptol enhances the superoxide dismutase and glutathione-s-transferase enzymatic activity, which play a role in the protection mechanism of *S. scabiei* mites. Therefore, *E. cardamomum* leaves essential oil can be used as an alternative to cure and effectively control *S. scabiei* (Sharma et al., 2020).

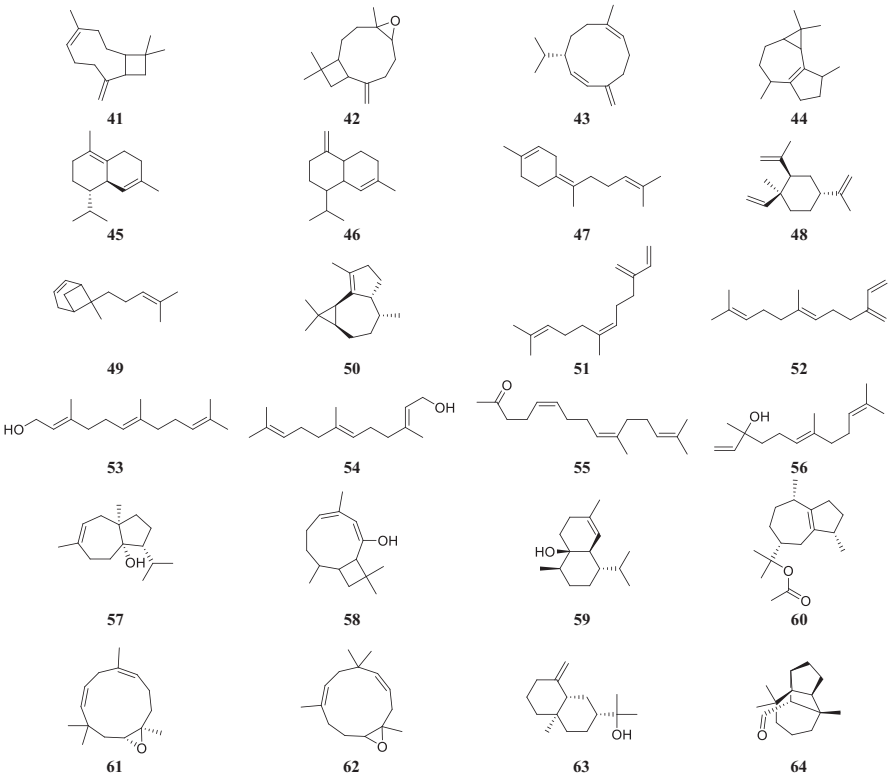

Fig. 12.3 Chemical structures of sesquiterpenes reported in cardamom leaves

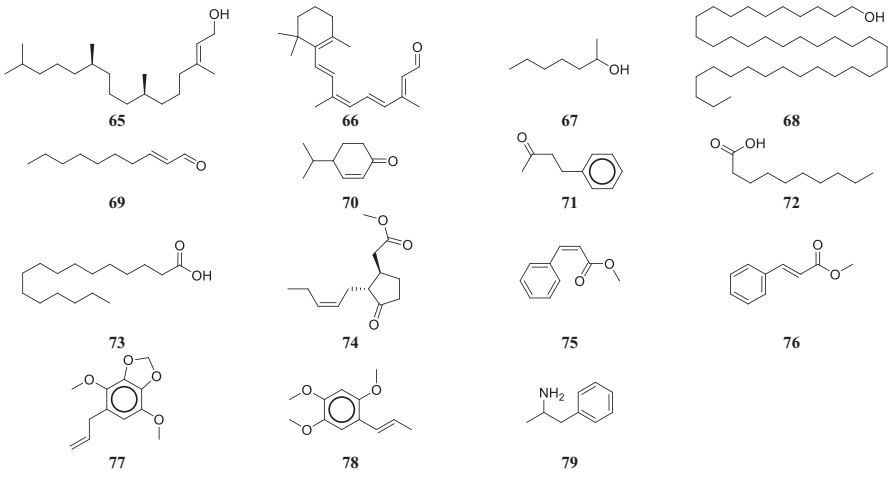

Fig. 12.4 Chemical structures of diterpenes, alcohols, aldehydes, ketones, acids, esters, other phenolic and nitrogenous compounds reported in cardamom leaves

12.4.2 Antioxidant and Antiaging Activities

Oxidative stress is a major causative factor of several chronic and degenerative diseases such as diabetes, cancer, immune dysfunction, and Parkinson's disease (Ashokkumar et al., 2020). Natural and synthetic antioxidants can free radical scavenging and suppress chronic and degenerative diseases. Natural antioxidants are preferred and considered safer than synthetic forms (Saeed et al., 2014, Zahra et al., 2016, Ashokkumar et al., 2020). In this sense, Zahra et al. (2016) screened the potency of several Zingiberaceae leaves as an antioxidant and antiaging agent. They extracted the leaves of species of Zingiberaceae, including *Elettaria cardamomum,* with ethyl acetate and methanol sequentially, determined the total phenolic and flavonoid contents of the extracts spectrophotometrically and tested antioxidant activities by using DPPH and ABTS assays. The results showed that the flavonoid content of the ethyl acetate and methanol extracts of cardamom leaves is 13.0 and 0.84 g quercetin eq./100 g extract respectively, while the total phenolic is 22.0 and 20.4 g gallic acid eq./100 g extract respectively. The methanol extract of cardamom leaves showed antioxidant capacity in the DPPH· (IC_{50} 671 mg/mL) and TEAC (7.93 mg/g) assays. It bears noting that the antiaging activity was tested using the antiglycation method and was attributed to the high flavonoid content of the leaves. At the same time, Asra et al. (2019) studied the antioxidant activity of the ethanol extract, hexane, ethyl acetate, and water fractions of cardamom leaves (*Elettaria cardamomum* (L.) Maton). The antioxidant activity examination was determined using reagent DPPH· using UV-Visible spectrophotometry and gallic acid as standard. The results of the antioxidant activity examination of ethanol extract, hexane, ethyl acetate, and water of cardamom leaves showed the IC_{50} of 4058 μg/mL, 8419 μg/mL, 2281 μg/mL, and 3889 μg/mL respectively. The results of phytochemical screening of cardamom leaves showed that ethanol extract and water fraction contain phenol, flavonoids, tannins, and saponins, for ethyl acetate fraction contains phenol, flavonoids, and tannins, for hexane fraction the results were negative for all the compounds tested.

12.4.3 Antidiabetic, Weight Loss, and Hypocholesterolemic

A current study showed that the diabetic rats given the *E. cardamomum* leaves extract for 7 consecutive days decreased their blood glucose level from 221.6 to 122.2 mg/dL (Winarsi et al., 2014). The blood glucose levels of these treated rats continuously decreased when the E. cardamomum leaves extract was given for a longer time (14 days) of 201.7 to 102.8 mg/dL compared to those of diabetic group rats given feed only (Ashokkumar et al., 2020). *E. cardamomum* leaves extract phenolic compound has a similar structure to flavone, flavanone, flavonol, flavanonol, catechin, anthocyanidin, and isoflavone with anti-diabetic properties. In the reduction of absorption activity of sodium-dependent glucose in order to control blood glucose levels, flavonoids might also take another route by slowing down glucosidase-alpha activity in the colons or by reduction of glucose 6-phosphatase and

fructose 1,6-bisphosphatase significantly in the liver and tissues or by inducing secretion of insulin in the diabetic rats. Flavonoid is an antioxidant compound which can reduce cell damage due to diabetes. Flavonoid was also reported to stimulate the synthesis of glycogen in the rat tissues through a mechanism of transduction of insulin signals, then showed a double effect as anti-hyperglycemic or insulin secretion and insulin mimetics or glycogen synthesis. Another possibility is stimulating glucose absorption in the peripheral tissues and regulating enzymes of carbohydrate metabolism. Flavonoids could also affect insulin secretion due to their insulin-mimetic characteristics, which might be related to the pleiotropic mechanism. In a long period of intervention, this *E. cardamomum* leaves extract containing flavonoid, given to the diabetic rats per oral, showed a decrease of glucose in the blood plasma up to 70%, but in the diabetic rats were not given the compound reduced to 47%. Similar to this research, other types of flavonoids, hesperidin and naringin, could also reduce the blood glucose level of diabetic rats. Those flavonoids might take cellular or molecular mechanisms (Winarsi et al., 2014).

12.4.4 Antibacterial Activity

The ethanol extract of *E. cardamomum* leaves was evaluated for their antibacterial activity compared with standard antibiotic penicillin (10 μg/mL) *in-vitro* by disc diffusion method using *Escherichia coli* and *Klebsiella pneumonia* as test organisms. The results were recorded by measuring the growth inhibition zone surrounding the disc. The experiments were done in triplicate. The ethanolic extract of *E. cardamomum* leaves showed a maximum zone of inhibition against *K. pneumonia* (16 mm at 100 μg/mL) when compared to that of standard (Penicillin) (Priyanka et al., 2015). The essential oil from *Elettaria cardamomum* leaves possesses potent antimicrobial properties (Batubara et al., 2016). The antibacterial activity is due to major phytochemicals like retinal 9-cis in the plant leaves (Priyanka et al., 2015). The most prospective essential oil is from *Elettaria cardamomum* leaves with eucalyptol as the active compound (Batubara et al., 2016). The aqueous leaf extracts and crude oil of cardamom possess potent antimicrobial properties (Jebur et al., 2014). Several studies reported that cardamom has potent antimicrobial activity against various human pathogenic Gram-positive and Gram-negative bacteria according to the zone of inhibition results, including the aqueous leaves and crude oil extracts. The inhibiting activities reported against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia, Enterobacter spp., Acinetobacter, E.coli, Serratia* spp. and *Salmonella typhi* (Jebur et al., 2014). The cardamom's antibacterial activity is comparable to ciprofloxacin's (Jebur et al., 2014). Antimicrobial characteristics of the *Elettaria cardamomum* leaves are due to various chemical compounds, including volatile oils, alkaloids, and tannins in their tissues. The chemical composition of *Elettaria Cardamomum* varies considerably with the product's variety, region, and age.

12.4.5 Antifungal Effect

Comparative analysis of antifungal efficacy of *E. cardamomum* leaf extracts green AgNPs and commercial agricultural fungicides combined with different synthetic fungicides. When used in combination, synergistic interaction was observed between green AgNPs and fungicides, carbendazim, mancozeb, and thiram (Jamdagni et al., 2018). *Fusarium oxysporum* was the most sensitive fungus, and *Penicillium expansum* was the least sensitive for almost all tested combinations. MIC analysis Agar well diffusion was used to study fungal growth inhibition. Fungal growth failed to occur at high concentrations of nanoparticles and increased steadily at lower concentrations (Jamdagni et al., 2018). Cardamom extract contains antifungal compounds against *Colletotrichum gloeosporioides* with acceptable limits phytotoxicity (Yulia et al., 2006). Surprisingly, the leaf extract of *E. cardamomum* nanoparticles showed good antifungal activity against all the tested fungal phytopathogens and could serve as potential antifungal agents (Jamdagni et al., 2021).

12.5 Green Synthesis of Silver Nanoparticles By Cardamom Leaves

Nanotechnology has magical research attractiveness worldwide because of the extreme difference and uniqueness in physical and chemical properties of nanostructures compared to their bulk equivalents. Nanoparticles are one of the various nanostructures which show multiple applications in many fields of our lives nowadays (Jamdagni et al., 2021). The basic nanoparticle synthesis techniques are "top-down" and "bottom-up" techniques. "Top-down" techniques are "break down" applications of mechanical and physical technologies such as atomization, laser ablation, etc. "Bottom-up" techniques are "build-up" technologies depend on chemical principles such as pyrolysis, sol-gel process, etc., for collecting atoms into more complex nanostructures. Physical and chemical methods require multi-step, tedious, environmentally polluting processing. So, researchers are forced to shift towards greener and safer nanoparticle synthesis techniques (Jamdagni et al., 2021). Biogenic synthesis of nanoparticles, a "bottom-up" technique, can use various biological entities, such as plant extracts, microbial cell filtrate, and isolated biomolecules, such as peptides, carbohydrates, and lipids. These biological entities and biomolecules act as efficient reducing agents and serve as capping agents and provide stability to the suspension. Various phytochemicals in plant extracts reduce the metal salt into nanoparticles and are adsorbed at the surface of newly synthesized nanoparticles, increasing the stability of nanoparticles'aqueous suspensions. On the other hand, the complexity of the components of plant extracts causes difficulty in elucidating the exact mechanism of reduction (Jamdagni et al., 2021). In this context, (Jamdagni et al., 2018,

Jamdagni et al., 2021) used the leaves extract of *Elettaria cardamomum* instead of seeds extract as a reducing and capping agent for nanoparticle green biogenic synthesis from the parent solution of silver nitrate to make the process more economical and eco-friendly and the synthesized AgNPs were tested as an agricultural fungicide. This study is based on the fact that silver has antimicrobial properties and can fight against infections. (Jamdagni et al., 2018) Silver nanoparticles (AgNPs) have better antimicrobial potential than the ionic form of the metal, making them one of the most popular metal nanoparticles used in antimicrobial applications. AgNPs are found to be active against several pathogens, including bacteria, fungi, yeasts, viruses, etc. So, they can help in the battle against the problem of antibiotic resistance development (Khatri et al., 2017, Jamdagni et al., 2018, Jamdagni et al., 2021).

Besides, Zaib et al. (2021) made a green synthesis of the carbon dots (CDs) from leaves of *E. cardamomum* using a simple ultrasonication technique and tested its Congo red (CR). Methylene blue (MB) dyes degradation activity indicates its ability to purify dyes in industrial wastewater. In ultrasonication technique synthesis of the carbon dots (CDs) from leaves of *Elettaria cardamomum* (E.C), the definite amount of dried leaves of *Elettaria cardamomum* was mixed with 20 mL of distilled water, then ultra-sonicated (40 kHz) for 45 min. After ultra-sonication, the greenish-brown product was centrifuged at 4500 rpm for 15 min. Ultimately, the supernatant was filtered through a 0.22 μm membrane filter to remove larger particles (Zaib et al., 2021). The confirmation of CDs was observed with an ultraviolet-visible spectrophotometer (the presence of carbon dots showing two absorption peaks at 220 and 272 nm). The synthesized CDs are characterized using Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Raman spectroscopy, and Photoluminescence spectroscopy (Zaib et al., 2021).

12.6 Conclusion

Although there are limited chemical and biological studies on cardamom leaves compared to fruits, the leaves are still part of the spices queen plant that carries its potential. For instance, green synthesized silver nanoparticles with cardamom leaf extract showed good antifungal activity against several fungal phytopathogens. Furthermore, cardamom leaves, aqueous extract, and essential oil possess potent antimicrobial activity against various human pathogenic Gram-positive and Gram-negative bacteria. Moreover, leaves extracts exhibit antihyperglycemic activity by reduction of glucose absorption and insulin-mimetic activity. In conclusion, leaf extract showed promising activities and needs more *in vivo* and clinical studies for those activities to emerge as pharmaceutical products for treating scabies, microbial infections, and diabetes mellitus.

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Chapter 13
Chemistry and Functionality of Black Cardamom (*Amomum subulatum*)

Rajan Sharma, Ahmed A. Hussein, Natascha Cheikhyoussef, and Ahmad Cheikhyoussef

Abbreviations

ACE	Angiotensin-converting enzyme
$AgNO_3$	Silver nitrate
Ang II	Angiotensin II
CE	Catechin equivalent
CH_3OH	Methanol
$CHCl_3$	Chloroform
DLA	Dalton's Lymphoma Ascites
DMF	Dose-modifying factor
DNA	Deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
G2/M	Growth 2 mitosis phase
GAE	Gallic acid equivalent
GC-MS	Gas chromatography mass spectrometry
GSH	Glutathione
$HAuCl_4.3H_2O$	Hydrogen tetrachloroaurate(III) trihydrate
HCl	Hydrochloric acid
Hg^{2+}	Mercury (II) ion
IC_{50}	Half-maximal inhibitory concentration

R. Sharma · A. A. Hussein (✉)
Chemistry Department, Cape Peninsula University of Technology, Cape Town, South Africa
e-mail: mohammedam@cput.ac.za

N. Cheikhyoussef
Ministry of Higher Education, Technology and Innovation, Windhoek, Namibia

A. Cheikhyoussef
Science and Technology Division, Multidisciplinary Research Service, Centre for Research Services, University of Namibia, Windhoek, Namibia

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IL-1	Interleukin-6
IL-1β	Interleukin-1-beta
LDL	Low-density lipoprotein
L-NAME	N^{ω}-Nitro-L-arginine methyl ester
MDA	Malondialdehyde
MEBC	Methanolic extract of black cardamom
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-κB	Nucelar factor kappa B
PARP-1	poly(ADP-ribose)polymerase-1
SOD	Superoxide dismutase
TBI	Total body irradiation
TNF-α	Tumor necrosis factor alpha
VLDL	Very-low-density lipoprotein

13.1 Introduction

Amomum subulatum Roxburgh (black cardamom or large cardamom), an aromatic and medicinal spice, is the dried fruit of a perennial herbaceous plant belonging to the family Zingiberaceae. It is native to the Eastern Himalayan Region comprising Nepal, Bhutan and India. It is the main cash crop of this region, with Sikkim as the leading producer. William Roxburgh first described this plant in his book "Plants of the Coast of Coromandel" (Roxburgh, 1820a) and "Flora Indica" (Roxburgh, 1820b). Locally, it is called *alainchi* and is one of the oldest spices, with its mention in Ayurvedic formulations where its Sanskrit name is *Brihadela*. It was known as *Amomum* to Greeks and Romans in the fourth century BC and was recorded by the Greek philosopher Theophrastus, the Greek father of botany (Sharma et al., 2009). The indigenous inhabitants of Sikkim are believed to be the first to collect large cardamom pods from the forests for medicinal purposes and as aromatic wild fruit. Today, India is the largest producer and exporter of large cardamom, producing 54% of the world's production, and Sikkim contributes to 88% of India's production (Abdullah, 2020).

Black cardamom is in the form of seed pods, which are dark brown to black. These pods are used as a spice, similar to green cardamom but possess different aromas and flavors. First, it has a strong smokey flavor and aroma derived from the traditional drying procedure over open flames.

13.2 Botany

Amomum is the second-largest genus of the Zingiberaceae family, with 150 species. It is cultivated in marshy and shady places across hills near water streams at 765 to 1675 above sea level with a rainfall of 3000–3500 mm per annum. It is a tall, evergreen monocot plant with subterranean rhizomes. The rhizomes produce several leafy shoots and panicles. The leaves are oblong and lancolate, 30–60 cm long. The

plant matures during the third year of its growth, and its height reaches up to 1.5 to 3 meters. Calyx and tube sections are sub-obtuse, and the upper one is cuspidate. The matured fruit is reddish brown, trilocular and contains a pinkish cultivated capsule. The capsules are 2 to 2.5 cm, oval to globular in shape, echinate containing several aromatic seeds in each cell with a sticky, sugary pulp (Kumar et al., 2012). Flowering occurs in the spring, and harvesting occurs between September and November. Apart from its aromatic and culinary applications, black cardamom has high medicinal values, as reported in the Ayurvedic and Unani systems of medicine.

13.3 Phytochemistry

Phytochemical screening of the methanol extract of black cardamom revealed the presence of many important classes of compounds with potential pharmaceutical importance (Drishya et al., 2022a). The qualitative determination of phytochemical constituents revealed a high concentration of flavonoids, terpenoids, and phenols in the methanol extract, whereas alkaloids, sugars, steroids, tannins and quinones were present in moderate concentration. The concentration of cardiac glycosides and saponins was very low, and there was no detection of anthroquinones in the preliminary screening of the extract.

There are reports of isolating important secondary metabolites like cardamonin and alpinetin among volatile and 1,8-cineole among volatile phytoconstituents from the black cardamom extract. The following sections focus on recent advances in black cardamom's phytochemical analysis and therapeutic applications.

13.4 Volatile Phytoconstituents

The volatile phytoconstituents from the black cardamom are represented by essential oils separated from different parts of the black cardamom plant. A recent GC-MS quantification of essential oil obtained by the supercritical fluid exchange technique demonstrated the presence of 1,8-cineole (**1**, 44.24%), α-terpenyl acetate (**3**, 12.25%), nerolidol (**11**, 6.03%) and sabinene (**5**, 5.96%) as major bioactive constituents (Algburi et al., 2021). The essential oil of fresh leaves of black cardamom by hydrodistillation indicated the presence of thirty-nine phytoconstituents with major constituents as terpinen-4-ol (**2**, 29.87%), 1,8-cineole (**1**, 18.69%), β-phellandrene (**6**, 7.97%), γ-terpinene (**7**, 6.67%), *p*-cymene (**8**, 6.20%) (Sharma et al., 2020). An analysis of the essential oil of freshly dried seeds of large cardamom obtained by hydrodistillation identified thirty-three phytoconstituents, and 1,8-cineole (**1**, 81.5–86%) was found to be the major component of essential oil (Rout et al., 2003). The pericarp (husk) of the black cardamom yielded 0.18% essential oil by hydrodistillation, and its GC-MS analysis identified thirty-seven phytoconstituents constituting more than 98% of the essential oil. The major compounds identified by the analysis were 1,8-cineole (**1**, 38.7%), β-pinene (**10**, 13.6%), α-terpineol (**9**, 12.6%), spathulenol (**12**, 8.3%), 4-terpineol (**4**, 4.5%), germacrene-D (**13**, 3.0%), α-pinene (**9**, 2.8%) and β-selinene (**14**, 2.7%) (Pura Naik et al., 2004).

The essential oil of black cardamom has been analyzed by researchers from different parts of the plant collected from different locations. Their composition may vary for obvious reasons, but their major and respective components show a similar trend, with 1,8-cineole (**1**, eucalyptol) being the major component (Fig. 13.1). 1,8-cineole imparts a pungent and harsh aroma to the black cardamom, whereas a sweet and earthy aroma comes from terpinyl acetate (**3**).

13.5 Non-volatile Phytoconstituents

Phytochemical analysis of a different extract of black cardamom has led to many pharmaceutically important bioactive compounds, leading to a better understanding of its reported ethnomedicinal applications. For example, cardamonin (**15**), and

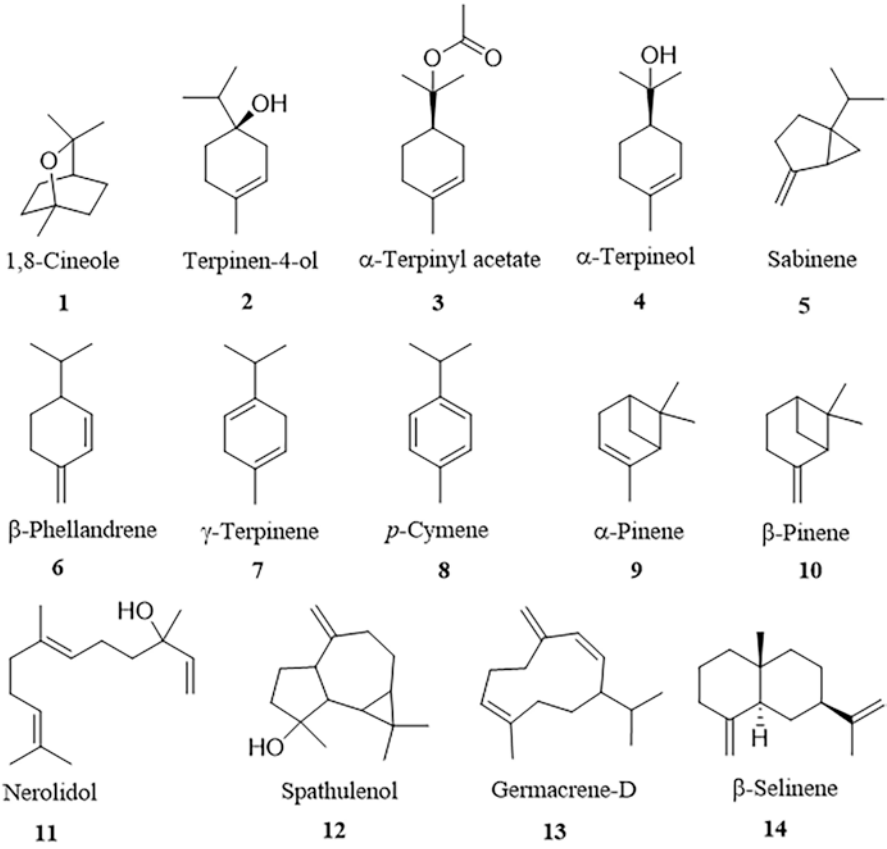

Fig. 13.1 Major phytoconstituents of essential oil of black cardamom

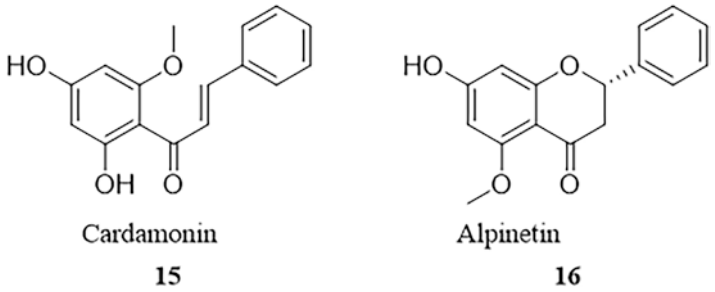

Fig. 13.2 Chemical structure of cardamonin and alpinetin

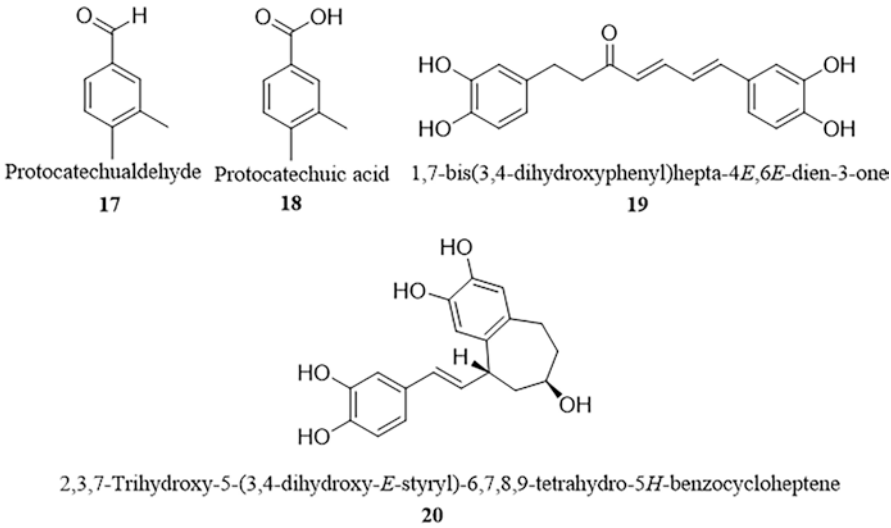

Fig. 13.3 Structure of four compounds (**17**–**20**) isolated for the first time from black cardamom

alpinetin (**16**), were reported to be isolated from the ethereal extract of the seeds of black cardamom (Fig. 13.2) (Rao et al., 1976).

Four compounds, **17–20** (Fig. 13.3)**,** have been isolated from the ethyl acetate soluble fraction of the black cardamom extract. This is the first report of isolating these compounds from black cardamom (Kikuzaki et al., 2001).

Extraction of fresh black cardamom pod husk with methanol HCl led to the isolation of a mixture of two deep pinkish-red pigments in the ratio of 1:2. These pigments (Fig. 13.4) were separated by paper chromatography to yield cyanidin-3-glucoside (**21**) and cyanidin-3,5-diglucoside (**22**) (Pura Naik et al., 1999).

A phytochemical investigation of the fruits of black cardamom led to the isolation of four new phytoconstituents viz. geranil-3(10)-en-9-olyl octadec-9-enoate (**23**), geranil-3(10)-en-9-carboxyl-β-D-arabinopyranoside (**24**), geranilan-9-carboxy-α-L-arabinopyranoside (**25**), stigmast-5-en-3β-ol-3β-D-arabinopyranosyl-2′-(3′-methoxy) benzoate-3′-octadec-9″,12″,15″-trienoate (**26**) and were given the common

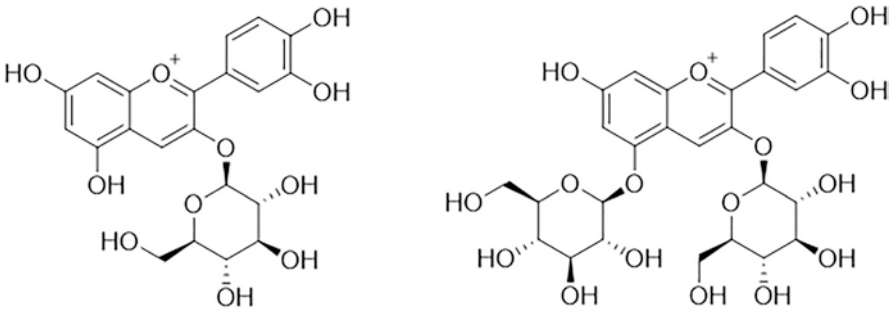

Fig. 13.4 Structure of pigments **21** and **22** isolated from the acidified methanol extract of the fresh black cardamom

names geranilenyl oleoate (**23**), geranilenyl carboxy arabinoside (**24**), geranilanyl carboxy arabinoside (**25**), β-sitosterol arabinosyl diester (**26**) (Fig. 13.5) (Kumar et al., 2014). This study also reported two known compounds, oleodilinolein and glyceryl trilinoleniate, from the methanol extract.

Another study of phytochemical analysis of a methanol extract of black cardamom reported three new compounds **(**Fig. 13.6**)** *n*-hexatriacont-16,18-diene (**27**), 3-methoxybenzyl octadec-9′,12′,15′-trienoate (**28**) and 1,4-naphthoquinone-2-olyl-β-D-arabinopyranosyl-2′-(2″,6″,10″,14″-tetramethylhexadecane)-1″-oate (**29**), and were given the common names hexatriacontadiene (**27**), methoxy benzyl linolenate (**28**) and lawsonyl arabinosyl phytoate (**29**) respectively along with a glyceride characterized as glyceryl-1-linoleate-2,3-dioleate (**30**) (Kumar et al., 2016).

13.6 Antioxidant Activity

In a study aimed to study the antioxidant effect of black cardamom on the oxidative stress in N^{ω}-Nitro-L-arginine methyl ester (L-NAME) induced hypertensive rats (Kanthlal et al., 2020), the administration of aqueous extract of black cardamom favorably altered the plasma oxidative markers such as superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione (GSH). The levels of plasma MDA were significantly decreased, whereas the GSH levels were increased dose-dependent, and a significant increase in SOD activity was observed. Treatment with an aqueous extract of black cardamom also affected the oxidative tissue stress markers favorably, where a decrease in the MDA levels in the heart, kidney and aorta was observed in a dose-dependent manner. A significant elevation in GSH was observed in the heart tissue at all doses.

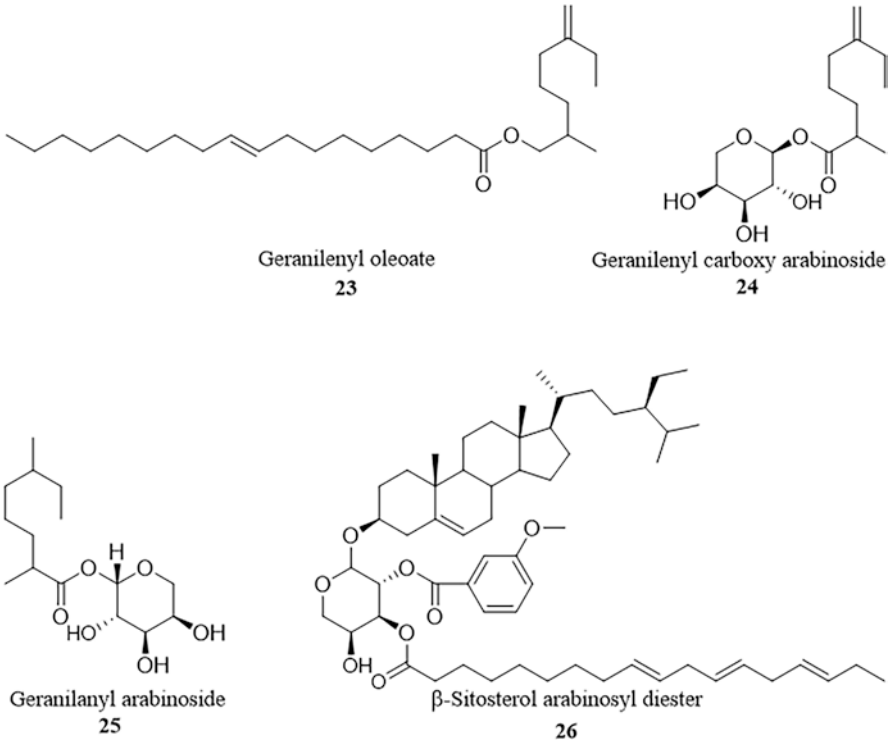

Fig. 13.5 Structure of four new compounds (**23–26**) isolated from the methanol extract of black cardamom fruit

Oral administration of black cardamom fraction (50:50; $CHCl_3$:CH_3OH) caused a significant decrease in lipid peroxidation, whereas a significant elevation in glutathione (GSH) and catalase activity was observed in the liver of atherogenic diet-fed rabbits as compared to the control group (Bairwa et al., 2011). Furthermore, an antioxidant assay of the essential oil of black cardamom revealed the average values of total phenolic contents and total flavonoids as 1325.03 mg Gallic acid equivalent (GAE) and100/g and 168.25 catechin equivalent (CE)/g, respectively, suggesting the essential oil with promising antioxidant potential (Algburi et al., 2021).

The ethyl acetate soluble fraction of the black cardamom extract exhibited a high radical-scavenging ability against 1,1-diphenyl-2-picrylhydrazyl (DPPH). In addition, compounds **17** and **19** displayed higher activity than the natural antioxidants α-tocopherol and L-ascorbic acid), whereas **18** and **20** were comparable to natural antioxidants (Kikuzaki et al., 2001).

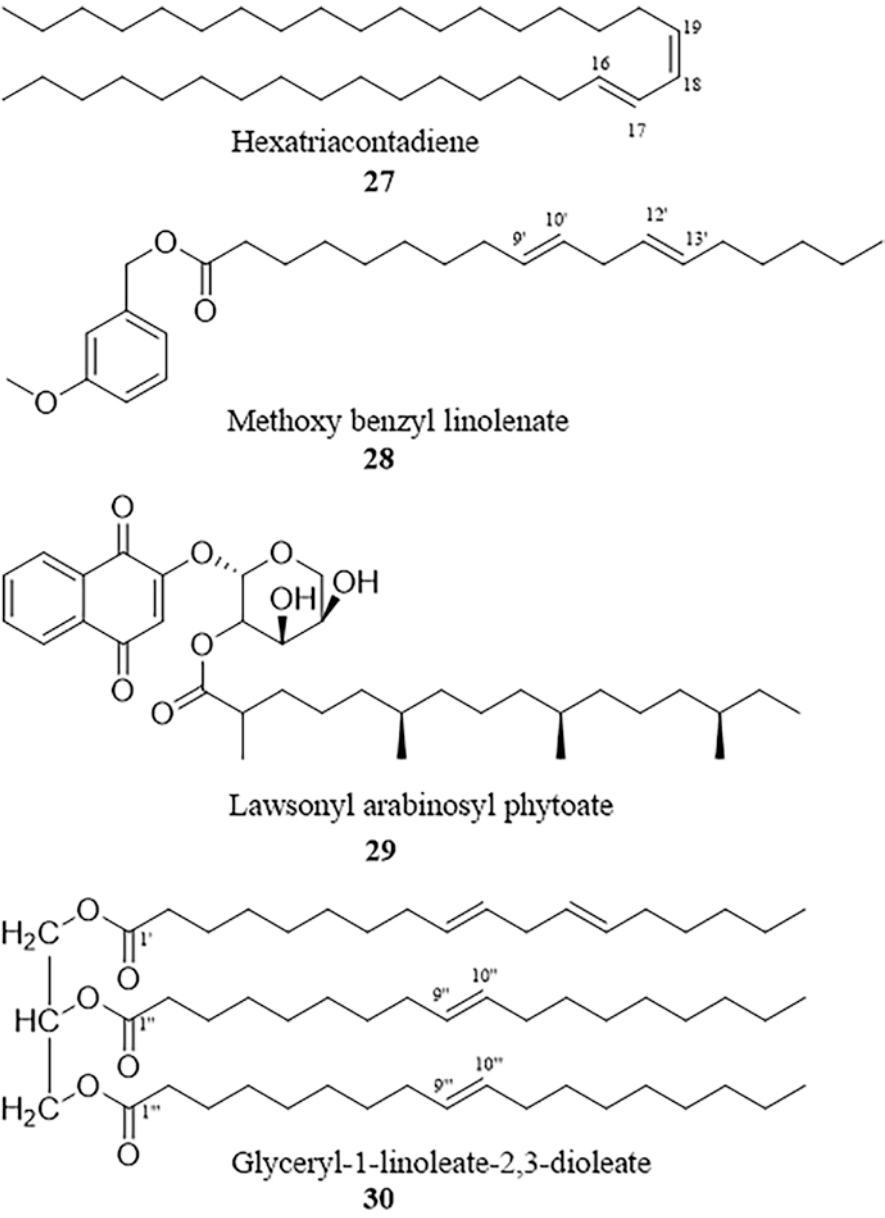

Fig. 13.6 Structure of three new compounds (**27**–**29**) and dioleo-linolein (**30**) isolated from the methanol extract of black cardamom

13.7 Antimicrobial Activity

The acetonic, methanolic and ethanolic extracts of black cardamom showed antimicrobial inhibitory activity against two bacteria, *Streptococcus mutans* and *Staphylococcus aureus* and two fungi, *Candida albicans* and *Saccharomyces cerevisiae* (Aneja & Joshi, 2009). In addition, the ethanol extract showed the highest zone of inhibition against *S. aureus*.

In a recent study of the antimicrobial potential of the essential oil of black cardamom by disk diffusion assay against pathogens *Candida albicans*, *Streptococcus mutans*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, and *Salmonella Typhimurium* showed inhibitory effect with following zones of inhibition 14.4, 13.2, 11.2, 11.0, 8.2 and 6.6 mm in diameter respectively indicating *C. albicans* as the most sensitive species compared to the others (Algburi et al., 2021).

13.8 Analgesic Activity

Methanol and ethyl acetate extract of black cardamom showed a significant analgesic effect as evaluated by the hot plate and writing method (Shukla et al., 2010). Among major phytoconstituents of black cardamom 1,8-cineole, β-myrcene is well reported to have analgesic properties.

13.9 Anti-inflammatory Activity

Radiation dose-modifying factor (DMF) and radioprotective efficacy of methanol extract of black cardamom (MEBC) dry fruits were studied in mice exposed to different doses of total body irradiation (TBI) (Drishya et al., 2022b). MEBC reversed X-ray-induced redox imbalance by enhancing antioxidant defense mechanisms. MEBC prevented TBI-induced hematopoietic damage by enhancing bone marrow cellularity, total white blood cell count, and Hb level. Treatment with MEBC also reduced inflammatory responses via reversing TBI-induced elevation in serum pro-inflammatory cytokine (TNF-α, IL-1β, IL-6) levels (Drishya et al., 2022a). It indicated that the methanol extract of black cardamom reduced TBI-induced oxidative stress and its associated inflammatory response by enhancing the antioxidant status and regulating the pro-inflammatory cytokines. In another study, the methanol extracts of seed and rind of black cardamom exhibited anti-inflammatory properties in various *in vivo* and *in vitro* anti-inflammatory models (Agnihotri, 2020). The rind extract of black cardamom showed better anti-inflammatory activity than the seed extract.

13.10 Antiulcer and Gastro-protective Effect

Black cardamom is used in the Ayurveda and Unani systems of medicine to treat gastrointestinal disorders. Crude methanol extract of black cardamom and its petroleum ether, ethyl acetate fractions, and essential oil significantly inhibited the gastric lesions induced by ethanol in rats (Jafri et al., 2001).

13.11 Hypotensive Properties

In a recent study, the aqueous extract of black cardamom significantly reduced the mean arterial pressure in Normotensive and Spontaneously Hypertensive rats compared to the control, suggesting the hypotensive effect of the black cardamom (Azmat et al., 2022). Furthermore, an *in vitro* and in *silico* study reported improvement in vascular function to evaluate the antihypertensive therapeutic potential of black cardamom (Arya et al., 2020). The aqueous extract of black cardamom showed a dose dependent angiotensin-converting enzyme (ACE) inhibitory and angiotensin II (Ang II) antagonistic activity. Results of the *in silico* molecular docking study performed on the six phytoconstiuents of black cardamom (cardamonin, alpinetin, petunidin-3-glucoside, cyanidin-3-glucoside, protocatecheuic acid and protocatecheuic aldehyde), showed that protocatecheuic acid and alpinetin had high-affinity binding with the target site than the standard used.

In the study of the vasorelaxant effect of the aqueous extract of black cardamom in L-NAME-induced hypertensive rats, treatment with aqueous extract of black cardamom prevented the increase in systolic, diastolic and mean arterial pressure due to L-NAME, administration (Kanthlal et al., 2020). Therefore, the reduction of blood pressure in the L-NAME-induced animal model of hypertension was proposed to result from the antioxidant activity of black cardamom extract.

13.12 Cardio-adaptogenic Properties

Black cardamom is reported to have cardiovascular beneficial properties. The cardio-adaptogenic property of black cardamom was demonstrated for the first time when evaluated for its protective effect against stress-induced myocardial damage in an experimental animal study (Verma et al., 2010). Although many substances of plant origin which show adaptogenic properties have been proposed to act at the cellular level by their antioxidant property, the cardio-adaptogenic property of the black cardamom may be attributed to a similar mechanism. Black cardamom fruit powder (seeds with pericarp) was evaluated for cardiovascular risk factors in patients with ischemic heart disease. Long-term dietary supplementation of black cardamom favorably improved the lipid profile and increased fibrinolytic activity and total antioxidant status (Verma et al., 2012).

13.13 Hypolipidemic Activity

Black cardamom fraction (50:50; $CHCl_3$:CH_3OH) hypolipidemic activity when studied for its effect on atherogenic diet-fed rabbits (Bairwa et al., 2011). Administration of the above black cardamom fraction caused a decrease in total cholesterol, triglyceride, phospholipid, low-density lipoprotein (LDL)- and very-low-density lipoprotein(VLDL)-cholesterol levels. The antiatherogenicity of black cardamom fraction could also be attributed to its direct antioxidative effects whereby activation of antioxidant enzymes can help prevent atherosclerosis which free radical oxidizing LDL-cholesterol can cause.

13.14 Anticancer Activity

Fruits of black cardamom are reported to be a component of the formulation used by the Garo tribe in Bangladesh to treat cancer (Ahmed et al., 2017). Cytotoxic activities of hexane and ethyl acetate extract of black cardamom were tested using human breast cancer (MCF-7) and cervical cancer (HeLa) cell lines, where the extracts exhibited IC_{50} value in the range of 510–798 μg/ml (Sharma et al., 2017). In a recent study, the anticancer therapeutic potential of the methanol extract of black cardamom was assessed in Dalton's Lymphoma Ascites (DLA) cells *in vitro* and in DLA-induced ascitic and solid tumor-bearing mice models *in vivo* (Sudarsanan et al., 2021). The methanol extract exhibited *in vitro* cytotoxicity and induced apoptosis in DLA cells. In *in vivo* investigation, the administration of methanol extract effectively reduced tumor burden and increased survival time, possibly *via* regulating NF-κB and pro-inflammatory cytokine expression and inducing tumor cell apoptosis. Another recent study highlights the effectiveness of black cardamom in anticancer therapy and its lung-specific efficacy, demonstrating the apoptosis-inducing potential of black cardamom fruit extracts and indicating that DNA damage is one of the causes of cell death (Makhija et al., 2022). In this study, the MTT screening of different cell lines against five different extracts prepared from sequential extraction revealed the dichloromethane extract as the most active extract and the lung cancer cell lines (A549 and H1299) the most sensitive target. The upregulation and overexpression of cleaved poly(ADP-ribose)polymerase-1 (PARP-1) in the lung cancer cell lines showed the failure of the DNA repairing mechanism leading to apoptosis.

In a study exploring the antiproliferative ability of cardamonin (**15**) on breast cancer cells, **15** inhibited the proliferation of MDA-MB 231 and MCF-7 breast cancer cells. Mechanistic study indicates that this cardamonin-induced antiproliferative effect is by G2/M phase arrest and apoptosis (Kong et al., 2020).

13.15 Nanoparticle Synthesis

The polyphenolic compound present in the extracts of black cardamom can act as a reducing and stabilizing agent for the successful formation of nanoparticles. Gold nanoparticles of black cardamom have been synthesized by green synthesis from the aqueous extract using $HAuCl_4.3H_2O$ (Singh & Srivastava, 2015). The dominant component of the extract, 1,8-cineole, is proposed to play a significant role in nanoparticle synthesis where oxidation of 1,8-cineole to 2-oxo-1,8-cineole takes place during the nanoparticle synthesis. Silver nanoparticles of black cardamom showed the largest inhibition zone of 23.4 mm against bacterial strain *Xanthomonas axonopodis* when compared with the silver nanoparticles prepared from aqueous extracts of black pepper (*Piper nigrum*), cloves (*Syzygium aromaticum*), red chili (*Capsicum annuum*), cinnamon (*Cinnamomum zeylanicum*) and tea (*Camellia sinensis*) (Javad et al., 2017). In a recent study, the silver nanoparticles synthesized using $AgNO_3$ as precursor and aqueous leaf extract of black cardamom as reducing agent selectively detect Hg^{2+} ions without interference from other heavy metals (Ismail et al., 2022). It can be a promising tool for further green method applications.

13.16 Other Applications

Essential oil of fresh leaves of black cardamom indicated anti-scabies potential when in vitro studies against *Sarcoptes scabiei* mites through the contact method resulted in 100% mortality within 60 min with its 10% concentration (Sharma et al., 2020). The antidiabetic potential of the dry fruit of black cardamom is reported in a study of α-amylase and α-glucosidase inhibition by aqueous extracts of seed and rind of black cardamom. Both extracts showed a dose-dependent inhibition of the enzymes. The IC_{50} value of α-amylase and α-glycosidase for seed extract (1.70 and 1.98 mg/mL) and rind extract (1.10 and 1.18 mg/mL) was less than that of the standard drug acarbose (2.1 mg/mL and 1.90 mg/mL) (Hussain et al., 2018).

Although the limited but nephroprotective role of black cardamom extract is also indicated, in a recent report, the anti-nephrotoxic potential of methanol extract of black cardamom is evidenced by a decrease in serum kidney toxicity indicators such as urea and creatinine when mice were administered with methanolic extract (Drishya et al., 2022a).

This ancient spice, *Amomum subulatum* Roxb. (large cardamom or black cardamom), has come a long way in its usage, from culinary uses in the kitchen to Ayurveda and Unani formulations, pharmaceutical and cosmeceutical applications up to modern nanotechnology. The modern scientific validation of the bioactivity of the black cardamom extracts and its phytoconstituents provides much-needed scientific support to ethnomedicine by linking the bioactivities with the ethnopharmacological applications.

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Part III
Cardamom: Technology, Processing, and Applications

Chapter 14
Effect of Processing on Cardamom Composition and Properties

Mohammad Rafiq Khan

Abbreviations

AGC	Alleppey Green Cardamom
BC	Bleached Cardamom
BWC	Bleached White Cardamom
CCS-1	Coorg cardamom Malabar Selection-1
CGC	Coorg Green Cardamom
GC-MS	Gas Chromatography-Mass Spectrometric
HBC	Half-bleached Cardamom
ICRI-1	Indian Cardamom Research Institute-1
ICRI-2	Indian Cardamom Research Institute-2
MC	Mixed Cardamom
MFPI	Ministry of Food Processing Industries
Mudigree-1	Name of a place that produces cardamom
NIFTEM	National Institute of Food Technology and Entrepreneurship Management
PV-1	Pods Variety-1
PV-2	Pods Variety-2
SEM	Scanning Electron Microscopic
SKP-14	A cardamom variety grown in Karnataka (K)

M. R. Khan (✉)
Department of Environmental Science and Policy, Lahore School of Economics, Lahore, Pakistan
e-mail: drrafiq@lahoreschool.edu.pk

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14.1 Introduction

Elletaria cardamom, honored as the" Queen of Spices," is a high-value spice being widely marketed all over the globe due to its distinctive aroma and flavor and its extensive uses in the field of Traditional Medicine. Considering its market status, keeping its and its products traits intact sounds imperative (Shirma et al., 2014). Although, Ashokkumar et al. (2020) adequately described different aspects of *E. cardamom,* including its processing, in their handbook published by NOVA. However, the effect of processing on cardamom's composition and properties sounds like an essential aspect of inquiry for its quality because that means its desired quality control. Before proceeding on this mission, it seems desirable to identify all the processes involved, from cultivation to the supply in the market for sale. Therefore, different processes are outlined as a guideline in the "Handbook of Agricultural Products" (Ashokkumar et al., 2020).

14.2 Cultivation

The choice of climate, nature of the soil, suitable varieties, etc., are extremely important as a prelude to the planning of Cardamom cultivation. These factors carry great importance to have an adequate crop yield and its desired traits. It is grown in areas where the annual rainfall ranges from 1500–4000 mm, with a temperature range of 10–35 °C and an altitude of 600–1200 m above Mean Sea Level. Rainfall distribution should be good, and summer showers during February–April are essential for panicle initiation. Deep black loam soil with high humus content found in the forest region is best suited for cultivation. It also grows on laterite soils, clay loams and rich black soils having good drainage. Sandy soil is not suitable (IndiaAgroNet.com). The popular varieties in India are Coorg cardamom Malabar Selection-1 (CCS-1), ICRI-1, ICRI-2, Mudigree-1, PV-1, and SKP-14.

Cardamom is propagated mainly through seeds and suckers, each consisting of at least one old and a young aerial shoot. Seedlings are usually raised in primary and secondary nurseries. Raised beds are prepared after digging the land to a 30–45 cm depth. The beds of 1 m in width and convenient length raised to a height of about 30 cm are prepared. A fine layer of humus-rich forest soil is spread over the beds. Seeds may be collected from well-ripe capsules. Immediately after harvesting, the husk is removed, and the seeds are washed repeatedly with water to remove the mucilaginous coating. Seeds should be sown immediately after extraction. The Reading Manual of National Institute of Food Technology Entrepreneurship and Management describes that 1 kg of seed capsules may produce 5000 seedlings (niftem online). Sowing is done in rows and may be taken up during November–January. Seedbeds should be dusted with chloropicrin or carbon disulfide. The germination commences in about 30 days and may continue for a month or two. After germination, the mulch should be removed.

14.2.1 Manuring

Manuring at the rate of "90g nitrogen (N), 60g phosphorus (P_2O_5) and 120g potash (K_2O) per bed of 5 × 1m dimension, in three equal split doses at an interval of 45 days is recommended to produce healthier seedlings. The first dose of fertilizer may be applied 30 days after transplanting in the secondary nursery" (niftem online).

14.2.2 Land Preparation

Pits of 45 × 45 × 30 cm are dug in April–May and filled with a mixture of topsoil and compost or well-decomposed farm yard manure. In slopy land, contour terraces may be made, pits may be taken along the contour, and a close planting (2 m × 1 m) is commendable.

14.2.3 Planting

The planting is carried out during the rainy season starting from June. Seedlings should be planted up to the collar region for better growth. Cloudy days with drizzle are the best for planting. Generally, in Kerala and Tamil Nadu, the seedlings are transplanted from March till May at 20 × 20 cm spacing and mulched immediately. Beds should be covered with an overhead pandal and should be watered regularly.

14.2.4 Fertilizer

A fertilizer dose of 75 kg nitrogen (N), 75 kg phosphorus (P_2O_5) and 150 g potash (K_2O) per hectare (ha) is required under irrigated conditions for high-yielding plantation yielding (100 kg/ha) and above and a dose of 30:60:30 kg/ha is required for gardens in the rain-fed condition. In addition, organic manures like compost or cattle manure may be given at 5 kg per clump. Fertilizer is applied in two doses. The first application in May will help in the production of suckers and the development of capsules; the second application in late September will help initiate panicles and suckers. Therefore, only half the dose of fertilizer should be applied during the first year, and a full dose should be given from the second year onwards. Application of fertilizer is done at a radius of 30 cm and covered with a thin layer of soil.

14.2.5 Irrigation

To overcome the dry spell during summer, it is essential to irrigate the crop to get maximum production as it helps initiate panicles, flowering and fruit set. These may be irrigated for 10–15 days until the onset of monsoon.

14.2.6 Intercultural Operations

These include mulching, weed killing, trashing, shade regulation, earthing, plant protection, etc. These are outlined below.

14.2.6.1 Mulching

It is an essential social practice in cardamom. Fallen leaves of the shade trees are utilized for mulching. Sufficient mulch should be applied during November–December to reduce the adverse effects of drought, which prevails for nearly 4–5 months during summer. In addition, exposing the panicle over mulch is beneficial for pollination.

14.2.6.2 Weed Killing

The first round of weed killing should occur in May–June, the second in August–September and the third in December–January. Weedicides like Paraquat at 625 mL in 500 liters of water may be sprayed in the interspaces between rows, leaving 60 cm around the plant base.

14.2.6.3 Trashing

Trashing consists of removing fold and drying shoots of the plant once a year with the onset of monsoon under rain-fed conditions and 2–3 times in high-density plantations provided with irrigated facilities.

14.2.6.4 Shade Regulation

The **c**ardamom plant is very sensitive to moisture stress. The shade helps regulate soil moisture and temperature and provides a congenial micro-climate for cardamom. However, excess shade is also quite detrimental and has to be regulated to provide 50–60% filtered sunlight. In order to provide adequate light during monsoon, shade regulation may be taken up before the onset of monsoon (niftem online).

14.2.6.5 Earthing

After the monsoon is over, a thin layer of fresh fertile soil, rich in organic matter, may be earthed up at the base of the clump, covering up to the collar region scraping between the rows or collecting soil from staggered trenches/check pits. This encourages new growth.

14.2.6.6 Plant Protection

Damages to the leaves, shoots, inflorescence, and thrips affected capsules fetch lower the product's price in the market. The shade is regulated in a thickly shaded area; spray Monocrotophos 0.025% from March to September. The shoots, panicles, and capsule/sorer larvae bore the unopened leaf buds and panicles, causing drying or feeding on young seeds, causing the empty capsules. Spraying Monocrotophos or Fenthion 0.075% at an early stage of infection kills this infection. Aphid's nymphs and adults suck the sap and act as a vector of the mosaic or 'Katte' Virus. The Spray of 0.05% Dimethoate controls these.

Parasitic nematodes cause poor germination and establishment in the nurseries, stunted and poor plant growth, and shedding immature capsules in the main field. Treat the plants in the nursery with Carbofuran 3 g @ 5 kg a.i/ha or in the main field with carbofurna 5 g a.i./clump and apply 0.5 kg of Neem cake per clump twice a year.

In the case of Katte diseases, spindle-shaped, slender chlorotic flecks appear on the youngest leaves; later, these develop into pale green discontinuous stripes ass leaves mature. Infected clumps are stunted and smaller, with slender tillers and shorter panicles. Healthy seedlings tend to rogue the infected plants.

Affected rot capsules turn brownish-black, often rotting, and extend to tillers and rhizomes. Do trashing, remove infected and dead plants etc., during premonsoon months, spray 1% Bordeaux mixture during May and repeat in August. Infected seedlings collapse at the collar region and die in patches; the entire clump dies in grown-up plants. It is called dumping. Pretreatment of the nursery with 1:50 formaldehyde drenches the soil after germination with 0.2% copper oxychloride.

14.3 Harvesting and Processing

Cardamom plants normally start bearing flowers 2 years after planting. In most areas, the peak harvest period is October to November. Picking is carried out at an interval of 15–25 days. The ripe capsules are harvested to get maximum green color during curing. After harvest, capsules are dried in a fuel kiln, electrical drier, or sun. It has been found that soaking the freshly harvested green cardamom capsules in 2% washing soda solution for 10 min before drying helps retain the green color during drying. When the drier is used, it should be dried at 45–50 °C for 14–18 h, while overnight drying at 50–60 °C is required for the kiln. The capsules kept for drying

are spread thinly and stirred frequently to ensure uniform drying. The dried capsules are rubbed with hands, coir mat, or wire mesh and winnowed to remove foreign matter. They are then sorted out according to size and color and stored in black polythene-lined gunny bags to retain the green color during storage.

14.4 Effect of Drying

This is the most important part of the process parameters, as it affects the quality of the final product. It is beneficial to dry the cardamom capsules soon after harvesting because drying soon after harvesting prevents the loss of flavor. The drying process should be as short as possible to avoid the growth of molds on the capsules and retain the product's bright green. The drying temperature should not be above 50 °C because a high temperature above 50 °C affects the color and delicate flavor of the marketable product. In most markets, cardamom capsules with an excellent green color can be sold at a comparatively higher price.

The moisture content of fresh cardamom capsules is about 85%. This needs to be reduced to 10% in the dried product to store the cardamom capsules securely. If the drying period is too long, molds can start growing on the cardamom. Several options are available to small-scale processors, depending upon the size of the business and the local weather conditions at the time of processing. Each method presents different advantages and disadvantages. Table 14.1 exhibits the temperature and time required to bring the moisture content of cardamom pods to 10 °C.

14.4.1 Sun Drying

It is a traditional drying method not only used for drying cardamom capsules but also widely applied on earth to dry minor to major bulky materials, and in this context, its application has a long history since antiquity. To dry cardamom, its capsules are spread on a concrete/hard mud floor to expose the stuff to be dried to the sun's natural heat. The capsules are placed away from direct sunlight to preserve the green color because the strong sunlight makes the color fade. This is the simplest and cheapest method but it cannot produce the highest quality product. It is only

Table 14.1 Types of driers/drying applied to bring the moisture content of cardamom to 10%

Type of drier/drying	Temperature	Time-Hours
Cross-flow electric dryer	55 °C	18–20
Solar cardamom dryers		3 days
Bin dryer	55 °C	10–12
Electric dryer	45–50 °C	10–12
Sun drying		5–6 days
Kiln dryer	45–50 °C	18–22

The construction and working of different drier are outlined below

successful in places where the climate is dry and hot. During the monsoon season, for example, drying is interrupted by rainfall which can cause molds to grow on the surface of the capsules. Moreover, the capsules may be contaminated by dirt and dust from their environment during drying.

14.4.2 Solar Drying

Using a solar dryer should improve the quality of the dried capsules as it is a cleaner, more controlled environment. However, it is not popular as the green color is lost during drying. In addition, the solar dryer is only helpful in dry, hot sunny climates. The capsules should be placed in the dryer, out of direct sunlight, and dried until they have a final moisture content of 10%. In places with high humidity, the solar dryer can only be used with an extractor fan to remove the humid air.

14.4.3 Wood-Fired Dryer

In India, cardamom capsules are traditionally dried in curing houses, using wood to provide heat. This method puts a huge demand on firewood. The smoke from the fire can give the capsules an unpleasant smoked flavor. The processor must ensure that the capsules closest to the heat source are not burnt or scorched. Cardamom capsules dried by this method are not of the highest quality.

14.4.4 Electric or Gas Dryer

A gas-fired electric dryer improves the use of a wood-fuelled fire and is the best choice for drying large quantities of cardamom, especially in places with rainfall during the drying season. It is the most expensive option but produces the highest quality product. The drying temperature must not exceed 50 °C. A range of dryers of different sizes is available depending upon the individual choice and budget. Fig. 14.2 shows a typical tray/dryer.

14.4.5 Humidity-Controlled Drying

A drying chamber has been developed that helps to reduce color loss and to produce high-quality pods. The cardamom capsules are placed in the drying chamber at a temperature of 50 °C. During the first 2 h of drying, the humidity builds up within the chamber. This allows the cardamoms to ‘cook’ an Fig. 14.2: A typical

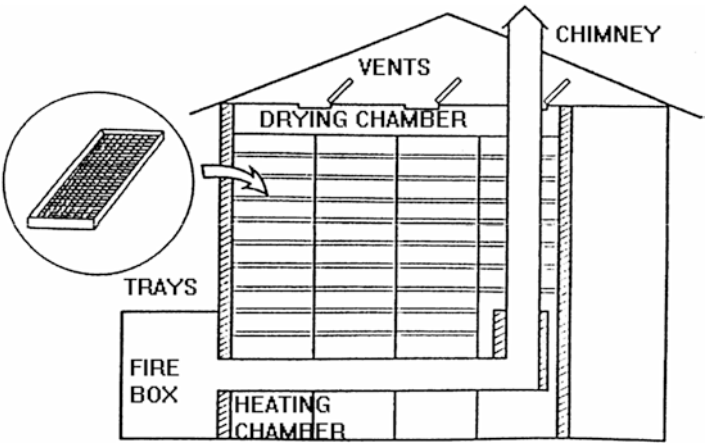

Fig. 14.2 A typical tray drier

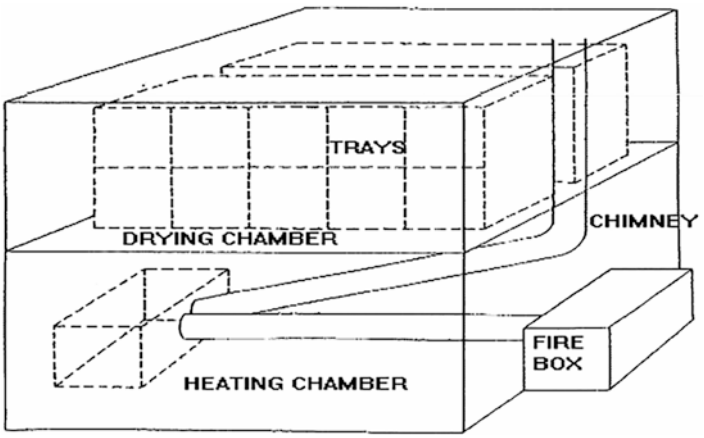

Fig. 14.3 A traditional drying chamber

tray dryer Cardamom processing simultaneously destroys the enzymes that break down the chlorophyll (chlorophyll gives the pods their green color). Therefore, no light is allowed into the drying chamber. After 2 h, the humid air is blown out of the chamber, reducing the humidity. The capsules are left in the chamber to dry until they have a final moisture content of 10%. Fig. 14.3 shows a traditional drying chamber.

The use of biomass gasifiers Electricity and liquefied petroleum gas (LPG) are clean and convenient fuels for drying but are not cheap or readily available in villages. Firewood, stubble and dry leaves are readily available in villages, but they are smoky and can contaminate the dried product. A gasifier is a device TERI (The

Tata Energy Research Institute in India) developed for cardamom drying. The gasifier uses briquettes made from firewood and other types of biomass and turns them into a gas that burns with a clean, smokeless flame. The main advantage of using a gasifier is that it is more efficient in terms of the fuel used. Biomass that burns in an open fire loses about two-thirds of its energy as smoke. This system, therefore, uses less fuel and produces a higher quality dried cardamom. The gasifier for drying cardamom, developed by TERI, can be made locally using recycled oil drums.

14.5 Effect of Processing Conditions on Quality of Cardamom Applying a Pre-drying Treatment with 2% Sodium Carbonate and at Different Drying Temperatures

Ilangantileke et al. (1993) conducted a study on the effects of processing conditions on the quality of cardamom using a pre-drying treatment with 2% sodium carbonate at drying temperatures 35 °C, 45 °C and 55 °C. The percentage of chlorophyll removal, total oil and essential oil content, percentage splits and percentage out-turn were determined for both chemically pre-treated and non-treated cardamom and compared. The- researchers claim that the chlorophyll content was best retained in the chemically treated cardamom at 45 °C drying temperature. The total oil content loss was minimal for the chemically pre-treated cardamom at a drying temperature of 45 °C, while maximum terpenoids were also retained at 45 °C in the untreated cardamom. The % splits were lowest for the untreated product continuously dried at 45 °C, and the % out-turn was highest for the chemically treated cardamom at 45 °C drying temperature. The research workers concluded that the favorable treatment conditions to meet trade quality standards were at 45 °C drying temperature and chemical pretreatment for continuously dried cardamom.

The Ministry of Food Processing Industries (MFPI), Government of India has documented, and the National Institute of Food Technology and Entrepreneurship Management and Ministry of Food Processing Industries, HSIIDC, Industrial Estate, Kundli, Sonipat, Haryana India has reported their effects (NIFTEM Online) as under:

> Soaking green (wet)-2 percent sodium carbonate solution for 10 min-Fixes green color. Sodium carbonate – Better retention of green color of c//ured capsules.
>
> A quick dip of capsules in hot water at 40 °C and dipping capsules for 10 min in 2% sodium carbonate- Better retention of the green color of cured capsules.
>
> Pre-soaking of capsules in copper formulations and chemicals like NAA, IAA, GA and magnesium sulfate- retain more chlorophyll.

14.6 Effect of Moisture Content on Physical Properties of Cardamom

Some of the moisture-dependent physical properties of cardamom seeds were studied by Gebreselassie (2012) at moisture contents 9.9%, 13.5%, 18.4%, and 23.2% wet basis (weight basis). He reported that The length, width, thickness, geometric mean diameter, thousand seed mass, and sphericity increased from 17.01 to 17.30 mm, 5.68 to 6.57 mm, 5.02 to 5.35 mm, and 7.86 to 8.47. Thus Cardamom capsules carry moisture levels of 70–80% at harvest. The initial moisture level must be brought down to 8–10% for proper storage. Cardamom capsules must be dried within 24 hours; further delay would result in deterioration of green color and appearance.

14.7 Effect of Enzyme Pretreatment on Extraction Yield and Quality of Volatile Oil of Cardamom Seeds

Baby and Ranganathan (2016) evaluated the effect of pretreatment on extraction yield and quality of volatile oil of cardamom seeds using various enzyme enzymes such as Celluclast 1.5 L, Pectinex Ultra SP.L, ViscozymeL and Protease. Scanning Electron Microscopic (SEM) was used to visualize the effect of the enzymes mentioned above on cardamom seed cell walls. The oil quality was further determined using Gas Chromatography and Mass Spectroscopy (GC-MS). The enzyme pretreatment resulted in "an increase in oil yield in the range of 7.23–7.83% as against 6.73% of the control sample. The enzyme pretreatment with Viscozyme-L proved to be most effective compared to all other tested samples. Scanning electron microscopic analysis of cardamom seeds indicated that the cell rupture in enzyme-treated seeds was smooth and open due to the hydrolysis of cell wall components, whereas water treatment resulted only in structural damage". It was further observed that enzyme pretreatment facilitated the release of oil from oil glands without affecting the oil's physicochemical properties or flavor profile. GC-MS studies indicated that the flavor profile of cardamom oil contains the major flavor compounds such as 1, 8 cineol and terpinyl acetate, which increased from 34.3 to 37.6% and 40.8–42.3%, respectively, with enzyme pre-treatment. From an economic point of view, the spice industry could use the study's results effectively to increase the extraction yield and quality of volatile oil from cardamom seeds.

14.8 Effect of Domestication on Cardamom

Kuriakose and Sinun (2009) compared wild and cultivated populations of cardamom in terms of vegetative and reproductive features to identify domestication syndromes and examine whether the two populations develop reproductive barriers. According to their report, "Several productive traits including the number of branches, number of inflorescences, and total number of flowers per clump, number of flowers that open each day, the duration of flowering, the length of the flower and the amount of nectar per flower are significantly greater in cultivated cardamom" Both wild and cultivated populations were found self-compatible, and there were no reproductive barriers between the two populations." The conclusion was that domestication in cardamom brings about significant changes in vegetative and reproductive traits and a shift in effective pollinators from native solitary bees to social bees.

14.9 Effect of Sorting and Storage

The seeds of Cardamom are sorted according to size and color and stored for long periods in black polythene-lined gunny bags to retain the green color during storage because the dried cardamom capsules must be stored in moisture-proof containers to keep them away from direct sunlight. The major objective protection of protection from direct sunlight is to protect the green color of cardamom, as otherwise, it will fade due to chemical changes in its chlorophyll content. Moreover, different grades, right from poor to the richest customers, are floated in the export markets. The deeper the green color and the larger the capsule size, the higher the grade. The grading of capsules at all levels is done by hand. An essential condition before storage is that the capsules are fully dried before filling in the gunny bags for storage. The presence of moisture in the bags causes the capsules to rot. Therefore, the stored cardamom should be inspected regularly for spoilage or moisture. If they are found with absorbed moisture, these must be re-dried to 10%moisture level.

"The storage room should be clean, dry, cool and free from pests. Mosquito netting should be fitted on the windows to prevent pests and insects from entering the room. Strong-smelling foods, detergents and paints should not be stored in the same room as they will spoil the delicate aroma and flavor of the cardamom.

Standards

	US Government requirements and ASTA	British Standard
Moisture (%)	<11.0	<13.0
Volatile oil (%)	<3.0	<4.0
Extraneous matter (% by weight)	0.5	
Mould (% by weight)	1.0"	

Table 14.2 Different grades of Cardamom MFPI

Serial	Grade	Color	Pod Size: Dia	Weight: Gm/Liter
1	Small	Green	5.5–6.5	385
2	Open/Split*	Greenish/pale yellow	6.5 and above	Undefined
3	Seeds	Black Brown	Undefined	550–650
4	Fruit-over matured pods	Yellowish	Undefined	425 and above a
Standard export grades				
1	AGEB Alleppey	Green extra	Bold size 7 mm	435
2	AGB Alleppey	Green bold	6 mm	415
3	AGS Alleppey	Green superior	5 mm	385
4	Alleppey green	Shipment Green1	4 mm	350
5	AGS-2 Alleppey green	Shipment Green-2	2–4 mm above	320

Open/Splits* is lower quality cardamom where over 60 percent of the pods are "open" (Seeds are exposed)

Different grades managed by MFPI are exhibited in Table 14.2.

Apart from the grades mentioned, MFPI has documented and reported that the internationally accepted and most commercially imported varieties of Cardamom are Malabar Cardamom, Sri Lankan Cardamom and Cambodian Cardamom, and these are narrated below.

(a) Grade of Guatemala Cardamom
(b) Jumbo Green (extra-large green)
(c) Imperial Best Green (large green)
(d) Fancy Green Extra (Extra green)
(e) Fancy Green (Medium size green)
(f) Imperial Mixed Green (large pale green)
(g) Mixed green (assorted colors)
(h) Mixed green split (medium size open green pods)
(i) Yellow mixed (yellow medium/large close pods)
(j) MYQ mixed yellow quality (medium light brown color for grinding)
(k) Cardamom seeds (decorticated)

"grading system for cardamom capsules separates them into different types:

- Alleppey Green Cardamom
- Coorg Green Cardamom
- Bleached or Half-bleached Cardamom
- Bleached White Cardamom
- Mixed Cardamom

Agmark Schedule I for Alleppey Green Cardamom

Grade	Trade name	Color	Empty and malformed capsules (%)	Immature and shrivelled capsules (%)	Blacks and splits (%)	Size (diameter of sieve hole mm)	Weight (G/L)	General characteristics
AGEB	Cardamom extra bold	Deep green or light green	2.0	2.0	0.0	7.0	435	Cardamoms are the dried capsules of Elletaria grew in South India. The capsules have 3 corners and a ribbed appearance The capsules are free of insect damage and visible mould. Thrip marks on the capsules do not mean the capsules are infested with insects.
AGB	Cardamom bold	As above	2.0	2.0	0.0	6.0	415	
AGS	Cardamom superior	As above	3.0	5.0	0.0	5.0	385	
AGS-1	Shipment green-1	As above	5.0	7.0	10.0	4.0	350	
AGS-2	Shipment green-2	As above	7.0	9.0	12.0	4.0	320	
AGL	Light	–	–	–	15.0	3.5	260	
AGN		–	–	–	–	–	–	

Agmark Schedule II for Coorg Green Cardamom

Grade	Trade name	Empty &malformed capsules (%)	Unclipped capsules (%)	Immature and shrivelled capsules (%)	Blacks and splits (%)	Size (diameter of sieve hole mm)	Weight (G/L)	General characteristics
CGEB	Extra bold	0.0	0.0	0.0	0.0	8.0	450	Cardamoms are the dried capsules of Elletaria grown in South India. Color range from greenish to brown. Global shape, skin ribbed or smooth, pedicels separated. The capsules have 3 corners and a ribbed appearance. The capsules are free of insect damage and visible mould Thrip marks on the capsules do not mean the capsules are infested with insects
CGB	Bold	2.0	0.0	3.0	0.0	7.5	435	
CG1	Superior	3.0	0.0	5.0	0.0	6.5	415	
CG2	Coorg green or Motta green	5.0	3.0	10.0	0.0	6.0	385	
CG3	Shipment	10.0	5.0	15.0	10.0	5.0	350	
CG4	Light	–	–	–	15.0	3.5	280	
CGN		–		–	–	–	–	

Agmark Schedule III for Bleached or Half-Bleached Cardamom

Grade	Empty and malformed capsules (%)	Immature and shriveled capsules (%)	Size (diameter of sieve hole) (mm)	Weight (G/L)	General characteristics
BL1	0.0	0.0	8.50	340	The cardamom is fully developed, dried capsules of Elleteria cardamom, bleached and/or half bleached by sulfuring. Color ranging from pale cream to white. Global or three-cornered with skin ribbed or smooth The capsules are free of insect infestation and visible mould Thrip marks on the capsules do not lead to the conclusion that the capsules are infested with insects
BL2	0.0	0.0	7.00	340	
BL3	0.0	0.0	5.00	300	
BL non specified	10.0	15.0	5.0		

Agmark Schedule IV for Bleached White Cardamom

Grade	Trade name	Empty and malformed capsules (%)	Immature and shrivelled capsules (%)	Size (diameter of sieve hole mm)	Weight (G/L)	General characteristics
BW1	Mysore/ Mangalore bleachable cardamom clipped	1.0	0.0	7.0	460	The cardamom is fully developed, dried capsules of Elleteria cardamom grown in Karnataka State. Reasonable uniform shade of white, light green or light grey color and suitable for bleaching The capsules are free from insect infestation and visible mould. Therefore, thrip marks alone do not lead to the conclusion that the capsules have been infested with insects
BW2	Mysore/ Mangalore bleachable cardamom unclipped	1.0	0.0	7.0	460	
BW3	Mysore/ Mangalore bleachable bulk cardamom clipped	2.0	0.0	4.3	435	
W4	Mysore/ Mangalore bleachable bulk cardamom unclipped					
BW Non specified						

14.10 Effect of Genetic Variability Parameters on genome Size

Anjali et al. (2016) analyzed the intraspecific variation in *Elettaria cardamomum* Maton (cardamom) using genome size, cytological studies and molecular marker data. Nuclear DNA content and molecular marker details furnished data on genome size and genetic diversity, respectively, among the studied accessions, and both complemented each other for evolutionary and taxonomic studies. The relative 2C genome size and total number of base pairs of cardamom were assessed through flow cytometric analysis using propidium iodide staining. The nuclear DNA content was estimated in various sections of the species representing individuals from wild and cultivar genotypes following *Zea mays* L. CE-777 (2C = 5.43 pg) as the internal reference standard. Chromosome number from the growing root tip was examined following standard protocols. Twenty-six ISSR primers that generated polymorphic bands were used for genetic diversity analysis of the thirty cardamom accessions. Estimated nuclear 2C DNA content ranged from 2.57 to 3.22 pg, demonstrating a 1.25-fold variation. The mean amount of 2C nuclear DNA of the cardamom was calculated as 2.87 pg, equivalent to 2806 Mbp as the diploid genome size. The chromosome number was found to be 2n = 48. Among the thirty cardamom accessions studied using ISSR markers, C53 (feral from Bonacaud) showed a very prominent level of genetic diversity and was lowest for C96 (Avinash-I, a released variety from Indian Institute of Spices Research, Kozhikode). The analyses revealed the existence of genetic variability within the studied cardamom accessions. The plant specimens also differed significantly in their genome size. However, the genetic variability parameters did not show any correlation with genome size.

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Chapter 15
Cardamom-Based Beverages

Niccolò Pilla, Vita Di Stefano, Paolo Gabrielli, Massimo Lucarini, and Alessandra Durazzo

15.1 Introduction

Besides the increasing demand for natural ingredients and improving appearance and health, the functional beverage industry represents the fastest-growing segment of the functional food market. Beverages are essential to nutritional intake, and their role in health has recently received much attention. Beverages meet consumers' demands regarding size, shape, storage, and desirable nutrients and bioactive compounds. Functional beverages consumption has a key role in human health, opening to possibility to incorporate a broad spectrum of nutrients and bioactive compounds into functional beverages, i.e., antioxidants, dietary fibers, prebiotics, proteins, peptides, unsaturated fatty acids, minerals, and vitamins (Ghoshal, 2019). The recent review by Valduga et al. (2019) summarized and analyzed species' pharmacological, phytochemistry and technological aspects with classical ethnobotanical and traditional use. Tailoring functional beverages from fruits and vegetables for specific disease conditions represents a great challenge (Dey & Sireswar, 2021). These functional beverages can be classified as dairy-based, probiotic, energy, sports, meal replacers, caffeinated, vegetable, and fruit (Corbo et al., 2014; Nazir et al., 2019; Dini, 2019). These functional beverages benefit one or more human body functions and their fundamental nutritional values. Ingredients of functional beverages could be minerals, vitamins, amino acids, dietary fibers, probiotics, and added raw fruits. Beverages can meet individual demands and nutrients, and bioactive compounds can be delivered conveniently.

N. Pilla · P. Gabrielli · M. Lucarini · A. Durazzo (✉)
CREA-Research Centre for Food and Nutrition, Rome, Italy
e-mail: alessandra.durazzo@crea.gov.it

V. Di Stefano
Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, Palermo, Italy

M. F. Ramadan (ed.), *Cardamom (Elettaria cardamomum): Production, Processing and Properties*, https://doi.org/10.1007/978-3-031-35426-7_15

Vegetables and fruit beverages are already used as ingredients for functional beverages (Sun-Waterhouse, 2011; Hui & Evranuz, 2012). In addition, fruits and vegetables constitute a new medium of probiotic transfer (Ayed et al., 2020). For example, fermented beverages based on fruits and vegetables are valid alternatives for people who are lactose intolerant or follow a vegan diet. Moreover, fermentation improves shelf life and safety (Ayed et al., 2020). Cardamom appears to be a valid candidate for the formulation of functional beverages thanks to its characteristic aroma, flavor, and bioactive components.

15.2 Historical and Traditional Aspects

Cultural tradition is linked to the territory, and local products' knowledge and valorization represent an essential objective for preserving local agro-biodiversity (Durazzo, 2019; Durazzo & Lucarini, 2021). Historically fruit, vegetables and spices have been used for preparing traditional beverages. The first evidence of modern fruit juice is dated to the late sixteenth century in Italy, with a lemonade. In the first half of the eighteenth century was identified the ability of citrus fruits to prevent scurvy by Dr. James Lind, and more than 100 years later, the Merchant Shipping Act of 1867 made carrying citrus juices on ocean voyages a legal requirement for British vessels, demonstrating the beneficial effect of functional beverage on the human health (Emmins, 2000).

Coffee and tea have a millenary tradition in central Asia and South America. Cardamom was utilized for the preparation of folk beverages by different cultures. After saffron and vanilla, cardamom is the third most expensive spice in the world (Singletary, 2022). The Arab tradition wants cardamom associated with coffee to reduce the power of the caffeine present in the latter. The resulting drink, *Qahwa*, is drunk on all festive occasions but also in daily life, both in the desert and in the cities, with family and friends and often consumed as folk medicine to combat headaches and stress (Alalwan et al., 2017). *Al-ward*, *Zafaran* and *Hail* are other traditional beverages typic of the Bahrain region. Hail is a cardamom extract consumed during the winter season as a cold beverage (Alalwan et al., 2017).

In Indian regions of Kerala and Tamil Nadu, cardamom is used to prepare a typical tea, popularly known as *Elakkai tea*, where crushed cardamom capsules are boiled with the tea in order to give a pleasant aroma (Ashokkumar et al., 2020). *Elakkai Tea* was also used to relieve tiredness due to overwork and depression (Ashokkumar et al., 2020). Moreover, in Ayurveda, cardamom was used as an ingredient in fermented beverages like *Arishta* and *Aasava* (Ashokkumar et al., 2020).

Thandai is a traditional milk-based beverage popular in Rajasthan and certain other Northern states of India. It is common to serve it during the summer and consume it as a delicacy during the festive season. It is prepared with nuts and flavoring agents like cardamom (Chawla et al., 2018). In Turkey, Sherbets are traditional popular beverages made with various fruits, flowers and spices, including cardamom (Tamer et al., 2019). Although Europeans do not use this spice in traditional

recipes, some exceptions exist, such as Swedish Kardemummabullar, very soft spiced brioches or Belgian Trappist beers. In addition, cardamom was sometimes used to prepare aromatized wines and vermouth (Tonutti & Liddle, 2010).

15.3 Alternative Use of Cardamom as a Beverage

In the growing interest and request for functional food, fruit and vegetables seem to be the consumers' favorite choices among functional food products or organic food commodities (Ferreira et al., 2015). In addition, blending different ingredients to formulate beverages seems to increase consumer acceptability and potential health benefits (Ogundele et al., 2016). For example, different cardamom concentrations were blended with coffee to produce a diverse product (Ariefandi & Rizki, 2015).

According to Durak et al. (2017), adding cardamom to coffee drinks gives flavor and aroma and could also affect biological activities (Durak et al., 2017). For example, Arabic coffee with two doses of cardamom was tested in healthy women, decreasing GGT, γ-glutamyl transpeptidase, and plasma levels (Badkook & Shrourou, 2013). Furthermore, different coffee mixtures with cardamom (2.5% w) and other spices were evaluated. The second ones seem to have a better nutritional value between commercial coffee and coffee blends, like the highest antioxidant activity. Moreover, the different blends reported a minor caffeine concentration, suggesting a role in the control of the assumption of caffeine in the cultures with high coffee consumption (Ragab & Yossef, 2020).

Ground coffee was also mixed with microencapsulated cardamom essential oil; the "cardamom coffee" had higher antioxidant activity than the original coffee (Kustyawati et al., 2022). Souza et al. (2020) developed a fruit juice made of 70% apple juice and 30% cardamom tea, evaluating the physiochemical characteristic and sensory acceptance. The blend was highly accepted, and the antioxidant activity increased compared to the apple juice and tea individually, demonstrating the synergistic effect.

Different blends of fruits juices, pomegranate, barberry, and grape juice with the addition of cardamom essence, ginger extract and hibiscus powder were evaluated, and organoleptic analysis and physiochemical properties were done (Sahraee et al., 2022). The results showed that according to the antioxidant properties, total phenol, anthocyanin, flavonoid, vitamin C content, and sensory analysis, the best fruit juice blend to produce a functional drink was 60% pomegranate juice, 20% grape juice, 20% barberry juice (Sahraee et al., 2022). Furthermore, adding cardamom essential oil influenced the total content of anthocyanin and flavonoids; instead, the ginger extract influenced the total phenol content (Sahraee et al., 2022).

Functional beverage based on toasted oat was assessed by Alemayehu et al. (2022), and the formulation also comprehended roasted black cardamom. The resulting composite beverage had a high nutritional value, and it could be recommended as part of a diet to improve nutritional status in people with limited resources, particularly in low-income countries (Alemayehu et al., 2022).

Cardamom beverages with whey were also evaluated; whey is another functional ingredient with lots of applications as a functional food ingredient (Macwan et al., 2016). Barukčić et al., 2019 evaluated whey-fermented beverages using active starting yeasts and adding different fruits and spices. Fermented cardamom whey guava beverage was evaluated, resulting in the most acceptance by a panel of judges (Barukčić et al., 2019).

Choudary and Sandey (2009) developed a whey-based mango-herbal (cardamom) beverage with different concentrations of cardamom extract. The beverage prepared with 2% cardamom extract showed excellent sensorial characteristics after 30 days at refrigeration temperature (Choudary & Sandey, 2009). Kishore et al. (2020) have developed a cold rehydrating beverage, having as ingredients jaggery (*Phoenix dactylifera*), tamarind (*Tamarindus indica*), cardamom (*Elettaria cardamomum*), *Aloe vera*, dried ginger, sodium benzoate, and water.

Winarsi et al. (2020) used cardamom rhizomes as the main ingredient for the formulation of functional beverages; the resulting drinks contain a high content of polyphenols (498.8 ppm) (Winarsi et al., 2020), more than others dinks based on ginger (447.9 ppm) (Ibrahim et al., 2015) and are an essential source of vitamin C 36 mg/100 g (Winarsi et al., 2020). The evaluation of a functional drink rich in antioxidant cardamom-rhizomes on the suppression of inflammation and the improvement of the lipide profile was taken by Winarsi and Susilowati (2018). A cohort of 30 women with atherosclerosis, age 40–65 years old, hypertension, hypercholesterolemia, and hypertriglyceridemia was recruited as research subjects. The group treated with the functional drink showed a significant plasma level decrease of IL-6, C-RP, total cholesterol, and LDL, and otherwise increased HDL (Winarsi & Susilowati, 2018).

15.4 Conclusion

The traditional culture of some regions, such as India and the Middle East, has always used cardamom as a flavoring agent in beverages and as a treatment for certain diseases. In conclusion, cardamom appears to be a valid ingredient for the formulation of functional drinks. The addition of cardamom in functional drinks is not only perceived by the consumer as a pleasant aroma, making the drink more accepted, but also, thanks to its content of bioactive compounds, it can contribute to the potential beneficial effects on human health by acting synergistically together to the ingredients.

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Chapter 16
Cardamom-Based Phytosomes

Uzma Tahir and Mohammad Rafiq Khan

Abbreviations

ACE	Angiotensin Converting Enzyme
DPPH	2, 2-diphenyl-1-picrylhydrazyl
RSM	Response Surface Methodology
SEM	Scanning Electron Microscope
SPC	Soy Phosphatidylcholine Complex

16.1 Phytochemicals

Several studies have reported that plants' nutritional value and medicinal benefits are naturally due to the polyphenolic compounds that are called phytochemicals. Phytochemicals define the basic properties of plants like flavor, smell and color. These natural bioactive compounds are absorbent to human bodies either used orally or topically (Kumar et al., 2017). The antioxidant and anti-inflammatory activity of phytochemicals extracted from plants makes them a popular constituent of several medicines, where they act as defense systems to prevent and treat diseases. Different studies have been conducted to identify the bioactivities of phytochemicals extracted from different plants to effectively use them in treating different diseases (Govindaraghavan et al., 2015).

U. Tahir (✉) · M. R. Khan
Department of Environmental Science and Policy, Lahore School of Economics, Lahore, Pakistan
e-mail: drrafiq@lahoreschool.edu.pk

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Herbal medicine is gaining popularity in both developing and developed countries as it is safer, biologically effective and cheaper than synthetic drugs (Ekor, 2014). Due to phytochemicals, antimicrobial and anti-inflammatory activity in herbal extracts enhances their efficacy in treating skin conditions like acne, eczema, burns and other bacterial and fungal skin infections (Kim & Lee, 2018). Phytochemicals in the form of bioactive chemical compounds extracted from terrestrial plants, such as polyphenols, steroidal saponins, flavonoids, vitamins and organosulphur compounds, are also found to help prevent and treat cardiovascular diseases (Vasanthi et al., 2012).

16.2 Bioavailability of Phytochemicals

The major constraint in achieving maximum output from phytochemicals in the form of medicine is their poor bioavailability to human bodies. Any medication, especially herbal, can only be helpful when it can transfer a significant portion of active compounds to the targeted being. The biological membranes easily absorb active compounds with high lipid solubility and low molecular size (Awasthi et al., 2011). However, most of the studied phytochemicals are polyphenolic compounds with high molecular weight and low lipid solubility. For example, alkaloids, terpene and flavonoids are hydrophilic, limiting their bioavailability (Lu et al., 2019). The polyphenols' multiple ring structure is the reason for large particle sizes and resulting higher molecular weights. These large compounds cannot be absorbed into the blood from the small intestine by a simple diffusion process. Some phyto-constituents are immiscible to oils and other lipids and thus fail to pass through the small intestine (Patel et al., 2009). Similarly, topical application of flavonoids such as silymarin and glycyrrhizic acid has a significant value in drugs and cosmetics, but low permeability through the skin due to the large molecular sizes hinders the optimal results (Alharbi et al., 2021).

Considering the health benefits of phytochemicals, it was essential to find a way to improve the bioavailability of active phyto-constituents by overcoming the transformation barriers. The formation of phytosomes results from diversified studies intended to enhance the absorption of herbal drugs to targeted bodies.

16.3 Phytosomes

Phytosomes, also called herbosomes, are basically nano forms of herbal products that are better absorbed and perform better than conventional herbal extracts. "Phyto" means plant, and "some" means very small or cell-like. Thus, phytosomes are cell-like plants because their nano size is easily absorbed into the skin in topical application. In the oral application of the drug, phytosomes provide a coating around bioactive components to protect them from degrading in the stomach's digestion process. These cell forms of phytochemicals easily become part of blood circulation

and bring the intended results effectively to treat fatal diseases (Jadhav et al., 2014). The phytosome technology is remarkable in herbal medicine as it enhances the bioavailability, brings significant clinical benefits, and ensures greater nutrient delivery to the tissues while protecting the nutrients from damage.

Water-soluble phyto-nutrients such as flavonoids and polyphenols can be transformed into phytosomes by reacting herbal drugs and phospholipids in an appropriate solvent. The phospholipids used in the process can be natural or synthetic. The resulting phyto-complexes, due to their improved ability to pass through the lipid-rich bio-membranes, are more bioavailable to the blood than conventional herbal extracts (Kareparamban et al., 2012).

16.4 Formation of Phytosomes

Generally, phytosomes are prepared by mixing phospholipids such as phosphatidylcholine, phosphatidylethanolamine or phosphatidylserine with herbal extracts in an aprotic solvent, for example, ethyl acetate, dioxane, acetone and methylene chloride. Specific stoichiometric ratios of all the constituents are used under certain conditions. The solvent evaporates eventually under a constant vacuum, resulting in an isolated lipid complex. The end product of the process is lipophilic, i.e., soluble to the polar and aprotic solvents providing enhanced stability and bioavailability (Kareparamban et al., 2012; Gaikwad et al., 2021).

Advances in phytosomes nanotechnology have significantly improved the bioavailability of bioactive phytochemicals by overcoming poor lipid solubility. For example, phytosomes of olive oil-based phytochemicals such as ginkgo and silybin are more easily absorbed into the administered body than the conventional extracts of olive oil. As a result, there are several commercially available phytosomes based pharmaceutical products. Table 16.1 enlists some of the available phytosomes in the market with their name, source and use.

16.5 Cardamom-Based Phytosomes

Medicinal plants have always been the focus of researchers worldwide as they are highly effective in treating several ailments with no or minimal side effects (Ho et al., 2018). The uses of herbal extracts or products are not limited to medicine, but they are significantly used for nutrition, flavoring, dyeing, fragrances, beverages and cosmetics. The major constraint in achieving optimized results from plant extracts is the low bioavailability and poor solubility of phytochemicals (Singh et al., 2017). As discussed previously that phytosomes formation is one way to increase the bioavailability of phytochemicals. Some other medicinal formulations that enhance bioavailability include liposomes, transferosomes, nano-emulsions, and nano-shells (Lewandowska et al., 2016). However, phytosomes which are phyto-phospholipid complexes, are the most effective solution to improve

Table 16.1 Commercially available phytosomes

No.	Phytosome	*Source plant*	Use
1.	Silybin	*Silybum marianum*	Hepatoprotective and antioxidant Activities for liver and skin
2.	Ginkgo	*Ginkgo biloba*	Brain and vascular protection, Anti-skin aging agent.
3.	Green Tea	*Thea sinensis*	Nutraceutical, systemic antioxidant, protection against cancer and damage to cholesterol
4.	Olive oil	*Olea europaea*	Anti-inflammatory, antioxidant, anti-hyperlipidemic activities and cardiovascular protection
5.	Centella	*Centella asiatica*	Used to treat Vein and Skin disorders
6.	Grape Seed	*Vitis vinifera*	Antioxidant activity protects against heart disease
7.	Curcumin	*Curcuma longa*	Anti-inflammatory, hepatoprotective activity
8.	Leucoselect	*Vitis vinifera*	Antioxidant activity, specific for the eyes, lungs, diabetes, veins, and protection against heart disease
9.	Hawthorn	*Carteagus*	species Antihypertensive activity treats heart disease and high blood pressure
10.	Bilberry	*Vaccinium myritillus*	Antioxidant improves capillary tone and reduces abnormal Blood vessel permeability
11.	Greenselect	*Camellia sinensis*	Antioxidant activity is the best choice for protection against cancer and damage to cholesterol
12.	Ruscogenin	*Ruscus aculeatus*	Anti-inflammatory

Patel et al. (2009), Gandhi et al. (2012), Kumar et al. (2017), Alharbi et al. (2021)

bioavailability. Many plants based phytosomes are commercially available, as mentioned in Table 16.1. However, there have not been many studies focusing on cardamom-based phytosomes.

The antioxidant, antimicrobial and anticancer activity of cardamom makes them highly useful medicine to treat cardiac, pulmonary and kidney diseases. The herb has also been proven to improve hypertension, depression and pregnancy discomfort (Singletary, 2022). Knowing the magical health benefits of the herb, cardamom-based phytosomes can achieve radical advances in medicine.

16.6 Formation of *E. Cardamom* Phytsomes

Response Surface Methodology (RSM) is one of the techniques to optimize different parameters to achieve synthesized phytosomes with maximum wavelength ultimately. Cardamom seeds identified by plant taxonomists can be used to form the herbal extract. Bibi and Jahan (2020) took 25 g of powdered *Elettaria cardamom* in a cellulose extraction thimble. Because defatting is usually done before further processing, the herbal product, 300 ml n-hexane solvent, was used to defeat the cardamom powder. Next, the Soxhlet apparatus was used to extract defatted powder

further, treating it with 300 ml of methanol. Finally, the extract from the Soxhlet apparatus was filtered and evaporated to get the final herbal extract. The preparation of *E. cardamom* phytosomes involves a thin layer hydration technique and thus was carried out as under:

1. In the first step, different amounts of *E. cardamom* and soy phosphatidylcholine from 100 to 500 mg were dissolved in 20 mL of ethanol to prepare phosphatidylcholine (SPC) complexes. Then, the mixtures were stirred for 120 min at 25 °C.
2. Ethanol evaporated and dried *E. cardamom* SPC complexes (0.2%) were again dissolved in anhydrous ethanol in a rotary evaporator, rotating at 180 rpm for 30 min at 40 °C.
3. The obtained residue was dissolved in phosphate-buffered saline (PBS) for hydration for 15–20 min. The swollen lipid complexes along the wall of the flask were then peeled off.
4. In the end, prepared phytosomes were sonicated for 4 min.

Bibi and Jehan 2020, studied the following parameters in the *Ellettaria cardamom* phytosomes thus prepared.

- Optimization of *E. cardamom* phytosomes
- Characterization of *E. cardamom* phytosomes
- Entrapment efficiency
- Antioxidant potential
- Antimicrobial activity
- ACE inhibition potential

16.6.1 Optimization of E. Cardamom *Phytosomes*

The thin layer hydration technique was used to prepare *Ellettaria cardamom* phytosomes to improve crude cardamom extract's bioavailability and medicinal efficacy. Response Surface Methodology (RSM) was further used to optimize the formulated phytosomes to improve their characterization and biological activities. The UV-visible spectrophotometer was used to scan different formulations of the *E. cardamom* phytosomes at 200–400 nm. As a result, optimized *E. cardamom* phytosome was obtained at a wavelength of 324 nm at 300 mg concentration of both *E. cardamom* and phospholipid when hydration time was 20 min. The maximum wavelength concentration of the product showed the highest amount of entrapped drug, resulting in optimal biological activities.

16.6.2 Characterization of E. Cardamom *Phytosomes*

The physical appearance of optimized E. cardamom phytosomes was studied by scanning electron microscope (SEM) images. Photomicrograph showed that phytosomes were spherical, and their surface was smooth without any crystalline

structure or impurity. The average particle size of optimized *E. cardamom* phytosomes was 577.8 nm. The polydispersity index was less than 0.5 (0.443), indicating that particle sizes were uniform and homogeneous. The small size of *E. cardamom* phytosomes improves the bioavailability and, thus, the effectivity of the drug, as they easily pass through cell permeable membrane (Maryana et al., 2016).

16.6.3 Entrapment Efficiency

Standard ascorbic acid was used to find the entrapment efficiency of optimized phytosomes prepared with 300 mg of *E. cardamom* extract and 300 mg of soy phosphatidylcholine complex (SPC). Free drug concentration was determined with the help of a standard ascorbic acid curve. Entrapment of the drug was directly proportional to the concentration of phospholipid. Optimized *E. cardamom* phytosome showed up to 74% of entrapment efficiency. Entrapment efficiency is highest when equal concentrations (1:1) of plant extract and phospholipid are used to formulate phytosomes.

16.6.4 Antioxidant Potential

The antioxidant potential of *E. cardamom* phytosomes was found to be significantly higher than crude E. cardamom extract. In order to find the antioxidant activity, different assays were performed on *E. cardamom* phytosomes and natural *E. cardamom* extract: e.g., DPPH· radical scavenging assay and lipid peroxidation inhibition assay (Rahman et al., 2015).

The presence of antioxidants can be determined by the degree of color change (decolorization) of the methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH·). More percentage of radical scavenging activity demonstrates that the sample has strong antioxidant potential. The results showed that the radical scavenging activity percentage of the DPPH· assay of *E. cardamom* phytosome was 72%, whereas *E. cardamom* extract showed 65% of radical scavenging activity at 100 µg/mL concentration of the sample. Similarly, the lipid peroxidation inhibition assay performed on *E. cardamomum* phytosomes showed 50% antioxidant activity compared to crude E. cardamom extract, which was 42%. The antioxidant potential of *E. cardamom* phytosome was higher than the natural extract of the herb; however, standard ascorbic acid showed a much higher inhibition rate, i.e., 86% for DPPH· radical scavenging assay and 72% for lipid peroxidation inhibition assay.

The antioxidant potential of any drug is due to the presence of flavonoids and polyphenols. The higher antioxidant potential of *E. cardamomum* phytsomes is probably due to polar groups of phospholipids. Other contributors to antioxidant activity in cardamom phytosomes are thiamin, riboflavin, vitamin C and phytochemicals (Al-Ansari et al., 2019).

16.6.5 Antimicrobial Activity

The Agar well diffusion method was used to evaluate the antimicrobial activity of *E. cardamom* phytosomes against Gram-positive (*Bacillus subtilis*) and Gram-negative (*E. coli*) strains. At 150 mg concentration of the samples, *E. cardamom* phytosomes showed higher results (*B. subtilis* 13 mm; *E. coli* 14 mm) than the *E. cardamom* extract (*B. subtilis*, 10 mm; *E. coli* 11 mm). Thus, the antimicrobial activity of phytosomes was higher than the crude cardamom extract.

16.6.6 ACE Inhibition Potential

Angiotensin-converting-enzyme inhibitors are a medication used to treat high blood pressure and heart failure. The ACE inhibition potential of phenolic compounds makes them a highly effective alternative to synthetic medicine. The ACE inhibition potential of *E. cardamom* phytosomes was 46% higher than 39% of *E. cardamomum* extract at 4 mg/ml concentration.

16.7 Conclusion

From the discussion, it may be concluded that cardamom is a well-known herbal drug used to treat several health-related problems. The types of phytochemicals present in cardamom include flavonoids, terpenoids, lipids, carotenoids, essential oils and carbohydrates. The antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and anticancer properties of phytochemicals present in cardamom make it an extraordinary herb that can be an alternative to conventional synthetic drugs. However, limited absorption and, thus, low bioavailability of phytochemicals to human cells hinder optimal healing results from the herbal drug. Phytosomes are nano-engineered drug delivery systems that enhance the bioavailability of bioactive chemicals across biological barriers. Manufactured phytosomes of different herbs are available in the market, which are more bioavailable and effective than the crude extracts of the herbs. Therefore, cardamom-based phytosomes could be prepared to improve the herb‘s health benefits. *Elettaria cardamom* phytosomes have better antioxidant, antimicrobial and ACE inhibition activity than natural cardamom extract.

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Chapter 17
Cardamom Safety

Mohammad Rafiq Khan and Shamaila Aslam

Abbreviations

CEO	Cardamom Essential Oil
PTSD	Post-Traumatic Stress Disorder
EO	Essential Oil
PES	Petroleum Ether Soluble
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
TM	Transverse Myelitis
PS	Performance Status
PI	Principal Investigator
RCTS	Randomized Controlled Clinical Trials
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
Hba1c	Hemoglobin A1C
BMI	Body Mass Index
FBS	Fasting Blood Sugar
Mets	Metabolic Equivalents
CHF	Congestive Heart Failure
ALT	Alanine Transferase
AST	Aspartate Aminotransferase
ALP	Alkaline Phosphatase

M. R. Khan (✉)
Department of Environmental Science and Policy, Lahore School of Economics, Lahore, Pakistan
e-mail: drrafiq@lahoreschool.edu.pk

S. Aslam
Northwest Houston Clinical Research PLLC, Tomball, TX, USA

M. F. Ramadan (ed.), *Cardamom (Elettaria cardamomum): Production, Processing and Properties*, https://doi.org/10.1007/978-3-031-35426-7_17

17.1 Introduction

Cardamom has been significantly backed by science concerning health and safety. It has been extensively prescribed in Traditional Medicine in its processed forms: seeds, oils, and extracts that are believed to carry impressive curative effects on different diseases because cardamom possesses different medicinal traits such as antiseptic (pulmonary), antispasmodic (neuromuscular), aphrodisiac, expectorant, anthelminthic, cephalic, cardiotonic, diuretic, emmenagogue, antibacterial sialagogue, stomachic, etc. (Mukherjee, 1972, Nambiar, et al., 1994, Vijayan et al., 2002, HealthSite.com Online ScienceDirect. 2011, Sharifi-Rad et al., 2021; Sharifi-Rad, 2021). It has been dominantly used for centuries in countries such as India and Pakistan, where it cultivated.

17.1.1 Historical Background

In a book authored by Vijayan et al. (2002) and published by Imprint CRC Press (ISBN 9780429218637), Ayurveda and Sidha systems of medicine have been reported to be the founders of onset of applications of cardamom as a component of several therapeutic formulations. Chaakasamhita, the ancient Indian medical book, described cardamom as an antidote for food poisoning. This formed a component of Bhrahmi rasayana, used as a treatment for inflammations. It is also a constituent of many balms, ointments and therapeutic oils for treating cramps, rheumatic pain, inflammations, etc. In Ayurvedic books, the properties of cardamom seeds are described as aromatic, acrid, sweet, cooling, stimulant, carminative, diuretic, cardiotonic and expectorant. Cardamom is used as an ingredient in preparations used for the treatment of asthma, bronchitis, hemorrhoids, renal and vesicle calculi, cardiac disorders, anorexia, dyspepsia, gastropathy, debility and vitiated conditions of vata (Sukumaran, Online). An aqueous extract of seeds is given to nursing mothers to treat ringworm infection in children. Roasted seeds are boiled with betel leaves, and the extract treats indigestion and worm trouble. The major reason is that the current regular stream of medicine does not receive the benefits of traditional medicine. The trends are now changing due to the skyrocketing prices of pharmaceutical drugs and the side effects encountered all over the globe. The leading Journal of American Medical Association (JAMA) spares some space for some material on Traditional Medicine. Active scientific research backing seems to have been started in the second half of the twentieth century (Singh, 1978).

17.2 Spectrum of Medical Applications of Cardamom

A significant number of health benefits of cardamom, which are scientifically backed and authenticated, are narrated below with the bon, which its use qualifies as a cure for diseases discussed below.

17.2.1 Lowering of High Blood Pressure

E. cardamom exhibits antioxidant and diuretic characteristics and thus may lower blood pressure. Cardamom may be helpful for people with high blood pressure. In a study, research workers gave three grams of cardamom powder a day to 20 adults who were newly diagnosed with high blood pressure (Bhanote, Online). After 12 weeks, blood pressure levels had significantly decreased to the normal range. The promising results of this study may be related to the high levels of antioxidants in cardamom. The participant's antioxidant status had increased by 90% by the end of the study. Antioxidants have been linked to lower blood pressure. The research workers also suspect that the spice may lower blood pressure due to its diuretic action meaning it can promote urination to remove water that builds up in the body, which means less pressure exertion per unit area. Cardamom extract has been shown to increase urination and decrease blood pressure in rats.

17.2.2 Fighting Against Cancer Cells

Cardamom may help in fighting against cancer cells. The compounds in cardamom may help fight cancer cells. Studies in mice have shown that cardamom powder can increase the activity of certain enzymes that help fight against cancer cells. In addition, the spice may also enhance the ability of natural killer cells to attack tumors. In one of the studies, researchers (Ashokkumar et al.) exposed two groups of mice to a compound that causes skin cancer and fed one group 500 mg of ground cardamom per kg (227 mg per pound) of weight per day while the other was without any dose. Their condition was compared. The cancer cells were reduced significantly in the group given the dose. The major constituents of cardamom essential oil (CEO) are 1,8-cineole, α-terpinyl acetate, sabinene, and β-linalool. They can be used in food, aroma, and pharmaceutical applications.

17.2.3 Relief from Asthma

It prevents Asthma (Rana Online). Dr. Preyas Vaidya, Consultant Pulmonologist, Fortis Hospital, Mulund, India, says, "Black Cardamom has been studied in animals and has been found to have some Bronchodilator effects by acting on calcium channels in the airways. It may have some effect on asthmatics; however, human scientific confirmations are lacking. It has flavonoids that may also help with relaxation in the airways. However, this is not a substitute for medicine. It does not help in the treatment or management of asthma. However, it has weak airway dilatation properties, which may make an asthmatic feel better. Airway constriction is one of the components of this condition, and cardamom may help alleviate it to an extent." A Dietician, Dr. Jaee Khamkar, Fortis Hospital, Kalyan, says, "Black cardamom

provides many health benefits; it has antiseptic and antispasmodic properties, anti-inflammatory properties, and is an antioxidant reservoir, a homeostasis agent and a blood pressure regulator. This tiny yet powerful black seed can help warm the respiratory tract. It helps ensure easy passage of air through the lungs. Moreover, black cardamom seeds help combat cough, cold and sore throat. This is achieved by alleviating the mucous membrane while normalizing mucous flow through the respiratory tract."

17.2.4 Cure for Depression

It helps cure depression. Cardamom is used in Ayurvedic medicine to fight depression, stress and other mental health issues. According to Ayurvedic doctors, one can boil cardamom in water or add it to tea to get the benefits (HealthSite.com). A recent study by (Asoumi et al., 2016) evaluated the efficacy of *E. cardamomum* methanol extract on anxiety-like behavior in a rat model of Post-Traumatic Stress Disorder (PTSD) using adult male Wistar rats. The rats were given either saline or different dosages (200, 400, and 800 mg/kg) of *E. cardamomum* methanol extract before and after stress sessions. In addition, open field, elevated plus-maze, and rotarod tests were used to evaluate locomotion and anxiety-like behavior in the rats. The results showed that *E. Cardamomum* methanolic extract, particularly at 400 mg/kg, significantly improved anxiety-like behavior in a rat model of PTSD.

17.2.5 Enhancement of Oral Health

It enhances oral health. Cardamom tea helps protect dental health by inhibiting bacterial growth (Teeth Bank Online). Bacteria grow on the surface of teeth and cause dental caries-a common condition where acids break down the tooth enamel. These acids are produced when bacteria ferment carbohydrates. Cardamom tea can help neutralize these bacteria and prevent plaque buildup, cavities, and dental caries. The antibacterial properties of cardamom also effectively treat halitosis-more commonly known as bad breath. Bad breath is caused when bacteria build up in the mouth and begin to feed on food particles. Cardamom helps eliminate the bacteria to keep the breath fresh all day long (Sharma, 2012).

17.2.6 Protection of the Human Heart

It protects the heart and liver. This activity may be interpreted as its hepatoprotective action due to its components, such as cineole, terpinene, terpiniol, sabinine, α-pinene and limonene, because of their action that acts as a tonic for the heart and

liver. In addition, they also act as an appetizer and promoters by the elimination of bile and help in reducing congestion of the liver.

17.2.7 Protection and Improvement of Digestive System

It provides digestive aid and thus improves the digestive system due to its anti-ulcerative and anti-inflammatory action (Jamal et al. 2005). Cardamom tea has long been used as a digestive aid to soothe stomach ailments, including gas and bloating. It was used in Turkey and Arabic societies to treat intestinal worms. Crushed cardamom seeds have anti-inflammatory traits that soothe irritated stomach muscles. This helps to prevent the contractions that cause stomach pains. Cardamom is a natural carminative, relieving the victims of gas from the alimentary canal. Drinking cardamom tea during or after meals can help streamline digestion and prevent gas. Some researchers also show that cardamom tea may be beneficial in treating irritable bowel syndrome, although results have been inconclusive. Like ginger tea, cardamom tea can help treat nausea. Sipping hot tea before boarding a boat or plane, the boarder suffers from motion sickness. Drinking cardamom tea may also help ease morning sickness, but consult a physician before drinking cardamom tea if the drinker is pregnant.

Jamal et al. (2005) studied the effect of a crude methanol extract, essential oil (EO), petroleum ether soluble (PS) and insoluble fractions of methanol extract, were studied in rats at different doses for their ability to inhibit stomach lesions induced by aspirin, ethanol and pylorus ligature. In addition, their effects on wall mucus and gastric acid output were also recorded. All fractions (TM, EO, PS, PI) significantly inhibited gastric lesions induced by ethanol and aspirin but not those induced by pylorus ligation.

17.2.8 Improvement of the Quality of Human Hair

It improves the quality of human hair. Large cardamom fruit, commonly known as ‘Heel.

17.2.9 Gastroprotective Action

‘Bari Ilaichi’ is used in the Greek (Unani) system of medicine to treat gastrointestinal disorders (Mukherjee). A crude methanol extract and its different fractions, e.g., essential oil, petroleum ether (60–80 °C), ethyl acetate and methanol fractions, were studied in rats for their ability to inhibit gastric lesions induced by aspirin, ethanol and pylorus ligature. A direct protective effect of ethyl acetate fraction on the gastric

mucosal barrier was seen. The decrease observed in gastric motility brought about by essential oil and petroleum ether fractions suggests the gastroprotective action of the spice (Jamal et al. 2005). These investigations validate the use of large cardamom in gastrointestinal disorders by Unani physicians.

17.2.10 Anti-inflammatory Activity

Due to its anti-inflammatory activity, it finds its use as a cure for teeth and gum infections. The decrease observed in gastric motility to prevent and treat sore throat, congestion of the lungs and pulmonary tuberculosis, inflammation of eyelids and also digestive disorders (Teeth Bank).

17.2.11 Body Basal Metabolism

Asbaghi et al. (2020) published a systematic review and meta-analysis to summarize all the reported randomized controlled trials (RCTs) evidence and to evaluate the effect of green cardamom on lipoproteins, glycemic control and anthropometric parameters in healthy and/or with without disease types compared with the control. Triglycerides were significantly reduced after cardamom supplementation when compared with the control group. In addition, cardamom intake from 3 small studies resulted in a significant increase in BMI compared to the control group. However, compared with the control group, cardamom supplementation did not significantly affect total cholesterol, LDL-cholesterol, HDL-cholesterol, fasting plasma glucose and body weight. The conclusion was that green cardamom intake significantly reduced triglyceride levels.

A few recent reviews and meta-analyses, such as by Nameni et al. (2022) and Yahyazadeh et al. (2021), have appeared in literature with the claim that these have been conducted to investigate carefully the situation in the metabolic context in response to the controversial results reported by different experts. The former Nameni et al. (2022) conducted a meta-analysis of the effect of cardamom supplementation on glycemic indices and weight profile of randomized controlled clinical trials (RCTs). They concluded that Daily cardamom supplementation showed a significant effect in HOMA-IR and HbA1C and showed no significant effect in BMI, weight and WC and also no significant effect in FBS, insulin and QUICKI. The letter claims that cardamom has beneficial effects on treating MetS and its complications.

17.2.12 Antidote of Snake or Scorpion Venom

Cardamom is effective as an antidote for both snake and scorpion venom Vijayan et al. (2002).

17.2.13 Aiding Weight Loss

It may aid weight loss and prevent serious diseases. Cardamom tea may help accelerate weight loss by streamlining the body's digestive processes. Cardamom works to prevent the buildup of fat while helping the liver process waste products more quickly. Ground cardamom has been shown to help prevent obesity. In an animal study published in Lipids in Health and Disease, researchers found that cardamom improved glucose intolerance and prevented the deposit of abdominal fats. Cardamom was also shown to affect the liver by ameliorating fibrosis positively.

Rahman et al. (2017) conducted a study on 28 Male Wistar rats, concluding that cardamom powder supplementation can prevent dyslipidemia, oxidative stress and hepatic damage in HCHF diet-fed rats. The rats were divided into four different groups: such as Control, Control + cardamom, HCHF, HCHF + cardamom and fed with the HCHF diet. Oral glucose tolerance test, organs wet weight measurements and oxidative stress parameters analysis, and liver marker enzymes such as ALT), AST, and ALP activities were estimated on the tissues from the rats. Plasma lipids profiles were measured, and histological staining was performed to evaluate inflammatory cell infiltration and fibrosis in the liver. The results indicated that "HCHF diet feeding in rats developed glucose intolerance and increased peritoneal fat deposition compared to control rats. Cardamom powder supplementation significantly improved glucose intolerance and prevented abdominal fat deposition in HCHF diet-fed rats. HCHF diet feeding in rats also developed dyslipidemia, increased fat deposition and inflammation in the liver compared to control rats. Cardamom powder supplementation significantly prevented the rise of lipid parameters in HCHF diet-fed rats. Histological assessments confirmed that the HCHF diet increased fat deposition and inflammatory cell infiltration in the liver, which was normalized by cardamom powder supplementation in HCHF diet-fed rats.

Furthermore, the HCHF diet increased lipid peroxidation, decreased antioxidant enzyme activities and increased advanced protein oxidation product level significantly in plasma and liver tissue, modulated by cardamom powder supplementation in HCHF diet-fed rats. HCHF diet feeding in rats also increased the ALT, AST and ALP enzyme activities in plasma which were also normalized by cardamom powder supplementation in HCHF diet-fed rats. Moreover, cardamom powder supplementation ameliorated the fibrosis in the liver of HCHF diet-fed rats."

In another study by Fatemeh et al. (2017), eighty overweight or obese prediabetic women were randomly divided into intervention and placebo groups. The former was given 3 g of green cardamom and the latter 3 g of rusk powder for 2 months. Different indicators were measured. The results indicated that.

After the intervention, mean total cholesterol and LDL-C significantly decreased, and insulin sensitivity increased in the cardamom group. In contrast, in the control group, mean HDL-C significantly decreased after the study, but no significant decrease in systolic and diastolic blood pressure, glycemic indices, and serum lipids values in the cardamom group compared to the placebo group. The researchers concluded that green cardamom supplementation might have a protective effect on

HDL-C levels in pre-diabetic subjects. In addition, it improved some blood parameters in these subjects; its effects were not different from the placebo group.

17.2.14 Help in Quitting Smoking

It may help quit smoking (Al Hanbali et al., 2021) Cardamom tea may be helpful if you are trying to stop smoking. Research published in Addictive Behaviors examined the potential of cardamom gum to aid nicotine withdrawal. Results showed that vanilla and baked apple cardamom gums effectively reduced nicotine withdrawal symptoms, including dysphoria, anxiety, and tension.

17.2.15 Boosting of the Immune System

It boosts the immune system. Like many herbal teas, cardamom tea may help treat and prevent the common cold and flu. That is because it is packed with antioxidants and vitamins that fight off viruses, fungi, and bacteria (Ashokkumar et al., 2021).

17.2.16 Clearing of Skin

Cardamom can be considered a savior for acne-prone skin. It prevents breakouts and erases blemishes. Furthermore, it controls the flow of sebum and clears out all clogged pores. In addition, its anti-inflammatory elements work to get rid of skin irritation and even out skin complexion.

17.2.17 Softening of Lips

Chapped lips are no more concerned due to Cardamom's antioxidants. Cardamom essential oils are added to lip balms for the fragrance and to smooth lips.

17.2.18 Miscellaneous Uses

Large Illachi flavor cardamom cola, prepared by blending caramer acid and carbonating mixture. The glabrous fruit stalks, usually discarded by farmers, can be used as the base of agarbattis. A study published in Ethnobotanical leaflets found that cardamom may effectively prevent bacteria, including streptococcus, which causes

sore throat. In addition, researchers found cardamom effective against staph infections and fungal infections, including candida. Some evidence suggests that using cardamom as an essential oil may help improve air intake during exercise and reduce blood pressure (Bhanote, Online); Nutritional Value of Cardamom (Elaichi)/100 g) is as under:

Protein: 11 g, Cholesterol: 0 mg, Carbs: 68 g, Total Fat: 7 g and Calorific Value: 311 kcal (Healthify me, Online). Cardamom has plenty of disease-preventing phytonutrients. It is also a good source of calcium, potassium, magnesium and essential electrolytes.

It is a rich source of vital vitamins like vitamin C, niacin and riboflavin (Vitamin B_2). Adding this whole spice to the food enriches it with vast nutrients and minerals. Cardamom pods are small spindle-shaped with a triangular cross-section. These pods contain several seeds, which can be used as a whole, or the seeds can be ground to powder (Healthify me (Online). The diseases given below are due to nutritional deficiencies. It may be that

E. cardamom cures them by supplying essential vitamins.

17.3 Side Effects of Cardamom Tea

Cardamom tea has not been shown to have severe side effects when consumed in moderation. However, its herbal tea may interact with certain medications, and thus it seems better to have an idea to talk to a doctor before drinking cardamom tea if one has a health condition. Research shows cardamom may interact with blood thinning medications and some antidepressants, so limit or avoid use if you take these medications. Linda Decann (2022) has highlighted 9 surprising cardamom side effects for females.

People have used cardamom in the past to take its help as a digestive aid, but there is not enough evidence to back this up in to-days real world, basing every interpretation on principles of logic. Its seeds and oil have been used for centuries to treat many health conditions, including asthma, heartburn, constipation, insomnia, and high blood pressure. However, very little scientific evidence indicates that cardamom works for these ailments (Table 17.1).

17.4 Cardamom Side Effects

The main concern with using cardamom is that there is not enough information about its safety when used in amounts higher than those used for cooking purposes. When used at these doses, Cardamom can be taken orally as an extract or powder. It should be avoided in pregnant women due to the lack of known effects during

Table 17.1 Components of *E. cardamom* and their applications in medicine

Component	Percentage	Medical Application	Reference
Oxygenated monoterpenes	40.7–66.7%	Antimicrobial action	Chueca et al.
Monoterpene hydrocarbons	23.1–58.6%	Carvacrol: antimicrobial, antioxidant, and anticancer activities.	Sharifi-Rad, et al.
Sesquiterpenes	0.1–2.0%	Smoking cessation, pain relief, osteoporosis, contraception	Hanbali et al.
Among Monoterpenoids, Predominant Constituents			
α-Terpinyl acetate	29.9–61.3%	Antioxidant, Synergy	Tamili and Bhattacharjee
1,8-Cineole	15.2–49.4%	A potent antiseptic kills bacteria in bad breath and other infections, exhibits expectorant activity, and clears breathing passages.	Ashokkumar et al.
α-Terpineol	0.83–13.2%	Anti Covid Treatment	Panikar et al. (2021)
β-Linalool	0.44–11.0%	Enhancement of skin penetration of drugs	Nowak et al.
Sabinene	1.9–4.9%	Several activities: Antidiabetic, anti-proliferative, anti-cancer, etc.	Ashokkumar et al 06-Apr-2021
Two sesquiterpene constituents identified			
Nerolidol	–	Antibacterial Activity	Balakrishnan et al.
Ardinen	–	Smoking cessation, pain relief, osteoporosis, contraception	Al Hanbali et al.
p-Cresol (a phenol derivative)	–	Dental Disinfectant	Sharma (2012) and Drug Bank Online
Compositional data subjected to Euclidean-distance-based similarity analysis showed two major clusters composed of constituents shown below			
1,8-cineole, α-terpinyl acetate, sabinene, and β-linalol	The major constituents oil (CEO) are aroma, The major constituent of cardamom essential oil (CEO)	These can be used in food, aroma, and pharmaceutical applications.	Ashokkumar et al. 06-Apr-2021

pregnancy. Cardamom has also been applied directly to the skin. There is not enough reliable information about the safety of using it on the skin at doses greater than those found in foods. Side effects in women include irritation and redness if applied directly to the skin and diarrhea if taken by mouth. All the reported side effects are outlined below

17.4.1 Diarrhea

Excessive consumption of cardamom can lead to diarrhea and dehydration. According to the book "Healing Spices: How to Use 50 Every Day and Exotic Spices to Boost Health and Beat Disease," consuming large amounts of cardamom may produce flatulence-inducing effects. Take 2 grams of cardamom in capsule form daily to get its benefits without worrying about side effects

17.4.2 Nausea

The U.S. National Institutes of Health reports that nausea is one of the possible side effects of consuming high amounts of cardamom. Nausea usually occurs when an individual consumes more than 1 gram per day of the spice. Be sure to follow your doctor's instructions if you take cardamom as a supplement to avoid exceeding this amount

17.4.3 Dizziness

According to the NIH, too much cardamom can cause dizziness. Cardamom is a spice that may impact your health and well-being, with potential benefits such as boosting digestion. However, these benefits come with side effects when consumed in high doses. In addition, the caffeine in cardamom may also become problematic if you overeat the spice and are sensitive to caffeine. Possible side effects of consuming large amounts of cardamom include headaches, dizziness, insomnia, and vomiting

17.4.4 Menstrual Cramps

Cardamom treats various digestive issues, including stomach spasms. However, the same properties in cardamom that help relieve one type of stomach spasm can worsen another. If you suffer from painful menstrual cramps, cardamom may increase the intensity of your cramps. Increase the amount of water you drink during your period to avoid dehydration and lessen the severity of your menstrual cramps

17.4.5 Dehydration

Drinking enough water is crucial to maintaining good overall health and avoiding dehydration. If consumed in large quantities, cardamom can have a mild diuretic effect, causing you to urinate more frequently than usual. In addition,

cardamom dehydration can happen if you do not drink more water while eating cardamom-flavored foods or taking cardamom supplements, so make sure you drink more water

17.4.6 Pregnancy

Pregnant women should avoid cardamom because it might stimulate the uterus, causing miscarriages or premature labor. Cardamom supplements also increase stomach acid levels and heartburn, which some pregnant women already experience without adding herbs that worsen these symptoms

17.4.7 Blood Thinning

According to the Memorial Sloan-Kettering Cancer Center, Cardamom may increase the risk of bleeding in people who take anticoagulant medications because it appears to have blood-thinning properties. Examples of anticoagulants include warfarin, clopidogrel, and aspirin. If you take one of these medications, do not use cardamom without consulting your doctor

17.4.8 Diabetes

Cardamom may increase insulin levels and lower blood sugar levels in people with diabetes. According to an article published in "Food Chemistry" in April 2007, "hypoglycemia" can cause hypoglycemia in some people. If you have diabetes and take medication that lowers blood sugar levels, do not use cardamom without first consulting your doctor, as it could cause a dangerous drop in your blood sugar level

17.4.9 Surgery

Cardamom might affect blood sugar levels and interfere with blood sugar control during and after surgery. Cardamom might slow down the central nervous system. There is concern that it might cause general anesthesia to work less well. Stop using cardamom at least 2 weeks before a scheduled surgery

Here Are a Few Ways to Use Cardamom:

- Use it in curries, mulled wine, pilafs, custards, and other desserts.
- Add a pod or two to your next pot of coffee or tea for a subtly sweet and aromatic drink.
- Sprinkle ground cardamom over fruit salads or fruit compotes.

17.5 Conclusion

It seems that regular consumption of cardamom is probably very healthy. It has some serious advantages for human health. In order to avoid side effects, people need to know how much cardamom they are allowed to consume and if there are specific contraindications. If used correctly. They can certainly enjoy all the benefits without suffering any negative consequences. They should always seek medical help if they have any doubts about whether to use it or not. Pregnant women should avoid cardamom because it might stimulate the uterus, causing miscarriages or premature labor. Cardamom supplements also increase stomach acid levels and heartburn, which some pregnant women already experience without adding herbs that worsen these symptoms. Cardamom tea may cause allergic reactions in certain individuals. If someone experiences symptoms including runny nose, itchy throat, or difficulty breathing when drinking cardamom tea, he/she should stop using it immediately. One should not drink cardamom tea if you are allergic to either its seeds or to other parts of its plants.

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Chapter 18
Cardamom in Food Applications

Samy Mohamed Galal and Nashwa Fathy Sayed Morsy

Abbreviations

BHT	Butylated hydroxytoluene
DPPH·	2,2-diphenyl picrylhydrazyl
ECF	Encapsulated cardamom flavor
FRAP	Ferric reducing antioxidant power
GC-MS	Gas chromatography-mass spectrometry
GRAS	Generally recognized as safe
MDA	Malondialdehyde
PV	Peroxide value
SC-CO_2	Supercritical fluid-CO_2
TBARS	Thiobarbituric acid reactive substance
TBHQ	Tertiary butyl hydroquinone

18.1 Cardamom Flavor

Cardamom is the "Queen of Spices"(Ashokkumar et al., 2020). There are three different varieties of cardamom: green, black and white (Fig. 18.1).

Cardamom is used in different forms: seeds, essential oil, and oleoresin (Fig. 18.2).

The US Food and Drug Administration (FDA, 2022) classed cardamom (*Elettaria cardamomum* Maton) seed as generally recognized as safe (GRAS) for human consumption. To meet their domestic needs, Saudi Arabia and India consumed half of

S. M. Galal · N. F. S. Morsy (✉)
Food Science Department, Faculty of Agriculture, Giza, Egypt
e-mail: asadgalal@agr.cu.edu.eg; nanafsm@agr.cu.edu.eg

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Fig. 18.1 Varieties of cardamom

Fig. 18.2 Different forms and products of cardamom

the world's cardamom production (Anwar et al., 2016). Cardamom's flavor is a result of the components of its essential oil. The pleasant fruity smell is caused by α-terpinyl acetate, linalyl acetate, and linalool, while the camphoraceous odor is caused by 1.8-cineole. The 1,8-cineole, α-terpinyl acetate, and terpene alcohols are crucial for assessing cardamom's flavor quality (Salzer, 1975). The green pods smell like lemon and have a sweet, spicy floral flavor (Fig. 18.3).

A high α-terpinyl acetate/1,8-cineole ratio indicates the superior quality of cardamom essential oil (Morsy, 2015). However, cardamom essential oil flavor components are degraded upon exposure to oxygen during storage, and the oxidation products give food an unpleasant flavor (Najafi et al., 2011). Therefore, the usage of cardamom essential oil is restricted to food with short shelf life (Govindarajan et al., 1982).

Fig. 18.3 Cardamom different flavors

18.2 Food Applications

18.2.1 Cardamom as a Food Preservative

The cardamom essential oil at 3000 ppm showed potent inhibition against (*Aspergillus terreus*, *Fusarium graminearum*, *Penicillium purpurogenum*, and *Penicillium madriti*), food-borne gram-positive bacteria (*Staphylococcus aureus*, and *Bacillus cereus*), and food-borne gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*). In addition, the cardamom ethanol oleoresin at the same level (3000 ppm) exhibited high antifungal activity compared to cardamom oleoresins obtained by other solvents (chloroform, methanol, and diethyl ether) (Singh et al., 2008).

The minimum inhibitory concentration value of the green cardamom essential oil against food pathogens (*Staphylococcus aureus* and *Salmonella typhimurium*) was 10 mg/mL. The major components of this essential oil, according to the gas chromatography-mass spectrometry (GC-MS) analysis, were α-terpinyl acetate (38.4%), 1,8-cineole (28.7%), linalyl acetate (8.42%), sabinene (5.21%), and linalool (3.97%) (Abdullah et al., 2017). The green cardamom supercritical fluid extract (obtained with 99.8% CO_2 at 30 °C, 300 bar, and 60 min) at a concentration of 0.12% in ethanol 95% (*v/v*) inhibited *Escherichia coli* O157:H7 (*E. coli*) and *Salmonella typhimurium* (*S. typhimurium*) JSG 1748 biofilm formation by 85.5%, and 100%, respectively. Likewise, black cardamom extract at a 5 mg/mL concentration in ethanol 95% (*v/v*) prevented *E. coli* O157:H7 and *S. typhimurium* JSG 1748 biofilm formation by 84.6% and 50.1%, respectively. The major compounds in the green and black cardamom supercritical fluid extracts were: α-terpinyl acetate (34.9%, 12.2%), 1,8-cineole (25.3%, 44.2%), and sabinene (5.48%, 5.96%), respectively (Abdullah et al. 2021a, b).

The antibacterial activity of the nanoemulsion composites (ginger, cinnamon, and cardamom essential oils) against *S. aureus* and *E. coli* was higher than that of each investigated essential oil, either in its pure or in the nanoemulsion form. The nanoemulsion obtained the highest antibacterial activity with an oil phase of 10% ginger, 22% cardamom, and 68% cinnamon essential oils (Jafarizadeh-Malmiri et al., 2022).

The antibacterial efficacy of cardamom seed ethanolic extract against the food-poisoning bacterial strains *S. aureus* and *E. coli* was stronger than other extracts (water, ethyl acetate, and *n*-hexane). Furthermore, it had significantly ($p < 0.05$) greater antibacterial activity against *S. aureus* than the antibiotic (Norfloxacin). Therefore, this extract was recommended as an efficient food preservative, avoiding synthetic preservative side effects (Yassin et al., 2022). In addition, cardamom fruits and products can be used as a natural antioxidant and food preservative due to the high polyphenols content (Anwar et al., 2016).

The antioxidant activity of the Java cardamom (*Amomum compactum* Soland ex. Maton) essential oil samples obtained from different parts [leaves, stems, rhizomes, fruits (pods and seeds)] by hydrodistillation was evaluated using 2,2-diphenyl picrylhydrazyl (DPPH·) and ferric reducing antioxidant power (FRAP) assays. The essential oils of cardamom fruit (pods and seeds) showed the highest FRAP antioxidant activity. However, the antioxidant activities of the four parts of cardamom essential oil samples against DPPH· radicals were not significantly ($p > 0.05$) different (Arista et al., 2022).

18.2.2 Meat Products

The antioxidant and antimicrobial effects of freeze-dried extracts (obtained by aqueous ethanol, 50%) of allspice, bay leaf, black seed, caraway, cardamom, clove, or nutmeg in raw ground pork meat stored at 4 °C for 12 days were investigated. Each spice extract was added to raw ground pork meat at 0.5% (*w/w*). Aqueous ethanol extract of cardamom recorded the highest antioxidant activity (measured by DPPH· and FRAP assays) compared to other spices. The peroxide values (PV) of caraway, cardamom, nutmeg, and clove-treated samples after 12 days of storage were lower than 50% of the 5-day stored control sample (15.7 meq active O_2/kg meat). On the 12th day of storage, the thiobarbituric acid reactive substance (TBARS) values of cardamom and caraway pork samples were less than 0.4 mg malondialdehyde (MDA)/kg meat. The control sample exceeded this level after only 3 days of cold storage. On the other hand, the total viable counts of the raw ground pork meat treated with allspice, clove, black seed, and cardamom at the end of refrigerated storage (4 °C) were significantly ($p < 0.05$) lower than those of the control sample (Muzolf-Panek et al., 2019).

18.2.3 Dairy Products

The taste threshold (minimum detectable limit) of cardamom aroma in water, sugar solution (2.5%) and milk (diluted with an equal quantity of water) using cardamom oleoresin was found to be 4.04 ppb, 4.48 ppb and 13.97 ppb, respectively. The

difference in perception was attributed to the ability of proteins to bind odor molecules (Senthil & Bhat, 2011).

Sensory characteristics (aroma, texture and taste) of the probiotic yogurt containing oleoresins of cardamom at 0.5% (*v/w*) and strains of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. Panelists highly accepted *lacti*s. The presence of cardamom oleoresin did not affect the population of probiotic strains in yogurt during refrigerated storage (4 °C) for 28 days. However, adding cardamom oleoresin increased the yogurt's antioxidant activity (measured by DPPH· assay) compared to control yogurt at all storage periods (Illupapalayam et al., 2014). The sensorial analysis of paneer (Indian soft cheese) incorporated with cardamom powder at 0.2, 0.4, 0.6, 0.8, and 1.0% by weight of the expected yield of paneer indicated that the most acceptable level was 0.6%. The products were stored at 7 °C for 28 days. Cardamom incorporation at 0.6% resulted in a lower paneer standard plate count by 2 logs after 14 days of storage. The plate count of control and cardamom samples exceeded the maximum level of the Indian standard (BIS, 1983) on days 7 and 21, respectively. Meanwhile, the coliform count of cardamom paneer samples remained within standards for 28 days of storage instead of 7 days for the control sample. On the other hand, the yeast and mold count in paneer samples, whether they contained cardamom or not, were similar and met the standards on day 7 of the storage (Eresam et al., 2015).

Custard was prepared with different levels (0.1, 0.2, and 0.3%, *w/w*) of 1,8-cineole-rich cardamom seed (*Elettaria cardamomum*) supercritical fluid-CO_2 (SC-CO_2) extract. The optimal conditions for obtaining the SC-CO_2 extract with the highest 1,8-cineole content (22.6%) were 200 bar, 50 °C, and a 90 min extraction time. The DPPH· radical scavenging activity (IC_{50} value) of the custard sample containing 0.1% (*w/w*) cardamom extract was 129.4 (μg/mL) instead of 241.9 (μg/mL) in the control sample. The sensory analysis of the custard samples indicated that the highest odor, color, aftertaste, and overall acceptability scores were recorded for the custard sample prepared with 0.1% (*w/w*) SC-CO_2 cardamom extract. In addition, the sensory panel reported that this product had a pleasant aftertaste. On the other hand, the aftertaste of the samples prepared with higher levels of cardamom extract was not accepted (Ghosh et al., 2015).

Fortification of soft cheese with cardamom at 0.2% and 0.4% (*w/w*) increased its taste scores significantly. Meanwhile, increasing the cardamom level to 0.4% (*w/w*) caused a significant ($p < 0.05$) decrease in the color scores of the product (Salih et al., 2021). Incorporating cardamom powder into inoculated milk at 0.25, 0.50, 0.75 and 1% (*w/w*) during the preparation of Labneh (concentrated yogurt) kept mold and yeast free for 30 days at 5 °C, compared to 10 days in the control sample. The preparation of Labneh with cardamom increased its oxidative stability due to the bioactive compounds released in the product. The addition of cardamom to Labneh at a 1% level did not significantly ($p < 0.05$) affect its sensory properties and acceptability during storage for 30 days. Including cardamom powder at levels higher than 0.5% significantly ($p \lesssim 0.05$) increased the product's hardness in the fresh state and after storage (Tawfek & Ali, 2022).

18.2.4 Vegetable Oils

Adding cardamom essential oil and ethanol extract (oleoresin) to mustard oil at 0.02% (*v/v*) reduced its oxidation rate to different extents during accelerated oxidation at 60 °C for 28 days of storage, compared with the control. Changes in the PV assessed oxidation. The antioxidant activity of essential oil was higher than that of oleoresin (Singh et al., 2008). The addition of 2000 mg/kg of methanolic cardamom extract to sunflower oil did not improve its oxidative stability upon heating at 85 °C (Beddows et al., 2000). On the other hand, the addition of cardamom essential oil to refined soybean oil at 0.4% (*v/v*) improved its oxidative stability by 10% during storage at 20 °C for 6 months. Rancimat measured the oxidative stability of oil at 110 °C. This level of addition did not negatively affect the sensory attributes of soybean oil (Dolati et al., 2016).

The encapsulated powder of SC-CO_2 extract of a polyherbal mix (1:1:2 tulsi leaves, bay leaves and cardamom seeds) was more efficient than synthetic antioxidants (tertiary butyl hydroquinone (TBHQ), and butylated hydroxytoluene (BHT)) at maintaining the oxidative stability of soybean oil during storage at 23 °C for 30 days and after being used for frying potato wedges. Therefore, each polyherbal extract and synthetic antioxidant was used at 0.01% of the oil content of the potato wedges. Potato wedges fried in control, BHT, and TBHQ soybean oil samples stored for 30 days had an unpleasant flavor; however, wedges fried in stored soybean oil enriched with the examined extract were acceptable (Ghosh et al., 2016).

18.2.5 Beverages

Crushed green cardamom capsules represent an essential component of a popular tea beverage in India (Ashokkumar et al., 2020) and are now gaining popularity in the United States. A large portion of the world's cardamom production is used to prepare coffee. It is a traditional beverage (Gahwa) in Arab countries (Raghavan, 2007). The freshly crushed whole green cardamom pods are necessary for the classic method of preparing the distinctive Gahwa. The proportion of ground cardamom and coffee used varies from 90:10 to 50:50 (*w/w*), respectively (Govindarajan et al., 1982). The odor, bitterness, acidity, spicy taste, and overall acceptability of brewed coffee supplemented with cardamom at concentrations of 0.5, 1, 1.5, 2, 2.5, and 3% were evaluated. Except for odor and acceptability, adding 3% cardamom to coffee significantly ($p < 0.05$) increased its sensory attributes. The control coffee beverage received the greatest rating for bitterness; however, cardamom at a 0.5% level received the highest rating from consumers (Durak et al., 2017). Extraction of cardamom roasted coffee with decoction led to an increase in α-terpinyl acetate level, compared to that produced by infusion. α-Terpinyl acetate was found to be responsible for the aromas (sweet, spicy and distinctive herbaceous) of cardamom coffee

beverages. On the other hand, the plain roasted coffee beverages prepared by either of the extraction methods (decoction or infusion) were rich in octyl acetate. The presence of cardamom improved the coffee aroma by masking the smoky odor of aromatic hydrocarbons (4-vinyl guaiacol) induced by heating (Abdelwareth et al., 2021).

Cardamom-flavored milk chocolate is widely accepted by combining butter, sugar powder, milk powder, cocoa mass, and emulsifiers with encapsulated cardamom flavor (Ravindran & Madhusoodanan, 2002).

Adding cardamom essential oil (10 μL in 90 μL of ethanol) to 70 mL of sweet orange juice extended its shelf life during storage at 4 °C. The total microbial count of the juice containing cardamom essential oil after 28 days of cold storage was lower than that of the fresh juice stored for 21 days. The addition of cardamom essential oil kept the ascorbic acid and acidity of the juice after 28 days higher than those of the control sample after 7 and 14 days of cold storage, respectively (Kapoor et al., 2011).

Five herbal mixture drinks were formulated from cinnamon and cardamom powders (80:20%; 60:40%; 50:50%; 40:60%, and 20:80%). The herbal drinks were prepared by brewing 50 g of the mixture with 500 mL of water. Based on sensory analysis, the highest scores of color and taste were recorded for the formula prepared from 60% cardamon and 40% cinnamon, while the most preferred aroma was recorded for the formula containing 40% cardamon and 60% cinnamon (Mardiana et al., 2020).

Functional drinks (each 500 mL) were formulated with different amounts of sliced dried cardamom rhizome (5, 7.5, 10, 12.5 and 15 g). In addition, the formulas contained fixed levels of other spices (wooden cup, cinnamon, cloves, star anise, crushed ginger, lemongrass, Tropicana slim and lime leaves). The formulas were boiled with 500 mL of water till total soluble solids reached 30–35%. The panelists preferred the color, taste, flavor, spiciness and aftertaste of the filtered drink, where a portion of 10 grams of cardamom rhizome was used. Adding spices improved the drink's aftertaste (Winarsi et al., 2020).

18.2.6 Bakery Products

The threshold levels of cardamom powder and essential oil in cookies were 1% and 0.05%, respectively. Additionally, high overall acceptability was recorded for cookies containing cardamom powder or volatile oil at concentrations ranging from 1.5 to 2 times their threshold levels (Badei et al., 2002). The oxidative stability of cookies prepared with small cardamom SC-CO_2 extract at 0.3% (*w/w* of dough weight) was significantly ($p < 0.05$) higher than that of the cookies with 0.2% extract. A pungent taste has characterized the cookies prepared with 0.4% extract. Formulated cookies with a post-extraction residual matrix of less than 5% of the dough weight did not improve their oxidative stability.

Meanwhile, concentrations higher than this level caused the cookies to become waxy (oily mouthfeel). Cookies formulated with either 0.3% cardamom extract or a 5% residual extraction matrix were packed in aluminum foil, flushed with nitrogen, and stored at room temperature (23 °C) for 200 days. The PV of the control samples exceeded 10 (meq/kg cookie) after 80 days of storage. Cardamom extract cookies did not reach this level after 200 days of storage, instead of 120 days in the case of residual extraction matrix cookies. The 1,8-cineole content of the 200-day-old stored cardamom extract cookies was higher than that of the fresh residual extraction matrix cookies. This compound degraded to a not detectable level after 80 days of storage of post-extraction sample matrix cookies (Dutta et al., 2017).

18.2.7 Other Food Products

Cardamom oleoresin reflects the flavor quality more than the distilled essential oil. It contains 52–58% essential oil (Purseglove et al., 1981). Packing sucrose cubes flavored with encapsulated cardamom oleoresin, at 1.2 g/Kg, in three-layer metalized laminate packaging materials protected their 1,8-cineole and α-terpinyl acetate for a $t_{1/2}$ value of 5 months at 25 °C and relative humidity of 33%. The half-life value represented the time required for the degradation of the characteristic constituents to 50% of their initial value (Sardar & Singhal, 2013; Sardar et al., 2013).

The odor, oral sense, and general acceptance attributes of candies prepared with cardamom essential oil, either in free or nano form, were not significantly ($p > 0.05$) different from those of the control sample. However, cardamom essential oil candy samples had significantly ($p < 0.05$) higher acceptance than thymol samples. In addition, the inhibition effect of the candies prepared with cardamom essential oil nanoemulsion against salivary *Streptococcus mutans* was significantly ($p < 0.05$) higher than that of the control candy (Karimi et al., 2020).

Cardamom is used at a level that ranges from 2–5% to impart a specific flavor in curry powder formulations (Nair, 2020). Cardamom essential oil incorporated at a 2% level in a nanoemulsion edible coating inhibited the growth of foodborne pathogens and extended the shelf life of ripened fresh tomatoes. Both coated and uncoated tomatoes were stored for 15 days at 25 °C and 60% relative humidity. At the end of storage, the total mesophilic bacteria, *E. coli*, and *L. monocytogenes* in the coated tomatoes were significantly ($p < 0.05$) lower by 2 logs than in the uncoated samples. In addition, the increase in MDA levels in tomatoes was reduced by coating. The MDA level of the coated tomatoes did not exceed 50% of that of the uncoated samples after 15 days of storage. During storage, coating kept the catalase and superoxide dismutase activities significantly ($p < 0.05$) higher than those of the uncoated tomatoes (Das et al., 2022).

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Chapter 19
Encapsulation of Cardamom Extracts

Ebru Kuyumcu Savan

Abbreviations

BAC	Bioactive compound
CEO	Cardamom essential oil
CS	Chitosan
EE	Encapsulation efficiency
EO	Essential oil
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
LC	Loading capacity
MRSA	*S. aureus*
NP	Nanoparticle
SAS	Supercritical antisolvent
SEE	Solar energy-based extraction
SFE	Supercritical fluid extraction
SFE	Supercritical fluid extraction
TPP	Tripolyphosphate

Since ancient times, plants have been used in food, medicine, and cosmetics. Their rich flavor and anti-allergic, antioxidant, antibacterial, anti-inflammatory, and antiviral properties make them attractive for various medicinal purposes. Many of these properties could be attributed to the high content of bioactive compounds such as polyphenols, isothiocyanates, etc. In addition, plants are rich in protein,

E. K. Savan (✉)
Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, İnönü University, Malatya, Turkey
e-mail: ebru.savan@inonu.edu.tr

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antioxidants, vitamins, and mineral nutrients. Numerous studies have reported that the rich and multifunctional nutraceutical ingredients of beneficial plants improve health and are used to produce food additives/supplements (Vincekovic et al., 2017).

Most liquid food flavors are volatile and chemically unstable in the presence of air, light, humidity, and high temperatures. This is also the case for cardamom essential oil (CEO) (Lawrence, 1979; Beristain et al., 2001). Cardamom (*Elettaria cardamom* Maton) is a plant species native to Southeast Asia, belonging to the Zingiberaceae family (Nahr et al., 2021). Cardamom is a little-known condiment in American cuisine but one of the most important spices for Scandinavian, Arabian, and Indian food products (Lawrence, 1979; Beristain et al., 2001). Its dried fruit has a pleasant aroma; it is used as a flavoring agent in food products and medicinal preparations (Nahr et al., 2021). In the pharmaceutical industry, cardamom tinctures are used to add flavor and aroma to many patented drugs, while in the cosmetics industry, cardamom oil finds its place in many exotic perfumes (Lawrence, 1979; Beristain et al., 2001). Cardamom essential oil, obtained from dried fruits by different extraction techniques such as steam distillation or solvent extraction, is the most important component of this plant. Essential oils (EO) are flavored in the food industry. Cardamom essential oil is a colorless or pale yellow liquid with an aromatic, penetrating odor and strong aromatic taste. CEO's main compounds are 1,8-cineol, α terpineol, terpinyl acetate, d-limonene, sabinene, and borneol. Cardamom essential oil shows antimicrobial, antifungal, antioxidant, anti-inflammatory, antiseptic, carminative, and diuretic activities. Cardamom essential oil can be a functional food ingredient, representing higher health benefits than ordinary food products (Nahr et al., 2021). CEO quickly loses flavor during storage through hydrolysis, rearrangement, polymerization, and oxidative reactions of terpenic and lipid components sensitive to acid, light, oxygen, or heat. The appropriate encapsulation of the CEO can minimize these changes. Encapsulation keeps the sensitive bioactive ingredients in the capsule's center and protects it with a shell or matrix. Also, encapsulation delays degradation and allows controlled release of the f CEO (Nahr et al., 2021).

19.1 Extraction Techniques

Natural products have attracted the attention of many researchers in recent years as complementary and alternative antimicrobial agents. Essential oils are products extracted from natural plants with complex mixtures of biologically active substances and offer potential new template molecules and bioactive compounds (BAC). Essential oils contain volatile secondary metabolites with antibacterial, antifungal, anti-inflammatory, antioxidant, anticancer, and antiviral activities. The extraction efficiency of EOs depends on their chemical composition, genotypes, and environmental and agricultural conditions. Recent research has highlighted the antimicrobial potential of EOs, with few reports against multidrug-resistant bacteria. In addition, the use of EOs as food preservatives has also been described by many to

control food pathogens. Essential oils are included in many dosage forms to provide flavor and natural preservation in the pharmaceutical field (Jamil et al., 2016). Essential oils are a diverse group of natural aromatic compounds mostly isolated from non-woody plant materials by hydro-distillation, solvent-solvent extraction, and liquid CO_2 extraction. Terpenoids include aliphatic hydrocarbons (low molecular weight), acids, alcohols, aldehydes, and esters, especially monoterpenes and sesquiterpenes, and diterpenes. They are characterized by the principal components present in higher concentrations rather than trace amounts of components (Majeed et al., 2015).

A unique BAC content characterizes each particular family, genus, and species of plants. Regarding their chemical structure, BACs could be classified into various categories (for example, polyphenols, alkaloids, terpenoids, organosulfur compounds, etc.) with great structural diversity and different physical/chemical properties (for example, most of them are thermally unstable). Many recent studies have focused on finding the most suitable extraction method and process to obtain high-quality BAC to extract various plant materials. The most commonly used techniques involve traditional extractions heated with solvents and agitation, mainly because of their simplicity, low cost, and versatility. However, selecting the most suitable technique is based on selecting the right solvent, as one of the most important factors defining extraction efficiency (Gupta, 2012).

On the one hand, conventional extractions are helpful because they are relatively simple methods, but on the other hand, they can be quite slow with poor extraction efficiency, consume large amounts of organic solvents and cause thermal degradation of target compounds (Wang & Weller, 2006). For this reason, it is recommended to apply advanced methods to reduce extraction time and solvent consumption and increase the yield and quality of the extracts. For example, extractions with the aid of microwaves, ultrasound and high pressure, compared to conventional extractions, allow highly selective and efficient recovery of high-quality BAC extracts from different plants. In addition, extractions with ultra-high pressure (Xi, 2015), negative pressure cavitation, high-voltage electrical discharges, pulsed electric fields, and mechanical-chemical methods have resulted in very efficient extractions. These techniques can provide valuable plant extracts in an environmentally friendly manner compatible with the "green concepts" preferred today. Furthermore, these procedures are fast, convenient, economical, sustainable, and efficient and have great potential for industrial scaling (Vincekovic et al., 2017).

Different extraction techniques have been used to extract bioactive components from the cardamom plant, including traditional extraction methods (e.g., hydro distillation and Soxhlet apparatus) and advanced procedures. Supercritical fluid extraction (SFE) has also been used among the various extraction techniques, which has many advantages. Characterization and quantification of cardamom content vary depending on the extraction method, genotype, maturity level, growing area, and moisture content. While applying extraction techniques, the pretreatment applied to the sample, pulverizing the sample, extraction time and extraction solvent are essential parameters. The extraction solvent should be carefully selected according to the extraction methods. In combined SFE and enzyme-assisted extractions, if apolar

and polar effects are in question, it may be necessary to use solvents such as hexane and acetone together. The presence of cuticular wax and the thermal degradation of α-terpinyl acetate causes organic solvent pollution. Particle size is a critical factor in SFE for higher yields of bioactive compounds. The degree of the size reduction or pulverization is an essential factor significantly influencing the extraction efficiency, which reduces the surface area by influencing the release of desired components within tissues and cells (Abdullah et al., 2022).

After extraction processes, various chromatographic techniques are used to characterize and quantify bioactive components. Bioactive components, fatty acids, tocopherols, phytosterol fractions and lipids found in plant extracts and their parts can be identified by gas chromatography (GC), high-performance liquid chromatography, and column chromatography. However, the gas chromatography-mass spectrometry (GC-MS) technique is most frequently used to diagnose and determine bioactive compounds (Abdullah et al., 2022).

19.1.1 Traditional Extraction Methods

Extraction procedures, such as hydrodistillation, steam distillation, Soxhlet extraction, or conventional extraction techniques, have been used to isolate bioactive components from cardamom. Traditional extraction methods have broader applicability due to ease of application. However, it has disadvantages such as being time-consuming, requiring a large amount of solvent, and having low extraction efficiency. Also, it is not suitable for heat-sensitive phytochemicals as heat is applied during extraction (Abdullah et al., 2022).

Hydrodistillation or steam distillation is widely used due to its ease of use, broad applicability and use of water as a solvent. This method is based on the principle that the combined vapor pressure at boiling temperatures equals the atmospheric pressure. Typically, the Clevenger apparatus is used. Increasing the vapor density, the cold water in an external system travels in a narrow, cooled container. The condensed steam is collected in a container with less dense oil at the top. Soxhlet (solvent) extraction, a traditional extraction approach, is performed with a Soxhlet apparatus consisting of a balloon to which the sample will be added, a heating system and a reversible cooler. The most commonly used organic solvents are hexane, ethyl alcohol, methanol, petroleum ether, dichloromethane, acetone, chloroform, or mixtures (Abdullah et al., 2022).

19.1.2 Advanced Extraction Methods

Advanced extraction methods include extraction procedures such as enzyme-assisted, controlled pressure drop, microwave-assisted, pressurized liquid, solar energy-based, supercritical liquid and ultrasound-assisted extractions. Advanced

extraction techniques are mainly applied due to their advantages, such as applying several techniques simultaneously, short extraction time, low solvent consumption, high extraction efficiency, being physically stable, and low risk of degradation of bioactive components. In recent years, combinations of traditional procedures and advanced extraction techniques have been created to isolate bioactive components. For example, ultrasound-assisted extraction as a pretreatment can be combined with hydrodistillation in the next step (Abdullah et al., 2022).

Microwave-assisted extraction approach is also used for oil extraction from plants and plant parts. A rotating microwave diffuser is used to ensure the even distribution of microwaves, and temperature monitoring is done with a shielded thermocouple. Supercritical fluid extraction (SFE) is a method with advantages such as a low risk of thermal decomposition of samples and bioactive compounds, easy cleaning, and environmental friendliness due to not using toxic solvents. It is also a technique in which the extraction efficiency could be increased by controlling the operating conditions such as time, temperature and pressure to liquefy carbon dioxide as the supercritical fluid. Subcritical extraction is an advanced method in which propane is used as the extraction solvent to extract bioactive components. The pressurized liquid extraction method is used for lipid fraction with the help of response surface methodology. Compared to other techniques, such as the pressurized liquid extraction method, Soxhlet or hydrodistillation extractions, remarkably high yields are obtained. The extraction of bioactive components is also carried out with the combined technique of enzyme-assisted hydrodistillation. In this technique, the structure of the cell walls is first disrupted by enzyme pretreatment, and free bioactive molecules are released. Then, SFE is applied by optimizing parameters such as co-solvent, enzyme type, temperature, pressure and time. An instant controlled pressure drop technique has recently been applied to increase oil yield as a pretreatment prior to hydrodistillation. An environmentally friendly, clean and green solar energy-based extraction (SEE) technique has been used as a new alternative. Compared to conventional techniques, SEE solar radiation achieves high efficiency, and the extraction time is shortened (Abdullah et al., 2022).

As a result, many extraction techniques have been used to extract bioactive components from different parts of cardamom. Table 19.1 summarizes some of the techniques used for the extraction of cardamom. Factors such as the extraction technique, the solvents used, the time, the geography of the plant, and the moisture content affect the extraction yield. Although solvent or Soxhlet extractions are also used from traditional extraction techniques, the hydrodistillation technique is frequently used. Advanced extraction methods are the most used techniques because of their advantages, such as shortening the extraction time, reducing the solvent consumption and increasing the yield (Abdullah et al., 2022).

High value-added BACs from vegetable and plant sources, such as essential oils, antioxidants and volatile compounds, often exhibit outstanding properties ranging from nutritional and medicinal properties to antimicrobial and antioxidant activities and can produce functional foods (Vincekovic et al., 2017). Recently, interest in the biological activities of EOs has increased. However,

Table 19.1 Comparison of traditional and advanced extraction methods in cardamom extract yields

	Parameter			
Extraction technique	Extraction time (hour)	Solvent	Extraction yield (%)	References
Conventional extraction				
Steam distillation	8	Steam	3.5	Lawrence (1970)
Hydro distillation	3	Water	1.0	Savan and Küçükbay (2013)
Soxhlet extraction	24	Petroleum ether	7.3	Sontakke et al. (2018)
Advance extraction				
Microwave-assisted extraction	4	Water	3.3	Mande and Sekar (2021)
Simultaneous distillation extraction	1	Dichloromethane	3.68	Noleau et al. (1987)
Supercritical fluid extraction	1	CO_2	5.5	Abdullah et al. (2021)
Sub-critical	–	Propane	6.8–7.2	Hamdan et al. (2008)
Enzyme-assisted hydro distillation	2	Water	2.5	Chandran et al. (2012)
Pressurized liquid extraction	–	75% ethanol	11	Asadollahi-Baboli and Mani-Varnosfaderani (2014)
Ultrasound-assisted hydrodistillation	–	Water	6.9–7.4	Morsy (2015)
Controlled pressure-drop	–	Steam	4.4	Teresa-Martínez et al. (2022)
Solar energy-based extraction	0.45	–	4.4	Al-Hilphy et al. (2022)

despite the excellent antimicrobial activity of EOs against pathogenic microorganisms, EOs and BACs are low in water solubility, unstable to environmental factors such as heat, light, humidity, and oxygen, exhibit strong off-flavors/odors, and common processing and storage conditions. Therefore, their use is limited because they decompose easily (Majeed et al., 2015; Jamil et al., 2016; Vincekovic et al., 2017). As a result, nano-sized formulations emerged to improve water dispersion and protect EOs from degradation (Jamil et al., 2016). Encapsulation is a technology that enables the controlled and targeted release of BACs and delivery and protection in food systems (Vincekovic et al., 2017). Also, nano-encapsulation has improved the antibacterial activity of some antibiotics (Jamil et al., 2016). Therefore, attempts have been made to preserve EO and BACs by encapsulation in various colloidal systems such as microcapsules, nanospheres, nanoemulsions, liposomes, and molecular inclusion complexes. The encapsulation efficiency, particle size and physical stability of EOs encapsulated in colloidal systems depend on the type of technique and the type and concentration/ratio of emulsifier/wall material used (Majeed et al., 2015).

19.2 Encapsulation Techniques

Encapsulation is one of the most exciting areas designed to preserve active ingredients. Encapsulation is a high-quality product that protects the components extracted from plants or waste materials (antioxidant bioactive compounds, vitamins, enzymes, microbial cells, aromas, acid regulators etc.) against adverse environmental conditions, ensures their controlled release in the target regions and increases their stability, bioavailability and bio-efficiency. It is a technology specifically applied for the supply of value-added compounds. It is common practice to preserve or enhance the bioactivity of natural extracts (Vincekovic et al., 2017). Encapsulation of bioactive oils by various methods (for example, interconnected establishment or use with EO systems) has been achieved due to their low water solubility, strong organoleptic flavor and low stability (Chiriac et al., 2021). Encapsulation of EOs also prevents oxidative degradation and loss of volatile compounds from maintaining their biological activities (e.g., antibacterial, antiviral, antifungal, anticancer, antidiabetic, anti-inflammatory, antioxidant, antiprotozoal and insect repellent) and composition. In addition, there are studies on the potential of EO encapsulation in various synthetic or natural polymer systems to improve human health and well-being (Chiriac et al., 2021). In recent years, encapsulation has received significant attention from the food, pharmaceutical, nutraceutical and cosmetic industries due to its wide application in the design of functional products such as foods and/or food ingredients (Vincekovic et al., 2017).

Encapsulation is entrapping a core material (i.e., the active ingredient, filler, internal phase or charge phase) in another immiscible solid or liquid, producing capsules ranging from approximately 10 nm to 10 mm (Vincekovic et al., 2017). Encapsulation consists of two important things, core (bioactive) and wall material/emulsifier (protecting bioactive). The wall material is very important because the core's stability and release behavior depend on its physicochemical nature as well as on the type and parameters of the encapsulation technique (Majeed et al., 2015). Core materials include solid particles, liquid droplets or gas bubbles. Immiscible may also be defined as the carrier(s), wall material, shell, coating, membrane, outer phase, or matrix (Vincekovic et al., 2017). The wall material is very important because the core's stability and release behavior depend on its physicochemical nature as well as on the type and parameters of the encapsulation technique (Majeed et al., 2015). In general, the two main forms and structures of encapsulated systems could be illustrated in Fig. 19.1. In the core-shell type, the core material forms a continuous phase enclosed in a shell, while the matrix type has the core material uniformly dispersed in a homogeneous solid phase matrix. In addition to these basic morphologies, capsules can also be found in different morphologies (Vincekovic et al., 2017).

It is necessary to protect the oil resin against environmental factors such as oxygen, light, heat, oxygen, and humidity, which cause deterioration. The microencapsulation technique is the most common approach to overcome this problem (Krishnan et al., 2005; Chiriac et al., 2021). Encapsulation of EOs into polymer-based formulations, e.g., micelles, micro and nanocapsules, films, or hydrogels, is

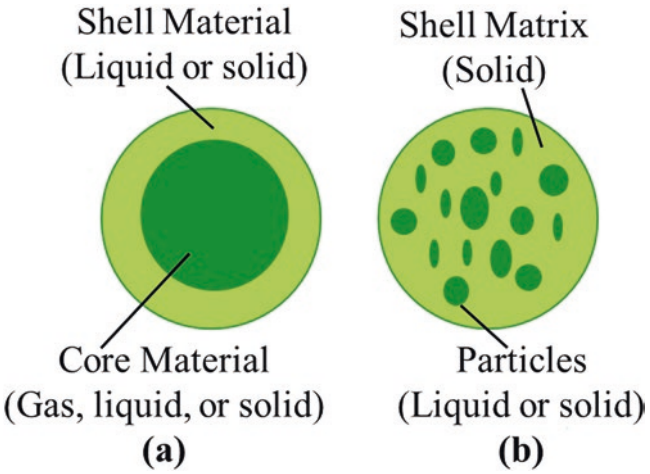

Fig. 19.1 (**a**) Capsule (core-shell) and (**b**) active ingredients encapsulated with the carrier material. (Drawn with permission from the study by Vincekovic et al., 2017)

done to provide reduced volatility, improved stability, and water solubility. In this way, the properties of these active ingredients are preserved without changing, and even their activity is increased, and, if necessary, control over the release of the encapsulated molecules can be achieved. Some structures used to encapsulate bioactive oils are given in Fig. 19.2 (Chiriac et al., 2021). Different encapsulation processes can be classified as mechanical, physical and chemical (Vincekovic et al., 2017).

In the studies, microcapsules, nanoparticles, nanostructured lipid carriers and nanoliposomes were used as carriers of cardamom bioactive components (Abdullah et al., 2022). In addition, many materials such as carbohydrates as hydrolyzed starches, emulsifying starches, gums (especially gum acacia), polysaccharides, gum-based emulsifiers, synthetic emulsifiers, proteins, lipids and gums have been used as encapsulating wall agents of CEOs and extracts (Beristain et al., 2001; Krishnan et al., 2005; Majeed et al., 2015; Abdullah et al., 2022). For example, among carbohydrates, β-cyclodextrin was used to prepare emulsions loaded with oregano essential oil, which were spray-dried to obtain stable microcapsules with sizes in the 1.07–38 mm range. Similarly, various researchers have used β-cyclodextrin to prepare an inclusion complex that protects hydrophobic EOs against oxidation and heat damage and enhances their antibacterial activity over a longer period. Similarly, Span 60, Tween 80 & Tween 20, Montanov 82, and Triton X-100 encapsulated EOs (Majeed et al., 2015). In addition, with increasing research, natural, productivity-enhancing and inexpensive alternative polymers such as gum Arabic and mesquite gum are being developed as encapsulating materials.

Beristain and Vernon-Carter (Beristain & Vernon-Carter, 1995) encapsulated orange peel oil using a mixture of gum Arabic and mesquite gum. In addition, there are studies in which encapsulation was carried out with a mixture of maltodextrin and mesquite gum (Beristain et al., 1999, 2001). The encapsulation is prepared by

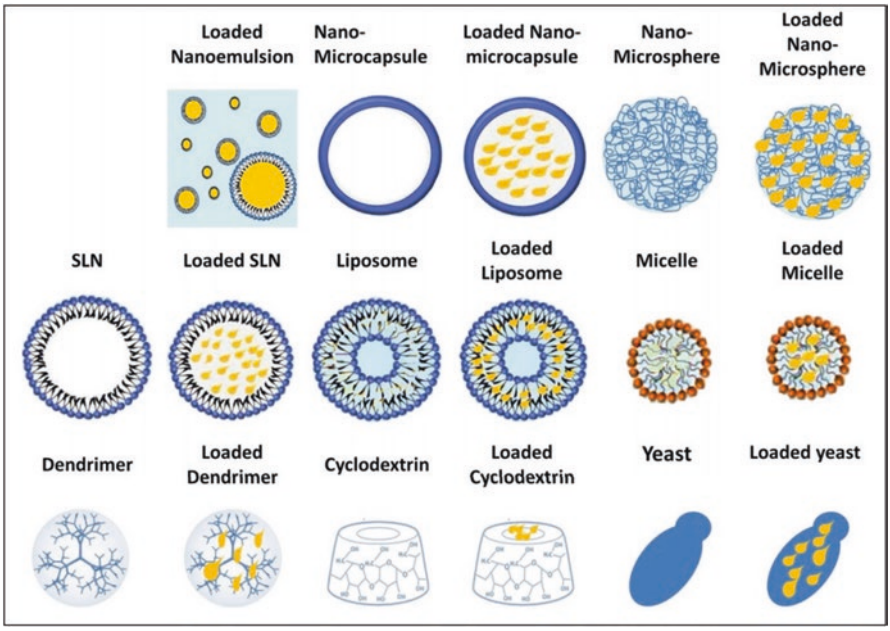

Fig. 19.2 Structures used in encapsulation of bioactive oils. (Permission obtained from Chiriac et al., 2021)

locking the extracts into the special matrix, which improves heat stability and shelf life. Gum Arabic is a very effective encapsulation agent due to its protective colloid function. It produces stable emulsions over a wide pH range with most oils. It also creates a visible film on the oil interface. Although gum Arabic is compatible with most gums, starches, carbohydrates, and protein, its use is limited due to its high cost and difficulty in supply. Therefore, maltodextrins and modified starches are also used as alternative carrier materials (Krishnan et al., 2005). Chitosan (CS) has also been reported to be used as a cost-effective carrier material for many pharmaceutical agents. Features such as biocompatibility, biodegradability, usability, cationic charge, high antimicrobial potential and safety have made chitosan an ideal carrier system. It was stated that the yet unreported EO potential of cardamom at the nanoscale to treat multidrug-resistant pathogens was discovered in the study investigating the microscopic, physicochemical and cytotoxic properties of the CEOs of functionalized designed chitosan nanoparticles. It was suggested that this potential could provide a bio-based alternative to overcome the current therapeutic challenges posed by multidrug-resistant pathogens (Jamil et al., 2016).

Different encapsulation and delivery strategies are used, such as microcapsules (increased stability and controlled release), nanoparticles (higher absorption and bioactivity), nanostructured lipid carriers (improved physicochemical stability), and nanoliposomes with their unique properties (higher solubility and bioavailability) as effective carriers of cardamom essential oils and extracts (summarized in Fig. 19.3) (Abdullah et al., 2022).

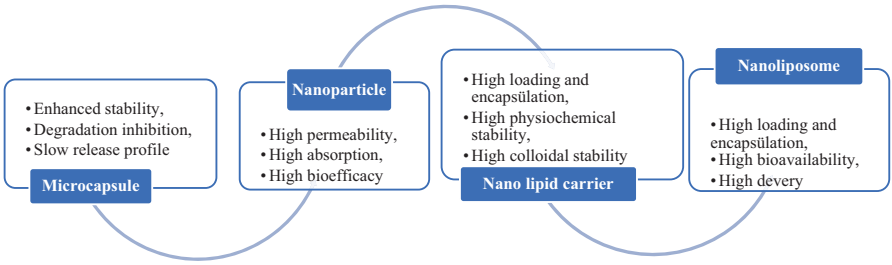

Fig. 19.3 Different encapsulation and delivery strategies

Microcapsules

A microcapsule is a hollow reservoir system consisting of a core and a wall (matrix shell) containing bioactive substances, with an average particle size ranging from 0.2 to 5000 μm, and having different shapes depending on the materials and methods of preparation (Krishnan et al., 2005; Abdullah et al., 2022). Microcapsule application as a delivery vehicle has outstanding advantages such as improved stability, inhibition of degradation, ease of transport, slow release characterization, targeted efficient delivery, and protection of bioactive molecules against the external environment with its hard shell. These properties make them attractive preservative and delivery vehicles for food, cosmetic, nutraceutical, and pharmaceutical applications (Abdullah et al., 2022). Studies in which the microencapsulation technique is used in the transport and protection of bioactive components in cardamom extracts and essential oils are included in the literature. Studies were carried out to obtain high microencapsulation efficiencies, well-controlled release, high antioxidant activity, highly stable extract, and volatile content in which this technique was applied.

In the study of Beristain et al., oil extraction was carried out from cardamom seeds by steam distillation technique. First, emulsions were prepared by homogenizing 300 g of mesquite gum/kg of deionized water with essential oil in different oil: gum ratios. Then, microcapsules were prepared from them by the spray-drying method. The microcapsules'moisture content, surface oil, total oil retention, bulk, and particle density were analyzed. The highest encapsulation strength of 83.6% was achieved for an oil: gum ratio of 1:4 with a powder surface oil concentration of 2590 mg/kg. It was suggested that mesquite gum had excellent emulsifying properties and good flavor encapsulation ability, which characterized it as an alternative encapsulation medium. This study stated that microcapsules could be easily used as food (Beristain et al., 2001).

Krishnan et al. conducted a study aimed at microencapsulation of oleoresins in cardamom by spray drying using binary and ternary mixtures of gum Arabic, maltodextrin and modified starch as wall materials. The content and stability of microencapsulated 1,8-cineole and α-terpinyl acetate volatiles were monitored and recorded for six weeks. Looking at the half-life times ($t_{1/2}$), they found that gum Arabic, maltodextrin and modified starch mixture provided better protection than gum Arabic as wall materials (Krishnan et al., 2005). Ghosh et al. obtained extracts of a

mixture of tulsi leaf, bay leaf and cardamom seeds using the supercritical carbon dioxide (SC-CO_2) technique. Microencapsulation of these extracts was carried out with maltodextrin and gum Arabic wall materials using spray drying technology. According to scanning electron microscopy results, it was stated that microcapsules with smooth, round morphology were obtained, and they had high antioxidant activity, phenolic content, reducing power and anti-inflammatory power. Encapsulated blend extract was evaluated for its possible use as a natural antioxidant in soybean oil. Storage and frying stability studies of soybean oil samples applied separately with encapsulated and unencapsulated extracts and commercial antioxidants were conducted. According to the results of this study, it was stated that the encapsulated extract had the best antioxidant activity. It was suggested that this encapsulation allowed for the controlled release of the antioxidant, thus making it a promising "green" antioxidant for soybean oil (Ghosh et al., 2016). In the study by Dutta & Bhattacharjee, 2017, cardamom seeds were subjected to α-amylase enzyme-assisted supercritical carbon dioxide extraction at 200 bar, 50 °C and 135 min. With this extraction technique, a 29.5% increased yield of 1,8-cineol was obtained in the extract. This extract was microencapsulated by spray drying, and its parameters were optimized according to the microencapsulation efficiency. In the optimized parameters, the inlet air temperature was 130 °C, and the wall material composition was maltodextrin: gum Arabic, 70:30. As a result of scanning electron microscopy analysis, it was suggested that the mean particle diameter of the capsule was 7.76 μm. In addition, the half-life of the encapsulate obtained with this encapsulation technique was 6.12 times higher than that of the extract. In addition, as a result of accelerated stability studies, it was suggested that the encapsulate had 7.88 times higher stability than the extract (Dutta & Bhattacharjee, 2017).

Nanoparticles

A nanoparticle has all sizes and structures in the nanoscale range (1–1000 nm). In recent years, applications of nanoparticles have emerged as efficient delivery vehicles due to their high surface/volume ratio, ultra-small size, easy penetration, absorption and bioactivity enhancement (Abdullah et al., 2022). In the study of Jamil et al., chitosan nanoparticles were prepared using the ionic gelation process. For this purpose, CS and tripolyphosphate (TPP) solutions were prepared. Upon addition of TPP aqueous solution to the chitosan solution, nanoparticles (NPs) spontaneously formed. To prepare cardamom oil-loaded CSNPs, 4 mL of cardamom oil was mixed with 4 mL of TPP-CS solution mixture. It has been suggested that zeta potential studies predict long-term stability as the zeta potential values obtained were greater than 50 mV in both empty and cardamom oil-loaded CSNPs. The potential cytotoxic effects of cardamom oil-loaded CSNPs were nvestigated through their potential to induce hemolysis and morphological changes in mammalian cells. Both null and CEO-loaded CSNPs did not show any hemolytic effects. In addition, it was stated that no necrotic damage or any change in cell morphology was observed in monolayer cell lines. According to the antimicrobial activity study results, it was found that cardamom oil alone could not inhibit both pathogens since cardamom oil was included below the minimum inhibitory concentration. Both

empty CSNPs and CEO-loaded CSNPs have been suggested to effectively control the growth of pathogens during the first 48 h. However, growth appeared on empty CSNPs after 48 h, while EO-loaded CSNPs were found to retain their antimicrobial potential for up to 7 days. The antimicrobial potential of CEOs encapsulated in CSNPs on multidrug-resistant *S. aureus* (MRSA), and *E. coli* was also determined. *S. aureus* has been confirmed to be MRSA due to resistance to cefoxitin disc. It was found that the rate of methicillin-resistant *S. aureus* isolates increased rapidly and was accepted as a nosocomial pathogen. Cardamom EO-loaded CS nanocapsules were highly effective in controlling multidrug-resistant *E. coli* and oxacillin (methicillin) resistant MRSA in vitro without showing any toxicity to human cells (Jamil et al., 2016).

Nanostructured Lipid Carriers

The nanostructured lipid carrier is a spherical nanoparticle with a lipid core consisting of a mixture of solid and liquid lipids surrounded by a lipophilic bilayer membrane, which exhibits the potential to combine lipophilic and hydrophilic bioactive. Nanostructured lipid carriers have many special advantages, such as high encapsulation efficiency, more controlled release for encapsulated components, high loading efficiency, good physicochemical stability (less mobility of bioactive material in the solid matrix), minimized excretion, and enhanced local deposition of bioactive compounds, higher solid lipid density, and high colloidal stability. The nanostructured lipid carriers are dissolved in the liquid lipid held in the bioactive, solid lipid in nanostructured lipid carriers, and an outer surfactant shell stabilizes the entire system. Compared to solid lipid nanoparticles, nanostructured lipid carriers' less ordered crystal structure provides higher bioactive loading, stable bioactive incorporation during storage and controlled release of bioactive material. Therefore, the rational design of the nanostructured lipid carrier greatly influences the physical and functional properties and the release behavior of the encapsulated compounds. These unique properties provide a high potential for industrial production. Nanostructured lipid carriers are advanced-generation delivery vehicles that can control release, prolong residence time, and increase permeability to maximize the health-promoting benefits of phytochemicals. Among these colloidal carriers, nanostructured lipid carriers derived from oil/water nanoemulsions have become the most promising delivery systems for bioactive compounds. Recently, nanostructured lipid carriers have been used to encapsulate functional food ingredients such as rutin, quercetin and hesperetin (Abdullah et al., 2022; Nahr et al., 2021).

In the study of Keivani Nahr et al., cocoa butter and olive oil were used as nanostructured lipid carriers in the encapsulation of CEO. The developed nanostructured lipid carrier particles were found to be round with a small size (<150 nm) with high retention efficiency (>90%). Furthermore, it was stated that there was no chemical interaction between nanostructured lipid carriers and cardamom essential oil components. According to in vitro release study, encapsulations had 40 to 55% release in 40 days. The results also showed that encapsulation of CEO with nanostructured lipid carriers could preserve antioxidant activity after 30 days, with a reduction of 5.7% and 12.32%, respectively, compared to CEO emulsion. As a result, it has been suggested that encapsulated essential oil can be used as a food supplement (Nahr

et al., 2021). The nanostructured lipid carriers' encapsulation efficiency (EE) and loading capacity (LC) of cardamom essential oil are determined by measuring free and free + encapsulated CEO. EE and LC are determined by Eqs. (19.1) and (19.2), respectively (Nahr et al., 2021).

$$\text{EE\%} \quad \frac{\text{Amount of encapsulated CEO}}{\text{Amount of encapsulated} \quad \text{free CEO}} \quad 100 \tag{19.1}$$

$$\text{LC\%} \quad \frac{\text{Amount of encapsulated CEO}}{\text{Amount of total lipids} \quad \text{CEO}} \quad 100 \tag{19.2}$$

Nanoliposomes

A liposome is a spherical structure consisting of interactions of phospholipids such as phosphatidylcholine with bioactive compounds of plant origin in a suitable solvent. When a liposome is dispersed in an aqueous solution, phospholipids immediately produce vesicles with a bilayer membrane that carries hydrophobic and hydrophilic bioactive compounds. Moreover, the bilayer membrane increases lipophilic compounds' water solubility and acts as a strong barrier against adverse environmental conditions (such as pH, oxygen and light). When the size of the liposome is in the nano-scale range (1–1000 nm), the solubility in water increases due to the increased surface area of the particles produced in the newly dispersed phase, the bioavailability increases due to the effective crossing of permeability barriers and subsequently improves distribution (Abdullah et al., 2022). Liposomes are usually prepared by mixing lipids in organic solvents and then by rotary evaporator, spray drying or lyophilization (Majeed et al., 2015).

Encapsulation is widely used to preserve bioactive compounds, target delivery and enhance biological functions. Encapsulation not only provides controlled release but also increases the bioavailability of bioactive compounds/drugs. The same trend increased antimicrobial activity when different EO components (peppermint oil, eugenol, carvacrol and thymol) were nano-encapsulated compared to unencapsulated EO. To achieve such advantages, EOs have been encapsulated using a variety of chemical, physicochemical and mechanical procedures. Molecular inclusion, coacervation and complex coacervation, spray drying, emulsification, ionic gelation and emulsion extrusion between these liposomes have been used by many researchers to encapsulate EOs (Majeed et al., 2015). With the emergence of several encapsulation processes over the years, encapsulation has been classified into mechanical (physical) and chemical methods (Reis et al., 2022).

19.2.1 Chemical Methods

Chemical methods involve obtaining microcapsules associated with precursor materials such as monomers or prepolymers by polymerization reactions or chemical interactions (Reis et al., 2022). Among chemical approaches, liposomes are

widely used to encapsulate EOs (Majeed et al., 2015). Chemical methods include coacervation, ionic gelation, liposome incorporation, and miniemulsion polymerization.

Coacervation is a physicochemical process involving separating one or more hydrocolloids from the solution, then depositing the newly formed coacervate phase around the active ingredient suspended in the same reaction medium (Majeed et al., 2015). Coacervation is widely used to encapsulate lipophilic materials. However, this technique also has a high potential for encapsulating hydrophilic substances. Numerous coating materials, such as gelatin, alginate, modified starch, gums and proteins, can be used. Coacervation is a colloidal phenomenon related to phase separation, in which a colloid-rich liquid phase is separated from a solution due to the reduction of solubility by chemical or physical means. The new phase appears as liquid droplets that eventually coalesce to form a continuous layer forming the capsule's wall. The wall hardens at the end of the process, and the capsules are isolated. Coacervation is recommended to encapsulate essential oils, aroma compounds, nutrients, vitamins, preservatives and enzymes (Reis et al., 2022).

The encapsulation technique by ionic gelation relies on the ionic cross-linking of a polymer in the presence of polyvalent cations. A polymeric or hydrocolloid solution with a hydrophobic active ingredient is dropped into an ionic solution under continuous agitation. It can be applied in ionic gelling, encapsulation of bioactive compounds or tissue control in foods, drugs, cosmetics, and probiotics. The primary coating materials used are alginate and chitosan. This technique has superior advantages for encapsulation as it uses non-toxic materials, is highly biocompatible, and has good mechanical resistance. In ionic gelation, positively charged polymers form a reversible intermolecular and intermolecular physical crosslinking by electrostatic interactions with certain polyanions. It also offers superior advantages in simplicity, convenience, low cost, mild processing conditions, the use of organic solvents, and eliminating the possibility of toxicity of chemical reagents and other undesirable toxic effects. However, the production of highly porous particles is the biggest disadvantage of this technique, as it intensifies the diffusion rate of the core material. This obstacle can be overcome by incorporating polyelectrolytes, such as proteins, into the gel structure, which can form a coating layer on the surface of the particles (Plati & Paraskevopoulou, 2022; Reis et al., 2022). Studies have been carried out on eugenol and carvacrol grafted chitosan nanoparticles prepared by Schiff base reaction and CH-NPs using the ionic gelation method (Majeed et al., 2015).

The *liposome incorporation* technique is applied to carriers for delivering pharmaceuticals, cosmetics and food ingredients and additives. Liposome microspheres have a spherical aqueous phase surrounded by one or more phospholipid bilayers in vesicles (Reis et al., 2022). Thanks to the amphiphilic nature of phospholipids, a lipid bilayer is formed where the hydrophilic "heads" face the aqueous phase, and the lipophilic chains are opposite each other, thereby protecting themselves from water. This structure enables liposomes to be used as carriers of EOs within bilayers (Plati & Paraskevopoulou, 2022). They are frequently preferred because they have a high natural potential to encapsulate essential oils, amino acids, vitamins, minerals, dyes, enzymes, microorganisms and fatty acids (Reis et al., 2022).

In the *miniemulsion polymerization* technique, monomer droplets are formed continuously by homogenization in high-power equipment. These droplets can act as nanoreactors, which are the site of the polymerization reaction, or as a monomer deposits to be transported to micelles resulting in the formation of polymeric particles (Reis et al., 2022). Nanoemulsions are oil-in-water systems with extremely small sizes (<200 nm) and superior properties to conventional emulsions in terms of optical clarity and kinetic stability. In addition, the high surface area provided by the nanometric size increases the bioactivity and can affect the antimicrobial activities by providing better diffusion of the active ingredients. More specifically, they may cause increased interaction with the cytoplasmic membrane, increased intracellular penetration and thus, antimicrobial activity. However, as with micro-emulsions, the rapid release of the encapsulated material is their major drawback due to its liquid nature. High-energy (or mechanical) methods such as high-pressure homogenization, micro-liquefaction, and ultrasonication are the most common ways to form nano-emulsions. Apart from these, low-energy (or non-mechanical) approaches (for example, the phase inversion temperature method) that result in droplet sizes smaller than 100 nm due to simpler equipment and lower energy costs have also been used (Plati & Paraskevopoulou, 2022). The miniemulsion polymerization encapsulation technique has been applied in many fields, including drugs and cosmetics, vegetable oils, aroma compounds and fragrances, and essential oils (Reis et al., 2022).

19.2.2 Mechanical Methods

Physical methods do not involve polymerization reactions since the materials used are already characterized as a type of polymer. Thus, only the formation of the microcapsule shape takes place mechanically. However, mechanical methods include extrusion, fluidization, lyophilization, solvent removal, spray dryer processes, and supercritical fluid technology (Reis et al., 2022). Mechanical methods for encapsulating EOs include spray drying, which is a low-cost, commercially available process that is often used. In spray drying, the core material is dispersed in the polymer solution and sprayed into a hot air chamber (Majeed et al., 2015). Liposomes are typically prepared by mixing lipids in organic solvents, that is, by chemical methods. In addition, after preparation by chemical techniques, they are dried by rotary evaporator, spray drying or lyophilization (Majeed et al., 2015).

Extrusion encapsulation is mainly used to encapsulate volatile, unstable and organic compounds such as essential oils. It is a technique used almost exclusively with glassy carbohydrate matrices. In addition to the food field, this technique is widely applied in the pharmaceutical and cosmetic industries. It is based on dispersing the core material in a melt. The mixture is then forced through a matrix towards a dehydrating liquid that hardens the coating (Reis et al., 2022).

The *fluidization* technique relies on the upward flow of a fluid through a bed of particles at sufficient speed to be suspended from the fluid flow without being expelled. In this process, the particles to be encapsulated are fluidized using hot air

in a coating chamber. Encapsulation by fluidization or fluidized bed is considered adequate for preserving other ingredients in enriched foods, especially for water-soluble solid particles, especially in low pH active compounds. Although it was originally a technique developed for the pharmaceutical industry, it has been frequently applied in functional additive compositions in the food industry (Reis et al., 2022).

Lyophilization is suitable for encapsulating sensitive compounds such as natural oils, flavor and aroma compounds, and pharmaceuticals. It is characterized as a multi-stage process consisting of freezing, sublimation (primary drying), desorption (secondary drying) and storage, yielding a dry product. In addition, lyophilization is a non-thermal process that preserves foods'nutritional and sensory properties and functional properties while preserving their natural color and flavor and preventing spoilage due to oxidation or chemical modification (Reis et al., 2022).

The *solvent evaporation* or *solvent removal* technique involves dissolving a compound and wall material in a suitable solvent. The solution is emulsified and heated to a temperature higher than the melting point of the encapsulating material. Low melting point lipids such as natural waxes are used as coating materials in most applications. This process is a suitable technique for encapsulating hydrophilic or hydrophobic particles associated with the continuous phase, where the molecules have no affinity, and is often used to encapsulate proteins and peptides. The microsphere formation process is affected by various parameters such as the nature and volume of the solvent, the type and concentration of the emulsifier, the rate of solvent removal, the ratio of the phase volume, and temperature (Reis et al., 2022).

Spray drying is one of the first methods often used to encapsulate compounds. It consists of preparing an aqueous emulsion of EOs with a biopolymer wall material, homogenization, atomization of the formed droplets in a heated air chamber, and dehydration of the particles. The result is small particles collected in a cyclone. Emulsion properties such as droplet size distribution and viscosity are crucial for the successful encapsulation of EOs. Therefore, selecting wall materials is particularly important, which must be characterized by high solubility in water, low viscosity at high concentrations, good emulsifying and film-forming activity, and effective drying behavior. Since it is almost impossible for a single coating material to cover all these properties, a mixture of two or more separate components is often used. Wall materials commonly used for spray drying are gums, maltodextrin, proteins from whey and soy, polysaccharides such as sodium caseinate, modified starch, gelatin and chitosan. The high temperature applied in this method is considered its main disadvantage.

Nevertheless, it is a relatively low-cost technique widely used in the food industry. The use of spray drying for powder food production has attracted the attention of industries due to its easy availability, reduced packaging, storage volume, reduced transportation costs, and increased stability of encapsulated compounds. It is also a short, flexible and continuous technique that can be applied to various food ingredients, including sweeteners, vitamins, minerals, carotenoids, oils and fats (Plati & Paraskevopoulou, 2022; Reis et al., 2022).

Supercritical antisolvent (SAS) and *solution-enhanced dispersion* with supercritical fluids are relatively new techniques. They are based on supercritical technology, the use of anti-solvent liquids and the supersaturation of compounds. The supercritical CO_2 is injected into a high-pressure column, followed by an emulsion containing the core compound, a shell material, and an organic solvent. The organic solvent is extracted from the emulsion with CO_2 with a small loss of core compound. Supercritical fluid extraction of emulsions is a variation of the SAS technique and combines traditional emulsion techniques with the unique properties of supercritical fluids to prepare micro and nanoparticles (Reis et al., 2022). Essential oil microspheres can also be prepared by applying supercritical solvent impregnation with supercritical carbon dioxide and two different starch types (sorghum and rice). This particular method is achieved by the high diffusion of CO_2 in starch, limiting the degradation of EO during storage and increasing its antioxidant activity. In general, supercritical solvent impregnation is considered an environmentally friendly process, where the most commonly used supercritical carbon dioxide is a "green" solvent. Due to the properties of CO_2, such as inert structure, low viscosity and surface tension, and higher mass transfer, the procedure is faster and more suitable for heat-sensitive compounds, and products are more homogeneous compared to cases where liquid solvents are included. The encapsulation process depends on the phase behavior of the respective polymers, supercritical fluid and active compound. However, the main disadvantages are the lack of a universal method suitable for all matrices and the need for experienced analysts (Plati & Paraskevopoulou, 2022).

Some applications of encapsulated cardamom are summarized in Table 19.2.

Encapsulation and Its Benefits

Encapsulation of essential oils for controlled release: Encapsulation represents a convenient and efficient approach to increase the physical stability of EOs, protect against evaporation, and provide a controlled release and enhanced bioactivity due to the narrow size range.

Encapsulation of essential oils for increased bioavailability: In addition to their controlled release properties, several researchers have also reported increased bioavailability of EOs after encapsulation in variable matrices (Majeed et al., 2015).

Encapsulation of essential oils for greater stability: Encapsulation provides controlled release and enhanced bioaccessibility of EOs and increases their stability as they are susceptible to transformation and degradation after exposure to environmental stresses (Majeed et al., 2015). Also, benefits after encapsulation, such as bioavailability, controlled release and protection of EOs against environmental stresses, are discussed. Encapsulated EOs are promising agents that can be used to increase the antimicrobial, antifungal, antiviral and pesticide activities of EOs in real food systems, to study their mechanism of action, and to provide non-lethal therapeutic agents to treat various diseases (Majeed et al., 2015).

Table 19.2 A summary of the encapsulation applications of CEOs

Technique used	Emulsifier type/wall material	Characteristic	Encapsulation efficiency (%)	Quantity	Particle size	References
Spray drying	EO and gum ratios	Microcapsules	83.6%	1: 4 w/w	–	Beristain et al. (2001)
Spray drying	Skim milk powder and HI CAP 100	Spherical	75–86	5% wt/w	13.97–21.87 mm	Najafi et al. (2011)
Spray drying	Maltodextrin and gum arabic	Microcapsules	71% (eugenol) and 56% (1,8-cineol)	60:40	3–25 µm	Ghosh et al. (2016)
Ionic gelation	Chitosan nano-particles and tripolyphosphate solution	Nanoparticules	>90%	4% v/v	50–100 nm	Jamil et al. (2016)
Emulsification	Sodium alginate and calcium chloride	–	–	3% and 2.5% (w/v)	–	Ganapathi et al. (1994)
Miniemulsion polymerization	Protein isolate, guar gum and carrageen	Microcapsules	98.5%	0.2–0.5% (w/v)	d43; $r = -0.2$	Mehyar et al. (2014)
Emulsification/ internal gelation	Alginate and whey protein concentrate	Microcapsules	83.67%	2% and 8% (w/v)	15–110 µm	Zandi et al. (2017)
Low energy nano-emulsification	Cocoa butter and tween 80	Nanostructured lipid carriers	>90%	(330 mg and 500 mg) / 25 mL water	<150 nm	Nahr et al. (2018)
Nanoemulsion polymerization	Chia seed mucilage	Nanofibers	41%	16 (mg/mL)	200–300 nm	Dehghani et al. (2020)
Co-crystallization	Gum acacia	Cubic crystal	35.23% (1,8-cineole) and 67.18% (α-terpinyl acetate)	2.5%	–	Sardar and Singhal (2013)

19.3 Conclusions and Future Perspectives

Phytochemicals such as alkaloids, carotenoids, organosulfur compounds, phenolics and phytosterols are health-promoting bioactive components that help prevent and alleviate physiological disorders and microbial infections. Researchers used traditional extraction techniques such as hydro distillation, steam distillation and Soxhlet extraction, and advanced extraction techniques such as enzyme-assisted, instantaneous controlled pressure drop, microwave-assisted, pressurized liquid-, solar-based, subcritical, supercritical liquid, and ultrasound-assisted extractions to extract the bioactive components of cardamom. Studies with advanced extraction methods have yielded promising results for future strategies for extracting bioactive components from plant material. In addition, the quality of the bioactive components of cardamom extracted using more environmentally friendly advanced techniques was superior to traditional methods. Different identification techniques such as GC, and GC-MS revealed that 1,8-cineol and α-terpinyl acetate is the major bioactive components in black and green cardamom.

Regarding the therapeutic potential of bioactive cardamom components, studies have suggested that they effectively alleviate foodborne pathogens, oxidative stress, and cardiovascular and gastrointestinal diseases. Encapsulation and delivery of essential oils and plant extracts with bioactive components via microcapsules, nanoparticles, nanostructured lipid carriers and nanoliposomes have been used as effective strategies with promising advantages, including increased stability, permeability, controlled release, bioavailability and bioactivity of embedded bioactive substances (Abdullah et al., 2022). Encapsulation has been used as a practical approach to protecting essential oils and plant extracts from light, air and moisture, interactions that lead to oxidation or evaporation and reduction of biological activities. In addition, with the encapsulation method, the solubility of oils and extracts was increased, controlled release was ensured, and they became more bioavailable. The most versatile and commercially available spray drying and emulsification techniques have been widely used to encapsulate cardamom essential oils and extracts. Encapsulated cardamom essential oils and extracts have enhanced antimicrobial, antifungal, antioxidant, antiviral and pesticidal activities (Majeed et al., 2015).

Including encapsulated cardamom, essential oils and extracts in food can be promoted due to its polyphenol profile, which can help alleviate oxidative stress and various lifestyle-related disorders and improve the antimicrobial status, shelf life, and quality of products. The literature review has shown that the bioactive components of cardamom have biotherapeutic potential. Therefore, some important points need to be taken into account. For example, issues such as the optimum dose of cardamom-derived bioactive compounds required to derive health benefits, physical form for supplementation to ensure maximum bioavailability at target sites, and potential health risks associated with any bioactive ingredient may need to be thoroughly investigated. Using encapsulated cardamom essential oils and extracts in food, cosmetics and pharmaceuticals can provide an economic benefit and address

consumer safety concerns. Products can be developed to promote the use of encapsulated cardamom essential oils and extracts in cosmetics and pharmaceuticals.

Further, research is needed to support recent analytical approaches to gain a deeper understanding of oxidation, isomerization, and thermal rearrangement processes and avoidance strategies. In addition, the identification of products produced from these processes seems to be a worthwhile future goal. Furthermore, encapsulated cardamom essential oils and extracts can be used in real food systems to increase their bioactivities, study their mechanism of action on cell membranes, and provide non-lethal therapeutics to treat various diseases.

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Chapter 20
Cardamom Oleoresin

Neelesh Kumar Nema, Baby Kumaranthara Chacko, Jerin Joseph, and Viju Jacob

20.1 Introduction

Elettaria cardamomum (L.) Maton, small or green cardamom, also known as the "Queen of Spices," which belongs to the ginger family (Zingiberaceae), is one of the world's rarest and most expensive spices due to its warm nature, slightly pungent properties, pleasant aroma, camphoraceous flavor, and magnificent applicability (Govindarajan et al., 1982; Nair, 2020). Seeds of the fruit (capsule) are the third priciest spice after saffron and vanilla, which is used in both sweet and savory foods. Seeds are produced from tropical perennial herb plants with strong root stalks that may reach 6 to 10 feet. It grows naturally between 700 and 7000 feet above sea level in the Indian Cardamom Hills (ICH) area located in Southern Western Ghats (Ashokkumar et al., 2020; Murugan et al., 2022) in India.

The highly referenced publications for the term "*Elettaria cardamomum* oleoresins"were extracted from multiple standard electronic databases (Google Scholar, Scopus, Web of Science, and PubMed) published, analyzed and presented to understand various factors relevant to oleoresins.

N. K. Nema (✉)
Nutraceutical, Synthite Industries Pvt. Ltd., Synthite Valley, Kadayirup, Kerala, India
e-mail: neeleshk@synthite.com

B. K. Chacko · J. Joseph · V. Jacob
CVJ Creative Centre, Synthite Industries Pvt. Ltd., Synthite Valley, Kadayirup, Kerala, India
e-mail: babykc@synthite.com; jerin@synthite.com; viju@synthite.com

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20.1.1 Cardamom – An Important Spice

The first harvest of cardamom from a plant needs 3 years to mature. Before yields decline, their commercial production lasts for 4–6 years. Large, perennial herbaceous plants having two different aerial growths-leaf and flower shoots-and a creeping rootstock. The multi-flowered spirals that make up the blossoms are on long-stalked panicles. Flowers are 30 to 35 mm long, are white or light green, and have blue or violet striations around the central lip. Along with the panicle, the pods contain tiny brown or black seeds (Jadav & Mehta, 2018). The plant, flower, flashy capsule, pod and seeds are depicted in Fig. 20.1.

20.1.2 Traditional Uses

Because of its aromatic characteristics, Cardamom is a well-liked alternative used for a long time to freshen breath. It is used in teas, desserts, baked products, and gourmet items because of its pleasant flavor. Cardamom is one of the main components of "*garam masala*" (*garam* sense "hot" while *masala* sense "spices"; a blend

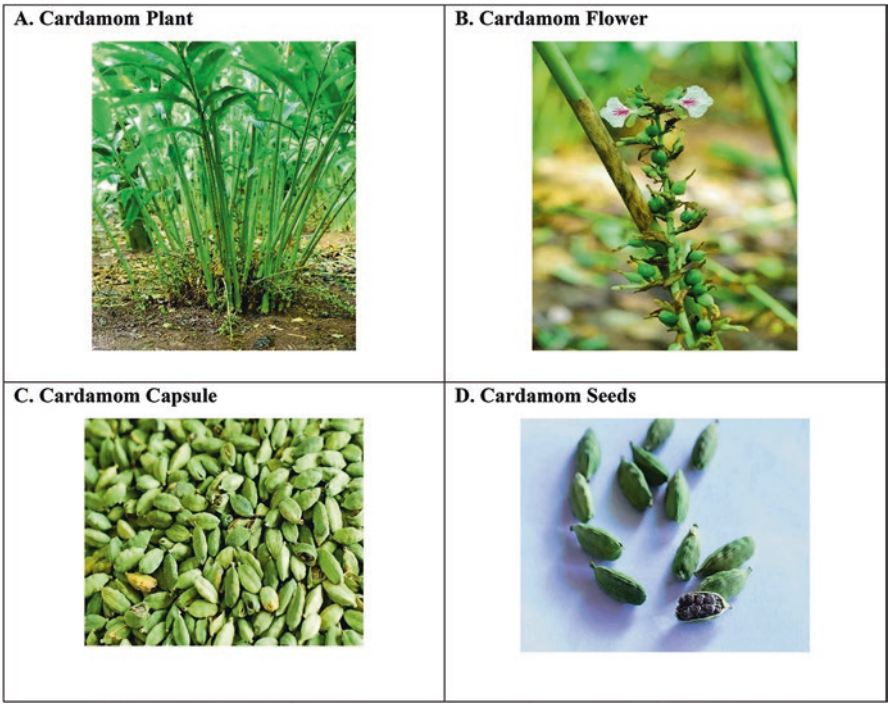


Fig. 20.1 Small/green cardamom

of diverse spices for warm flavor and aroma) in Indian cuisine (Korikanthimathm et al., 2001). Due to its carminative and anti-emetic properties, Cardamom is also eaten internally for health and well-being to alleviate indigestion, nausea, and vomiting (Peter, 2006). Additionally, it is used as a laxative to relieve gas and bloating. It works wonders to get rid of coughs and colds. It is used to treat a wide range of ailments in some indigenous communities, including bronchitis, depression, dysentery, influenza, urinary tract infections, and anorexia. Additionally, it aids in immune system support (Czarra, 2009; Parthasarathy et al., 2008).

20.1.3 Cardamom Oil and Oleoresin Market: Domestic and Global Level

The most recent information from the UN Comrade database shows that Guatemala is the world's top exporter of cardamom, with a trade value of $659,139,679. Indonesia comes second ($274,912,095), while India is third ($236,220,162). A total of 134,961,159 and 47,196,152 USD were also exchanged in commerce between the United Arab Emirates and Nepal, respectively. The primary markets for cardamom include the Middle East, South Asia, South East Asia, and Europe. Saudi Arabia is the leading importer of cardamom, valued at $223,269,995, followed by China and the United Arab Emirates, with USD values of 157,871,312 and 147,404,669, respectively (Comtrade, n.d.). India produces a considerable amount of cardamom that is used locally. On 69,190 Hectares of land, India produced 9,895,279 kg of cardamom in 2021; this amount climbed to 14,958,285 kg in 2022. The three primary states that produce cardamom in India are Kerala (70%), Karnataka (20%), and Tamil Nadu (10%). Cardamom is traded in a graded and bulk form. Cardamom is sorted using sieves and commands prices based on size, color, and freshness. The grade with the elegant green color 7 mm and above is more expensive than others. Malabar and Mysore are the two most important cultivars traded internationally. The third intermediate, Vazhukka, mostly grown in Kerala, India, is also recognized as a worldwide cardamom product (Alagupalamuthirsolai et al., 2020; Comtrade, n.d.; Parthasarathy et al., 2008; Spices Board, n.d.).

20.2 Essential Oil

Essential oils are volatile fragrant, highly concentrated substances collected from oil 'sacs' of flowers, leaves, stems, roots, seeds, bark, resin, or fruit rinds. They are frequently called plants' "vital strength or life force." These complex precious liquids are readily evaporated essences that give plants a pleasing aroma. Unlike fatty oils, they are present in lower concentrations ranging from 0.01 to 10%. Tons of plant matter only provide a few hundred pounds of oil. These oils include a diverse

range of therapeutic components as well as powerful antibacterial and analgesic capabilities. These oils are often used in various products, including meals, pharmaceuticals, and cosmetics, for their flavor and medicinal or odoriferous benefits. Olfactory receptors recognize the chemical characteristics of floating essential oil particles via chemoreceptors, aiding in fragrance detection and sensory enhancement (Nema et al., 2022).

20.2.1 Essential Oils and Their Phytoconstituents

The chemical composition of cardamom oil differs considerably with the product's variety, region, and age (Parthasarathy et al., 2008). According to AOAC's analysis of cardamom capsules from 2005, the approximate composition was as follows: carbohydrate 68–69%, protein 10–11%, fat 2–3%, and ash 5–6%. In addition, essential mineral components, i.e., calcium (approximately 92–93 mg), phosphorus (183 mg), sodium (17 mg), potassium (124 mg), and iron (12–13 mg) for typical human physiological functions daily were also identified in 100 g of capsule seeds (Nikam et al., 2009; Sarac, 2021; Sontakke et al., 2018). Furthermore, cardamom capsules also included flavonoids (catechin, myricetin, quercetin and kaempferol) and carotenoids (lutein and β-carotene) compounds (Alanazi et al., 2022). The volatile oil content in the seeds strongly depends on storage conditions, with an average yield of 2 to 5%. The volatile oil contains triterpenoids of about 1.5% α-pinene, 2.8% sabinene, 1.6% myrcene, 11.6% limonene, 36.3% 1,8-cineole, 3% linalool, 2.5% linalyl acetate, 2.6% α-terpineol, 31.3% α-terpinyl acetate, and 2.7% *trans*-nerolidol. The others are either equal to or less than 1.5%. According to one research, six primary oxygenated molecules, namely 1,8-cineole, α-terpinyl acetate, linalool, linalyl acetate, α-terpineol, and terpin-4-ol, account for over 90% of the aromatic compounds in essential oils (Peter, 2006).

A unique mix of the main components, 1, 8-cineole, and the esters, α-terpinyl and linalyl acetate, produces the basic cardamom aroma (Korikanthimathm et al., 2001). The distillation procedure from diverse seed sources used the solvent dichloromethane, yielding 3.68% cardamom oil. The GC-MS research discovered 100 to 122 distinct chemical components (Noleau et al., 1987).

A typical chromatogram of GC spectra of cardamom seed's essential oil is presented in Fig. 20.2.

The oil was conquered by oxygenated molecules and contained little mono- or sesquiterpenes hydrocarbons (Alanazi et al., 2022). Major triterpenes available in the essential oil are highlighted in Table 20.1.

Researchers from Madikeri, Karnataka's Indian Institute of Spices Research analyzed six genotypes of green cardamoms (*Elettaria cardamomum* (L.) Maton) from the Malabar, Mysore, and Vazhukka ecotypes between 2018 and 2019. The genotypes employed were Appangala-1, PV-1, ICRI-2, FGB-34, PV-2, and Green Gold. They assert that the genotype Green Gold showed higher levels of the molecules that give them their sweet, pleasant aroma, including 1, 8-cineole (40.8%), and

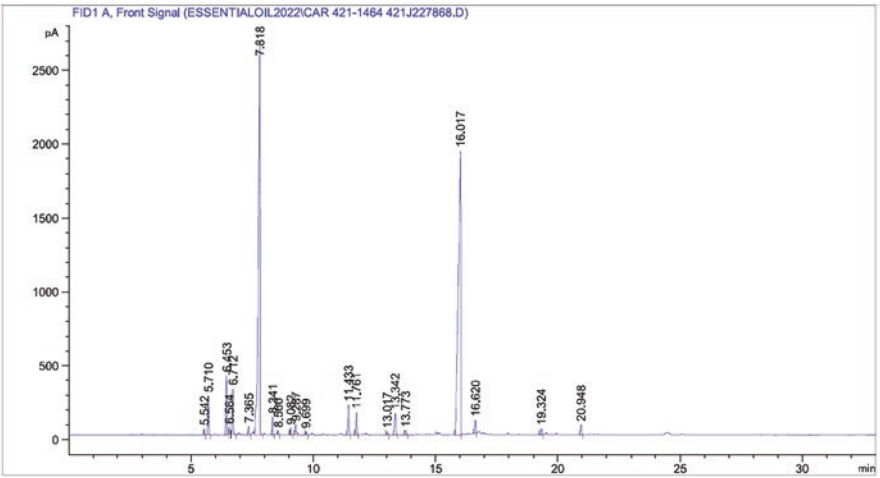


Fig. 20.2 Chromatogram of GC spectra of cardamom seed's essential oil

α-terpinyl acetate (35%), than other genotypes. Green Gold is a physiologically superior and high-producing genotype of cardamom from the Vazhukka ecosystem. The study's findings indicated that another genotype PV-2 is a higher quality genotype due to its superiority in pleasant sweet aroma due to the presence of α-terpinyl acetate (37.9) and linalool (11.1%) (Alagupalamuthirsolai et al., 2020). Again another study, oxygenated monoterpene molecules were found to be the most abundant in 22 potential cardamom accessions, followed by monoterpene hydrocarbons and sesquiterpenes (Ashokkumar et al., 2021).

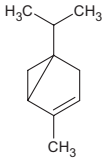
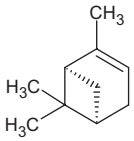
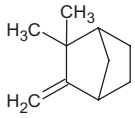
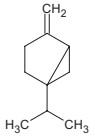
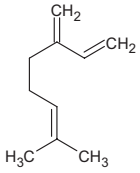
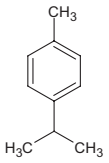
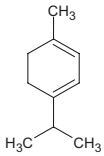
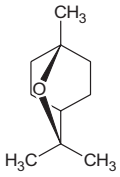
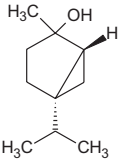
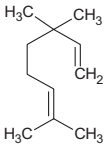
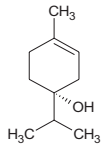
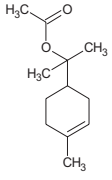
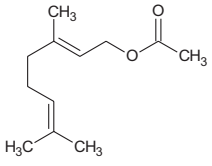
An aqueous extract found 11 major compounds, in that α-terpinyl acetate (54.4%) was maximum by GC-MS/MS-based phytochemical analysis possessed anti-inflammatory effects (Cárdenas Garza et al., 2021).

Through the hydro-distillation procedure, cardamom seeds yield 3.74% (v/w) essential oil. The GC/MS analysis found 19 chemical ingredients, accounting for 98.2%. In addition, Monoterpene components such as 1, 8-cineole (34.3%), α-terpinyl acetate (23.3%), and α-pinene (17.7%) were detected in high concentrations (Alanazi et al., 2022).

20.2.2 Extraction and Processing

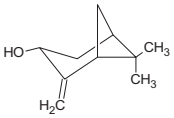
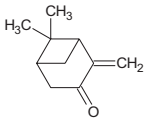
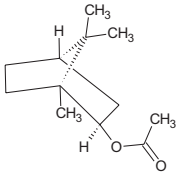
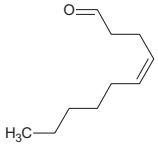
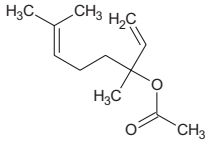
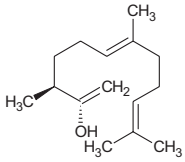
The essential oil and other bioactive metabolites accumulated in cardamom capsules contribute to their characteristic aroma and are utilized in functional food, beverages, pharmaceutical, nutraceutical, perfumery, and cosmetic products. Essential oils could be extracted using various methods, i.e., maceration, cold pressing, solvent extraction, enfleurage, hydro distillation, super critical CO_2 extraction, turbo distillation extraction, steam distillation, and enzyme assisted extraction, etc.

Table 20.1 Triterpenes present in the essential oil of Cardamom

Composition	Structure	Structure
Monoterpenes hydrocarbons	 α-Thujene	 α-Pinene
	 Camphene	 Sabinene
	 Myrcene	 β-Cymene
	 γ-Terpinene	
Monoterpenes oxygenated	 1,8-cineole/eucalyptol	 *Trans*-sabinene hydrate
	 Linalool	 Terpinen-4-ol
	 α-Terpinyl acetate	 Geranyl acetate

(continued)

Table 20.1 (continued)

Composition	Structure	Structure
Bicyclic monoterpenoids	 *trans*-pinocarveol	 Pinocarvone
	 Bornyl acetate	
Non-terpenes	 *cis*-4-decenal	
Acyclic monoterpenoids	 Linalyl acetate	
Oxygenated Sesquiterpenes	 (E)-Nerolidol	

(Fig. 20.5). Cardamom seeds are hydro-distilled to produce a pale yellow essential oil. Since enzyme pre-treatment helps to disturb the fundamental structure of cell walls for better liberation of free bioactive chemicals, advanced methods like enzyme-assisted hydro-distillation (EAHD) are now widely used for oil and oleoresin extractions (Chandran et al., 2012). Cardamom volatile oil extraction yield and quality could be improved by pre-treating the oil with enzymes such as Celluclast 1.5 L, Pectinex Ultra SP.L, Viscozyme L, and Protease. The enzyme pre-treatment raised the yield from the control sample's 6.73% to 7.23–7.83%. In the study enzymes, ViscozymeL was the most efficient enzyme for the pretreatment process, followed by Pectinex, Celluclast, and Protease (Baby & Ranganathan, 2016). The effects of traditional hydro-distillation (HD) and solvent-free microwave

extraction on the cardamom essential oil (*Elletaria cardamomum* L.) were also studied (SFME). Scanned electronic microscopy was used to investigate cardamom seeds. A statistical study shows that the oxygenated component, which is more valuable and mainly consists of aromatic compounds with potent fragrances, makes up most of the essential oils supplied by SFME. Scanning electron microscopy of the cardamom seeds after SFME treatment reveals the increased breakdown of flavor cells compared to the normal hydro-distillation process (Lucchesi et al., 2007).

Additionally, the recently introduced extraction technique, pressurized liquid extraction (PLE), was examined along with conventional methods, i.e., hydrodistillation and Soxhlet extraction procedures for essential oil composition. The quantity of desired oxygenated components like 1,8-cineole and α-terpinyl acetate were higher in PLE than in the hydrodistillation and Soxhlet methods under ideal circumstances, and it was found that PLE had the greatest extraction yield (Eikani et al., 2013). There are currently some new options for extraction to increase yield, i.e., instantly controlled pressure drop (DIC) or immediate, controlled pressure drop (ICPD). By these methods, the yield of green cardamom may increase up to 4%. In addition to the methods mentioned above, green cardamom essential oil can also be extracted using the solar energy-based methods known as solar energy extraction (SEE) technology which is 23–43% greener than the conventional method. One study found that optimal solar radiation of 1000 W/m^2 and a (slightly longer extraction time of 0.45 h than with traditional hydro-distillation) can increase the extraction yield of cardamom seeds. This method is also sustainable and reduces energy consumption (Al-Hilphy et al., 2022). Another study examined the extraction yield using various solvent systems by relating the simple and steam distillation methods. N-hexane solvent was found that n-hexane solvent enhanced the essential oil yield (6.3%) compared to ethyl acetate in a simple distillation process while processing for 6 h (Raissa et al., 2020).

20.3 Oleoresin

There are two parts to oleoresin: volatile oil and resin part. Figure 20.3 shows the visible distinction between volatile oil and oleoresin. The oleoresins are highly concentrated substances that contain all of the volatile (aromatic essential oil- 52-58% volatile oil) and non-volatile components (including color, fat, spicy ingredients, and waxes) of the spices that mostly mimic the flavor and aroma of the whole fresh spice.

The full flavor impact of spice is not exposed until the oil and resin are combined. The great strength of the active ingredients in oleoresins makes it possible to use them in modest amounts. Steam or hydro-distillation produces volatile oil, while solvent extraction is used to obtain resin. Oleoresins have significant local and foreign markets. They are used in various products, including beverages, food preparations, soup powders, curry powders, confectioneries, noodles, canned meats, chicken products, and sauces. Except for applications where dried spice powder is

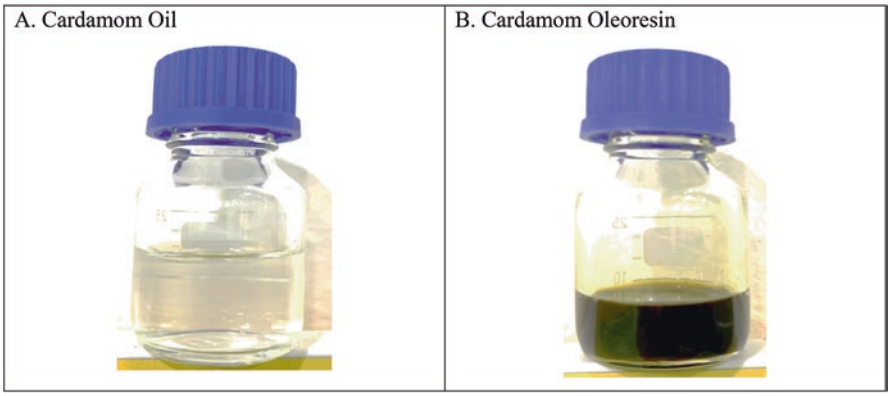


Fig. 20.3 Cardamom oil and cardamom oleoresin

necessary, oleoresins are given priority, and they are expected to eventually replace ground spices without compromising flavor. Oleoresin cardamoms are favored due to their microbiological benefits, similar properties to spices, identical taste and pungency, and ease of storage and transportation. Oleoresins may also survive heat better than raw spices and have a longer shelf life because of their lower moisture content. End-user industries are continuously growing and will endure doing so as long as customers desire high-quality products.

So far as product quality is concerned, oleoresin will undoubtedly have an emerging market in the upcoming days. In the upcoming years, it is projected that oleoresins will take the role of pulverized spices without compromising flavor (Govindarajan et al., 1982; Nair, 2020; Ravindran & Madhusoodanan, 2003). Cardamom oil and oleoresin can be combined in a specific way depending on how the final product will be used. Flavor and taste can be used to control sensory attributes.

20.3.1 Oleoresin and Its Phytoconstituents

Cardamom oleoresins include 60% essential volatile oils (isoprenoids and benzenoids) and 40% non-volatiles (fixed oils, alkaloids, carotenoids, and anthocyanins). (Boelens et al., 2000). The majority of the lipid components of cardamom were identified by Gopalakrishnan et al. in 1990. They concluded that the waxes, or n-alkanes (C21, C23, C25, C27, C29, C31, and C33) and n-alkenes (C21, C23, C25, C27, C29, C31 and C33) and sterols, contributes much. When the fixed oil was analyzed, it was shown to include 2.2–15.3% linoleic and 5.8% linolenic acid, caproic 5.3, and stearic 3.2. It also contained 42.5–44.2% oleic acid and 28.4–38.0% palmitic acid. Less than 2% of myristic, arachidic, hexadecanoic, pentadecanoic, and lauric was also determined in the fixed oil (Gopalakrishnan et al., 1990; Ravindran & Madhusoodanan, 2003). According to one research, cardamom resin

contains significant fatty acids such as palmitic (C16:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3), which account for 64% of the total fatty acids. Palmitic acid (C16:0) was the most prevalent fatty acid found, ranging from 10 to 30%, followed by oleic acid (C18:1), which varied from 20 to 25%, along with linoleic acid (C18:2), 5 to 15% and linolenic acid (C18:3), which ranged from 10 to 15% (Arpitha et al., 2019).

20.3.2 Extraction and Processing

Cardamom oleoresin has a much-specialized market for consumption. Even while cardamom oil accounts for practically the whole flavor and aroma of the capsules, the "richness" that is linked to the presence of nonvolatile substances, on the other hand, contributes notable sensory attributes and due to this, oleoresin often costs more money (Govindarajan et al., 1982). Green cardamom yields a semi-solid liquid ranging in hue from greenish to brown, containing triglyceride and steroid components that give flavor and aroma. Cardamom, either freshly ground, cardamom powder devoid of essential oils, or cardamom powder from which oil has been removed, are all used to make oleoresin. Choosing the right raw material, grinding it to the ideal extraction particle size, selecting the appropriate solvent, selecting the extraction procedure, miscella distillation, and mixing are just a few of the crucial factors that need to be carefully taken into account when making oleoresin preparations (Nair, 2020). To assist the breakdown of flavor cells and enable fast solvent extraction, the cardamom seed is crushed into a coarse powder with particle sizes ranging from 500 to 700. It is preferable to avoid fine grinding since it not only results in the loss of volatiles but also slows down extraction because of compact lagging. The powdered cardamom is introduced to the extractor with a suitable solvent, such as acetone, alcohol, methanol, ethyl acetate, ethyl methyl ketone, or a mixture of these solvents. Batch countercurrent extraction or soxhlet extractions are methods used to produce industrial oleoresins (Ahmad et al., 2022). The discharged and wasted material, rich in other nutrients, including starch, fiber, carbohydrates, and protein, is used to manufacture crop manure and animal feed. The cardamom oleoresin has a great hue, from brown to greenish brown. The oleoresins have been very seasonal, with the yield mounting up to 4% using solvent extraction.

The chloroform-extracted oleoresins contain 21.8% α-terpinyl acetate, whereas methanol contains around 25.9%. The most prevalent component of ethanol oleoresin is 5-hydroxymethylfurfural (28.9%). Methanol and ethanol oleoresins are the most effective antifungal agents against the food-borne fungus *Aspergillus terreus* (Singh et al., 2008). The industrial production flow diagram of oil and oleoresin is depicted in Fig. 20.4.

Advance Extraction Techniques

Since current techniques have several shortcomings, creating effective, unique, and resilient methods for extracting bioactive phytochemicals and essential oils is

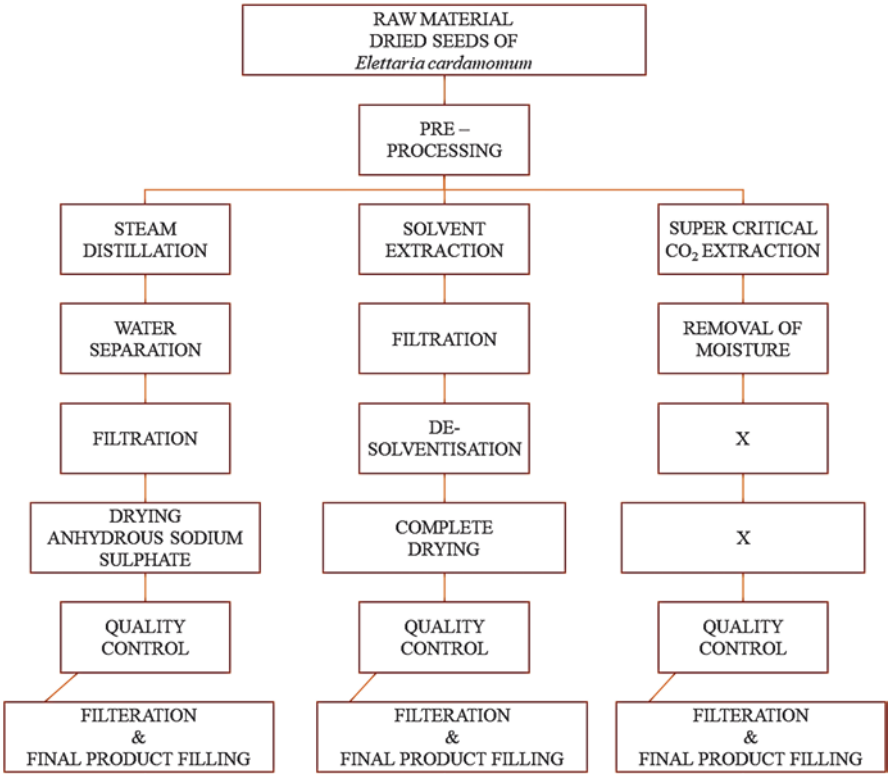


Fig. 20.4 Production flow of essential oil and oleoresin

imperative. Modern methods for extracting phytochemicals from cardamom, innovative extraction techniques, such as Ultrasonic assisted extraction (UAE), enzyme-assisted extraction (EAE), immediate, controlled pressure drop (ICPD), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), subcritical and supercritical fluid extraction (SFE), have been used in recent years in addition to conventional techniques like hydro-distillation (HD) and steam distillation (SD) (Ahmad et al., 2022; Ravindran & Madhusoodanan, 2003) (Fig. 20.5).

Ultrasonic-assisted extraction (UAE) of *Elettaria cardamomum* L. seeds, followed by hydro-distillation, expedited the extraction process and generated high-quality cardamom phytochemicals with the best sensory characteristics. GC-MS was used to identify the major components of cardamom, which included elevated levels of α-terpinyl acetate (22.9% to 40.5%) and 1,8-cineole (26.5% to 39.3%) (Morsy, 2015). The phytochemicals in green cardamom can also be extracted using the microwave-assisted extraction (MAE) technique, which involves applying microwave radiation to an insulated thermocouple reactor. Extraction yield is affected by moisture content, extraction time, and microwave power (Ahmad et al., 2022; Lucchesi et al., 2007). An advanced supercritical fluid extraction technique

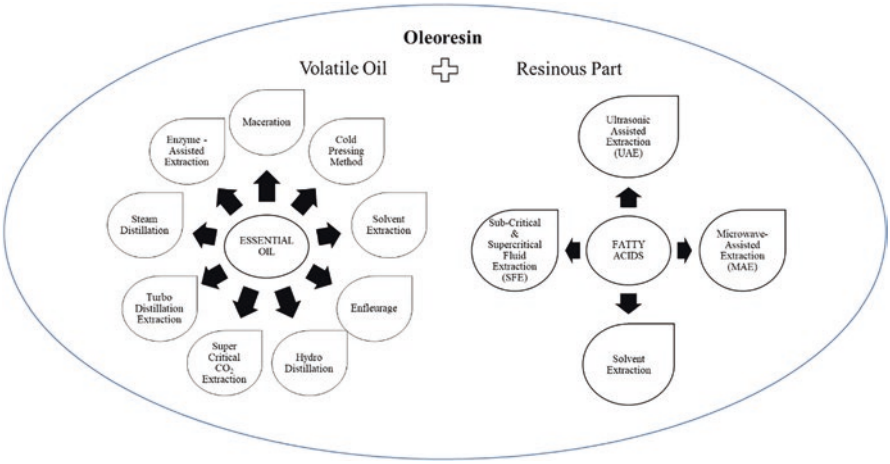


Fig. 20.5 Extraction processes for essential oil and oleoresin of cardamom Seeds

has potential advantages such as decreased sample degradation, effective material transfer, heat denaturation of bioactive chemicals, no sample cleanup, and environmental friendliness. Cardamom phytochemicals were successfully extracted by Paul et al. from cardamom seeds (25 g) by utilizing 99.8% pure carbon dioxide as a solvent (supercritical fluid) at a constant temperature of 50 °C, a pressure of 200 bar, and a flow rate of 2 liters/min of CO_2 (Paul et al., 2021). Hamdan et al. used modern analytical techniques to extract cardamom seeds using supercritical fluid carbon dioxide (SF-CO_2) and subcritical propane and to identify their quality components, including volatiles, fatty acids, pigments, and antioxidants. It was discovered that even with just one extraction, oil from cardamom seeds may be readily recovered using sub- or supercritical fluids. Applying propane in sub-critical conditions can significantly lower the cost of extraction (Hamdan et al., 2008). Oleoresin can be obtained from organic solvents. Hydrocarbon solvents generate oleoresin that contains 10–20% fixed oil, whereas polar solvents such as alcohol are used to make a fat-free product. Kasturi and Iyer extracted 4% fixed oil using volatile oil-free cardamom seeds and carbon tetrachloride solvent (Kasturi & Iyer, 1955; Roopan & Madhumitha, 2018).

20.4 Therapeutic Activity and Potential Health Benefits

Cardamom oil‘s/oleoresin’s active ingredients, including steroids, oil, lipids, and carbohydrates, have antioxidant, anti-inflammatory, antibacterial, antidepressant, and anticancer properties. In addition, it can remove toxins from the body, thus helping in detoxification (Korikanthimathm et al., 2001; Kumar et al., 2016; Roopan & Madhumitha, 2018).

20.4.1 Antioxidant and Anti-inflammatory Activity

Antioxidants are natural or synthetic chemicals that fight free radicals by scavenging them, slowing down the pace at which essential components oxidize, altering their biochemical characteristics, and promoting health. Oleoresins are superior to butylated hydroxytoluene as antioxidants (Halliwell, 2000; Kapoor et al., 2008; Mamdapur et al., 2021). Non-volatile (resin) cardamom seeds, such as α-terpinyl acetate, polyphenols, fatty acids, and sterols, were found to have anti-inflammatory properties in paw edema-induced Wistar rats in one research (Arpitha et al., 2019; Cárdenas Garza et al., 2021; Singh et al., 2008).

20.4.2 Antimicrobial and Antibacterial Activity

The essential oil in cardamom has a high degree of antiseptic and antimicrobial properties. The cardamom infusion treats a sore throat and pharyngitis, relaxes uvula, and hoarseness during the infective stage of influenza. The EO of cardamom has robust broad-spectrum antibacterial anti-yeast activities against various microorganisms, *Staphylococcus aureus, Escherichia coli*, *Salmonella typhi*, *Saccharomyces cerevisiae*, *Streptococcus mutans,* and *Candida albicans* (Ashokkumar et al., 2020). In addition, cardamom essential oil used in the study showed antibacterial properties against *subtilis* and *E. coli* species (Pourkhosravani et al., 2021). The ability of the oil to damage the cell membranes of some bacteria may be the cause of its antibacterial effects. Therefore, one examiner concluded that this oil might be used in upcoming antibacterial drugs.

20.4.3 Metabolic Syndrome

Cardamom may help with metabolic syndrome, which includes heart disease and type 2 diabetes, with defining pathophysiological conditions like high blood sugar, obesity, hypertension, high triglycerides, high cholesterol, and low "good" cholesterol levels, according to several studies (Noumi et al., 2018). In a different trial, 83 people with type 2 diabetes received green cardamom or a placebo. Cardamom users reported better insulin and hemoglobin A1c levels after ten weeks (Aghasi et al., 2019). Women who were obese or overweight had pre-diabetes and/or elevated cholesterol. Their research revealed that the levels of C-reactive protein, inflammatory proteins, and other indicators that can lead to health issues were lower in the women who took cardamom for 8 weeks (Kazemi et al., 2017). Cardamom is good for your heart. Cardamom may help to avoid heart attacks. According to a 2017 study by Nagashree et al., antioxidant benefits may help to improve cardiac function and control metabolic syndrome (Nagashree et al., 2017).

20.4.4 Oral Health

Cardamom seeds can be chewed to raise the pH of the saliva and the plaque. Cardamom seeds can lubricate and moisturize the mouth while protecting those particularly prone to developing cavities (Swathi et al., 2016).

20.4.5 Liver Health

In one study, green cardamom supplements improved liver health indicators and treated nonalcoholic fatty liver disease (Daneshi-Maskooni et al., 2018; Elguindy et al., 2016). In other animal studies, scientists fed rats a high-fat, high-carbohydrate diet and measured certain liver health markers. Cardamom-treated rats experienced less liver stress than control rats who consumed an un-supplemented diet after 8 weeks. According to this research, cardamom may prevent some forms of liver damage (Rahman et al., 2017).

20.4.6 Anti-Carcinogenic Properties

In vitro research using cardamom essential oil revealed anti-carcinogenic properties. According to Bhattacharjee et al. (2007), 1, 8-cineole and limonene, two phytochemicals found in CEOs, have a preventative effect on cancer growth. Furthermore, they added that aqueous cardamom extract might lessen lipid peroxidation and enhance the activity of the detoxifying enzyme glutathione S-transferase (Bhattacharjee et al., 2007; Elguindy et al., 2016).

20.4.7 Miscellaneous Activities

Al-Zuhair et al. (1996) noted that in addition to its diuretic effects, cardamom phytochemicals also have antispasmodic activity through receptor blockade. Cardamom can be used in conjunction with gentamicin to avoid the nephrotoxicity it causes. Gentamicin's structural and functional alterations to the rat kidney are protected by cardamom (Al-Zuhair et al., 1996; Elkomy et al., 2015). Additionally, it suppresses gastrointestinal disorders, obesity, and urinary issues (Khan et al., 2011; Kumar & Kumari, 2021). Cardamom is used as a bronchodilator in the treatment of asthma by acting as a Ca^{++} antagonist (Khan et al., 2011) and chemo-preventive agent against 7,12-dimethylbenz [a] anthracene (DMBA)-induced skin carcinogenesis (Qiblawi et al., 2012). Additionally, cardamom oil protects against aluminum chloride-induced neurotoxicity by inhibiting AChE activity and reducing oxidative damage

(Auti & Kulkarni, 2019). *E. cardamomum* essential oil displayed its acaricidal and repellent activity by inhibiting anti-acetylcholinesterase AChE and glutathione S-transferase GST (Alanazi et al., 2022). Cardamom essential oil (EO) has insecticidal properties that are effective against many insect pest life stages (Goudarzvand Chegini & Abbasipour, 2017).

20.5 Oleoresin and Volatile Oil: Food and Pharmaceutical Applications

Ancient Greek and Roman physicians and Indian Ayurvedic practitioners have used green cardamom to treat different ailments (Roopan & Madhumitha, 2018). The uses of drugs manufactured from natural substances in the medical profession vary. Cardamom seeds have an inhibiting impact on microorganisms and have antibacterial properties due to the presence of terpenoids. The volatile oil of cardamom seeds more effectively inhibits the growth of the microbial species under investigation than fixed oil, and it has been demonstrated that the inhibitory action against some pathogenic fungi is stronger with increasing volatile oil concentration. In addition, cardamom seeds are said to have stimulating, cooling, and carminative qualities as well as diuretic, cardio-tonic, anti-carcinogenic, and cytotoxic characteristics (Huang et al., 2000). Because of its soothing properties, cardamom oil is also mixed with massage oil, perfumes, and lotions. Applying cardamom oil makes the body feel light and relaxed. Cardamom oil is also used in cosmetics due to its cooling nature and a pleasant aroma. Due to its widespread acceptance, it is a clear, colorless liquid easily included in a wide range of cosmetic items.

As flavoring ingredients in a variety of sweets, frozen desserts, bakery and baked goods, liquids including alcoholic and non-alcoholic beverages, candies and condiments, puddings, foods, rice, pickles, gravies, and meat products, cardamom seeds and volatile oils are used in addition to its therapeutic use.

Cardamom oil and oleoresin are frequently used in milk and dairy products. Several items are available on the market for kids and adults, with cardamom flavors being a preferred option (Jadav & Mehta, 2018).

20.6 Contribution of Various Technology to Developing Newer Formats

Creating new forms is vital and very much required in the food processing industry. Traditional preparation and cuisine employ volatile oil for taste and aroma, but because these oils have low boiling and flash points, small amounts of their volatile components are more likely to evaporate when food is baked, boiled, or even merely kept at high temperatures. Oleoresin is being used instead of volatile oils and is in

great demand to stop these issues. Even oleoresins contain natural antioxidants of the corresponding spices. They are rich in phenol and alcohol components, making them more stable however, they have oxidation problems because of exposure to active components in the presence of oxygen, light, and moisture and undergo color changes. To accomplish this goal, several academics and industrialists are presently developing new formats to meet market demand.

Micro-encapsulated cardamom oleoresin might be the next-generation food flavoring solution and technique. Cardamom oil is the key ingredient in most culinary goods, including pastries, biscuits, cakes, and a range of Ready to Cook (RTC) and Ready to Eat (RTE) semi-processed food items. Because oil has some limitations when frying the food, encapsulated oleoresin coating with gum or modified starch might be a practical option. In addition, the micro-encapsulation process protects the product from oxidation and makes it heat resistant while frying or baking (Dima & Dima, 2015; Mani et al., 2017).

Similarly, to solve the oxidation problem, cardamom oleoresin was co-crystallized with a sugar matrix, resulting in cardamom being imprisoned in the sugar environment and continually releasing the flavor. This activity is beneficial in making the candidate feasible for use in ready-to-drink drinks such as tea and other culinary products (Sardar et al., 2013; Sardar & Singhal, 2013).

Nanoparticles were produced as another attempt to manage antibiotic-resistant bacterial strains. TiO_2 nanoparticles were created using the sol-gel process and then loaded with natural cardamom essential oil to form the potential solution. The resultant solution coined CEO@TiO_2 might be a strong candidate for various pharmaceutical and food processing applications (Ouerghi et al., 2021). Using cardamom oleoresin, the experiment can be repeated to create a more stable formulation that retains the sensory attributes. Nanostructured lipid carriers (NLCs) were created using cocoa butter and olive oil, similar to the method described above. In this new approach, cardamom essential oil (CEO) was treated in a fatty environment. After 30 days of testing and assessment, this carrier retained its antioxidant activity (reduction of just 5.7%) compared to CEO emulsion (reduction of 12.3%). The results backed up the usage of CEO-loaded NLC as a dietary supplement (Keivani Nahr et al., 2020). Cardamom essential oil can be substituted for cardamom oleoresin to produce a comparable nano-sized encapsulated product with a longer shelf life. Gold nanoparticles were synthesized using *Elettaria cardamomum* extracts by reduction of an aqueous solution of HAuCl4 at room temperature. Reduction of Au3+ to gold nanoparticles can be observed by changing the color, PH changes, and UV spectroscopic analysis. Particle sizes can be confirmed by observing XRD analysis; it indicates the greater reduction potential of *Elettaria cardamomum* extract. These extracts can synthesize other nanoparticles (Roopan & Madhumitha, 2018; Xin Lee et al., 2016). Silver nanoparticles were also prepared with different concentrations of $AgNO_3$ solution and *Elettaria Cardamomom* seed extract and further characterized by UV-VIS spectroscopy (Gnanajobitha et al., 2012; Krishnan et al., 2015).

20.7 Conclusion

Cardamom is a widely used spice in a wide range of cuisines worldwide. It is an ancient plant species native to India with amazing flavor and healing properties. Cardamom oils have a niche market in terms of their use and cost; however, oil cannot always be utilized in products heated to high temperatures due to its low boiling and flash points characteristics. Since oleoresin is the primary solution, it also has a variety of market occupancies. Both ingredients are widely used in various goods, including beverages, culinary preparations, soup powders, curry powders, confectioneries, noodles, canned meats, chicken products, and sauces in domestic and international markets. In the upcoming years, newer cardamom oils and oleoresins developed through newer technologies are anticipated to replace traditional powdered spices without sacrificing flavor and fragrance.

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Chapter 21
Non-Food Applications of Cardamom

Niccolò Pilla, Vita Di Stefano, Paolo Gabrielli, Massimo Lucarini, and Alessandra Durazzo

21.1 Introduction

In recent years, research increasingly requires a multidisciplinary approach. The study of food and raw materials is no longer limited to foods and nutraceuticals fields, but new applications must be evaluated and applied (Durazzo & Lucarini, 2019). Novel analytical techniques have been developed to elucidate the structure of active molecules (Ramadan et al., 2021). Furthermore, new and innovative applications appear possible in line with technological advances. Nanotechnologies are new and promising frontiers in various scientific disciplines, i.e., pharmaceutical, nutraceutical, and agricultural ones (Souto et al., 2020; Yeung et al., 2020; Blanco-Llamero et al., 2022; Zielińska et al., 2020; Santos et al., 2023). Nanoparticles'biological synthesis is considered an ecofriendly and easily scaled-up technology (Singh et al., 2016). On the other hand, then environmental sustainability and the recovery of waste are a further necessity, in line with the biorefinery approach and circular economy.

Using chemical products in industries substantially impacts the environment and human health, and the replacement of these with natural products could be an important solution (Ahsan et al., 2020). For example, plant extracts as an insecticide could be essential in public health and urban pest management and as crop protectants for medicinal and food crops grown in controlled environments (Isman, 2020).

N. Pilla · P. Gabrielli · M. Lucarini (✉) · A. Durazzo
CREA-Research Centre for Food and Nutrition, Rome, Italy
e-mail: massimo.lucarini@crea.gov.it

V. Di Stefano
Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy

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Again, the recovery of waste products is another strategy to reduce the environmental impact and exploit the potential of plants (Lucarini et al., 2019, 2020a, b). The following chapter illustrates the possible non-food applications of cardamom.

21.2 Insecticidal Activity

Plant essential oil has the potential to control pest infestation (Divekar, 2023). Many essential oils have various effects, such as ovicidal, repellent, antifeeding, and biocidal activities against various arthropod pests (Isman, 1999; Gong & Ren, 2020; Marsin et al., 2020). Additionally, some plant extracts or phytochemicals are highly effective against insecticide-resistant insect pests (Ahn et al., 1997; Chowański et al., 2016). The insecticidal constituents of many plant extracts and essential oils against stored product insects are mainly monoterpenoids such as limonene, linalool, terpineol, carvacrol, and myrcene (Ahn et al., 1997; Pavela et al., 2020; Abdelgaleil et al., 2021; Liu et al., 2022). Due to its chemical composition, cardamom is a potential candidate substitute for chemical pesticides, and its potentiality has already been assessed.

In particular, cardamom essential oil has been tested against *Tuta absoluta,* a pest of the Aolenacee family. Goudarzvand Chegini and Abbasipour (Goudarzvand Chegini & Abbasipour, 2017) tested cardamom essential oil against the egg, second larval instars (inside leaf, outside leaf) and adult of *T. absoluta,* concluding that the LC_{50} values were 351.1, 7.88, 1.55 and 1.88 μl L^{-1} air, respectively (Goudarzvand Chegini & Abbasipour, 2017).

Elettaria cardamomum seed essential oil showed good adulticidal activity against *C. maculatus, T. castaneum,* and *E. kuehniella*, confirming its usefulness as a candidate for the control of insect pests in stored products (Abbasipour et al., 2011). Recently, cardamom essential oil was tested against *Oryzaephilus surinamensis* (Kumar & Hemalatha, 2021). The cardamom essential oil was encapsulated in nanoparticles, and tested its insecticidal activity was evaluated. The results showed that essential oil nanoparticles had a high contact and fumigant activity, confirming that cardamom could effectively substitute chemical pesticides in controlling stored product grain pests (Kumar & Hemalatha, 2021).

An alcoholic extract produced from cardamom waste (leaves and stems) was tested against the growth of rice weevil. The rise weevil was treated under different exposure times and stored for three weeks (Widiyaningrum & Candrawati, 2021). The insecticidal activity was evaluated in terms of the weight loss difference assumed as feed consumption. Compared with another waste extract (*Zingiber zerumbet*), cardamom extract is more effective, demonstrating the potential use as a bioinsecticide (Widiyaningrum & Candrawati, 2021).

Furthermore, different amounts of cardamom seed powder were evaluated against red pal weevil *Rhynchophorus ferrugineus*. Data revealed that 5 mg of cardamom is the most effective, showing 93% of mortality after one exposition and 100% mortality after 2 days (Mona, 2020).

21.3 Other Alternative Use of Cardamom

The textile industry is a large user of chemical products, especially for printing and dyeing. However, some of these chemicals were harmful to both humans and the environment. Therefore, it is a significant challenge to find valid substitutes that are sustainable and green. Singh and Srivastava (2017) tried to obtain a natural dye from the peel of black cardamom. They found that black cardamom has good serviceability for dyed; furthermore, it represents low-cost and eco-friendly products that could be used on a large scale (Singh and Srivastava, 2017).

Habib et al. (2021) evaluated new plant-based mordants for cotton dyeing. The bio-mordant formulation includes several plant extracts and cardamom for its metamio-mordant function. These mordants improved color strength and made the coloration process more eco-friendly (Habib et al., 2021).

Cardamom biomass was also evaluated as a potential natural sorbent for removing toxic contaminants from wastewater (Itankar & Patil, 2021). In particular, cardamom tamarind pod cover and cardamom tamarind seed had a percentage of Cr(VI) biosorption of 28.9 and 59.2, respectively (Itankar & Patil, 2021).

Another example of the uses of cardamom waste is shown by Chandraju et al. (2014); cardamom husk, considered a waste product, was efficiently converted to reducing sugar with a yield percent of about 45–50% (Chandraju et al., 2014).

The study of Souza et al. (2022) is worth mentioning in evaluating cardamom Pickering emulsions stabilized with cellulose nanocrystals or cellulose nanofibers. The resulting study showed an interaction between some compounds of the essential oil and the nanocellulose, resulting in a strong solid phase adsorption, which protects the active compounds from the environments and preserves their physiochemical characteristics and biological activities, improving cardamom's application in a different sector, such as cosmetics, biomedical, and others (Souza et al., 2022).

Fruits of *A. villosum* and *E. cardamomum* were utilized to synthesize gold and silver nanoparticles (Soshnikova et al., 2018). The nanoparticles were synthesized with green methods using an aqueous extract of the two species. The methodology of the green synthesis results in being inexpensive, ecological, and safe. In addition, an *in vitro* assay of the nanoparticles was done, and they showed antioxidant, catalytic, antimicrobial, and cytotoxicity activities (Soshnikova et al., 2018).

21.4 Conclusion

Foods industry is still the main application for cardamom. Despite this, continuous research in the technological field has allowed the use and exploitation of cardamom in various fields. Furthermore, environmental concerns and rising awareness drive research toward green solutions. Using plant extracts as insecticide, for example, is a convenient choice, because they are inexpensive, biodegradable, and respect

the environment. Further studies are therefore necessary to develop new and efficient applications. Another emerging research direction is the utilization of cardamom wastes, in line with the biorefinery approach and throughout nanotechnologies processes.

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