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Molecular Chemical and Pharmacological Characterization of Cardamom Varieties

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Abstract: *Cardamom (Elettaria cardamomum and related varieties) is one of the most valuable medicinal and aromatic spices widely used in traditional and modern therapeutic systems. The present study focuses on the molecular, chemical, and pharmacological characterization of different cardamom varieties to evaluate their bioactive constituents and biological potential. The research aims to compare the phytochemical profile, essential oil composition, and pharmacological activities of selected green and black cardamom samples.*

In the molecular characterization, DNA-based techniques such as PCR amplification and genetic marker analysis were used to determine varietal differences and genetic diversity among cardamom species. Chemical characterization involved qualitative and quantitative phytochemical screening, including analysis of alkaloids, flavonoids, terpenoids, phenols, and volatile oil content using GC-MS (Gas Chromatography–Mass Spectrometry). The essential oil profile revealed the presence of key bioactive compounds such as 1,8-cineole, α-terpinyl acetate, limonene, and linalool, which are responsible for its characteristic aroma and therapeutic effects.

Pharmacological evaluation of cardamom extracts demonstrated significant antioxidant, anti-inflammatory, antimicrobial, gastroprotective, and cardioprotective activities. These biological effects are mainly attributed to its rich phytoconstituents and synergistic action of volatile compounds. Comparative analysis among different varieties showed variation in chemical composition and biological potency, indicating that environmental and genetic factors significantly influence the quality of cardamom.

The study concludes that cardamom is a potent natural source of bioactive compounds with strong therapeutic potential. Molecular and chemical profiling provides valuable information for standardization, quality control, and future drug development. Further in-depth research is recommended to isolate individual active constituents and explore their mechanisms of action for pharmaceutical applications.

Keywords: Phytoconstit, cardamon, bioactive.

I. INTRODUCTION

Cardamom, scientifically known as Elettaria cardamomum Maton, is one of the most important aromatic spice crops belonging to the family Zingiberaceae. It is widely recognized as the “Queen of Spices” due to its pleasant aroma, distinct flavor, and extensive medicinal importance. Apart from Elettaria cardamomum (green cardamom), other related species such as Amomum subulatum (black cardamom) are also used in traditional medicine and culinary practices. Cardamom is native to the evergreen forests of Southern India and is extensively cultivated in tropical regions such as India, Sri Lanka, Guatemala, and Tanzania.(1)

In traditional systems of medicine like Ayurveda, Siddha, and Unani, cardamom has been used for centuries to treat a wide range of ailments including digestive disorders, halitosis, bronchitis, asthma, kidney problems, and inflammation. It is considered a carminative, stomachic, and diuretic agent. The essential oil extracted from cardamom seeds is highly valued for its therapeutic properties such as antimicrobial, antioxidant, anti-inflammatory, gastroprotective, and

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cardioprotective activities. These biological effects are mainly attributed to its rich content of volatile oils and phytochemicals.(2)

From a phytochemical perspective, cardamom contains a complex mixture of bioactive compounds including essential oils (such as α-terpinyl acetate, 1,8-cineole, limonene, linalool), flavonoids, phenolic acids, tannins, terpenoids, and sterols. These constituents contribute not only to its medicinal properties but also to its strong fragrance and flavor. The concentration and composition of these compounds vary significantly depending on species, geographical location, soil conditions, climate, and post-harvest processing methods.(2)

In recent years, there has been increasing scientific interest in the molecular and chemical characterization of medicinal plants, including cardamom. Molecular characterization using DNA-based markers such as RAPD, ISSR, and PCR techniques has become an important tool for identifying genetic diversity, confirming species authenticity, and preventing adulteration. This is particularly important in the herbal drug industry, where quality and standardization are major concerns.

Chemical characterization using modern analytical techniques like Gas Chromatography–Mass Spectrometry (GC-MS), High Performance Liquid Chromatography (HPLC), and UV-Visible spectroscopy helps in identifying and quantifying the bioactive constituents responsible for pharmacological activity. These techniques provide scientific validation for the therapeutic claims associated with traditional medicinal plants.(4)

Pharmacological studies on cardamom have demonstrated a wide range of biological activities. Its antioxidant potential helps in reducing oxidative stress caused by free radicals, while its anti-inflammatory properties contribute to the management of inflammatory diseases. The antimicrobial activity supports its use in treating infections, and its gastroprotective effects justify its traditional use in digestive disorders. Additionally, emerging studies suggest potential benefits in cardiovascular protection, metabolic regulation, and neuroprotection.

Despite its wide usage and therapeutic importance, variation in chemical composition and pharmacological efficacy among different cardamom varieties remains a major concern. These variations can lead to inconsistencies in quality and therapeutic outcomes. Therefore, a systematic study involving molecular, chemical, and pharmacological characterization is essential for proper standardization, quality control, and scientific validation of cardamom varieties.(5,6)

The present study is designed to evaluate and compare different varieties of cardamom through molecular fingerprinting, phytochemical profiling, and pharmacological screening. This integrated approach helps in understanding genetic diversity, identifying active chemical constituents, and correlating them with biological activities. The findings of this study are expected to contribute to the development of standardized herbal products and support the pharmaceutical application of cardamom in modern medicine.

Cardamom, botanically classified as Elettaria cardamomum Maton (green cardamom) and Amomum subulatum Roxb. (black cardamom), is a perennial herb belonging to the family Zingiberaceae. It is one of the most ancient and highly valued spices in the world, often referred to as the “Queen of Spices” due to its unique aroma, therapeutic importance, and extensive commercial demand. Cardamom seeds are enclosed within small triangular pods, which contain a rich reservoir of essential oils and bioactive compounds responsible for its characteristic fragrance and medicinal properties. It is predominantly cultivated in tropical and subtropical regions, especially in India (Kerala, Karnataka, Tamil Nadu), Sri Lanka, Guatemala, and parts of Africa.

Historically, cardamom has been used for thousands of years in traditional medicinal systems such as Ayurveda, Siddha, and Unani. In Ayurveda, it is classified under “Tridosha shamak” herbs, meaning it helps in balancing Vata, Pitta, and Kapha doshas. It is commonly used as a carminative, digestive stimulant, mouth freshener, diuretic, and expectorant. Traditional formulations containing cardamom are prescribed for conditions such as indigestion, flatulence, nausea, vomiting, respiratory congestion, urinary tract disorders, and oral infections. Its use in herbal teas and formulations highlights its importance as both a functional food and a medicinal agent.(4,5)

Modern pharmacological research has validated many of these traditional claims, revealing that cardamom possesses a wide spectrum of biological activities including antioxidant, anti-inflammatory, antimicrobial, gastroprotective,

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hepatoprotective, cardioprotective, and antispasmodic effects. These activities are mainly attributed to its rich phytochemical composition, particularly volatile oils and polyphenolic compounds. The major volatile constituents include α-terpinyl acetate, 1,8-cineole (eucalyptol), limonene, linalool, sabinene, and terpinene derivatives. These compounds are responsible for both the aroma and the pharmacological efficacy of cardamom.

In addition to volatile oils, cardamom also contains non-volatile phytoconstituents such as flavonoids, phenolic acids, tannins, sterols, proteins, carbohydrates, and lipids. These compounds contribute significantly to its antioxidant and anti-inflammatory potential by scavenging free radicals and modulating enzymatic pathways involved in oxidative stress and inflammation. The synergistic interaction between volatile and non-volatile constituents enhances the overall therapeutic effectiveness of cardamom.(5,6)

However, significant variation exists in the chemical composition and biological activity of cardamom depending on its species, geographical origin, soil type, altitude, harvesting stage, drying methods, and storage conditions. For example, green cardamom (Elettaria cardamomum) generally contains a higher percentage of oxygenated monoterpenes such as α-terpinyl acetate, whereas black cardamom (Amomum subulatum) contains more smoky, camphor-like compounds due to different processing techniques. These variations directly influence the medicinal quality, flavor profile, and pharmacological potency of the plant material.(12)

In the context of modern herbal drug research, standardization and authentication of medicinal plants have become extremely important. Adulteration, substitution, and variability in raw drug quality are major challenges in the herbal pharmaceutical industry. To address these issues, molecular characterization techniques such as Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), and Polymerase Chain Reaction (PCR)-based DNA barcoding are widely used. These techniques help in identifying genetic diversity, confirming species authenticity, and ensuring quality control of plant materials at the DNA level.

Alongside molecular methods, advanced analytical techniques play a crucial role in chemical characterization. Gas Chromatography–Mass Spectrometry (GC-MS) is extensively used to identify volatile oil components, while High Performance Liquid Chromatography (HPLC) and UV-Visible spectroscopy are used for quantification of flavonoids, phenols, and other phytochemicals. These methods provide a scientific basis for correlating chemical composition with pharmacological activity, which is essential for drug development and standardization.

Pharmacological evaluation of cardamom has gained significant attention in recent years. The antioxidant activity helps in preventing oxidative stress-related diseases such as cancer, diabetes, and cardiovascular disorders. The anti-inflammatory activity supports its role in treating arthritis and inflammatory bowel diseases. Its antimicrobial activity makes it effective against a range of bacterial and fungal pathogens. Furthermore, gastroprotective effects support its traditional use in managing gastric ulcers and indigestion, while cardioprotective and lipid-lowering effects suggest its potential in managing cardiovascular health.(11,12)

Despite extensive research, there remains a gap in the integrated study of molecular diversity, chemical composition, and pharmacological activity across different cardamom varieties. Most studies focus on either phytochemical analysis or biological activity independently, without correlating genetic variation with chemical and pharmacological differences. Therefore, a comprehensive approach combining molecular, chemical, and pharmacological characterization is necessary to obtain a complete scientific understanding of cardamom.

The present study aims to evaluate and compare different varieties of cardamom using an integrated scientific approach. It involves molecular fingerprinting to assess genetic diversity, phytochemical and GC-MS analysis to identify and quantify bioactive constituents, and pharmacological screening to determine biological activities. This holistic investigation is expected to contribute to the development of standardized herbal formulations, improve quality control measures, and support the pharmaceutical utilization of cardamom in modern medicine.

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II. NEED OF STUDY

• To scientifically evaluate different varieties of cardamom (Elettaria cardamomum and Amomum subulatum) for their molecular, chemical, and pharmacological differences.
• To authenticate cardamom varieties using molecular techniques and prevent adulteration and substitution in the herbal drug market.
• To study genetic diversity among different cardamom samples using DNA-based markers such as RAPD, ISSR, or PCR techniques.
• To standardize cardamom based on phytochemical composition for ensuring consistent quality in pharmaceutical and nutraceutical applications.
• To identify and compare the major bioactive constituents such as essential oils, flavonoids, and phenolic compounds in different varieties.
• To correlate chemical composition with pharmacological activities like antioxidant, antimicrobial, anti-inflammatory, and gastroprotective effects.
• To evaluate the impact of geographical location, soil conditions, and processing methods on the quality and potency of cardamom.
• To provide scientific validation for traditional medicinal uses of cardamom in Ayurveda and other systems of medicine.
• To explore cardamom as a potential natural source for development of herbal drugs and pharmaceutical formulations.
• To support quality control and standardization protocols in the herbal and spice industry.
• To bridge the gap between traditional knowledge and modern scientific research through integrated molecular and pharmacological studies.
• To contribute data for future research on isolation, purification, and drug development from cardamom bioactive compounds.
• o establish a scientific foundation for comparing different genetic varieties of cardamom using molecular characterization techniques.

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• To ensure proper identification and authentication of genuine cardamom species at the DNA level for pharmaceutical use.
• To reduce confusion arising from morphological similarity between closely related species and varieties.(13)
• To develop reliable quality control parameters for cardamom raw material used in herbal formulations.
• To assess intra-species and inter-species genetic variation that may influence phytochemical content and biological activity.
• To determine chemotypic differences among cardamom varieties based on essential oil composition and secondary metabolites.
• To standardize raw drug material according to pharmacopoeial requirements for consistency in medicinal preparations.
• To evaluate seasonal, geographical, and environmental influence on phytochemical variation in cardamom.
• To support industrial demand for high-quality standardized cardamom in pharmaceutical, food, and cosmetic sectors.
• To investigate the relationship between genotype and chemotype of cardamom for better selection of high-yield varieties.
• To enhance reliability of herbal medicines by minimizing variability in active constituent concentration.
• To scientifically validate traditional claims regarding therapeutic effectiveness of different cardamom varieties.
• To explore the pharmacological significance of minor phytoconstituents that may contribute to synergistic activity.
• To support the development of fingerprinting databases for medicinal plants used in quality assurance.
• To encourage sustainable utilization and cultivation of superior cardamom varieties with higher medicinal value.
• To provide baseline data for future clinical and preclinical studies on cardamom-derived compounds.
• To integrate modern analytical tools with ethnopharmacological knowledge for holistic plant evaluation.
• To support regulatory frameworks for herbal drug approval by providing molecular and chemical evidence.(15)
• To identify potential markers that can be used for quick detection of adulteration in commercial samples.
• To contribute to the global scientific literature on spice pharmacology and medicinal plant standardization.

III. AIM

The aim of this study is to perform a comprehensive molecular, chemical, and pharmacological characterization of selected cardamom varieties (Elettaria cardamomum and related species) in order to evaluate their genetic diversity, phytochemical composition, and biological activities, and to correlate these parameters for standardization, authentication, and assessment of their therapeutic potential.

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IV. OBJECTIVES

• To collect and authenticate different varieties of cardamom (Elettaria cardamomum and Amomum subulatum).
• To perform molecular characterization of cardamom varieties using DNA-based techniques such as RAPD, ISSR, or PCR markers.
• To analyze genetic diversity and establish molecular fingerprinting profiles of selected cardamom samples.
• To carry out preliminary phytochemical screening for detection of major secondary metabolites such as alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids.
• To estimate quantitative phytochemical parameters including total phenolic content, total flavonoid content, and essential oil yield.
• To analyze essential oil composition using Gas Chromatography–Mass Spectrometry (GC-MS) for identification of major volatile constituents.
• To compare chemical composition among different cardamom varieties and identify chemotypic differences.
• To evaluate antioxidant activity of cardamom extracts using in vitro methods such as DPPH and FRAP assays.
• To assess anti-inflammatory activity using suitable in vitro models like protein denaturation and membrane stabilization methods.
• To evaluate antimicrobial activity against selected Gram-positive, Gram-negative bacteria and fungal strains.
• To study the relationship between phytochemical constituents and pharmacological activities of different varieties.
• To assess the influence of environmental and geographical factors on chemical and biological variation.
• To provide scientific validation for the traditional medicinal uses of cardamom.
• To support standardization and quality control of cardamom for pharmaceutical and nutraceutical applications.(16)
• To identify potential bioactive markers useful for future drug development and research.

V. REVIEW OF LITERATURE

Cardamom (Elettaria cardamomum Maton and Amomum subulatum Roxb.) is one of the most extensively studied medicinal spices due to its pharmacological importance and commercial value. A large number of scientific studies have been conducted on its phytochemistry, molecular diversity, and biological activities. The available literature indicates that cardamom contains a rich profile of volatile and non-volatile bioactive compounds, which are responsible for its therapeutic potential.

1. Traditional and Ethnopharmacological Studies

KIRTIKA KR et al; (2011) Several ethnopharmacological surveys report that cardamom has been widely used in Ayurveda, Unani, and Siddha systems of medicine. It is traditionally prescribed for digestive disorders such as dyspepsia, flatulence, and indigestion. It is also used as a remedy for respiratory conditions including asthma, bronchitis, and cough. Earlier literature suggests that cardamom acts as a carminative, stomachic, and diuretic agent. In traditional formulations, it is commonly combined with other spices to enhance digestive and detoxifying effects.

2. Phytochemical Studies

KOKATE C K et al ; (2015) Numerous phytochemical investigations have revealed that cardamom seeds contain a wide range of bioactive constituents. Early studies identified essential oils as the major chemical component, accounting for the characteristic aroma and medicinal properties. Later research using chromatographic techniques confirmed the presence of monoterpenes and oxygenated terpenoids such as α-terpinyl acetate, 1,8-cineole, limonene, sabinene, and linalool.
Studies also report the presence of flavonoids, phenolic acids, tannins, sterols, proteins, carbohydrates, and fixed oils. Among these, phenolic compounds and flavonoids are mainly responsible for antioxidant and anti-inflammatory activities. GC-MS analysis in various research works has shown that the composition of essential oil varies significantly depending on species, origin, and processing methods.

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3. Molecular Characterization and Genetic Diversity

SINGH G et al ; (2015) Recent studies have focused on molecular characterization of cardamom to understand genetic variability among different cultivars. Techniques such as Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), Amplified Fragment Length Polymorphism (AFLP), and DNA barcoding have been used.

Research findings indicate significant genetic diversity within Elettaria cardamomum populations across different geographical regions. Molecular markers have proven useful in distinguishing high-yielding and disease-resistant varieties. Studies also highlight that molecular fingerprinting is an effective tool for authentication and prevention of adulteration in commercial cardamom samples.

4. Chemical Profiling and Analytical Studies

GUPTA AND SHARMA P et al; (2018) Several analytical studies using Gas Chromatography–Mass Spectrometry (GC-MS) have identified key volatile compounds in cardamom essential oil. α-terpinyl acetate is consistently reported as the major constituent, followed by 1,8-cineole and limonene. High Performance Liquid Chromatography (HPLC) studies have been used for quantification of flavonoids and phenolic compounds.

Literature shows that environmental factors such as altitude, rainfall, soil composition, and harvesting stage significantly affect essential oil yield and composition. Post-harvest drying methods also influence the stability and concentration of volatile oils.

5. Pharmacological Studies

AHMED S et al ; (2016) Extensive pharmacological research has demonstrated multiple biological activities of cardamom extracts and essential oils:

• Antioxidant Activity: Studies using DPPH, ABTS, and FRAP assays confirmed strong free radical scavenging ability due to phenolic and flavonoid content.

• Anti-inflammatory Activity: Research indicates inhibition of inflammatory mediators such as prostaglandins and cytokines.

• Antimicrobial Activity: Cardamom extracts show effectiveness against Gram-positive and Gram-negative bacteria including Staphylococcus aureus, Escherichia coli, and fungal strains like Candida albicans.

• Gastroprotective Activity: Animal studies suggest that cardamom protects gastric mucosa by reducing acid secretion and enhancing mucus production.

• Cardioprotective Effects: Some studies indicate lipid-lowering and antihypertensive potential due to antioxidant mechanisms.

6. Comparative Studies on Cardamom Varieties

KHAN M A et al ; (2014) Comparative research between green cardamom (Elettaria cardamomum) and black cardamom (Amomum subulatum) shows significant differences in chemical composition and pharmacological effects. Green cardamom contains higher levels of oxygenated monoterpenes, while black cardamom contains more smoky and camphor-like compounds due to drying over open fire.

Studies also report that green cardamom generally exhibits stronger antioxidant and antimicrobial activity compared to black cardamom due to higher α-terpinyl acetate content. However, black cardamom has been found more effective in respiratory disorders due to its expectorant properties.

7. Gaps Identified in Literature

MISHRA P et al; (2018)Although extensive research is available, several gaps still exist:

• Limited integrated studies combining molecular, chemical, and pharmacological evaluation in a single framework.

• Lack of standardized fingerprint databases for cardamom varieties.

• Insufficient correlation between genetic variation and phytochemical differences.

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• Limited clinical validation of pharmacological effects in humans.
• Variability in extraction methods leading to inconsistent results across studies.

CHAUDHARI N V et al; (2016) Cardamom (Elettaria cardamomum Maton and Amomum subulatum Roxb.), belonging to the family Zingiberaceae, has been extensively investigated in traditional medicine, phytochemistry, molecular biology, and pharmacology. The literature reveals that it is not only a culinary spice but also a complex medicinal plant with diverse bioactive compounds and significant therapeutic potential.

1. Historical and Traditional Literature Reports

TIWARI P et al ; (2011) Historical texts of Ayurveda such as Charaka Samhita and Sushruta Samhita describe cardamom as a "Tridosha balancing" spice. It has been used since ancient times in formulations like Trikatu churna, Chyawanprash, and digestive tonics. Traditional Arabic and Unani literature also describe its use as a breath freshener and digestive stimulant.

Ethnobotanical studies conducted in India, Sri Lanka, and Southeast Asia consistently report the use of cardamom seeds for treating gastrointestinal disorders, urinary tract infections, halitosis, and respiratory congestion. Tribal communities also use decoctions of cardamom pods for detoxification and fever management, indicating long-standing empirical medicinal knowledge.

2. Early Phytochemical Investigations

BALKRISHNA A et al ; (2013) Initial phytochemical studies on cardamom seeds identified the presence of volatile oils as the primary active fraction. Early chemical isolation studies reported compounds such as cineole and terpinyl acetate as key aroma contributors. Later research expanded this understanding by identifying a broader spectrum of terpenoids and phenolic compounds. Investigations using solvent extraction methods revealed that methanolic and ethanolic extracts contain higher concentrations of flavonoids and phenolic acids compared to aqueous extracts. These compounds were linked with antioxidant and free radical scavenging properties.

3. Advanced Phytochemical and GC-MS Studies

AGARWAL B et al ;(2006) Modern analytical studies using Gas Chromatography–Mass Spectrometry (GC-MS) have provided detailed profiling of cardamom essential oils. The major constituents reported across multiple studies include:

• α-terpinyl acetate (dominant compound)
• 1,8-cineole (eucalyptol)
• limonene
• sabinene
• linalool
• α-pinene and β-pinene

WALES T et al ; (2005) Research shows that α-terpinyl acetate content can vary significantly depending on geographic origin and post-harvest drying techniques. Studies from southern India report higher concentrations compared to African and Central American samples.

HPLC and spectrophotometric analyses further confirmed the presence of flavonoids such as quercetin derivatives and phenolic acids like gallic acid and caffeic acid, which contribute to antioxidant potential.

4. Molecular Biology and Genetic Diversity Studies

EVANS W C et al ; (2009) Molecular characterization of cardamom has gained importance due to increasing demand for authentication and quality control. Several studies have used RAPD, ISSR, AFLP, and SSR markers to assess genetic diversity.

Research findings indicate that Elettaria cardamomum populations exhibit moderate to high genetic variability, which is influenced by altitude, climate, and cultivation practices. Cluster analysis and dendrogram studies have grouped cardamom accessions based on geographical origin.

DNA barcoding studies using ITS (Internal Transcribed Spacer) and matK gene sequences have been successful in distinguishing closely related species, especially between Elettaria and Amomum genera. These molecular tools are now considered essential for preventing adulteration in commercial spice markets.

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5. Environmental and Agronomic Influence Studies

KAILASH N P et al ; (2012) Agronomic research highlights that environmental factors significantly affect cardamom yield and phytochemical composition. Studies show that:

- High altitude cultivation results in higher essential oil concentration
- Shaded plantation systems improve volatile oil stability
- Soil organic content influences phenolic compound accumulation
- Harvesting stage (green vs. fully mature pods) affects oil yield and composition

Post-harvest drying methods (sun drying vs. controlled drying) also impact the retention of volatile compounds. Excessive heat exposure leads to loss of α-terpinyl acetate and reduction in aroma quality.

6. Pharmacological Research Developments

HENDRICKS M et al ; (2012) Pharmacological studies have expanded significantly over the last two decades, demonstrating multiple biological effects:

Antioxidant Activity:

Studies using DPPH, ABTS, and nitric oxide scavenging assays show strong antioxidant capacity, mainly due to phenolic and flavonoid content.

Anti-inflammatory Activity:

Research indicates inhibition of cyclooxygenase (COX) and lipoxygenase pathways, reducing production of inflammatory mediators.

Antimicrobial Activity:

Essential oils show broad-spectrum activity against bacteria and fungi, including resistant strains. Gram-positive bacteria are generally more sensitive than Gram-negative due to cell wall differences.

Gastroprotective Effects:

Animal studies demonstrate reduced gastric lesions, increased mucus secretion, and decreased acid production.

Metabolic and Cardiovascular Effects:

Emerging studies suggest antihypertensive and lipid-lowering properties, possibly due to antioxidant-mediated vascular protection.

7. Comparative Studies Between Varieties

BABU K N et al ; (2014) Comparative scientific studies highlight clear differences between green and black cardamom:

- Green cardamom shows higher essential oil concentration and sweeter aroma profile
- Black cardamom contains smoky phenolic compounds due to drying over open fire
- Pharmacological studies indicate stronger antimicrobial and antioxidant activity in green cardamom
- Black cardamom shows better expectorant and respiratory benefits

These differences confirm that each variety has distinct chemical and therapeutic profiles.

8. Industrial and Quality Control Studies

RANI R et al ;(2010) Industrial research emphasizes the importance of standardization in the spice and pharmaceutical industries. Adulteration and substitution are major concerns in global trade. Studies recommend the use of:

- GC-MS fingerprinting for essential oil profiling
- DNA-based authentication methods for raw material verification
- Spectroscopic techniques for rapid quality assessment

These approaches help ensure consistency, purity, and therapeutic reliability of commercial cardamom products.

9. Identified Research Gaps

KRES W J et al (2005) Despite extensive research, several limitations remain:

- Lack of integrated studies combining molecular, chemical, and pharmacological data
- Insufficient correlation between genotype and chemotype variations
- Limited clinical trials validating pharmacological effects in humans

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• Absence of standardized global fingerprint database for cardamom
• Incomplete understanding of synergistic interactions between phytoconstituents

7. Role and Classification

1. Role of Cardamom

 Cardamom (Elettaria cardamomum and Amomum subulatum) plays an important role in traditional medicine, modern pharmacology, food industry, and nutraceutical applications due to its rich phytochemical profile and therapeutic potential.

A. Role in Traditional Medicine

• Used as a carminative agent to relieve gas, bloating, and indigestion.
• Acts as a stomachic, improving appetite and digestion.
• Used as an expectorant in cough, cold, and bronchial congestion.
• Functions as a mouth freshener and helps in treating bad breath (halitosis).
• Employed in Ayurvedic formulations for balancing Vata and Kapha doshas.
• Used in herbal teas and decoctions for detoxification and general wellness.

B. Pharmacological Role(20)

• Acts as a strong antioxidant, protecting cells from oxidative stress.
• Exhibits anti-inflammatory activity, reducing swelling and inflammatory mediators.
• Shows antimicrobial action against bacteria and fungi.
• Provides gastroprotective effect by reducing gastric acid and protecting mucosa.
• Shows potential cardioprotective and antihypertensive effects.
• Demonstrates antispasmodic activity useful in abdominal cramps.

C. Nutraceutical and Functional Food Role

• Used as a natural flavoring agent in food and beverages.
• Added to sweets, bakery products, and dairy formulations for aroma enhancement.
• Used in herbal formulations and dietary supplements.
• Acts as a natural preservative due to antimicrobial properties.

D. Industrial Role

• Used in pharmaceutical formulations like syrups, digestive tonics, and tablets.
• Important in cosmetic and perfumery industries for fragrance.
• Used in essential oil extraction industry for aroma compounds.

2. Classification of Cardamom

Cardamom can be classified based on botanical source, size, color, and usage.

A. Botanical Classification

• Kingdom: Plantae
• Division: Angiosperms
• Class: Monocotyledons
• Order: Zingiberales
• Family: Zingiberaceae
• Genus: Elettaria / Amomum
• Species:
o Elettaria cardamomum (Green cardamom)
o Amomum subulatum (Black cardamom)

B. Commercial Classification

• Green Cardamom (Small Cardamom / True Cardamom)
o Scientific name: Elettaria cardamomum
o Color: Green

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o Aroma: Sweet, pleasant, strong
o Uses: Food flavoring, medicine, beverages
• Black Cardamom (Large Cardamom)
o Scientific name: Amomum subulatum
o Color: Dark brown to black
o Aroma: Smoky, strong, camphor-like
o Uses: Spices, respiratory medicines
C. Based on Size
• Small cardamom (green type)
• Large cardamom (black type) Based on Processing
• Sun-dried cardamom – traditional drying under sunlight
• Flame-dried cardamom – dried over fire (black cardamom acquires smoky flavor)
• Machine-dried cardamom – modern controlled drying method
D. Based on Quality Grades (Commercial)
• Extra Bold Grade – large pods with high oil content
• Bold Grade – medium size, good quality
• Small/Choti Grade – smaller pods, lower oil content
• Split/Inferior Grade – broken or damaged pods
E. Based on Chemical Composition (Chemotype)
• High α-terpinyl acetate type – high aroma and medicinal value
• High 1,8-cineole type – stronger medicinal and respiratory action
• Mixed terpene type – balanced composition
B. Solvents and Analytical Grade Chemicals
• Petroleum ether (defatting)
• Ethyl acetate (semi-polar extraction)
• n-hexane (volatile oil enrichment)
• Acetone (polyphenol extraction)
• Standard reference compounds (gallic acid, quercetin, rutin)
• Microbial culture media (Muller Hinton agar, potato dextrose agar)
• Phosphate buffer solution (pH maintenance for assays)
C. Microbial Strains Used
• Staphylococcus aureus (Gram positive)
• Escherichia coli (Gram negative)

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• Bacillus subtilis (Gram positive)
• Candida albicans (fungal strain)
• Strains were maintained under aseptic conditions in microbiology laboratory.

D. Molecular Biology Reagents
• CTAB extraction buffer (for DNA isolation)
• Taq DNA polymerase enzyme
• dNTP mix (deoxynucleotide triphosphates)
• $MgCl_2$ solution (PCR cofactor)
• Agarose powder (gel preparation)(21)
• Ethidium bromide or safer alternatives (DNA staining dye)
• Molecular weight DNA ladder (100 bp/1 kb marker)

2. Methods

A. Sample Pre-Treatment and Standardization
• Cardamom pods were air-dried under controlled temperature (below 40°C).
• Seeds were separated and pulverized using a mechanical grinder.
• Powder was passed through mesh sieve (40–60 mesh size) for uniform particle size.
• Moisture content was determined to ensure stability before analysis.

B. Defatting and Fractionation Process
• Powdered samples were first defatted using petroleum ether in a Soxhlet apparatus.
• Sequential solvent extraction was performed in increasing polarity order:
1. Petroleum ether fraction (non-polar compounds)
2. Ethyl acetate fraction (medium polarity compounds)
3. Methanol fraction (polar phytochemicals)(22)
• Each fraction was concentrated separately for specific activity testing.

C. Physicochemical Evaluation
• Loss on drying (LOD): to determine moisture content
• Total ash value: to estimate inorganic impurities
• Acid-insoluble ash: to check soil/silica contamination
• Volatile oil percentage: determined using Clevenger apparatus
• Swelling index and foaming index: for saponin-related behavior

D. Advanced Phytochemical Profiling
• High-resolution thin layer chromatography (HPTLC) was used for fingerprint profiling.
• Retention factor (Rf values) were recorded for comparison between varieties.
• UV spectral scanning was used to detect characteristic absorbance peaks of flavonoids.
• Total antioxidant capacity was measured using phosphomolybdenum method.

E. Volatile Oil Isolation (Hydrodistillation)
• Fresh crushed seeds were subjected to Clevenger-type hydrodistillation for 3–4 hours.
• Oil layer was separated and dried over anhydrous sodium sulfate.
• Yield percentage was calculated based on dry weight of plant material.
• Oils were stored in amber bottles at 4°C to prevent degradation.

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F. Advanced GC-MS Profiling
• Column: capillary column (non-polar phase)(23)
• Carrier gas: helium
• Injection: split mode
• Mass range: 40–500 m/z
• Compounds were identified using:
o NIST spectral library
o Retention index comparison
• Relative percentage of each compound was calculated using peak area normalization.

G. DNA Isolation Quality Check
• Extracted DNA was checked for purity using A260/A280 ratio.
• Integrity of DNA was confirmed by agarose gel electrophoresis.
• Only high-quality DNA samples were used for PCR amplification.

H. PCR Optimization Conditions
• Initial denaturation: 94°C
• Annealing temperature: optimized based on primer type (RAPD/ISSR)
• Extension: 72°C
• Number of cycles: 35 cycles
• Final extension: 72°C for complete amplification(24)
• Reproducibility was ensured by repeating reactions thrice.

I. Data Analysis for Molecular Studies
• Gel images were analyzed using software-based band scoring.
• Binary data matrix (1/0 scoring) was prepared for presence/absence of bands.
• Similarity index was calculated using Jaccard coefficient.
• Cluster analysis was performed using UPGMA method to construct dendrogram.

J. Biological Activity Screening (Additional Approaches)
1. Reducing Power Assay
• Evaluates electron-donating ability of extracts.
• Absorbance increase indicates higher antioxidant potential.
2. Total Antioxidant Capacity (TAC)
• Phosphomolybdenum method used for overall antioxidant estimation.
3. Time-Kill Kinetic Study (Antimicrobial)
• Measures bacterial inhibition over time rather than single-point zone measurement.

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VII. MATERIALS AND METHODS

A. Materials Used

1. Plant Materials

Green cardamom (Elettaria cardamomum) Black cardamom (Amomum subulatum)

Cardamom seeds and pods collected from local markets and authenticated sources.

2. Chemicals and Reagents Methanol Ethanol

Petroleum ether Ethyl acetate Distilled water

Folin–Ciocalteu reagent DPPH reagent

Ferric chloride Aluminum chloride Sodium carbonate Ascorbic acid Gallic acid Quercetin standard

3. MicrobialStrains Staphylococcus aureus Escherichia coli Bacillus subtilis Candida albicans

4. Instruments and Equipment Soxhlet apparatus

Clevenger apparatus UV-Visible

spectrophotometer GC-MS instrument

HPLC

system PCR machine Centrifuge Hot air oven pH meter Electronic balance Mechanical grinder Incubator

B. Methods

1. Collection and Authentication

Different varieties of cardamom were collected from local spice markets and authenticated by a pharmacognosy expert.

The samples were cleaned, shade dried, and powdered for further studies.

2. Preparation of Extract

The powdered cardamom seeds were subjected to Soxhlet extraction using methanol and ethanol.

Extracts were concentrated using rotary evaporator and stored in airtight containers.

3. Phytochemical Screening

Preliminary phytochemical tests were carried out for detection of:

Alkaloids Flavonoids Phenols Tannins Saponins Terpenoids Glycosides

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4. Essential Oil Isolation
Volatile oil was isolated by hydrodistillation using Clevenger apparatus for 3–4 hours. Oil yield was calculated and stored at 4°C.
5. GC-MS Analysis
GC-MS analysis was performed to identify major volatile constituents such as: α-terpinyl acetate
1,8-cineole limonene linalool
6. Molecular Characterization
DNA was isolated using CTAB method. PCR amplification and RAPD/ISSR markers were used to study genetic diversity among cardamom varieties.
7. Antioxidant Activity
Antioxidant activity was evaluated by: DPPH
assay FRAP
assay ABTS
assay

8. Anti-inflammatory Activity
Anti-inflammatory activity was determined using:
Protein denaturation assay Membrane stabilization method
9. Antimicrobial Activity
Agar well diffusion method was used against bacterial and fungal strains. Zone of inhibition was measured in millimeters.
10. Statistical Analysis
All experiments were carried out in triplicate and results were expressed as mean ± standard deviation.

9. Collection and Authentication of Materials
Collection of Plant Materials
The collection and authentication of plant materials are critical steps in any pharmacological and phytochemical study because the quality, purity, and identity of the raw drug directly influence the accuracy and reliability of experimental results. In the present study on molecular, chemical, and pharmacological characterization of cardamom varieties, a systematic and standardized approach was followed for collection and authentication to ensure scientific validity.
A. Selection of Cardamom Varieties
• Different commercially and medicinally important varieties of cardamom were selected, mainly:
o Elettaria cardamomum (small/green cardamom)
o Amomum subulatum (large/black cardamom)
• The selection was based on:
o Medicinal importance in traditional systems
o Market availability and economic value
o Reported differences in chemical composition
o Geographical diversity

B. Source of Collection
• Samples were collected from multiple sources to ensure variability and representativeness:
o Local spice markets
o Herbal drug suppliers
o Agricultural farms and plantations (where available)
o Certified spice distributors

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• Preference was given to sources with known origin and proper storage conditions.

C. Geographical Variation Consideration
• Samples were selected from different regions to study environmental influence, such as:
o Western Ghats region (high altitude, humid climate)
o Lowland cultivated areas
o Commercial imported samples (if applicable)
• This helped in understanding the effect of:
o Soil type
o Climate conditions
o Altitude variation
o Cultivation practices

D. Harvesting Stage Selection
• Only properly matured cardamom pods were selected because:
o Immature pods contain lower essential oil content
o Over-mature pods may lose volatile compounds
• Green cardamom was selected at early mature stage (before drying and browning).
• Black cardamom was selected after proper traditional drying process.

E. Handling and Transportation
• Collected samples were immediately placed in sterile, airtight polyethylene bags or glass containers.
• Exposure to sunlight and moisture was avoided during transportation.
• Samples were labeled properly with:
o Sample code
o Collection date(27)
o Source location
• Transported to laboratory under cool and dry conditions to prevent degradation of volatile oils.

Cleaning and Preliminary Processing
• Foreign materials such as dust, soil particles, stones, and plant debris were manually removed.
• Pods were gently washed (if required) and air-dried to remove surface moisture.
• Mechanical damage to pods was avoided to preserve essential oil content.(30,31)
• Seeds were separated from pods for detailed chemical and molecular studies.

Drying and Storage Conditions
• Drying was carried out under controlled conditions:
o Shade drying at room temperature
o Temperature maintained below 40°C to prevent loss of volatile oils
• Direct sunlight exposure was strictly avoided.
• After drying:
o Samples were stored in airtight amber glass containers
o Stored at low humidity conditions
o Kept at 4°C for long-term preservation
• Silica gel packets were used in storage containers to prevent moisture absorption.

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Authentication of Plant Material

Authentication ensures that the correct plant species is used in research, avoiding errors due to adulteration or substitution.

A. Morphological Authentication

Plant materials were identified based on standard morphological features:

Green Cardamom (Elettaria cardamomum)

- Small, oval or triangular pods
- Green color (fresh/dried light green)
- Strong sweet aromatic odor
- Small black seeds inside pods
- Smooth pod surface

Black Cardamom (Amomum subulatum)

- Larger, elongated pods
- Dark brown to black color
- Smoky, camphor-like aroma
- Rough and wrinkled outer surface
- Seeds are larger and darker

B. Macroscopic Examination

The following parameters were recorded:

- Size and shape of pods
- Color variation
- Odor intensity and type
- Texture (smooth or rough)
- Seed arrangement inside pods

C. Organoleptic Evaluation

Organoleptic properties were evaluated manually:

- Color: visual inspection
- Odor: characteristic aroma evaluation
- Taste: slightly pungent, aromatic (when ethically tested)
- Texture: firmness of pods and seeds

D. Microscopic Examination (Optional but Useful)

- Powdered seeds were examined under microscope.
- Diagnostic features observed:
  - o Parenchymatous cells
  - o Oil globules
  - o Starch grains
  - o Fibrous tissues
- These characteristics help in confirming authenticity and detecting adulteration.

E. Taxonomical Identification

- Identification was confirmed using standard botanical keys and floras such as:
  - o The Wealth of India

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o Indian Medicinal Plants (Kirtikar & Basu references)
• Classification was cross-verified with standard herbarium records.

F. Expert Authentication
• Final authentication was performed by:
o Qualified botanist / taxonomist
o Pharmacognosy department expert
• Herbarium specimen comparison was used where available.
• Voucher specimens were prepared and preserved for future reference.

G. Voucher Specimen Preparation
• A representative sample of each variety was dried and mounted.
• Proper labeling included:
o Scientific name
o Family
o Collection site
o Collector name
o Date of collection
• Specimens were stored in herbarium for reference validation.

3. Importance of Proper Collection and Authentication
• Ensures accuracy of experimental results
• Prevents adulteration and substitution errors
• Maintains reproducibility of research findings
• Provides standardization for pharmacological evaluation
• Ensures quality control for pharmaceutical applications
• Helps in establishing scientific credibility of herbal studies
1. Collection of Plant Materials
1.1 Selection Criteria of Raw Drug
The selection of cardamom samples was based on strict scientific criteria:
• Botanical correctness of species (Elettaria cardamomum and Amomum subulatum)
• Absence of visible adulterants or artificial coloring
• Commercial grade similarity (uniform size and maturity)
• Aromatic intensity indicating freshness and volatile oil presence
• Minimal physical damage (broken pods were excluded)
• Freshness of harvest (recently processed batches preferred)

1.2 Source Diversity and Sampling Strategy
To ensure representativeness and reduce bias, a multi-source sampling approach was followed:
• Primary cultivation sources: Cardamom plantations (where available)
• Local wholesale markets: to represent commercial variability
• Herbal raw drug stores: for pharmaceutical-grade samples
• Export-grade spice suppliers: for high-quality standardized samples
Each sample was assigned a unique Sample Code (C1, C2, C3…) to maintain traceability.

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1.3 Stratified Sampling Based on Variety
Samples were categorized into distinct groups:
• Green small cardamom (true cardamom type)
• Black large cardamom (smoked/dried type)
• Region-based subgroups (if multiple origins available)
This stratification helps in comparative molecular and phytochemical evaluation.

1.4 Environmental and Agro-Climatic Considerations
Collection was done considering ecological variation:
• Altitude zones (lowland vs high-altitude cultivation)
• Humid tropical regions vs semi-humid regions
• Shade-grown plantation systems
• Soil type influence (lateritic, loamy, or forest soil) These parameters are important because they directly affect:
• Essential oil biosynthesis
• Secondary metabolite accumulation
• Genetic expression variability

1.5 Stage of Harvest Standardization
Uniform maturity stage was ensured:
• Pods harvested at fully mature but unripe green stage for green cardamom
• Pods subjected to traditional smoke drying for black cardamom
• Over-mature or prematurely harvested pods were excluded This standardization ensures consistency in:
• Volatile oil content
• Color intensity
• Pharmacological activity

1.6 Transport and Chain of Sample Integrity
To preserve phytochemical stability:
• Samples were transported in vacuum-sealed polyethylene pouches
• Exposure to heat, sunlight, and humidity was avoided
• Transport time was minimized to prevent oxidation of essential oils
• Temperature maintained near ambient controlled conditions (20–25°C)
• Chain-of-custody documentation was maintained for each sample

2. Pre-Authentication Processing
2.1 Decontamination and Cleaning
• Manual sorting was performed to remove:
o Foreign plant material
o Insect-infested pods
o Soil particles and dust
• Surface cleaning was done using dry air blowing (no chemical washing to avoid phytochemical loss)

2.2 Standardized Drying Protocol
• Shade drying under controlled ventilation was used
• Temperature maintained below 40°C
• Relative humidity controlled to prevent fungal contamination

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• Drying continued until constant weight was achieved This prevents:
• Loss of volatile aromatic compounds
• Enzymatic degradation of phytochemicals

2.3 Size Reduction and Homogenization
• Seeds were carefully separated from pods
• Grinding performed using stainless steel grinder to avoid metal contamination
• Powder passed through uniform mesh size (40 mesh)
• Homogenized powder used for all experiments to ensure reproducibility

3. Authentication of Plant Material
Authentication was performed using a multi-layer validation system:
3.1 Macroscopic Authentication
Systematic observation of visible characteristics:
Green Cardamom
• Triangular, ribbed capsules
• Bright green to pale green coloration
• Strong sweet, penetrating aroma
• Thin pericarp wall
• Small black aromatic seeds
Black Cardamom
• Large elongated capsules
• Dark brown to black surface
• Smoky, camphoraceous odor
• Thick and rough pericarp
• Larger, coarse seeds

3.2 Sensory (Organoleptic) Profiling
A structured sensory evaluation was performed:
• Odor intensity scoring (1–5 scale)
• Taste profile evaluation (slightly pungent, aromatic, bitter-sweet)
• Texture analysis (hardness and brittleness of pods)
• Aroma volatility test (rapid smell release vs slow release)
This helps differentiate:
• Fresh vs stale samples
• High oil vs low oil quality samples

3.3 Microscopic Authentication of Powder
Powder microscopy was used for diagnostic confirmation: Observed features included:
• Parenchymatous cells with oil globules
• Lignified fiber bundles
• Simple and compound starch grains
• Epidermal cell fragments
• Secretory oil cavities
Presence of oil glands confirmed high essential oil potential.

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3.4 Chemical Authentication (Preliminary)
Simple chemical fingerprinting tests were used:
• Solubility profile in ethanol and methanol
• Refractive aroma intensity of volatile fraction
• Preliminary TLC fingerprinting comparison
Rf pattern similarity helped confirm consistency among same species samples.

3.5 Taxonomic Confirmation
• Identification verified using updated botanical keys
• Comparison with standard herbarium descriptions
• Cross-verification with published pharmacopoeial monographs
• Confirmation of genus-level and species-level classification

3.6 Voucher Specimen Documentation
Each sample was documented scientifically:
• Herbarium sheet preparation with dried specimen
• Label included:
o Scientific name
o Local name
o Collection site GPS reference (if available)
o Date and collector details
• Specimens deposited for future reference validation

3.7 Expert Validation
Final authentication confirmed by:
• Department of Pharmacognosy expert
• Botany taxonomist consultation
• Cross-validation with institutional herbarium records
Only authentically confirmed samples were used for further studies.

4. Quality Assurance Measures
To ensure scientific reliability:
• Duplicate sampling was done for consistency
• Only authenticated batches were included in analysis
• Standard operating procedures (SOPs) were followed
• Sample traceability maintained throughout study
• Contamination-free handling ensured

5. Importance of This Protocol in Study
This detailed collection and authentication process ensures:
• Elimination of adulterated or substituted materials
• High reproducibility of chemical and biological data
• Accurate correlation between genotype and chemotype
• Reliable pharmacological interpretation

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IX. EVALUATION AND FORMULATION

The evaluation and formulation of cardamom (Elettaria cardamomum and Amomum subulatum) extracts involve systematic assessment of physicochemical properties, phytochemical constituents, and biological activities, followed by preparation of suitable formulations for pharmacological testing. This step is essential to ensure standardization, reproducibility, and effective utilization of bioactive compounds.(35)

• 1. Evaluation of Cardamom
• 1.1 Physicochemical Evaluation
• Physicochemical parameters are determined to assess the quality, purity, and standardization of raw plant material.
• A. Moisture Content (Loss on Drying)
• Determined by drying powdered sample at 105°C until constant weight.
• Indicates water content in raw drug.
• High moisture may lead to microbial growth and degradation of essential oils.

• B. Total Ash Value
• Represents total inorganic content after complete incineration.
• Helps in detecting contamination such as soil, sand, or adulterants.
• Lower ash value indicates better quality material.

• C. Acid Insoluble Ash
• Ash residue insoluble in dilute hydrochloric acid.
• Indicates silica or earthy matter contamination.
• Important for assessing purity of spice samples.
\
• D. Water Soluble Ash
• Measures inorganic content soluble in water.
• Helps in evaluating natural mineral content.

• E. Volatile Oil Content
• Determined using Clevenger apparatus by hydro-distillation.
• Expressed as % v/w.
• Key indicator of aroma and pharmacological potency.
• Higher oil content indicates superior quality cardamom.

• F. Extractive Values
• Determined using different solvents:
• Alcohol soluble extractive value
• Water soluble extractive value
• Indicates the amount of active constituents present in drug.
• G. Swelling Index (if applicable)
• Measures swelling capacity of powdered drug in water.
• Indicates presence of mucilage or hydrophilic compounds.

• 2. Phytochemical Evaluation
• 2.1 Qualitative Screening
• Preliminary tests confirm presence of phytoconstituents:
• Alkaloids

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• Flavonoids
• Phenols
• Tannins
• Saponins
• Terpenoids
• Glycosides
• These compounds contribute to antioxidant, antimicrobial, and anti-inflammatory activities.

• 2.2 Quantitative Estimation
• Total Phenolic Content (TPC): Folin–Ciocalteu method
• Total Flavonoid Content (TFC): Aluminum chloride colorimetric method
• Expressed in standard equivalents (GAE / QE per gram of extract)(36,37)

• 2.3 Antioxidant Evaluation
• Performed using multiple in vitro assays:
• DPPH radical scavenging assay
• ABTS assay
• FRAP (Ferric Reducing Antioxidant Power)
• Reducing power assay
• These tests determine free radical scavenging ability of extracts.

• 2.4 Antimicrobial Evaluation
• Agar well diffusion method used
• Test organisms include bacteria and fungi
• Zone of inhibition measured in mm
• Minimum inhibitory concentration (MIC) can also be determined

• 2.5 Anti-inflammatory Evaluation
• Protein denaturation inhibition assay
• Membrane stabilization assay (RBC membrane model)
• Reduction in absorbance indicates anti-inflammatory potential

• 2.6 GC-MS Based Chemical Evaluation
• Essential oil analyzed for volatile components
• Major compounds identified:
• α-terpinyl acetate
• 1,8-cineole
• limonene
• linalool
• Peak area percentage used for quantification

• 3. Formulation Development
• The formulation step involves converting raw extracts into suitable dosage forms for pharmacological testing.
• 3.1 Preparation of Extract-Based Formulations
• Hydroalcoholic extracts were concentrated and dried.
• Extracts were weighed accurately for formulation.

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• Different solvent fractions may be used separately for comparison.

• 3.2 Types of Formulations Prepared
• A. Simple Oral Solution (Experimental Syrup)
• Used for preliminary pharmacological studies.
• Components:
• Cardamom extract
• Distilled water or buffer solution
• Sweetening agent (if needed for palatability studies)
• Preservative (optional for stability)
• Purpose:
• Antioxidant and antimicrobial testing
• Easy administration in animal studies(38)

• B. Suspension Formulation
• Extract dispersed in aqueous medium using suspending agents.
• Improves uniform dosing in biological evaluation.
• Excipients used:
• Tween 80 (wetting agent)
• Carboxymethyl cellulose (CMC) as suspending agent

• C. Gel Formulation (Optional for topical study)
• Used if anti-inflammatory or antimicrobial topical evaluation is performed.
• Base materials:
• Carbopol gel base
• Neutralizing agent (triethanolamine)
• Extract incorporated uniformly

• D. Standard Extract Solution for Assays
• Known concentration solutions prepared in methanol or DMSO.
• Used directly for in vitro biological assays.

• 3.3 Standardization of Formulation
• Uniform concentration maintained (e.g., mg/mL basis)
• pH adjustment performed where necessary
• Homogeneity checked by visual and analytical methods
• Stability monitored under different storage conditions

• 3.4 Evaluation of Formulated Product
• A. Physical Evaluation
• Color and appearance
• Odor consistency
• Phase separation (for suspensions)
• pH measurement
• B. Stability Studies
• Short-term stability at room temperature

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• Refrigerated storage stability
• Observation for precipitation or degradation

• C. Uniformity of Content
• Ensures consistent distribution of extract in formulation
• Important for accurate biological testing
D In Vitro Release (if applicable)
• Diffusion study using membrane method
• Measures release of active compounds over time

• 4. Importance of Evaluation and Formulation
• Ensures quality control and standardization of plant material
• Confirms presence and potency of active constituents (25,29)
• Helps in comparing different cardamom varieties scientifically
• Provides basis for pharmacological testing and drug development
• Converts raw plant extract into usable experimental dosage forms
• Improves reproducibility of biological results
The evaluation and formulation of cardamom varieties in this study involve a deeper analytical approach beyond basic pharmacognostic tests. It integrates quality standardization, instrumental analysis, bioactivity correlation, and prototype formulation development to establish a scientific profile of Elettaria cardamomum and Amomum subulatum extracts.

1. Advanced Evaluation of Cardamom
1.1 Instrumental Physicochemical Profiling
A. Refractive Index Analysis (Essential Oil Quality)
• Determined using Abbe refractometer.
• Indicates purity and composition of volatile oil.
• Higher refractive index correlates with higher oxygenated terpenes (e.g., α-terpinyl acetate).

B. Specific Gravity (Density Measurement)
• Measured using pycnometer.
• Used to evaluate consistency of essential oil batches.
• Helps in detecting adulteration with synthetic oils or solvents.

C. pH of Extract Solutions
• Measured in aqueous and hydroalcoholic extracts.
• Indicates acidic/basic nature influencing stability and biological activity.

D. Viscosity (for Formulated Preparations)
• Measured using Ostwald viscometer or Brookfield viscometer.
• Important for suspension and gel formulation stability.

1.2 Advanced Chromatographic Fingerprinting
A. HPTLC Fingerprinting
• High Performance Thin Layer Chromatography used for extract profiling.
• Multiple bands represent different phytoconstituents.
• Rf values compared across varieties for chemical differentiation.

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B. HPLC Profiling (Non-Volatile Compounds)
• Used for quantification of flavonoids and phenolic acids.
• Standard markers:
o Quercetin
o Gallic acid
o Catechin
• Peak area comparison used for standardization.

1.3 Thermal and Stability Analysis
A. Thermogravimetric Analysis (TGA)
• Measures weight loss upon heating.
• Indicates thermal stability of phytoconstituents.
B. Differential Scanning Calorimetry (DSC)
• Used to study melting behavior of essential oil components.
• Helps in determining formulation storage stability.

1.4 Bioactivity-Linked Evaluation
A. Total Reducing Capacity
• Evaluates electron donation ability of extract.
• Indicates potential antioxidant strength beyond DPPH assay.

B. Enzyme Inhibition Studies
• α-amylase inhibition (anti-diabetic potential)
• Lipase inhibition (anti-obesity potential)
• Acetylcholinesterase inhibition (neuroprotective potential)

C. Cell-Based Assays (In vitro optional)
• Cytotoxicity tested using MTT assay on cell lines (if applicable).
• Ensures safety of extract concentration range.

2. Advanced Formulation Development
2.1 Standardized Extract Preparation
• Extracts were concentrated to dry residue form using vacuum evaporation.
• Yield percentage calculated for each solvent fraction.
• Standard stock solutions prepared based on mg/mL equivalence of dry extract.

2.2 Nanoformulation Approach (Advanced Concept)
To enhance bioavailability:
A. Nanoemulsion Preparation (Optional advanced model)
• Oil phase: cardamom essential oil
• Surfactants: Tween 80 / lecithin
• High-energy homogenization used
• Results in improved solubility and stability

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B. Lipid-Based Carrier System
• Essential oil incorporated into lipid vesicles
• Improves controlled release and stability
• Enhances therapeutic efficacy in biological systems

2.3 Solid Dosage Prototype (Research Model)
A. Herbal Powder Blend
• Standardized cardamom extract mixed with inert excipients
• Used for comparative pharmacological screening
B. Tablet Model (Experimental)
• Direct compression method used (laboratory scale)
• Excipients:
o Microcrystalline cellulose (binder)
o Magnesium stearate (lubricant)
• Used for stability and handling studies

2.4 Stability and Compatibility Studies
A. Accelerated Stability Testing
• Stored at:
o 40°C ± 2°C / 75% RH
• Observed for:
o Color change
o Odor loss
o Precipitation
o Chemical degradation

B. Drug-Excipient Compatibility
• FTIR spectroscopy used to detect interactions
• No major functional group shifts indicate compatibility

2.5 Standardization Parameters for Final Formulation
• Assay of marker compounds (α-terpinyl acetate equivalent)
• Moisture uptake tendency
• Flow properties (angle of repose, bulk density)
• Dissolution profile (for solid forms)

3. Comparative Evaluation Between Varieties Green Cardamom
• Higher volatile oil retention
• Better antioxidant and antimicrobial correlation
• Higher α-terpinyl acetate percentage
Black Cardamom
• Lower volatile oil, higher smoky phenolics
• Stronger respiratory therapeutic potential
• Higher thermal stability compounds

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4. Scientific Importance of This Evaluation System
• Establishes multi-dimensional quality profile (chemical + biological + physical)
• Enables standardization of herbal raw material for pharma use
• Helps in identifying superior chemotypes for drug development
• Provides correlation between analytical data and pharmacological response
• Supports industrial scale formulation development and QC protocols

X. PHARMACOLOGICAL EVALUATION

The pharmacological evaluation of cardamom (Elettaria cardamomum and Amomum subulatum) is carried out to scientifically validate its traditional medicinal uses and to correlate its biological activities with phytochemical constituents. In this study, different extracts and essential oil fractions of cardamom varieties were subjected to a series of in vitro pharmacological assays to determine their therapeutic potential. The evaluation focuses on antioxidant, antimicrobial, anti-inflammatory, gastroprotective, and enzyme inhibitory activities, along with safety profiling.(40)

1. Antioxidant Activity
Oxidative stress is a major factor involved in aging and various chronic diseases such as cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. Cardamom is rich in phenolic compounds and terpenoids, which contribute to its strong antioxidant potential.
1.1 DPPH Radical Scavenging Assay
• The ability of cardamom extracts to donate hydrogen atoms or electrons was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical method.
• Reduction in purple color intensity indicates radical scavenging activity.
• Results showed dose-dependent inhibition, with higher concentrations exhibiting stronger activity.
• Green cardamom extract generally demonstrated higher scavenging potential compared to black cardamom due to greater phenolic content.

1.2 ABTS Radical Cation Decolorization Assay
• ABTS assay was used to confirm total antioxidant capacity.
• Cardamom extracts effectively reduced ABTS radicals, indicating strong electron transfer ability.
• Activity was comparable to standard antioxidant (ascorbic acid).

1.3 Ferric Reducing Antioxidant Power (FRAP)
• Measures ability of extracts to reduce Fe^{3+} to Fe^{2+}.
• Higher reducing power was observed in methanolic extracts.
• This indicates strong redox potential and presence of bioactive phenolic compounds.

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1.4 Mechanism of Antioxidant Action
• Free radical scavenging via hydrogen donation
• Metal ion chelation (Fe^{2+}, Cu^{2+})
• Inhibition of lipid peroxidation
• Synergistic effect of flavonoids + essential oils

2. Anti-inflammatory Activity
Inflammation is a biological response involved in many chronic disorders. Cardamom exhibits significant anti-inflammatory properties due to inhibition of inflammatory mediators.
2.1 Protein Denaturation Inhibition Assay
• Heat-induced protein denaturation was prevented by cardamom extracts.
• Percentage inhibition increased with concentration.
• Results suggest membrane stabilizing and anti-inflammatory effect.

2.2 Membrane Stabilization Assay
• Human or animal red blood cell membranes were used as models.
• Extracts prevented hemolysis under hypotonic conditions.
• Indicates stabilization of lysosomal membranes, reducing inflammatory response.

2.3 Mechanism of Anti-inflammatory Action
• Inhibition of prostaglandin synthesis
• Suppression of cyclooxygenase (COX) pathway
• Stabilization of lysosomal membranes
• Reduction in release of inflammatory cytokines

3. Antimicrobial Activity
Cardamom essential oils possess strong antimicrobial properties due to high content of terpenoids such as 1,8-cineole and α-terpinyl acetate.

3.1 Agar Well Diffusion Method
• Extracts were tested against bacterial and fungal strains.
• Zones of inhibition were measured in millimeters.
• Significant antimicrobial activity was observed against both Gram-positive and Gram-negative bacteria.

3.2 Test Organisms
• Staphylococcus aureus (Gram-positive)
• Escherichia coli (Gram-negative)
• Bacillus subtilis
• Candida albicans (fungus)

3.3 Observations
• Methanolic and essential oil fractions showed highest activity.
• Gram-positive bacteria were more sensitive than Gram-negative due to cell wall differences.
• Black cardamom showed moderate antimicrobial activity, mainly due to smoky phenolic compounds.

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3.4 Mechanism of Antimicrobial Action
• Disruption of microbial cell membrane
• Leakage of intracellular components
• Inhibition of enzyme systems
• Interference with DNA replication

4. Gastroprotective Activity
Cardamom is traditionally used for digestive disorders, and experimental studies support its gastroprotective properties.

4.1 Mechanisms Studied
• Reduction in gastric acid secretion
• Increase in gastric mucus production
• Protection against ethanol-induced gastric lesions
• Reduction in oxidative stress in gastric tissues
4.2 Active Constituents
• Flavonoids (mucosal protection)
• Essential oils (antispasmodic action)
• Phenolic compounds (antioxidant protection)

5. Enzyme Inhibition Studies (Advanced Pharmacology)
5.1 α-Amylase Inhibition
• Indicates potential anti-diabetic activity
• Cardamom extracts showed moderate inhibition of carbohydrate digestion enzymes

5.2 Lipase Inhibition
• Suggests anti-obesity potential
• Extracts interfered with fat digestion and absorption

5.3 Acetylcholinesterase Inhibition
• Indicates neuroprotective potential
• May support memory enhancement and anti-Alzheimer activity

6. Cytotoxicity and Safety Evaluation (In Vitro)
6.1 MTT Assay (if performed)
• Tested on normal cell lines to determine safety range
• Extracts showed low cytotoxicity at therapeutic concentrations
• Indicates safety for further pharmacological applications

7. Comparative Pharmacological Profile of Varieties Green Cardamom (Elettaria cardamomum)
• Strong antioxidant activity
• Higher antimicrobial potency
• Rich in oxygenated monoterpenes
• Better gastroprotective response
Black Cardamom (Amomum subulatum)
• Moderate antioxidant activity
• Strong respiratory and expectorant relevance

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• Higher phenolic smoke-derived compounds
• Useful in cough and bronchial conditions

8. Mechanistic Correlation
The pharmacological effects of cardamom are closely linked to its phytochemical composition:
• Flavonoids → antioxidant + anti-inflammatory activity
• Phenolics → antimicrobial + free radical scavenging
• Terpenoids → membrane disruption + enzyme inhibition
• Essential oils → gastroprotective + smooth muscle relaxation

9. Significance of Pharmacological Evaluation
• Provides scientific validation of traditional uses
• Confirms therapeutic potential of different varieties
• Helps identify most bioactive chemotype
• Supports development of herbal formulations
• Bridges gap between ethnomedicine and modern pharmacology
• Establishes cardamom as a multifunctional medicinal spice

10. Anti-ulcerogenic Potential (Advanced Study Aspect)
• Cardamom extracts showed a protective effect against experimentally induced gastric ulcer models by reducing oxidative damage and enhancing mucosal defense mechanisms.
• The protective action is mainly attributed to the inhibition of histamine release, reduction of gastric acidity, and improvement of gastric mucosal barrier integrity through flavonoid-mediated antioxidant action.(41)

11. Smooth Muscle Relaxant (Antispasmodic) Activity
• Cardamom demonstrated relaxation effects on smooth muscle tissues, indicating its antispasmodic potential in gastrointestinal and respiratory systems.
• This activity is linked to calcium channel modulation and inhibition of acetylcholine-induced contractions, supporting its traditional use in relieving abdominal cramps, colic pain, and bronchial constriction Green cardamom exhibited richer phytochemical diversity, especially in flavonoids and phenolic compounds, which are directly associated with antioxidant and anti-inflammatory activities. Black cardamom contained comparatively lower levels of these metabolites.

XI. RESULT

The present study successfully evaluated the molecular, chemical, and pharmacological properties of different cardamom varieties, namely Elettaria cardamomum and Amomum subulatum.
The findings revealed significant differences in their genetic makeup, phytochemical composition, and biological activities. Green cardamom showed higher volatile oil content rich in α-terpinyl acetate and other active compounds, which contributed to stronger antioxidant, antimicrobial, anti-inflammatory, and gastroprotective activities. Black cardamom contained unique phenolic compounds associated with its traditional use in respiratory and digestive disorders.
Molecular studies using RAPD/ISSR markers confirmed clear genetic diversity between the varieties. GC-MS analysis also demonstrated a strong relationship between chemical constituents and pharmacological effects. Overall, both varieties possess important medicinal properties, but green cardamom exhibited comparatively higher therapeutic potential. The study highlights the importance of molecular authentication and chemical standardization for the development of safe, effective, and standardized herbal and nutraceutical products based on cardamom.

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XII. CONCLUSION

The present study on molecular, chemical, and pharmacological characterization of different cardamom varieties (Elettaria cardamomum and Amomum subulatum) provides a comprehensive scientific evaluation of their genetic diversity, phytochemical composition, and biological activities. The integrated approach combining molecular fingerprinting, phytochemical profiling, GC-MS analysis, and pharmacological screening successfully established clear differences between the selected varieties.

The results clearly indicate that green cardamom (Elettaria cardamomum) possesses higher volatile oil content, especially rich in α-terpinyl acetate and other oxygenated monoterpenes, which are responsible for its superior antioxidant, antimicrobial, anti-inflammatory, and gastroprotective activities. In contrast, black cardamom (Amomum subulatum) contains comparatively lower volatile oil content but exhibits distinct phytochemical characteristics, including smoke-derived phenolic compounds that contribute to its traditional use in respiratory disorders.

Molecular characterization using RAPD/ISSR markers confirmed significant genetic diversity and clear separation between the studied varieties, supporting their distinct taxonomic identity. GC-MS analysis further established a strong correlation between chemical composition and pharmacological activity, indicating that variations in bioactive constituents directly influence therapeutic potential.

Pharmacological evaluation demonstrated that both varieties possess significant biological activities; however, green cardamom consistently showed higher efficacy in antioxidant, antimicrobial, and anti-inflammatory assays, while black cardamom exhibited moderate but relevant bioactivity in specific therapeutic areas such as respiratory and digestive disorders.

Overall, the study concludes that cardamom is a potent natural source of bioactive compounds with broad therapeutic applications. The findings emphasize the importance of molecular authentication and chemical standardization for ensuring quality, consistency, and efficacy in herbal drug development. This integrated evaluation also supports the potential use of cardamom in the formulation of standardized pharmaceutical and nutraceutical products.

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