Antimicrobial Activity and Phytochemical Profiling of *Cinnamomum Zeylanicum* and *Elettaria Cardamomum* against Selected Bacterial Strains

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Abstract: The study was planned to evaluate the phytochemical screening and antimicrobial activity of cinnamon bark powder (*Cinnamomum zeylanicum)* and cardamom (*Elettaria cardamomum*) seed ethanolic extract. Phytochemical profiles of cinnamon bark exhibited the presence of phenols, alkaloids, flavonoids, tannins, and steroids. In contrast, the cardamom seed extract contained phenols, flavonoids, tannins, and steroids, with alkaloids notably absent. These findings contribute to the phytochemical standardization of these commonly used spices and underscore their potential as sources of bioactive compounds for further pharmacological investigation. Screening of selected spices extracts for antimicrobial sensitivity against *Escherichia coli*, Coagulase-negative staphylococcus (CoNS), *Staphylococcus aureus*, Klebsiella species, Proteus species, *Pseudomonas aeruginosa* was carried out. Antimicrobial activity of spices extract were examined by well diffusion technique. Ethanoilc extract of cinnamon powder and cardamom seed showed no antimicrobial activities against all tested microorganisms.

Keywords: Antimicrobial activity, *Cinnamomum zeylanicum*, *Elettaria cardamomum*, Flavonoids

I. INTRODUCTION

Natural products, particularly those derived from plants, have played a vital role in maintaining human health since the earliest records of medicinal practice. Over the past century, plant-derived phytochemicals have served as a critical foundation for pharmaceutical development. The efficacy of traditional medicinal systems is primarily attributed to the biological activities of these natural compounds, which form the basis of many herbal preparations used in the treatment of various human diseases [1]. Herbs, commonly used as food additives, have been employed for centuries not only as flavoring and seasoning agents but also as traditional remedies and natural preservatives to enhance the sensory attributes of food. These botanical substances contribute characteristic taste, aroma, pungency, and color to culinary preparations, thereby stimulating appetite and improving palatability. Beyond their culinary applications, many herbs have been traditionally recognized for their pharmacological properties, including carminative, stomachic, tonic, antispasmodic, and antihelminthic activities [2]. Plants exhibiting anti-pathogenic properties and prebiotic activity have garnered significant interest in recent scientific research due to their potential applications in promoting health and preventing disease. Medicinal plants are a rich source of diverse secondary metabolites, including tannins, terpenoids, alkaloids, flavonoids, phenolic compounds, and quinones. These bioactive constituents have been extensively utilized in traditional medicine systems across the world for the treatment of various diseases and infections [3].

Phytochemicals are the primary bioactive compounds responsible for the medicinal properties of many potent medicinal plants. The pharmacological efficacy of these plants is largely determined by the specific phytoconstituents they contain [4]. Numerous studies have reported that various spices exhibit significant antimicrobial activity [5]. As a result, spices are commonly utilized in regions with warmer climates, where the prevalence of infectious diseases tends to be higher. The use of herbs and spices as antimicrobial agents has been an integral part of traditional medical practices across diverse cultures worldwide. The antimicrobial activities of various dietary spices are attributed to the presence of numerous naturally occurring bioactive compounds [6]. Plant-derived therapeutics are generally considered to be less toxic and associated with fewer adverse effects compared to their synthetic counterparts [7]. A growing body of evidence has demonstrated that many spices possess antioxidant, anti-inflammatory, and immunomodulatory properties, which may contribute to their potential role in both the prevention and treatment of various cancers, including breast, lung, cervical, and prostate cancers [8]. Cinnamon (*Cinnamomum zeylanicum*), widely recognized for its culinary and therapeutic uses, has been utilized in food preparations and traditional medicine since ancient times, particularly by Egyptian and Chinese civilizations. Approximately 250 species within the cinnamon genus have been identified globally. Cinnamon is commonly employed as a flavoring agent and is known for its pharmacological properties, including antimicrobial and antioxidant activities, as well as its role as a natural food preservative. Medicinally, cinnamon has been used in the management of conditions such as diarrhea, flatulent dyspepsia, influenza, cough, bronchitis, angina, palpitations, and infections, and it has demonstrated efficacy in lowering blood glucose levels in individuals with diabetes. Additionally, cinnamon exhibits anti-inflammatory, antifungal, insecticidal, and anticancer properties, making it a valuable natural therapeutic agent [9]. Various parts of the *C. zeylanicum* tree contain distinct profiles of bioactive compounds. The leaves are rich in eugenol (70–95%) with lower concentrations of cinnamaldehyde (1–5%), whereas the bark predominantly contains cinnamaldehyde (65–80%) along with lesser amounts of eugenol (5–10%) [10]. Studies have demonstrated that the antimicrobial activity of

cinnamaldehyde is primarily attributed to its lipophilic nature, characteristic of terpenoids and phenylpropanoids. This lipophilicity facilitates the compound's ability to penetrate microbial cell membranes, allowing it to access intracellular targets and disrupt essential enzymatic systems of the microorganisms, ultimately leading to cell death [11]. Evidence suggests that *C. zeylanicum* is well-tolerated and exhibits minimal or no adverse effects, supporting its potential role as a safe adjunctive therapy in the improvement of patient health [12].

Cardamom (*Elettaria cardamomum*) is a perennial herbaceous plant belonging to the family Zingiberaceae. It is characterized by subterranean rhizomes that give rise to multiple leafy shoots and flowering panicles. India is recognized as the largest global producer of cardamom. The volatile oil extracted from cardamom seeds is composed of a complex mixture of terpenoids and other aromatic compounds. Major constituents include 1,8-cineole (36.3%) and α-terpinyl acetate (31.3%), followed by limonene (11.6%), linalool (3.0%), trans-nerolidol (2.7%), α-terpineol (2.6%), linalyl acetate (2.5%), and sabinene (2.8%). Other components present in smaller quantities include α-pinene (1.5%), myrcene (1.6%), terpinen-4-ol (0.9%), β-pinene (0.2%), α-phellandrene (0.2%), γ-terpinene (0.7%), terpinolene (0.5%), citronellol (0.3%), nerol (0.5%), geraniol (0.5%), and methyl eugenol (0.2%). This diverse phytochemical profile underpins the pharmacological activities attributed to cardamom essential oil [13]. *E. cardamomum* exhibits a wide range of potential therapeutic applications. It has been traditionally utilized for its stomachic and diuretic properties, as well as for its reported abortifacient activity. Additionally, cardamom possesses notable antimicrobial, antifungal, and antiviral properties. It has been used in the management of various gastrointestinal disorders, including constipation, colic, diarrhea, dyspepsia, and vomiting, as well as in the treatment of neurological conditions such as headache and epilepsy. Furthermore, it has shown potential benefits in addressing cardiovascular disturbances [14]. From the previous evidence, the present study was aimed to evaluate the antimicrobial activity of *C. zeylanicum* and *E. cardamomum* on selected bacterial species.

II. MATERIALS AND METHODS

Plant materials

The dried bark of *C. zeylanicum* and *E. cardamomum* dry fruits were collected locally and were authenticated by a botanist.

Preparation of the extract

Cinnamon bark samples were thoroughly washed with deionized water and air-dried under direct sunlight for two days. The dried barks were subsequently oven-dried at 40 °C for 30 minutes and ground into a fine powder using a mechanical grinder. To prepare the extracts, 10 g of cinnamon powder was mixed with 50 mL of either absolute ethanol or methanol in a 250 mL Erlenmeyer flask. The flasks were sealed with aluminum foil and incubated on a rotary shaker at room temperature for 48 hours. After incubation, the mixtures were centrifuged at 3,500 rpm for 20 minutes, and the supernatants were filtered through Whatman No. 1 filter paper. The resulting filtrates were evaporated in a dry oven at 40 °C until a semi-solid mass was obtained, which was further dried in a crucible at 45 °C to yield the final extract. The dried extracts were stored at -20 °C until further use. Prior to application, both methanol and ethanol extracts were reconstituted in dimethyl sulfoxide (DMSO) to enhance solubility and cellular permeability [15]. The dried fruits of *E. cardamomum* were thoroughly washed under running tap water to remove surface dust and epiphytic contaminants, followed by a final rinse with sterilized distilled water. The cleaned samples were air-dried on sterile filter paper at room temperature and subsequently ground into a fine powder using a sterilized mortar and pestle under aseptic conditions. The resulting powder was then subjected to extraction using both aqueous and organic solvents [16].

Determination of Moisture content

The moisture content of the powdered samples was determined using the Dean and Stark distillation method. Accurately weighed 5 g of each powdered sample was transferred into a clean round-bottom flask. Sufficient xylene was added to the flask to completely cover the sample. The flask was then assembled with a Dean and Stark apparatus connected to a water condenser. The setup was heated using a heating mantle to initiate co-distillation. As the temperature increased, moisture present in the sample co-distilled with xylene, condensed through the water condenser, and was collected in the graduated receiver tube of the apparatus. Heating was continued until the water level in the receiver remained constant, indicating complete removal of moisture. The volume of water collected was recorded, and the moisture content of the sample was calculated.

Determination of volatile oil content

The volatile oil content of the powdered sample was determined by hydro-distillation using a Clevenger-type apparatus, following standard protocols. Accurately weighed 5 g of powdered plant material was transferred into a round-bottom flask fitted with a Clevenger apparatus and connected to a water condenser. A total of 100 mL of distilled water was added to the flask. The mixture was subjected to continuous boiling, during which the steam carried the volatile oil components into the condenser. The resulting distillate was collected in the graduated arm of the Clevenger apparatus, where the aqueous phase was automatically separated and returned to the boiling flask, while the oil layer accumulated in the calibrated tube. The distillation was continued until no further increase in the oil volume was observed. The volume of volatile oil obtained was recorded directly from the graduated tube and expressed as a percentage relative to the weight of the original sample.

Determination of water-soluble and alcohol-soluble extractive values

Accurately weighed 5 g of powdered sample was macerated with 100 mL of distilled water and 100 mL ethanol in a closed conical flask for 24 hours for water soluble extractive and alcohol soluble extractive value respectively, with occasional shaking during the first 6 hours. The mixture was filtered, and 25 mL of the filtrate was evaporated to dryness in a pre-weighed dish. The residue was dried at 105 °C to constant weight, and the percentage of water-soluble and alcohol-soluble extractive was calculated with reference to the air-dried sample.

Phytochemical screening

Phytochemical analysis of the sample extract was carried out to test for the presence of steroids, phenols, flavonoids, alkaloids, and tannins as per standard protocols [17-18].

Test for steroids (Salkowki test)

About 5 g of crude extract of the samples was mixed with 2 mL of chloroform, followed by the addition of concentrated H2SO4 to form two distinct layers. The upper layer was turns into red and sulphuric acid layer showed yellow with green fluorescence. It indicates the presence of steroids.

Test for phenols (Ferric chloride test)

To 0.5 mL of the sample, a few drops of 0.5% neutral ferric chloride solution were added. The development of a dark green coloration indicated the presence of phenolic compounds.

Test for flavonoids (Ammonia test)

Ammonia solution was added (1:5) in a test tube containing 1 mL of the sample, followed by the addition of concentrated sulphuric acid. The appearance of a yellow coloration that fades upon standing indicates a positive test for flavonoids.

Test for alkaloids (Mayer's test)

Addition of Mayer's reagent to 0.5 mL of sample yields a white or creamy precipitate, which indicates the presence of alkaloids.

Test for tannins (Ferric chloride test)

1 mL of extract was boiled with 5 mL distilled water, followed by addition of 0.1% ferric chloride, yielding a brownish green or blue-black color confirms the presence of tannins.

Microorganisms

Standard strains of microorganisms used in the present study (*Escherichia coli*, Coagulase-negative staphylococcus (CoNS), *Staphylococcus aureus*, Klebsiella species, Proteus species, *Pseudomonas aeruginosa*) were obtained from culture collection of Public Health Laboratory, Thiruvananthapuram. The bacterial cultures were maintained on nutrient agar slants at 4°C under refrigerated conditions. Prior to antimicrobial susceptibility testing, each strain was reactivated by inoculating into sterile nutrient broth and incubating overnight at 37°C to achieve optimal growth.

Determination of antimicrobial activity

Antimicrobial activity of sample extracts was determined by agar well diffusion method [19]. 0.1 mL of freshly cultured test organisms was uniformly spread onto the surface of sterile Mueller-Hinton agar plates using a sterile spreader. Wells were prepared in the agar using a sterile gel puncture, with five wells made per plate. Each well was loaded with 50 µL of ethanolic and aqueous plant extract. Amoxyclav discs (30 µg/disc) were employed against Gram-negative bacterial isolates, while ciprofloxacin discs (5 µg/disc) were used for Gram-positive bacterial cultures. The plates were left undisturbed at room temperature for a brief period to allow for pre-diffusion of the extracts into the medium, followed by incubation at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition surrounding each well was measured to assess antimicrobial activity.

III. RESULTS

Percentage of moisture content and volatile oil content

The physico-chemical parameters analyzed included moisture content and volatile oil content (Table 1). The moisture content of cinnamon was determined to be 18%, whereas that of cardamom seeds was 4%. The volatile oil content was found to be 2% for cinnamon and 5% for cardamom, respectively.

Table 1. Physicochemical Parameters of *Cinnamomum zeylanicum* (Cinnamon) and *Elettaria cardamomum* (Cardamom)

Parameter	Cinnamon (%)	Cardamom (%)
Moisture Content	18	4
Volatile Oil	2	5

Phytochemical analysis

The results of phytochemical screening revealed the presence of various phytochemical constituents including phenols, alkaloids, flavonoids, tannins, and steroids in the extract of cinnamon bark, whereas the seed extract of *E. cardamomum* showed the presence of flavonoids, tannins, steroids, and phenolic compounds (Table 2). Alkaloids were not detected in the cardamom seed extract.

Table 2. Phytochemical Screening of *Cinnamomum zeylanicum* (Cinnamon) and *Elettaria cardamomum* (Cardamom) Extracts

Phytochemical Tested	Cinnamon	Cardomom
Phenols	Present	Present
Alkaloids	Present	Absent
Flavonoids	Present	Present
Tannins	Present	Present
Steroids	Present	Present

Antimicrobial activity

A qualitative well diffusion method was performed to evaluate the antimicrobial activity of *C. zeylanicum* and *E. cardamomum* against selected bacterial strains. The antimicrobial potential of the cinnamon bark extract was evaluated against six bacterial strains: *E. coli*, Klebsiella spp., *P. aeruginosa*, Proteus spp., *S. aureus*, and CoNS. The methanolic extract of cinnamon bark exhibited no inhibitory activity against any of the tested organisms, as evidenced by the absence of inhibition zones (Figure 1). This indicates that the extract, at the concentration used in the study, was ineffective in suppressing the growth of both Gram-negative and Gram-positive bacteria.

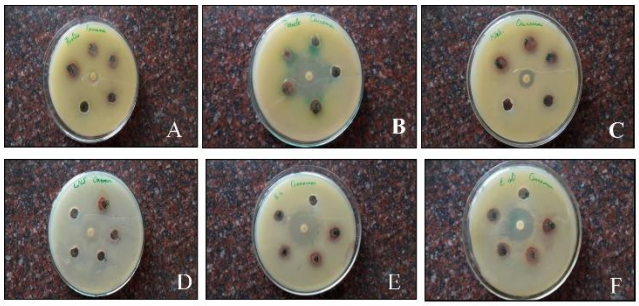


Figure 1. Antimicrobial sensitivity test of Cinnamon bark extract against standard bacterial strains
A) Proteus spp., B) *P. aeruginosa* C) Klebsiella spp., D) CoNS E) *S.aureus* F) *E.coli*

To validate the sensitivity of the test organisms, standard antibiotic discs were used as positive controls. Amoxyclav (30 µg/disc), targeting Gram-negative bacteria, produced distinct zones of inhibition: 26 mm for E. coli, 15 mm for Klebsiella spp., 10 mm for Proteus spp., and 38 mm for *Pseudomonas aeruginosa*. For Gram-positive bacteria, ciprofloxacin (5 µg/disc) exhibited a zone of inhibition of 28 mm against S. aureus and 19 mm against CoNS (Table 3). These results confirmed the susceptibility of the tested strains to conventional antibiotics, while demonstrating complete resistance to the cinnamon bark extract under the conditions employed.

Table 3. Antimicrobial activity of Cinnamon bark extract against selected Bacterial strains

Microorganisms	Zone of Inhibition					Positive control	
	MD (mm)	1 (mm)	2 (mm)	3 (mm)	Negative Control	Amoxyclav (30µg/disc)	Ciprofloxacin (5µg/disc)
E.coli	R	R	R	R	R	--	26mm
Klebsiella Species	R	R	R	R	R	--	15mm
Proteus Species	R	R	R	R	R	--	10mm
Pseudomonas aeruginosa	R	R	R	R	R	--	38mm
S. aureus	R	R	R	R	R	28mm	-
CoNS	R	R	R	R	R	19mm	-

'R' -Resistant

The antimicrobial efficacy of the alcoholic extract of cardamom seeds exhibited no inhibitory activity against any of the tested organisms (Figure 2), indicating complete resistance of all strains to the concentration of extract used in this study.

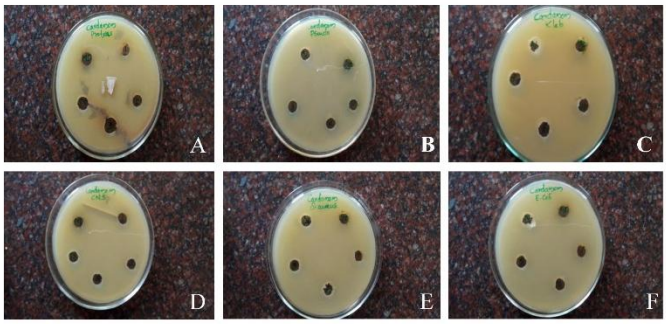


Figure 2. Antimicrobial sensitivity test of Cardamom seed extract against standard bacterial strains
A) Proteus spp., B) *P. aeruginosa* C) Klebsiella spp., D) CoNS E) *S.aureus* F) *E.coli*

To confirm the responsiveness of the bacterial isolates, standard antibiotic discs were employed as positive controls. Amoxyclav (30 µg/disc), tested against Gram-negative bacteria, produced clear zones of inhibition measuring 26 mm for *E. coli*, 15 mm for Klebsiella spp., 10 mm for Proteus spp., and 38 mm for *Pseudomonas aeruginosa*. For Gram-positive organisms, ciprofloxacin (5 µg/disc) demonstrated inhibition zones of 28 mm against *S. aureus* and 19 mm against CoNS (Table 4). These results confirm the sensitivity of the test organisms to conventional antibiotics and their resistance to the cardamom seed extract under the experimental conditions applied.

Table 4. Antimicrobial activity of cardamom seed extract against selected Bacterial strains

Microorganisms	Zone of Inhibition					Positive control	
	MD (mm)	1 (mm)	2 (mm)	3 (mm)	Negative Control	Amoxyclav (30µg/disc)	Ciprofloxacin (5µg/disc)
E.coli	R	R	R	R	R	--	26mm
Klebsiella Species	R	R	R	R	R	--	15mm
Proteus Species	R	R	R	R	R	--	10mm
Pseudomonas aeruginosa	R	R	R	R	R	--	38mm
S. aureus	R	R	R	R	R	28mm	-
CoNS	R	R	R	R	R	19mm	-

IV. DISCUSSION

Spices have been integral to the cultural and medicinal practices of various regions since ancient times, serving diverse roles as natural dyes, flavoring agents, preservatives, and therapeutic compounds. The bioactivity of these spices is primarily attributed to the presence of phytochemicals with pharmacological potential. Among them, *C. zeylanicum* is widely recognized not only as a culinary ingredient but also for its medicinal properties. In traditional Ayurvedic medicine, cinnamon has been utilized for the management of respiratory and gastrointestinal disorders, reflecting its longstanding application in herbal therapeutics. The traditional use and scientific evaluation of *E. cardamomum* have gained considerable attention due to their demonstrated anti-inflammatory properties. Numerous studies have reported significant anti-inflammatory effects associated with bioactive compounds present in these plants, supporting their ethnopharmacological relevance and potential therapeutic applications.

The moisture content of cinnamon bark was found to be higher (18%) compared to *E. cardamomum* seeds (4%), indicating a lower water retention capacity in the latter. In contrast, the volatile oil content was greater in cardamom seeds (5%) than in cinnamon bark (2%), suggesting a higher concentration of essential oil constituents in the seed matrix. This difference may be attributed to the inherent anatomical and phytochemical composition of seeds, which are often richer in oil than bark tissues. Furthermore, the solubility profile of the two spices differed with respect to solvent polarity. Cardamom demonstrated greater solubility in alcohol, whereas cinnamon exhibited higher solubility in water. This variation may be due to the presence of a higher proportion of alcohol-soluble phytoconstituents and relatively fewer

water-soluble components in cardamom seed extracts. These observations are consistent with the findings reported by Philip *et al*., (2011), who highlighted the influence of phytochemical composition on solvent-specific solubility behavior [20]. Phytochemical screening of the methanolic extract of cinnamon bark revealed the presence of phenols, alkaloids, flavonoids, tannins, and steroids. These findings are consistent with those reported by Abdullah, 2013, who also documented a similar phytochemical profile in cinnamon bark extracts [21]. In contrast, the methanolic extract of *E. cardamomum* seeds showed the presence of phenols, flavonoids, tannins, and steroids, while alkaloids were absent. This observation aligns with the findings of Harborne, 1967, who reported the presence of phenolic and flavonoid compounds and the absence of carbohydrates, glycosides, and alkaloids in cardamom seed extracts [22]. Flavonoids are a diverse group of polyphenolic compounds known for their extensive biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, which contribute significantly to human health and the prevention of various chronic diseases [23]. Tannins, another class of polyphenols, exhibit cytotoxic effects and function as potent free radical scavengers. Their ability to neutralize oxidative stress highlights their potential therapeutic role in managing degenerative conditions such as cancer, atherosclerosis, and age-related disorders [24]. Alkaloids, characterized by their nitrogen-containing structures, are pharmacologically active compounds widely utilized in the development of life-saving medications. Their therapeutic applications span a range of conditions, including hypertension, cardiovascular diseases, and certain forms of cancer, owing to their broad spectrum of biological activities.

The antimicrobial activity of alcoholic extracts derived from the bark of *C. zeylanicum* and the seeds of *E. cardamomum* was evaluated against six clinically significant bacterial strains, encompassing both Gram-positive and Gram-negative organisms. Neither extract exhibited measurable antimicrobial activity against any of the tested strains. In contrast, standard antibiotics, amoxyclav and ciprofloxacin produced clear zones of inhibition, confirming the susceptibility of the bacterial strains to conventional antimicrobial agents. The absence of antibacterial activity in the plant extracts may be attributed to several factors, including the low susceptibility of the test organisms to the phytoconstituents, the nature of the solvent used for extraction, and the potential inadequacy of the extract concentration. It is also possible that the bioactive compounds responsible for antimicrobial effects are present in insufficient quantities in the alcoholic extracts or may require alternative extraction methods to be effectively isolated and active. These findings suggest the need for further investigation into optimized extraction protocols, higher extract concentrations, or alternative solvents to fully assess the antimicrobial potential of these medicinal plants.

V. CONCLUSION

The overall findings of this study indicate that the selected spices *C. zeylanicum* and *E. cardamomum* contain a range of phytochemical constituents, including phenols, flavonoids, steroids, tannins, and alkaloids (absent in cardamom). Despite the presence of these bioactive compounds, the alcoholic extracts of both spices exhibited no antimicrobial activity against the tested bacterial strains. This lack of efficacy may be attributed to the low susceptibility of the microorganisms to the phytochemicals present, suboptimal extract concentrations, or limitations related to the extraction solvent. These results highlight the importance of optimizing extraction methods and concentrations, as well as the need for further studies to evaluate the antimicrobial potential of individual phytoconstituents.

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