ANTIMICROBIAL POTENTIAL OF FRUIT EXTRACTS OF *ELETTARIA CARDAMOMUM* MATON (CHHOTI ELAICHI) AGAINST THE PATHOGENS CAUSING EAR INFECTION

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Summary

The antimicrobial activity of *Elettaria cardamomum* fruit extracts were tested by agar well diffusion method against the six ear pathogens causing ear infection namely, *Staphylococcus aureus, Proteus mirabilis, Escherchia coli, Pseudomonas aeruginosa, Acinetobacter* sp. and *Candida albicans* in different solvents. Organic fruit extracts displayed antimicrobial activity against five tested ear pathogens and aqueous extracts were unable to exhibit any antimicrobial activity. The highest antimicrobial activity of *E. cardamomum* fruit was found against *S.aureus* (19.3mm) in acetonic extract with an MIC of 25 mg/ml. This study reveals that the organic fruit extract of *E. cardamomum* showed good antimicrobial activity and can be used for developing novel herbal ear drop.

Keywords: *Elettaria cardamomum*, ear infection, antimicrobial activity, minimum inhibitory concentration

Introduction

Ear infection is amongst the most common diseases encountered in medical practice today, affecting people of all ages from neonate to geriatric groups [1]. It is mainly caused by *Pseudomonas aeruginosa, Staphylococcus aureus, S. epidermidis, Streptococcus pneumoniae, Escherichia coli, Proteus mirabilis, Aspergillus niger, A. fumigatus, A. flavus, Candida albicans* [2,3,4,5].

An increase in multi drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases which has triggered immense interest in the search of new drugs or preparations from the natural sources including plants. Antimicrobial activity of medicinal plants is widely spread and a large number of its secondary metabolites showed antimicrobial activity. There is a great structural diversity exist among antimicrobial phytocompounds. Major groups of phytocompounds include alkaloids, anthraquinones, cardiac glycosides, saponins, tannins and polyphenols [6,7,8].

Elettaria cardamomum Maton, commonly called choti elaichi or Queen of all spices (family Zingiberaceae), is a perennial shrub with thick, fleshy and lateral roots which can grow to a height of 8 feet [9]. This shrub natively belongs to India and Srilanka and is commercially cultivated in southern India, Sri Lanka, Tanzania and Guatemala [10,11].

Leaves of *E. cardamomum* are lanceolate, green or dark green, glabrous on both surfaces with acuminate apex. The flowers are borne on panicles and they emerge directly from the underground stem on long floral stalks and appear white or pale green in colour. The fruit are tri-ocular, ovoid, oblong or greenish-brown capsules containing about 15-20 reddish brown seeds, which are covered by aril [10,12]. Fruits and seeds are economically important parts of *E. cardamomum*. The seed contain essential oil, which is strongly dependent on storage conditions, with an average yield from 2 to 5%.The seeds and essential oil are used as flavouring components in a variety of foods, including alcoholic and non-alcoholic beverages, frozen desserts, candies, baked goods, puddings, condiments, gravies and meat products [13,12].

E. cardamomum was traditionally used in various gastrointestinal, cardiovascular and neural disorders. It is used as a powerful aromatic, carminative, diuretic and stimulant. In India, it is used for many conditions, including asthma, bronchitis, kidney stones, anorexia and general debility, urinary tract disorders. Studies have revealed its use as an effective skin penetration enhancer for certain actives, as an anti-carcinogenetic agent, anti-ulcerogenic agent and anti-microbial and anti-convulsant agent[14,15,12].There have already been a number of studies on the chemical and antimicrobial properties of *E. cardamomum* [10,11,12,15].However, to the best of our knowledge, this is the first report on the antimicrobial activity of different solvent extracts of *E. cardamomum* fruits on the pathogens causing ear infection. Therefore, the objective of this research was to validate the antimicrobial potential of *E. cardamomum* fruit extracts against the bacterial and fungal pathogens causing ear infection, isolated from the local patients of Kurukshetra, with a view of searching a herbal extract as a remedy for treating ear infections and other resistant microorganisms.

Material and methods

Plant collection

The fruits of *Elettaria cardamomum* were obtained from the local market of Kurukshetra, Haryana. The taxonomic identity of this plant was confirmed by Dr. B.D. Vashishta of Botany Department, Kurukshetra University, Kurukshetra.

Extraction of plant material

The samples were carefully washed under running tap water followed by sterile distilled water and air dried at room temperature (40^0C) for 4-5 days and then homogenized to a fine powder using a sterilized mixer grinder and stored in air tight bottles. Four different solvents namely ethanol, methanol, acetone and aqueous (hot and cold) were used for extraction. A 10 g amount of homogenized fruit was separately soaked in conical flasks each containing100 ml of acetone, ethanol, methanol (95%) and sterile distilled water. Also the same amount (i.e. 10 g) of homogenized fruit was immersed separately in 100 ml of hot sterile distilled water in conical flasks and allowed to stand for 30 min on a waterbath with occasional shaking followed by keeping all the flasks on rotary shaker at 200 rpm for 24 h [16,17,18]. Each preparation was filtered through a sterilized Whatman No. 1 filter paper and finally concentrated to dryness under vacuum at 40^0C using rotaevaporator. The dried extract thus obtained was sterilized by overnight UV-irradiation and checked for sterility on nutrient agar plates and stored at 4^0C in labelled sterile bottles until further use [19, 20].

Test microorganisms

Five bacteria namely *Staphylococcus aureus* (HM626197)*(Gram-positive), *Acinetobacter* sp. (HM626198), *Proteus mirabilis* (HM626199), *Escherchia coli* (HM626200),

Pseudomonas aeruginosa (HM626201) (Gram-negative) and one yeast, *Candida albicans* used in this study, were isolated from the patients having ear infection from the local ENT clinics of Kurukshetra [21]. Bacterial strains were identified on the basis of staining, biochemical and molecular characteristics (16S rRNA sequencing) [22] and yeast on the basis of staining, morphological and cultural characteristics [23,24]. The bacterial isolates were subcultured on Nutrient agar and yeast on Malt yeast agar and incubated aerobically at 37^{o}C.The media were procured from Hi Media Laboratory Pvt. Ltd., Bombay, India. **(* Nucleotide sequence of all the five bacteria have been submitted to GenBank database, which have provided GenBank accession number, HM626197-HM626201).**

Ear drops

Three commonly prescribed ear drops by otolaryngologist, two allopathic ciplox (antibacterial), candid (antifungal), and a herbal ear drop bilwa tel (antimicrobial), used in this study, were procured from the local market of Kurukshetra.

Screening for antimicrobial activity

The acetone, methanol, ethanol, hot and cold aqueous *E. cardamomum* fruit extracts were used for evaluation of the antimicrobial activity by the agar well diffusion method [25, 26]. In this method, pure isolate of each microbe was subcultured on the agar media plates at 37^{0}C for 24 h. One plate of each microorganism was taken and a minimum of four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10^{6} cfu/ml (standardized by 0.5McFarland standard) and used as the inoculum for performing agar well diffusion assay. One hundred microlitre (100µl) of inoculum of each test organism was spread onto the agar plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells of 8mm were made with a sterile borer in the inoculated agar plates and the lower portion of each well was sealed with a little specific molten agar medium. The dried extracts were reconstituted in 20% dimethylsulphoxide (DMSO) for the bioassay analysis [27]. A 100µl volume of each extract was propelled directly into the wells (in triplicates) of the inoculated agar plates for each test organism. The plates were allowed to stand for 1hr for diffusion of the extract into the agar and incubated at 37^{O}C for 24h [28,19]. Sterile DMSO (20%) served as the negative control and ciplox (for bacteria), candid (for fungi) and Bilwa tel (antimicrobial) ear drop served as the positive control. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 8mm [29]. The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with ± standard deviation were calculated.

Determination of minimum inhibitory concentration (MIC)

MIC for each test organism was determined by following the modified agar well diffusion method. A twofold serial dilution of each extract was prepared by first reconstituting the dried extract (100 mg/ml) in 20% DMSO followed by dilution in sterile distilled water (1:1) to achieve a decreasing concentration range of 50mg/ml to 0.39mg/ml. A 100 µl volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100µl of standardized inoculum (10^{6} cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37^{o}C for 24 hrs and observed for the inhibition zones. MIC, taken as the lowest concentration of the test extract that completely inhibited the growth of the microbe, showed by a clear zone of inhibition (>8mm), was recorded for each test organism [19,30,31,26].

Results

The antimicrobial activity of *Elettaria cardamomum* fruit extracts on the agar plates varied with the type of solvent used for extraction and the microorganisms tested for susceptibility assay. Positive controls produced significantly sized inhibition zones against the tested bacteria and yeast, however, negative control produced no observable inhibitory effect against any of the test organism as shown in Table 1.

The present study reveals that all the three organic solvents (ethanol, methanol and acetone) extracts of fruit possessed antimicrobial activity against the five tested ear pathogens while organic fruit extracts did not exhibit any activity against the bacterium, *P.aeruginosa.* However, the aqueous extracts, both hot and cold of *E. cardamomum* fruit lacked antimicrobial activity against all the tested ear pathogens.

The acetonic fruit extract was found to be most effective against *Staphylococcus aureus* (19.3mm) followed by *Proteus mirabilis* (18.6mm), *Acinetobacter* sp (17.6mm), *Escherchia coli* (15.6) and *Candida albicans* (15.3).The inhibiton zones produced by the ethanolic and methanolic extracts against *S. aureus* ranged between 18.3mm and 17.6 mm. *S. aureus* was found to be most sensitive pathogens which survived upto lowest concentration of 12.5 mg/ml of all the organic extracts (Table 2), thus having an MIC of 25 mg/ml. The inhibition zone produced by the ethanolic and methanolic fruit extracts against *P. mirabilis* and *Acitenobacter* sp. ranged between 15mm and 16mm. *P. mirabilis* and *Acitenobacter* sp. were found to be moderately effective as they survived up to 25mg/ml (methanolic and ethanolic extracts), thus having an MIC of 50 mg/ml. The zone of inhibition produced by the ethanolic and methanolic fruit extracts against *E.coli* and *C.albicans* was almost equal and ranged between 13mm and 14mm, thus having an MIC of 50mg/ml.

Table 1. Antimicrobial activity of *Elettaria cardamomum* fruit extracts on ear pathogens determined by agar well diffusion method.

Solvent extracts (mg/ml)	**Diameter of growth of inhibition zones (mm)**					
	Staphylococcus Aureus	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Acitenobacter* sp.	*Escherchia coli*	*Candida albicans*
Methanol	17.6[a]±0.57[b]	15.6±0.57	-	16.3±0.57	14±0	13.6±0.57
Ethanol	18.3±0.57	16.3±0.57	-	16±0	14.6±0.57	14.3±0.57
Acetone	19.3±0.57	18.6±0.57	-	17.6±0.57	15.6±0.57	15.3±0.57
Hot Aqueous	-	-	-	-	-	-
Cold aqueous	-	-	-	-	-	-
DMSO	0	0	0	0	0	0
Ciplox ear drop	56.3±0.57	46.3±0.57	34±0	32.6±0.57	36±0	nt
Bilva tel ear drop	13.6±0.57	-	-	11.6±0.57	-	-
Candid ear drop	nt	nt	nt	nt	nt	21.3±0.57

- No activity, nt = not tested, [a] Values, including diameter of the well (8mm), are means of three replicates [b]± Standard deviation

Table 2. MIC of *Elettaria cardamomum* fruit extracts on ear pathogens determined by modified agar well diffusion method

Solvent extracts	**MIC (mg/ml)**				
	Staphylococcus aureus	*Proteus mirabilis*	*Escherchia coli*	*Acitenobacter* sp.	*Candida albicans*
Methanol	25	50	50	50	50
Ethanol	25	50	50	50	50
Acetone	25	25	50	25	50

Discussion

E. cardamomum is widely used in various parts of the world's traditional medicine system and it has been used in India since ancient times [15]. The antimicrobial potential of this plant extracted in different solvents (eg aqueous, methanol, ethanol, acetone, chloroform, hexane, ethyl acetate, diethyl ether) had been evaluated against different bacterial and fungal human pathogens and had reported variable activities in different parts, seeds, pods, fruits in different solvents [11,26,13,10].

In our study, the organic fruit extracts of *E. cardamomum* displayed good to moderate activity against the five ear pathogens namely *Staphylococcus aureus, , Proteus mirabilis*, *E. coli, Candida albicans, Acitenobacter* sp. and did not exhibit any activity against *Pseudomonas aeruginosa*. Among the tested microorganisms, *S.aureus* was found to be most sensitive ear pathogen against all the tested organic extracts. The antimicrobial activity of fruit extracts against the tested ear pathogens may be due to the presence of secondary metabolites mainly, terpenoids belonging to the class of monoterpenes (linalyl acetate, nerol, neryl acetate, geranyl acetate, geraniol, citronellol, linalool, cisocimene and methylheptenone) and sesquiterpenes (t-caryophyllene, valencene, Nerolidol, farnesol)[15].

The aqueous fruit extracts of *E. cardamomum* lacked antifungal and antibacterial activity. The absence of antimicrobial activity in the aqueous extracts might either be due to the polarity of antimicrobial compounds make them more readily extracted by organic solvents as compared to aqueous extract or active compound may be present in insufficient amount in the crude extract to show activity with the dose level employed and lastly, if the active principle is present in high quantities, there could be other constituents present in the extract exerting antagonistic effects of the bioactive compounds[32,33].

It may, therefore, be concluded from the above investigation that the crude extracts obtained from the fruits of the *E. cardamomum* may be used to treat the bacterial and fungal ear infections. Out of the three organic extract tested, acetonic extract has been found to be best in inhibiting the growth of ear pathogens. At last, the need of the hour is to perform more and more screening of the natural products or plant parts as such screening experiments form a primary platform for further phytochemical and pharmacological studies that may open the possibilities of finding new clinically effective antifungal and antibacterial compounds against the ear pathogens and the resistant bacterial and fungal pathogens.

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