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Antimicrobial Activity of *Amomum subulatum* and *Elettaria cardamomum* Against Dental Caries Causing Microorganisms

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Abstract

The *in vitro* antimicrobial activity of *Amomum subulatum* and *Elettaria cardamomum* fruit extracts were studied against *Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Candida albicans* and *Saccharomyces cerevisiae*. The acetone, ethanol and methanol extracts of the selected plants exhibited antimicrobial activity against all tested microorganism except *L. acidophilus*. The most susceptible microorganism was *S.aureus* followed by *S.mutans, S.cerevisiae* and *C.albicans* in case of *Amomum subulatum* while in the case of *Elettaria cardamomum; S.aureus* was followed by *C.albicans, S. cerevisiae* and *S.mutans*. The largest mean zone of inhibition was obtained with the ethanolic extract of *A. subulatum* and acetonic extract of *E.cardamomum* against *Staphylococcus aureus* (16.32mm and 20.96mm respectively). Minimum inhibitory concentrations (MIC) of the extracts were also determined against the four selected microorganisms showing zones of inhibition ≥10mm. This study depicts that ethanol and acetone extracts of fruits of *Amomum subulatum* and *Elettaria cardamomum* can be used as a potential source of novel antimicrobial agents used to cure dental caries.

Keywords: Dental caries, *Amomum subulatum, Elettaria cardamomum*, zone of inhibition, minimum inhibitory concentration.

Introduction

Dental caries is a very common problem that affects all age groups. It is a process in which the enamel and the dentine are demineralised by acids produced by bacterial fermentation of carbohydrates (de Soet and de Graaff, 1998). In real life, it is the most common infectious disease affecting human beings (Balakrishnan *et al*., 2000). Medicinal plants since ancient times have been employed for prophylactic and curative purposes (Amadi *et al*., 2007). This study reports the antimicrobial effects of *Amomum subulatum* (Badi elaichi) and *Elettaria cardamomum* (Chhoti elaichi) on dental caries causing microorganisms.

The large cardamom (*Amomum subulatum* Roxb., family Zingiberaceae) is one of the major cash crops cultivated between elevations of 600 and 2000 m in tropical wet evergreen forests of Eastern Himalayas in India (Sikkim and Darjeeling areas), Nepal and Bhutan. The fruit is a trilocular, reddish brown to dark pink, many seeded capsule and seeds contain 2–3% essential oils, which possess medicinal properties and are used as adjuncts to various medicinal preparations (Gupta *et al*., 1984; Sinu and Shivanna, 2007; Hussain *et al*., 2009). The seeds have properties similar to those of true cardamom (*Elettaria cardamomum*) but are much larger in size and are used as a condiment for culinary and other preparations. *Amomum* seeds are used as spices and their plant parts are used in traditional medicine for curing toothache, dysentery, diarrhoea, rheumatism, vomiting, dyspepsia and lung diseases (Dutta *et al*., 2000; Sabulal *et al*., 2006).

The small cardamom *Elettaria cardamomum* also belongs to family Zingiberaceae and is historically known as the "Queen of all Spices". It grows in the understory of tropical rain forests at elevations of 762-1524m, where it rains about 381cm per year. It is cultivated commercially in India, Sri Lanka, Guatemala and Tanzania. The fruits are thin walled, smooth skinned, oblong, green capsules containing 15-20 aromatic reddish brown seeds. The seeds contain a volatile oil, used for flavouring cakes, curries, bread and other culinary purposes, like flavouring coffee and confectionery. It was traditionally used in various gastrointestinal, cardiovascular and neural disorders (Arora and Kaur, 2007; Dhulap *et al*., 2008). Studies have revealed its use as an effective skin penetration enhancer for certain drugs, anticarcinogenetic agent, anti ulcerogenic agent and anti microbial and anti convulsant agent (Dhulap *et al*., 2008).

Materials and Methods

Amomum subulatum and *Elettaria cardamomum* fruits were obtained from the local market of Delhi, India (February, 2009). The plants were identified and authenticated taxonomically by a botanist of the Department of Botany, Kurukshetra University, Kurukshetra. The samples were carefully washed under running tap water followed by sterile distilled water and then air dried for two days, pounded using a mixer grinder and stored in airtight bottles.

Extraction of plant material

Four different solvents namely ethanol, methanol, acetone and water (at two different temperatures hot and cold) were used for extraction.

Cold aqueous extraction: Ten grams of fruit powder was soaked in 100ml cold sterile distilled water in a conical flask and left undisturbed for 24h, then filtered off using a sterile Whatman filter paper no1 (Ogundiya *et al*., 2006). The filtered extract was concentrated under vacuum below 40ºC using a rotaevaporator (Heidolph, VE-11). The weight of the solid residue was recorded and taken as the yield of crude extract (Bag *et al*., 2009).

Hot aqueous extraction: Hot aqueous extract was prepared by boiling 10g of fruit powder in 100ml of sterile distilled water for 30min and kept undisturbed in a conical flask for 24h. The other steps were same as followed in the case of cold aqueous extract.

Organic solvent extraction: Ten grams of fruit powder was kept in 70% methanol, ethanol and acetone for 3 consecutive days at room temperature and then filtered followed by concentrating under vacuum using the rotaevaporator (Bag *et al*., 2009).

The extracts thus obtained were stored in labelled sterile bottles and kept in the freezer at 4ºC until further use for the screening of antimicrobial activity. The extracts were reconstituted in 20% DMSO for the bioassay analysis (Rajasekaran *et al*., 2008).

Test Microorganisms

Three dental caries causing bacteria *Streptococcus mutans* (MTCC*497), *Staphylococcus aureus* (MTCC 740), *Lactobacillus acidophilus* (MTCC *447) and two yeasts *Candida albicans* (MTCC 227) and *Saccharomyces cervisiae* (MTCC 170) were procured from Microbial Type Culture Collection, IMTECH, Chandigarh. The microorganisms were subcultured on different media such as *S.mutans* on Brain heart infusion agar, *S.aureus* on Nutrient agar, *L.acidophilus* on Lactobacillus MRS agar, *C.albicans* and *S.cerevisiae* on Malt yeast agar (Himedia Laboratory Pvt. Ltd., Bombay) and incubated aerobically at 37^{O}C.

Antimicrobial assay

The antimicrobial activity was tested using agar well diffusion method. Agar plates were swabbed with 100µl of the respective broth cultures (1.5×10^{8}CFU/ml, standardized by 0.5 Mac Farland) and were kept at room temperature for 15 min for absorption to take place. Wells of 8mm size were made with sterile borer in the inoculated agar plates and loaded with 100µl of plant extracts. DMSO was used as a negative control whereas Ciprofloxacin (bacteria) & Amphotericin-B (yeast) were used as positive control. Prior to incubation at 37ºC for 24 hrs, the Petri dishes were kept at room temperature for 15min in order to promote diffusion of the extracts into the agar (Khokra *et al.*, 2008; Rios *et al*., 1988). All the tests were made in triplicate and the mean diameter of the inhibition zones in millimetre and the standard deviation was calculated.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined only for the three organic solvent extracts which showed positive antimicrobial activity against four tested microorganisms by modified agar well diffusion method (Okeke *et al*., 2001). In this technique, a twofold serial dilution of the extracts was prepared by first reconstituting in 20% dimethylsulphoxide (DMSO). They were diluted in sterile distilled water to achieve a decreasing concentration range of 10mg/ml to 0.04mg/ml. A 100 µl volume of each dilution was introduced in wells in the respective agar plates already seeded with the standardized inoculum (1.5×10^{8} cfu/ml) of the test microbial strain. All the test plates were incubated at 37ºC for 24 hrs. MIC was considered the lowest concentration of the *A.subulatum* and *E.cardamomum* fruit extracts that showed visible zone of inhibition (Nkere and Iroegbu, 2005).

Results and Discussion

We chose *Streptococcus mutans, Lactobacillus acidophilus* and *Candida albicans* as test microorganisms for our study because they have been implicated in dental caries (Lee *et al.,* 2004; Joshi and Joshi, 2005). *C.albicans* is the most common yeast isolated from the oral cavity, and is associated with fungal oral infections, endocarditis and septicemia (Bagg, 1999). *Staphylococcus aureus* a major human pathogen responsible for a number of hospital – acquired infections propagates mainly in mouth and hands in the hospital environment (Knighton, 1960; Lowy, 1998; Piochi and Zelante, 1975). We also chose *Saccharomyces cerevisiae* as a test organism for our study. While it is considered to be an opportunistic pathogen in the oral cavity, it may induce significant oral risks by acting as a tertiary colonizer in dental caries.

The results of antimicrobial activity of the five extracts of *Amomum subulatum* and *Elettaria cardamomum* by agar well diffusion method have been shown in Table 1 and Table 2 respectively. From the present data it is evident that the acetonic, methanolic and ethanolic extracts of *A.subulatum* showed antimicrobial inhibitory activity against two bacteria *S.mutans* and *S.aureus* and two fungi *C.albicans* and *S.cerevisiae*, with the mean of the highest zone of inhibition being 16.32mm and MIC of 2.5mg/ml (Table 3), shown by ethanolic extract of *A.subulatum* against *S.aureus* (Figure 1). The hot and cold water extracts of *A.subulatum* did not show any inhibitory effect against any of the five microorganisms tested. Similarly the acetonic, methanolic and ethanolic extracts of *E.cardamomum* showed inhibitory activity against *S.mutans, S.aureus, C.albicans* and *S.cerevisiae* with the mean of the highest zone of inhibition being 20.96mm and MIC of 1.25mg/ml (Table 4), shown by acetonic extract of *E.cardamomum* against *S.aureus*. The cold water extract of *E.cardomomum* showed antimicrobial activity against *S.mutans* and *S.aureus* while the hot water extract showed no activity at all.

The ethanol and acetone extracts of fruits of *Amomum subulatum* and *Elettaria cardamomum* showed greater antimicrobial activity than the corresponding water and methanolic extracts. This finding is interesting, because in the traditional method of treating a microbial infection, decoction of the plant parts or boiling the plant in water was employed. Whereas, according to the present study, preparing an extract with an organic solvent (acetone and ethanol) shows a better antimicrobial activity, in accordance with the results obtained by Nair *et al*. (2005).

Conclusion

Since the ethanol and acetone extracts of fruits of *Amomum subulatum* and *Elettaria cardamomum* were effective against the tested dental caries causing microorganisms, purification and toxicological studies of the plant and *in vivo* trials should be carried out so that it can be used as a potential source for the development of a phytomedicine to act against dental caries causing microbes. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration.

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Table 1: Antimicrobial activity of fruit extracts of *Amomum subulatum* on the test organisms.

Amomum subulatum extracts (mg/ml)	Diameter of growth of inhibition zones (mm)				
	Streptococcus mutans	*Staphylococcus aureus*	*Lactobacillus acidophilus*	*Candida albicans*	*Saccharomyces cerevisiae*
Acetone	12.31[a]±0.57[b]	16±0	-	29.30±1.15	16.65±0.57
Methanol	12.94±1	14.31±0.57	-	18.96±1	17.65±0.57
Ethanol	14.95±1	14.65±0.57	-	11.64±0.57	18.32±0.57
Hot water	10.64±0.57	14±0	-	-	-
Cold water	10±0	10.31±0.57	-	-	-
Ciprofloxacin (5 µg/ml)	27.32±0.57	34.66±0.57	25.65±0.57	nt	nt
Amphotericin B (100 units/ ml)	nt	nt	nt	13±0	11.94±1
DMSO	-	-	-	-	-

(-) = no activity, nt = not tested

[a] Values, including diameter of the well (8 mm), are means of three replicates

[b] ± Standard deviation

Table 2: Antimicrobial activity of fruit extracts of *Elettaria cardamomum* on the test organisms.

***Elettaria cardamomum* extracts (mg/ml)**	**Diameter of growth of inhibition zones (mm)**				
	Streptococcus mutans	***Staphylococcus aureus***	***Lactobacillus acidophilus***	***Candida albicans***	***Saccharomyces cerevisiae***
Acetone	12.94[a]±1[b]	20.96±1	-	13.31±0.57	11.94±1
Methanol	10.93±1	13.31±0.57	-	14.31±1	13.95±1
Ethanol	11.94±1	18.65±0.57	-	15±0	12.94±1
Hot water	-	-	-	-	-
Cold water	10.93±1	10.58±1.15	-	-	-
Ciprofloxacin (5 µg/ml)	27.32±0.57	34.66±0.57	25.65±0.57	nt	nt
Amphotericin B (100 units/ ml)	nt	nt	nt	13±0	11.94±1
DMSO	-	-	-	-	-

(-) = no activity, nt = not tested

[a] Values, including diameter of the well (8 mm), are means of three replicates

[b] ± Standard deviation

Table 3: MIC of fruit extracts of *Amomum subulatum.*

***Amomum subulatum* fruit extracts**	**MIC (mg/ml)**				
	Streptococcus mutans	***Staphylococcus aureus***	***Lactobacillus acidophilus***	***Candida albicans***	***Saccharomyces cerevisiae***
Acetone	5	2.5	nt	5	5
Methanol	5	2.5	nt	5	5
Ethanol	5	2.5	nt	5	5

(-) = no activity, nt = not tested

Table 4: MIC of fruit extracts of *Elettaria cardamomum.*

***Elettaria cardamomum* fruit extracts**	**MIC (mg/ml)**				
	Streptococcus mutans	***Staphylococcus aureus***	***Lactobacillus acidophilus***	***Candida albicans***	***Saccharomyces cerevisiae***
Acetone	5	1.25	nt	2.5	5
Methanol	5	1.25	nt	2.5	5
Ethanol	5	1.25	nt	2.5	5

(-) = no activity, nt = not tested

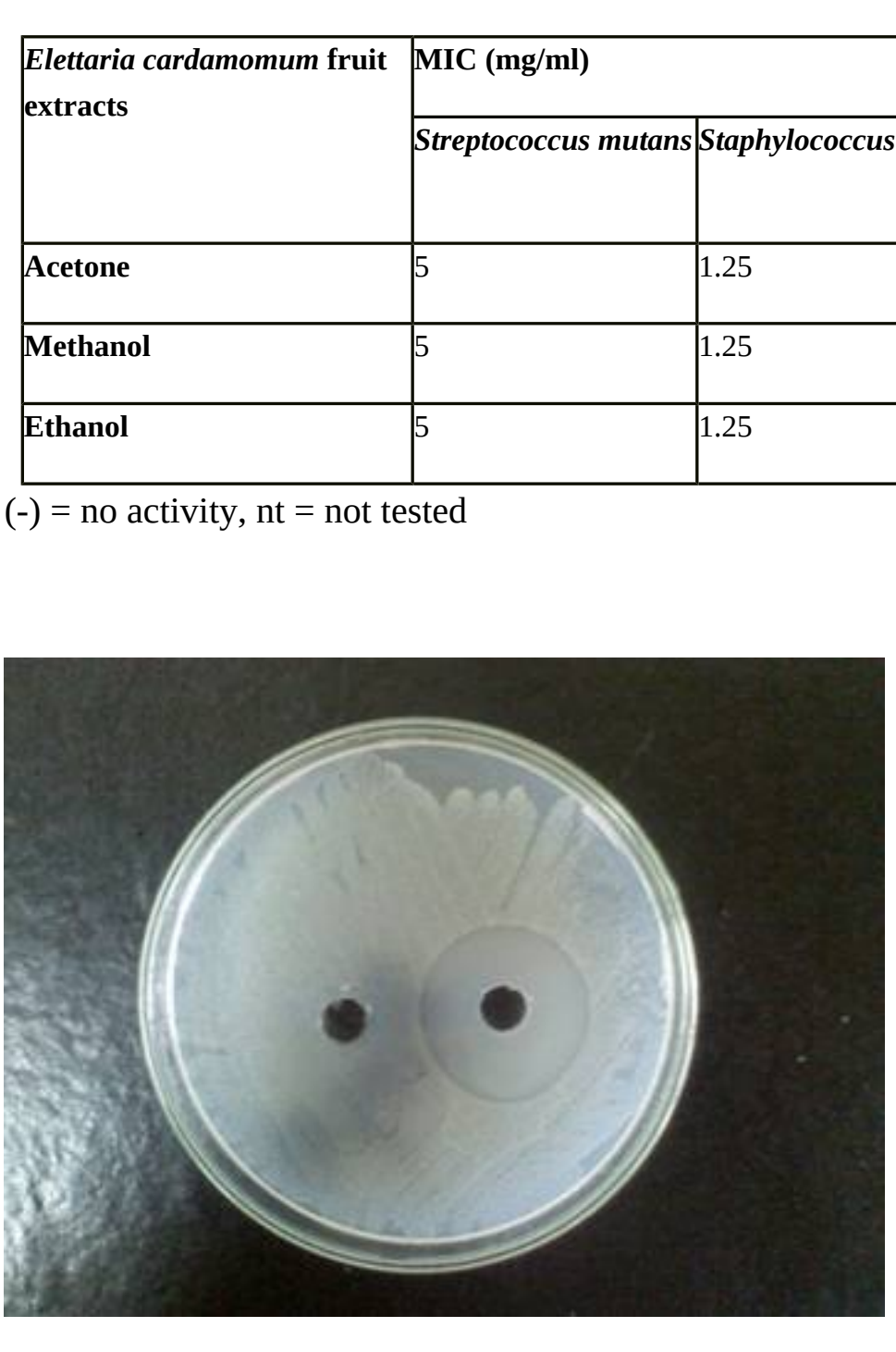

Figure 1: Zone of inhibition shown *Elettaria cardamomum* fruit acetone extract against *Staphylococcus aureus* and negative control (DMSO).

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