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Research Article

Antimicrobial Activity of Clove and Ginger Powder Extracts on *Streptococcus mutans*

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Abstract: The aim of present work is to evaluate the antimicrobial activity of clove and ginger powder extracts on Streptococcus Mutans. An in-vitro study was conducted to assess effectiveness of 5%, 10%, and 50% clove and ginger powder extracts on Streptococcus mutans. The ditch plate method was used to test the antimicrobial activity. Ditches were prepared on blood agar plates with the help of punch having 6-mm diameter. The plates were left for 1 hr at room temperature and then incubated at 37°C for 48 hours and examined for zone of inhibition. There was no zone of inhibition observed with 5% clove and ginger powder extracts. There was significant difference in mean diameter of zone of inhibition of 10% and 50% clove and ginger extract. Results showed that both clove and ginger powder extracts had antimicrobial activity against streptococcus mutans, while antimicrobial activity was significantly higher in clove aqueous extract than ginger aqueous extract.
Keywords: streptococcus mutans, clove buds, ginger, extracts, zone of inhibition, antimicrobial activity.

INTRODUCTION

Dental diseases are recognized as major public health problems throughout the world. Numerous epidemiological studies showed that tooth decay is the most common affliction of mankind [1]. Dental caries is one of the most common human diseases that affect the vast majority of individuals. Hence, there is an urgent need to promote traditional preventive measures that are acceptable, easily available, and cost effective[2].

Herbs and spices have been found to reduce inflammation, protect against infection, helps to detoxify the liver and cleanse the lungs and other organs and also protect from cell damage that can lead to rheumatoid arthritis, osteoporosis, heart disease and other degenerative diseases[3].

The use of herbs is the most ancient approach to healing known. The herbal medicines may be in form of powders, liquids, or mixtures, which may be raw or boiled, ointments, liniments, and incisions. Roots, barks, and leaves of various plants are employed in ethnomedicine [4].

Cloves are used in Indian Ayurvedic medicine, Chinese medicine, and western herbalism and dentistry where the essential oil is used as an anodyne (painkiller) for dental emergencies. Cloves are the aromatic flower buds of a tree in the family Myrtaceae, Syzygium aromaticu. Clove is one of the most valuable spices that has been used for centuries as food preservative and for many medicinal purposes[5].

Ginger or ginger root is the rhizome of the plant Zingiber officinale, consumed as a delicacy, medicine, or spice. Ginger is one of the most commonly consumed dietary condiments in the world[6].

As clove and ginger both are used in Indian context in cities, as well as in villages. Both are easily available and consumed on daily basis by Indian people. Although many studies have been conducted to explore medicinal uses of the clove and ginger, still there is grey area in relation to research pertaining to streptococcus mutans species. So we undertook this study to check the effect of clove bud extract and ginger extract on streptococcus mutans.

MATERIAL AND METHODS

The clove buds and ginger rhizomes were purchased from the market of Vadodara city.

Preparation of extracts

100 gm of each of clove buds and ginger rhizomes were used in the experiment. After drying properly, they were cut into small pieces and ground to

powder in a ball mill. The weighted powder i.e. 5 gm, 10 gm and 50 gm was kept separately in sterile, dry screw-capped bottles, which were stored in a dry cool place for one week before aqueous extraction. 10 ml of sterile water was added to each bottle of powder. The extracts were allowed to soak for 48 hours before the mixtures were centrifuged at 2,000 rpm for 10 minutes. The supernatants were passed through a 0.45 mm membrane filter; the extracts were prepared at 5, 10 and 50 % concentrations (v/v) and stored in 5 ml portions at 20°C.

Micro-organism

The test miro-organism S Mutans was obtained from Institute of Microbial Technology, Chandigarh, India.

Preparation of Culture Media

S Mutans was added to nutrient broth and then sub-cultured onto nutrient agar plate and incubated anaerobically at 37^0 for 24 hours. The inoculum for antimicrobial activity was prepared by adjusting the density of organism to approximately 10^8 colony forming units/ml with the help of 0.5 Mcfurland opacity standards. Lastly it was inoculated on blood agar plate by lawn culture method.

Antimicrobial susceptibility testing

The ditch plate method was used to test the antimicrobial activity. Ditches were prepared on blood agar plates with the help of punch having 6-mm diameter. On each petri dish, four ditches were made and labeled for various concentrations of clove buds and ginger rhizomes extract. 50 micro-litres each of 5%, 10% and 50% clove and ginger extracts were introduced into equal sized ditches made on petri dishes. Sterile distilled water was used as control. The plates were left for 1 hr at room temperature and then incubated at 37°C for 48 hours and examined for zone of inhibition. The average of those zones was recorded in millimeters.

Statistical analysis

The antimicrobial activity indicated by an inhibition zone surrounding the ditch containing the clove buds and ginger rhizomes extract was recorded if the zone of inhibition. The experiments were performed 4 times and the mean values of the diameter of inhibition zones with ± standard deviations were calculated. The significant mean differences of diameter of inhibition zones between two extract was analyzed by using unpaired t test. Statistical Package for Social Sciences (SPSS version 17, SPSS Inc., Chicago) was used for analysis. $P < 0.05$ was taken as statistically significant.

RESULTS

The inhibition zone of S. mutans for two extracts were observed at various concentrations (5%, 10% and 50%) for 24 h incubation period. There was no zone of inhibition observed with 5% concentration for both clove buds and ginger rhizomes extract. There was a statistical significant result ($P < 0.05$) when the mean diameter of inhibition zones for clove buds and ginger rhizomes extract were compared at concentration of 10% and 50%.

Table-1: Effect of various concentrations of clove bud extracts on streptococcus mutans

Concentration	Zone of inhibition (mm)				Mean ±SD
	Z_1	Z_2	Z_3	Z_4	
5%	0	0	0	0	0
10%	2.2	1.8	1.9	2.2	2.02±.20
50%	4.2	4	4	4.3	4.12 ± 0.19

Table-2: Effect of various concentrations of ginger extracts on streptococcus mutans

Concentration	Zone of inhibition (mm)				Mean ±SD
	Z_1	Z_2	Z_3	Z_4	
5%	0	0	0	0	0
10%	0.6	0.5	0.5	0.7	0.57 ± 0.09
50%	2	2.1	2.2	2	2.07 ± 0.09

Table- 3: Comparison of mean zone of inhibitions of various concentrations of CLOVE and Ginger aqueous extract

Concentrations	Clove extract Mean ± SD	Ginger extract Mean ± SD	t-value (df)	p-value
5%	0	0	0	
10%	2.02±.20	0.57 ± 0.09	13.22 (6)	0.00001 (S)
50%	4.12 ± 0.19	2.07 ± 0.09	19.50 (6)	0.0001 (S)

S = Significant df = degree of freedom

level of significane p-value <0.05

DISCUSSION

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects[7].

An attempt has been made to enrich the knowledge of antibacterial activity of 5%, 10%, and 50% crude extract of clove buds and ginger rhizomes on S Mutans. Through the extensive literature review it has been concluded the beneficial aspects of plant derived drugs as good source of antibiotics, antioxidants and anti-inflammatory agents[8,9].

In the present study maximum antimicrobial activity of CLOVE extract with mean zone of inhibition of 4.12 mm ± 0.19 mm was found at 50% concentration. Our results are in line with the findings of the study conducted by Rahim et al [10].

The antimicrobial activities of clove have been proved against several bacteria and fungal strains. Sofia et al. tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove. The only sampled that showed complete bactericidal effect against all the food-borne pathogens tested Escherichia coli (E. coli), Staphylococcus aureus and Bacillus cereus was the aqueous extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action[11].

Several constituents of clove has been identified, mainly eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone, acetyleugenol, alpha-humulene, methyl salicylate, isoeugenol, methyleugenol, phenyl propanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid. The main constituents of essential oil are phenylpropanoides such as carvacrol, thymol, eugenol and cinnamaldehyde[12-13].

We found that 10% ginger extract inhibited the growth of streptococcus mutans which is also confirmation of the findings of the study conducted by Giriraju A et al. [14]. The difference observed could be attributed to variations in the quality of ginger used, differences in the microbiological techniques used. "Spicy to the tongue, yet soothing to the digestive tract" are how herbalist Steven Foster describes the rhizome (or root) that's been prized for more than 4000 years. From its use in breads baked by ancient Greeks to ginger and spicy cuisine, ginger (Z. officinale) is a popular flavoring agent. The active compounds contained in ginger are divided into two groups: volatile essential oils and fragrant or harsh phenol compounds [15].

The results of present study have provided the justification for therapeutic potential of spices. The practice of using spices as supplementary or alternative medicine in developing countries like India will not reduce only the clinical burden of drug resistance development but also the side effects and cost of the treatment with allopathic medicine. Further clinical evaluation of spices in vivo experiments is required to be carried for low cost treatment with few side effects and for prevention of oral diseases.

Comparison of the degrees of inhibition of the various botanical extracts from different studies cannot be exactly justifiable, since the experiments were performed on different extracts (ie aqueous, ethanolic extracts) and not on pure compounds. Further experiments with a narrower range may be proved useful in determining the effective concentrations of the clove and ginger extracts.

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