Assessment of antimicrobial potential of 10% ginger extract against Streptococcus mutans, Candida albicans, and Enterococcus faecalis: An in vitro study

Anjan Giriraju, GY Yunus

ABSTRACT

Background: Streptococcus mutans, Candida albicans, and Enterococcus faecalis are the three oral microorganisms most commonly implicated in the causation of oral infections. All these oral microorganisms have shown resistant to routinely used antimicrobials. There is a need for an antimicrobial agent which is effective, safe, and economical. Zingiber officinale, commonly known as ginger is one such plant product which has been used from ancient time. It has been shown to possess promising inhibitory effect on many of the oral microorganisms. On review of dental literature, there was scarcity of studies which had tried to assess antimicrobial potential of ginger extract against S. mutans, E. faecalis, and C. albicans; hence, the present study was designed.

Aim: To evaluate the in vitro antimicrobial potential of 10% ginger extract against S. mutans, E. faecalis, and C. albicans.

Settings and Design: Laboratory setting and experimental design.

Materials and Methods: In the first part of the study, 10% ethanolic ginger extract was prepared in the laboratory of Pharmacy College. It was then subjected to microbiological assay to determine its zone of inhibition using Agar disk diffusion test and minimum inhibitory concentration (MIC) using serial broth dilution method against S. mutans, C. albicans, and E. faecalis.

Results: 10% ethanolic ginger extract showed: (a) Maximum zone of inhibition of 8 mm, 14 mm, and 11 mm against S. mutans, C. albicans, and E. faecalis respectively. (b) MIC of 1.25%, 2.5%, and 2.5% against S. mutans, C. albicans, and E. faecalis respectively.

Conclusion: 10% ethanolic ginger extract was found to possess antimicrobial potential against all the three pathogens used in the study.

Key words: Antimicrobial potential, 10% ethanolic ginger extract, Zingiber officinale

Streptococcus mutans (causative organism for dental caries), Candida albicans (causative organisms for candidiasis), and Enterococcus faecalis (causative agent for secondary root canal infection) have been considered very difficult to control as they have developed tolerance against various antimicrobial agents in routine use. This calls for an urgent need to explore novel bio-active compounds, which are safer and biodegradable. Ginger (Zingiber officinale) one such medicinal plant is having antimicrobial property against various human pathogens; however, less data is available on its antimicrobial potential against oral pathogen. Hence present study is an attempt to explore the antimicrobial potential of ginger against S. mutans, C. albicans and E. faecalis.

MATERIALS AND METHODS

Phytochemical standardization

Macroscopic characters

Color and appearance: Yellowish brown or light brown. Peeled rhizome with buff external surface showing longitudinal striations and occasional loose fibers, outer surface dark brown and, more or less covered with cork which shows conspicuous, narrow, longitudinal, and transverse ridges.

- Odor: Aromatic
- Taste: Pungent
Tests for extraneous material:
1. Foreign matter: <1.0%
2. Sand and silica: Absent
3. Insect infestation: Nil
4. Rodent contamination: Nil.

Physico-chemical analysis:
1. Acid content: <0.0% w/w
2. Acid insoluble ash: <1.0% w/w
3. Moisture content: <12.0% w/w
4. Volatile oil content: 2.0% w/w.

Alcohol soluble extractive value >6.0% w/w.
Water soluble extractive value >14.0% w/w.

Successive extractive value:
1. Petroleum ether extractive value: 2.2% w/w
2. Chloroform extractive value: 3.9% w/w
3. Methanol extractive value: 3.3% w/w.

Identification of crude drug by high performance thin layer chromatography
- Sample: Z. officinale crude drug [gingerol]
- Solvent system: n-hexane:ether = 40:60
- Sample preparation: The powdered sample extracted with chloroform. The extracted concentrated in chloroform.
- Detection: Vanillin sulfuric acid
- Densitometer scan: 254 nm
- Inference: 6 - gingerol - 1.2% w/w by high performance thin layer chromatography.

Preparation of ethanolic ginger extract
First 500 g of fresh ginger was procured from the local market and was cleaned using the distilled water. The cleaned ginger was then minced into fine pieces and was suspended in a sterile jar containing 1000 ml of 70% ethanol. The suspended minced ginger in steriele jar was then subjected to process of cold maceration (continuous shaking at constant intervals of time) for 48 h. After 48 h, the suspended minced ginger was subjected to the process of filtration using sterile muslin cloth. During the filtration process, residue of ginger and the filtrate was obtained. The filtrate so obtained was placed over steam bath apparatus for 5 days to facilitate evaporation of ethanol content from the filtrate. After 5 days, dried extract (total yield was 25 g) was obtained, which was pulverized into fine powder using a mortar and pestle.

Preparation of 10% stock solution of ethanolic ginger extract
10 grams of 10% ethanolic ginger extract powder was dissolved in 100 ml of Dimethyl sulfoxide (an inert solvent) to obtain 10% ethanolic ginger extract.

Microbiological tests
The stock solution of 10% ethanolic ginger extract obtained was subjected to microbiological tests namely disk diffusion test (to determine zone of inhibition) and Serial broth dilution test (to determine minimum inhibitory concentration [MIC]) in order to determine the antimicrobial potential against S. mutans, E. faecalis, and C. albicans.

The standard strains of the organisms used in the study were S. mutans (ATCC 25175), C. albicans (ATCC 2091), and E. faecalis (ATCC 35550). Brain Heart Infusion (BHI) broth, Sabouraud Dextrose broth, sterile MIC tubes, Micropipettes and 10% stock solution of 10% ethanolic ginger extract were other instruments used in the study.

Determination of zone of inhibition using 10% ethanolic ginger extract
Agar disk diffusion method was used to determine zone of inhibition. Various volumes of 10% concentration ginger extract measuring 75 µl, 50 µl, 25 µl, 10 µl, and 5 µl were tested. Interpretation of diffusion results were carried out by noting the presence or absence of zone of inhibition around the wells.

Determination of MIC using ethanolic ginger extract
Procedure: Revival of organisms: The selected bacterial and candida strains were revived by plating on blood agar medium. After overnight incubation at 37°C, isolated colonies were selected and identities of the organisms were confirmed. Isolated colonies were then transferred to sterile BHI broth and Sabouraud dextrose broth for the bacterial and Candida strains respectively and once again incubated overnight. The growth concentration was adjusted to 10⁷ organisms/ml by using 0.5 McFarland’s turbidity standard.[3]

An ethanolic solution of 10% concentration was prepared from the ginger powder as the stock solution. A volume of 200 µl of the BHI broth was added into each of the ten MIC tubes per bacterial strain. For the Candida strain, 200 µl of the Sabouraud’s broth was added in each of the ten MIC tubes. In the first MIC tube containing 200 µl broth, 200 µl of stock solution was added. After mixing well, 200 µl was transferred to the second MIC tube. This was continued till the last (10th tube). From the last tube, 200 µl final solutions were discarded. By following this serial dilution, the concentration of ginger powder achieved was 10%, 5%, 2.5%, 1.25%, 0.62%, 0.31%, 0.15%, 0.07%, 0.03%, and 0.01% respectively.

To each of the 10 such prepared MIC tubes with various concentrations, 200 µl of the earlier prepared strains of S. mutans was added such that the final volume per tube was 400 µl. The procedure was repeated for the E. faecalis and C. albicans strains. The tube was then incubated for 24 h at 35°C.
After the incubation, the MIC values were determined by visual inspection of the tubes. In each series of tubes, the last tube with clear supernatant was considered to be without any growth and taken as MIC value. Turbidity in the MIC tube indicated growth of bacterial/Candida strain implying that the organisms were resistant to ethanolic ginger extract.

RESULTS

Table 1 shows the zone of inhibition of 10% ethanolic ginger extract against E. faecalis, S. mutans and C. albicans respectively. For E. faecalis, maximum zone of inhibition was 14 mm at 75 µl and minimum zone of inhibition was 10 mm at 25 µl when compared to positive control (4.2% Sodium hypochlorite), which had zone of inhibition of 16 mm. For S. mutans, maximum zone of inhibition was 8 mm at 75 µl and minimum zone of inhibition was 6 mm at 50 µl when compared to positive control (0.2% Chlorhexidine), which had zone of inhibition of 15 mm. For C. albicans, maximum zone of inhibition was 11 mm at 75 µl and minimum zone of inhibition was 9 mm at 50 µl when compared to positive control (Flucanazole), which had zone of inhibition of 14 mm.

Table 2 shows MIC of 10% ethanolic ginger extract against E. faecalis, S. mutans and C. albicans respectively. MIC of 10% ethanolic ginger extract for E. faecalis was established at 2.5%, S. mutans at 1.25% and C. albicans at 2.5%.

DISCUSSION

“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. Members of the Zingiberaceae family are important components in traditional medicine for the treatment of many diseases. Ginger’s pungent components offer powerful anti-inflammatory and antioxidant activities, making it useful in arthritis, Alzheimer’s, cancer, and cardiovascular disease. The active compound responsible for this effect is zingibain, an enzyme that counteracts inflammation. Ginger also has shown real promise in preventing blood platelets from clumping, helping to fight heart disease and stroke. Research suggests this root may protect nerve cells from chemical toxins.

The active compounds contained in ginger are divided into two groups: volatile essential oils and fragrant or harsh phenol compounds. Among these volatile essential components, which constitute gingerol and shagelol have been accounted for antimicrobial activity of ginger.

In the present study, 10% ethanolic ginger extract showed antimicrobial activity against S. mutans exhibiting maximum zone of inhibition of 8 mm at 75 µl and MIC at 1.25% concentration. Similar result was obtained in a study conducted by Akihiro et al. where zone of inhibition of 8.2 mm was seen, supporting the findings of our present study.

In the present study, 10% ethanolic ginger extract showed antimicrobial activity against C. albicans exhibiting maximum zone of inhibition of 11 mm at 75 µl and MIC at 2.5% concentration. Similar result was obtained in the study conducted by Atai et al., where zone of inhibition of 11.2 mm and MIC at 2% concentration was seen, supporting the findings of this present study.

In another study conducted by Joe et al., using 10% ethanolic ginger extract, the zone of inhibition of 8 mm was seen indicating the present study had better results. The difference observed could be attributed to variations in the quality of ginger used, differences in the microbiological techniques used, variation in temperature and solvent used to prepare ginger extract.

In the present study, 10% ethanolic ginger extract showed antimicrobial activity against E. faecalis exhibiting maximum zone of inhibition of 14 mm at 75 µl and MIC at 2.5% concentration. Similar result was obtained in a study.
conducted by Rahman et al. where zone of inhibition of 12 mm was seen, supporting the findings of the present study.\textsuperscript{(12)}

In the present study, positive control was used against all the three organisms in order to compare the antimicrobial efficacy of ethanolic ginger extract. 10% ethanolic ginger extract showed zone of inhibition of 8 mm against \textit{S. mutans} when compared to positive control (0.2% Chlorhexidine solution) which showed zone of inhibition of 15 mm, indicating it was less potent than the positive control. 10% ethanolic ginger extract showed zone of inhibition of 11 mm against \textit{C. albicans} when compared to positive control (Flucanazole) which showed zone of inhibition of 14 mm, indicating it was comparative to positive control. 10% ethanolic ginger extract showed zone of inhibition of 14 mm against \textit{E. faecalis} when compared to positive control (4.2% NaOCl), which showed zone of inhibition of 16 mm, indicating it was comparative to positive control. This study was first of its kind where 10% ethanolic ginger extract along with positive control was used against all 3 microorganisms in order to compare the efficacy of ethanolic ginger extract.

CONCLUSION

10% ethanolic ginger extract showed good antimicrobial potential against \textit{S. mutans, E. faecalis} and \textit{C. albicans}. However, further studies are recommended at clinical and field setting to assess its practical and economic feasibility and to recommend its use in the clinical setting.

ACKNOWLEDGMENT

Authors would like to acknowledge:

a. Dr. Kishore Bhat, Professor and Head and his faculty members, Department of Microbiology, Maratha Mandal Dental College and Hospital, Belgaum, for their assistance in Microbiological procedures during the study.

b. Mr. Narayan Myskin, Professor and Mr. Praveen, Laboratory Technician, Department of Pharmacognany, Bapuji Pharmacy College, Davangere, for their assistance in preparation of extract.

REFERENCES

1. Nayak A, Nayak RN, Soumya BG, Bhat K, Kudlakar M. Evaluation of antibacterial and anti candidal efficacy of aqueous and alchoholic extract of neem [Azadirachta indica] – An in vitro study. Int J Res Ayurveda Pharmacol 2011;2:230-5.
2. Akhani SP, Vishwakarma SL, Goyal RK. Anti-diabetic activity of Zingiber officinale in streptozotocin-induced type I diabetic rats. J Pharm Pharmacol 2004;56:101-5.
3. Shukla Y, Singh M. Cancer preventive properties of ginger: A brief review. Food Chem Toxicol 2007;45:683-90.
4. Available from: http://www.shop.nationalgeographic.com/ngs/product/books/science-and-space/desk-reference-to-nature’s-medicine — hardcover. [Last accessed 2011 Apr 15].
5. Lantz RC, Chen GJ, Sarihan M, Solyom AM, Jolad SD, Timmermann BN. The effect of extracts from ginger rhizome on inflammatory mediator production. Phytomedicine 2007;14:123-8.
6. Manju V, Nalini N. Effect of ginger on bacterial enzymes in 1,2-dimethylylazidine induced experimental colon carcinogenesis. Eur J Cancer Prev 2006;15:377-83.
7. Nurtjahja-Tjendraputra E, Ammit AJ, Roufogalis BD, Tran VH, Duke CC. Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. Thromb Res 2003;111:259-65.
8. Available from: http://www.worldcat.org/title/essential-guide-to-herbal-safety/oclc/57535216. [Last accessed 2011 May 4].
9. Available from: http://www.umm.edu/altmed/articles/ginger-000246.htm. [Last accessed 2011 Apr 30].
10. Siddaraju MN, Dharmesh SM. Inhibition of gastric H+, K+ ATPase and Helicobacter pylori growth by phenolic antioxidants of Zingiber officinale. Mol Nutr Food Res 2007;51:324-32.
11. Westerterp-Plantenga M, Diepvens K, Joosen AM, Bérubé-Parent S, Tremblay A. Metabolic effects of spices, teas, and caffeine. Physiol Behav 2006;89:85-91.
12. Rahman SA, Thangaraj S, Salique SM, Khan KF, Natherer SE. Antimicrobial and biochemical analysis of some spices extract against food spoilage pathogens. Internet J Food Saf 2010;12:71-5.
13. Available from: http://www.globalsciencebooks.info/JournalsSup/08MAPSB_2_2.html. [Last accessed 2011 May 4].
14. Ohara A, Saito F, Matsushita T. Screening of antibacterial activities of edible plants against Streptococcus mutans. Food Sci Technol Res 2008;14:190-3.
15. Atai Z, Atapour M, Mohseni M. Inhibitory effect of ginger extract on Candida albicans. Am J Appl Sci 2009;6:1067-9.
16. Joe MM, Jayachitra A, Vijayapriva M. Antimicrobial activity of some common spices against certain human pathogens. J Med Plants Res 2009;3:1134-6.