ORIGINAL ARTICLE

Inhibitory effects of ginger extract on *Candida albicans*, *Staphylococcus aureus* and *Lactobacillus acidophilus*

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Antimicrobial activity, drug resistance, zingiberene

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Abstract

Background: The incidence of infections caused by yeast, particularly species of Candida and facultative anaerobic bacteria like *Staphylococcus aureus* and *Lactobacillus acidophilus* has increased dramatically during the past decade. Topical and systemic antifungal agents and anti microbial agents may be indicated to control oral candidiasis and periodontal and endodontic disease. The development of resistance is an emerging trend that may threaten the clinical effectiveness.

Aim: An *in vitro* study to evaluate the antimicrobial efficacy of ginger extract on *Candida albicans*, *S. aureus*, *L. acidophilus*, using five different polar organic solvents.

Materials and Methods: The crude solvent extracts of ginger were prepared and subjected to antimicrobial assay using the Agar disk diffusion method to determine the zone of inhibition. The solvent extract with a significant zone of inhibition is subjected to gas chromatography and mass spectrometry, to identify the bioactive constituent in the ginger extract.

Result: Hexane extract of ginger shows high antimicrobial property, and the bioactive component was found to be zingiberene.

Conclusion: The solvent extracts of ginger were proven to have effective antimicrobial property.

Introduction

Plant derived products have been used for medicinal purposes for centuries. It is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs. Spices and herbs are widely used in phytotherapy, which is using plants and their chemical constituents to eliminate certain health problems. This form of treatment is common in Europe, Italy, India, France, UK, and Spain.[1]

The incidence of infections caused by yeast, especially species of Candida and facultative anaerobic bacteria such as *Staphylococcus aureus*, *Lactobacillus acidophilus* has dramatically increased in the past decade. Topical and systemic antifungal agents and antimicrobial agents may be indicated to control oral candidiasis and pyogenic infections.

The development of resistance is an emerging trend that may threaten the clinical effectiveness. The traditional medicine serves as the only opportunity for health care and plants are the vital sources. Among the herbal therapeutics, ginger and its applications are well documented.[2]

Ginger (*Zingiber officinale*) belongs to *Zingiberaceae* family. The part of the plant used is rhizome. In Sanskrit, ginger is known as Sringavera, which has given way to Zingiberi in Greek and the Latin *Zingiber*. Ginger has been used as medicine from Vedic period and is called "*mahaaushadhi,*" means the great medicine.

In the fresh ginger rhizome, the gingerols were identified as the major active components and gingerol 5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-1 is the most abundant constituent in the gingerol series. The powdered rhizome contains 3-6% fatty oil, 9% protein, 60-70% carbohydrates, 3-8% crude fiber, about 8% ash, 9-12% water, and 2-3% volatile oil. The volatile oil consists of mainly mono and sesquiterpenes; camphene, beta-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, linalool, alpha-zingiberene (30-70%), beta-sesquiphellandrene (15-20%), beta-bisabolene (10-15%) and alpha-farmesene. In dried ginger powder, shogaol a dehydrated product of gingerol, is a predominant pungent constituent.[1]

The oral cavity is inhabited by more than 700 microbial species and many intrinsic, and extrinsic factors affect the composition,

metabolic activity, and pathogenicity of the highly diversified oral micro flora.[3] It includes pigmented and nonpigmented micrococci, some of which are aerobic, Gram-positive aerobic spore-bearing bacilli, colliforms, Proteus, and lactobacilli. The gingival crevices and the crypts of the tonsils have wide spectrum of anaerobic micrococci, microaerophilic and anaerobic streptococci, vibrios, fusiform bacilli, cornybacterium species, actinomyces, leptothrix, mycoplasma, neisseria, and bacteroids are all found in varying extents. Among fungi *Candida* and geotrichum have been reported.[4]

Candidiasis is a common oral and peri-oral opportunistic infection that usually results from overgrowth of endogenous *Candida* fungal micro-organisms. *Candida albicans*, a regular inhabitant of normal skin and oral, vaginal and intestinal mucosa, is the primary etiological agent. Other pathogenic members of the genus *Candida* often isolated from the oral environment are (in descending order of virulence) *Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida pseudotropicalis, Candida krusei,* and *Candida guilliermondi*.[5]

Staphylococci species are present in the environment and as normal flora of humans and animals. They are resistant to heat and drying and may be recovered from the environment months after contamination.[6] In normal healthy oral cavity, *Staphylococcus* has been isolated from supragingival and subgingival plaque. In oral diseases, *S. aureus* is frequently associated with angular cheilitis and also in infected jaw cyst, oral mucosal lesions, and denture-induced stomatitis, and less frequently in acute dentoalveolar abscess.[7]

L. acidophilus are Gram-positive, nonspore forming rods that grow best under microaerophilic conditions. They are present in high numbers in saliva, on the dorsum of the tongue, mucous membranes, the hard palate, in dental plaque, and in fewer numbers on tooth surfaces. Lactobacilli are found in root but also in deep dentinal caries associated with pulpitis. The association of lactobacilli and dentinal caries were reported by Goadby in 1899.[8]

Numerous antimicrobial assays using the extracts of ginger has been done. However, they reported the effects of aqueous and alcoholic extracts only. Studies using nonpolar organic solvents are very few. Polarity of the compounds being extracted by each solvent is different, and this influences their intrinsic bioactivity. Hence, this study is aimed to evaluate the antimicrobial properties of the organic solvent extracts of ginger and its bioactive constituents against *C. albicans*, *S. aureus,* and *L. acidophilus*.

Materials and Methods

The present *in vitro* study was conducted to evaluate the antimicrobial efficacy of crude solvent extract of ginger on *C. albicans*, *S. aureus*, and *L. acidophilus*. The antimicrobial bioassay of the selected plant (*Z. officinale)* was done in the Department of Microbiology, Meenakshi Ammal Dental College and Chennai. All the standard microbiological and advanced bio-instrumental procedures were done in the Hubert Enviro Care System (P) Ltd., Ashok Nagar, Chennai, Tamil Nadu, India.

Collection of ginger

Roots of ginger are cleaned and were minced into fine pieces with a sharp knife. The minced ginger was dried under sunlight for 1 week. The minced and dried ginger is ground to a coarse powder in a grinding machine at a standard grinding mill. The ground coarse powder was transferred to bottle with stoppers and the bottle was labeled with the name of the herb and was stored in a wooden cupboard.

Test organisms for the study

The following fungal and bacterial pathogens were procured from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Sector 39-A, Chandigarh - 160 036, India, as freeze dried forms for the antimicrobial bioassay of the selected plants. *C. albicans* (MTCC 183), *S. aureus* (MTCC 7443), and *L. acidophilus* (MTCC 447). The lyophilized cultures obtained from MTCC were stored in refrigerator at 4°C until use.

Crude solvent extraction

The various organic solvents selected for the study were hexane, ethyl acetate, chloroform, acetone, and diethyl ether. 25 g of the dried powder of ginger were mixed with 75 ml of the solvents selected for the study in separate brown color bottles. The bottles were labeled appropriately.

After 72 h, the crude extracts were obtained by filtering and the extracts collected were left for evaporation in a cool, dry place for 2 days. After complete evaporation, the plates were weighed again for detecting the amount of crude extract obtained from each solvent. The crude extracts were then reconstituted in dimethyl sulfoxide, which is an inert organic solvent, to prepare a concentration of 10 mg/ml.

Antimicrobial bioassay

Preparation of inoculums'

The lyophilized cultures were added to Sabouraud's dextrose agar for *C. albicans,* Brain Heart infusion agar for *S. aureus* and *L. acidophilus.* These were incubated at 37°C in 10 % CO_2 for 2 h in a candle jar. After incubation, the broths with turbidity were adjusted to 0.5 McFarland standards and were used as the inoculums'.

Results

The efficacy of herbal extracts of the medicinal plant ginger, *Z. officinale* was evaluated against three organisms, *C. albicans*, *S. aureus,* and *L. acidophilus*. The antimicrobial assay of the selected plant was done using the agar well-diffusion method.

The solvent extracts showing the zone of inhibition for each test organism were observed [Figures 1-3]. The zones were measured and recorded in millimeters. The mean value of three experiments was calculated. The data collected were analyzed by ANOVA and for multiple comparisons Tukey's *post hoc* test. All statistical analyses were done using SPSS version 14.0.

The mean zones of inhibition observed for *C. albicans* is 13 mm, 14.6 mm, 13.6 mm, 13.6 mm, and 13.3 mm for hexane, ethyl acetate, chloroform, acetone and diethyl ether extracts; for *S. aureus* includes 15.3 mm, 11.6 mm, 9.6 mm, 11.5 mm and 11 mm for hexane, ethyl acetate, chloroform, acetone and diethyl ether extracts, and for *L. acidophilus* includes 12 mm, 15 mm, 12.6 mm, 15.6 mm, and 15.3 mm was observed for hexane, ethyl acetate, chloroform, acetone and diethyl ether extracts, respectively.

There is no significant difference between the antimicrobial activities of the solvent extracts of ginger against the *C. albicans* and *L. acidophilus*, all the solvent extracts of ginger are equally effective [Table 1].

In *S. aureus* hexane, extract of ginger exhibits a significant difference when compared with ethyl acetate, acetone, and chloroform and diethyl ether extracts of ginger. Hence, hexane extract of ginger proved to exhibit high antimicrobial activity against *S. aureus*.

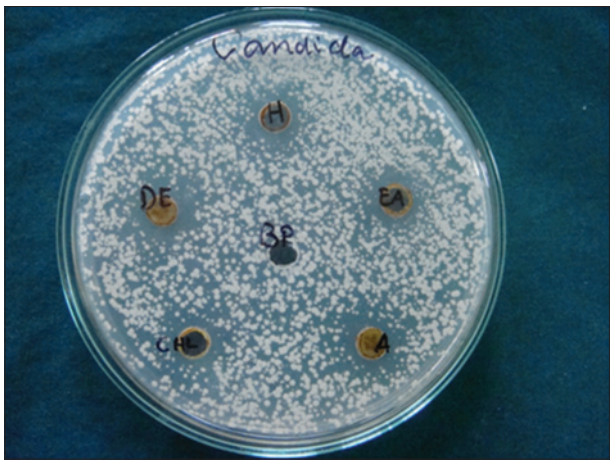


Figure 1: Zones of inhibition exhibited by solvent extracts of ginger on *Candida albicans*

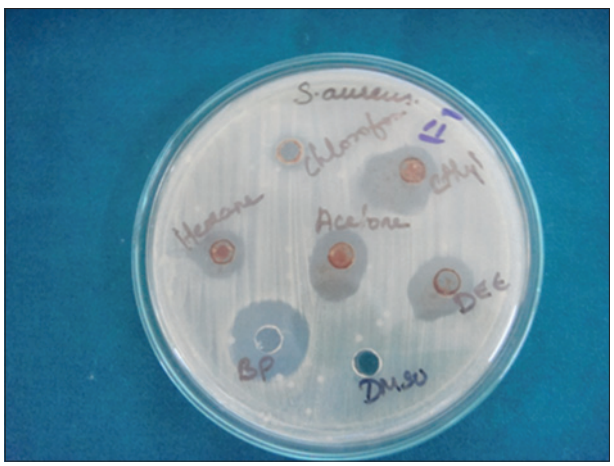


Figure 2: Zones of inhibition exhibited by solvent extracts of ginger on *Staphylococcus aureus*

Bio-active constituent of ginger extract

A weight of 100 g of dried ginger was extracted using hexane for 72 h in cold condition. It is then distilled and extract collected. TLC pattern of crude hexane extract showed four major spots. Bioassay-guided fractionation of the hexane extract using silica gel column chromatography yielded a single compound with solvent mixture hexane:ethyl acetate (1:1). The single compound obtained is identified using GC-MS technique as *zingiberene* [Graph 1]. The antimicrobial activity of the compound identified using disc diffusion method against *S. aureus* [Figure 4]. This compound showed maximum antimicrobial activity against *S. aureus*.

Discussion

The resistance to antimicrobial drugs has continued to grow in the last decades. The increased prevalence of their resistance is due to

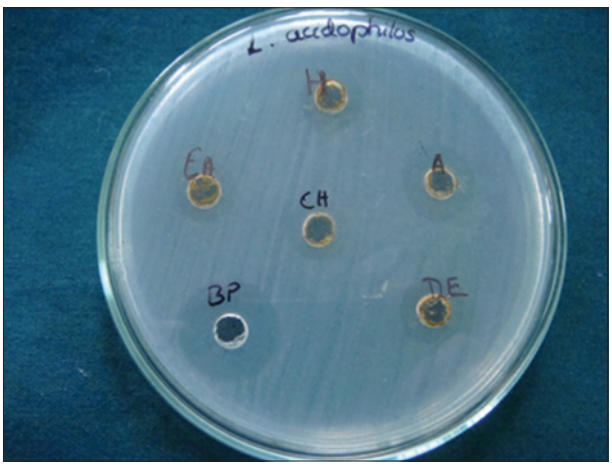


Figure 3: Zones of inhibition exhibited by solvent extracts of ginger on *Lactobacillus acidophilus*

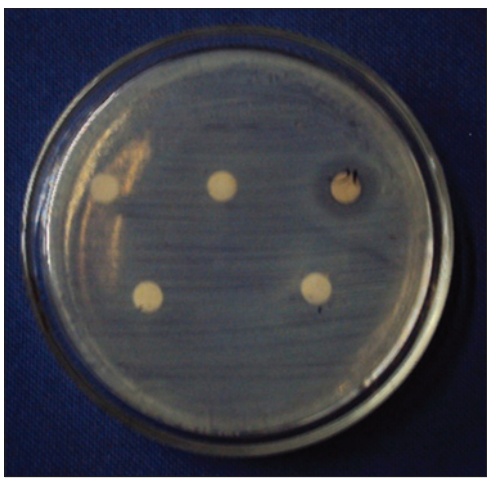


Figure 4: The antimicrobial activity of the bioactive compound obtained is identified by Agar disk diffusion method on *Staphylococcus aureus*

Table 1: There is no significant difference between the antimicrobial activities of five different solvent extracts of ginger

Multiple comparisons					
Dependent variable: *Staphylococcus aureus*					
Tukey HSD					
(I) VAR0009 (J) VAR00009	Mean difference (I-J)	Standard error	Sig.	95% Confidence interval	
				Lower bound	Upper bound
H					
EA	3.6667	0.51640	0.000	1.9672	5.3662
Chl	5.6667	0.51640	0.000	3.9672	7.3662
A	3.8333	0.51640	0.000	2.1338	5.5328
Die	4.3333	0.51640	0.000	2.6338	6.0328
EA					
H	-3.6667	0.51640	0.000	-5.3662	−1.9672
Chl	2.0000	0.51640	0.020	0.3005	3.6995
A	0.1667	0.51640	0.997	−1.5328	1.8862
Die	0.6667	0.51640	0.702	−1.0328	2.3662
Chl					
H	−5.6667	0.51640	0.000	−7.3662	3.9072
EA	2.0000	0.51640	0.20	3.6995	−0.3005
A	−1.8333	0.51640	0.033	3.5328	−0.1338
Die	−1.3333	0.51640	0.148	−3.0328	0.3662
A					
H	−3.8333	0.51640	0.000	-5.5328	−2.1338
EA	−0.1667	0.51640	0.997	−1.8662	1.5328
Chl	1.8333	0.51640	0.033	0.1338	3.5328
Die	0.5000	0.51640	0.863	−1.1995	2.1996
Die					
H	-4.3333	0.51640	0.000	−6.0328	−2.6333
EA	−0.6667	0.51640	0.702	−2.3662	1.0323
Chl	1.3333	0.51640	0.148	−0.3662	3.0328
A	−0.5000	0.51640	0.863	−2.1995	1.1096

The mean difference is significant at the 0.05 level

extensive use and misuse of antimicrobials.[8] Dramatic increases in the incidence of infections caused by *C. albicans, S. aureus,* and *L. acidophilus* which are the common cause of candidiasis, pyogenic infections, and dental caries in the oral cavity.[5]

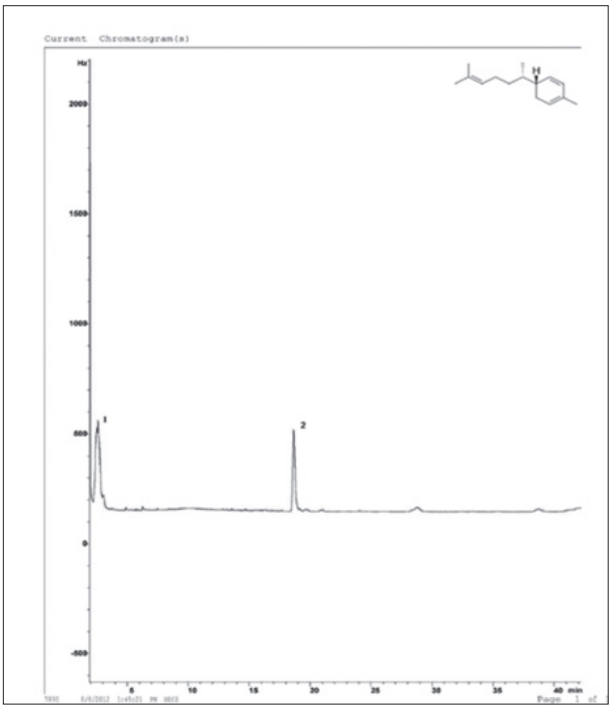
Ginger has been used as medicine from Vedic period and is used as an essential ingredient in traditional medicine. The *gingerols* were identified as the major active components and among the volatile oil present in ginger are sesquiterpenes, beta-sesquiphellandrene (15-20%), beta-bisabolene (10-15%), and alpha-farnesene. In dried ginger powder, shogaol a dehydrated product of gingerol, is a predominant pungent constituent.[1]

Zingiberene is one of the major volatile oil present in the ginger extract along with sequesterpene, which causes the fragrance of the spice.[9]

Successful prediction of bioactive compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. In addition to the intrinsic bioactivity of the compound, the polarity of the compounds extracted from the solvent determines the ability to dissolve or diffuse in the different media used in the assay.[10]

In this study, different organic solvent extracts of ginger were prepared using the solvents such as hexane, ethyl acetate, chloroform, acetone, and diethyl ether. The prepared extracts were screened for their antimicrobial activity against pathogens *C. albicans, S. aureus,* and *L. acidophilus* using agar well-diffusion method. The best suitable extract was further optimized from the results of the antimicrobial assay.

The results showed that the mean zone of inhibition of hexane extract of ginger shows a statistically significant difference among the five different solvent extracts of ginger used against *S. aureus* which can be correlated with the studies conducted by Etoh *et al*.[11] and Khanom *et al*.[12] and the bioactive constituent present in the hexane extract of ginger was found to be *Zingiberene.*



Graph 1: The bioactive compound in ginger is identified by gas chromatography and mass spectrometry technique as zingiberene

Similar results obtained from the studies conducted by Sasidharan *et al.*,[13] Yang *et al.*,[14] and El-Baroty *et al.*[15] to identify the major bioactive component from different solvent extracts of ginger.

This suggests that ginger extract can be used as a promising antimicrobial agent against infectious diseases. Further bioactive compound analysis can be done for the solvent extracts of ginger against *C. albicans* and *L. acidophilus* by advanced bio-instrumentation methods.

Conclusion

The global problem of antibiotic resistance has led to the isolation and characterization of new antimicrobial compounds in the plant products.[16] Despite the limited in vivo investigations, the available evidence indicates that there is potential for the discovery of novel and effective antimicrobial therapy using natural products. There is a clear need to conduct additional studies to evaluate the clinical efficacy and safety of these substances.

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