U. Santo Grace et al /J. Pharm. Sci. & Res. Vol. 9(9), 2017, 1417-1419

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Antimicrobial Activity of Ethanolic Extract of *Zingiber Officinale* – An *in vitro* Study

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Abstract:

Aim :To analyze the antimicrobial efficacy of ethanolic extract of *Zingiber officinale* (ginger) on *Staphylococcus aureus* and *Enterococcus feacalis*.

Materials and Methods: *Z. officinale* (rhizomes) was obtained commercially. Dried, ground rhizomes were extracted with ethanol by maceration. 12.5 g of plant material was soaked with 50 ml of ethanol for 24 hrs at room temperature. After that the resulting extract was filtered through filter paper (Whatman no.1). The filtrates obtained were evaporated to dryness by placing in the hot air oven at 40 °C for 24 hrs. The precipitate was made into a concentration of 100mg/ml. Then diluted in ethanol and different concentrations as 5µl, 10µl and 15µl were prepared. Erythromycin disc was placed as a standard.

Result : It was found that ethanolic extract of ginger is best effective against *S. aureus* when compared with *E. feacalis* when diluted upto 15 µl.

Conclusion: *Z. officinale* ethanolic extract has shown a wide range of influence on gram positive aerobic bacteria. This investigation of ginger has confirmed its significance, especially in the area of influence on tested staphylococci.

Keywords: ginger, antimicrobial efficacy, ethanolic extract, *Z. officinale.*

INTRODUCTION:

Zingiber officinale (ginger), a horizontal, branched, fleshy, aromatic white to yellow coloured perennial herb with leafy stem up to 60 cm, has long been used in the field of medicine. It belongs to the family Zingerberaceae. Its leaves are narrow being 20 cm long and 1.5-2cm wide. Dense spiked, yellow green with purple ending flowers are seen. (1) The rhizome is rich in secondary metabolites such as phenolic compounds (gingerol, paradol and shogaoal), volatile sesquiterpenes (zingiberene and bisabolene) and monoterpenoids (curcumene and citral)

Ginger has been widely used all over the world in ayurvedic medicine, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases (2). It has direct anti-microbial activity and thus can be used in treatment of bacterial infections (3). Ginger are relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. It has also been "Generally Recognized as Safe" (GRAS) by the US FDA (4).

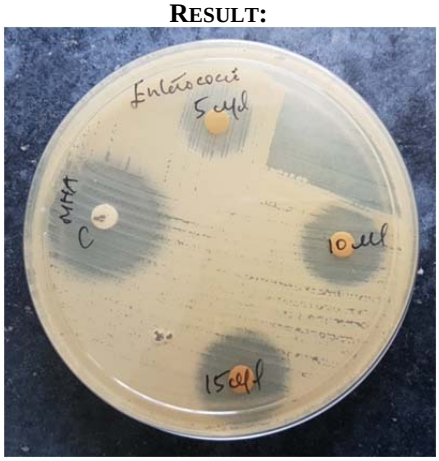
Periodontal disease is a multifactorial diseases characterized by inflammation and forming a biofilm by majority of gram negative and few gram positive bacteria Biofilm is a community of microbial cells attached to a surface producing colonies and embedded in the extrapolymeric substances. (5). These are the major sources of various problems in food industry, medicine and everyday life thereby leading to food spoilage, infections and disease transmission. (6,7) Bacteria present in the biofilm community are generally more resistant to antimicrobial agents than planktonic extracts. (8.9).

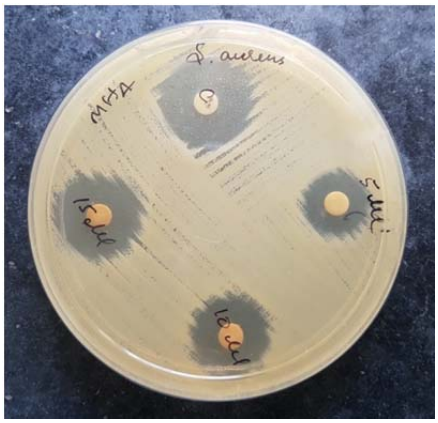
Various natural herbs and plant extracts have been used in ayurvedic medicine to eliminate the formation of biofilm. Previous studies have demonstrated that plant extracts and isolated compounds from *Z. officinale* possess strong antioxidant (10), antibacterial, antifungal, anticancer and anti-inflammatory effects (11). Quave et al, 2008 and Sandasi et al, 2010 have demonstrated the plant extracts in preventing biofilm formation and adherence (12,13). Since periodontal disease is characterized by inflammation and forming a biofilm, the applications of ginger may be useful in treating the disease. So the aim of the study was to analyze the antimcrobial efficacy of ethanolic extract of *Zingiber officinale* (ginger) on gram positive *Staphylococcus* and gram negative *Enterococcus*.

MATERIALS AND METHODS:

Z. officinale (rhizomes) was obtained commercially. Dried, ground rhizomes were extracted with ethanol by maceration. 12.5 g of plant material was soaked with 50 ml of ethanol for 24 h at room temperature. After that the resulting extract was filtered through filter paper (Whatman no.1). The filtrates obtained were evaporated to dryness by placing in the hot air oven at 40 °C for 24 hrs. The precipitate was made into a concentration of 100mg/ml. Then diluted in ethanol and different concentrations as 5µl, 10µl and 15µl was prepared. Erythromycin disc was placed as a standard. A lawn of each bacterial isolate was prepared on MHA plates using a sterile cotton swab from the inoculum showing growth of 0.5 MacFerland standard. Three filter paper discs were placed on dried MHA plates and ginger extracts (5 µl, 10µl 15µl and control) were added on each disc separately.

RESULT:





Dilution	*Staphylococcus aureus*	*Enterococcus faecalis*
5µl	17mm	18mm
10 µl	20mm	21mm
15 µl	23mm	24mm
Control (Erythromycin)	27mm	29mm

It was found that ethanolic extract of ginger is best effective against *S. aureus* when compared with *E. feacalis* when diluted upto 15 µl.

DISCUSSION:

Microbial biofilm plays a major role in causing periodontal destruction. Biofilms are composed of micro-colonies of bacterial cells (15-20% by volume) that are non-randomly distributed in a shaped matrix (Socransky SS). It is made of a wide array of gram positive and gram negative aerobic and anaerobic bacteria. Treating them is a challenge. There are various methods in eliminating biofilm. Plant extracts have been found effective in treating periodontal microflora. Ginger is an age old Ayurvedic plant used in the field of Indian medicine. Since periodontitis is characterized by formation of a biofilm, the use od plants extracts can be applied to treat it. This study was aimed at finding the antimicrobial efficacy of ginger in gram positive staphylococcus and gram negative enterococcus species.

Ethanolic extract of ginger was prepared by using dry ginger which was commercially available. Although, ethanol by itself has antimicrobial properties, this study is justified as the ehanol was evaporated when heated 40 °C for 24 hrs. The tested ethanolic extract of ginger showed marked antibacterial activity against *Staphylococcus aureus* and *Enterococcus feacalis.* The strongest inhibition activity of the ginger extract was observed against *Staphylococcus aureus* (23 mm zone) when diluted upto 15 µl when compared to *Enterococcus feacalis* Though there is not much difference comparing the zone of inhibition of *Staphylococcus aureus* and *Enterococcus feacalis,* it is clear that the ethanolic extract of ginger is more efficient in gram positive organisms. It showed stronger inhibitory effects against *Staphylococcus aureus* when compared to *Enterococcus faecalis.* Previous studies have shown that soya beam extract of ginger has shown good antimicrobial activity against food borne pathogens- *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio cholerae, Klebsiella spp.* and *Salmonella spp.*(14)

Malu et al, in their study suggested that the inhibition of bacterial growth activity of the extracts is dose dependent. The antibacterial activity and inhibition activity of ginger extracts could be attributed to the chemical properties of ginger. The main constituents of ginger are sesquiterpenoids with zingiberene as the main component. Other components include β- sesquiphellandrene, bisabolene and farnesene, which are sesquiterpenoids, and trace monoterpenoid fraction,(β-sesquiphellandrene, cineol and citral)(16). Antimicrobial activity of crude ginger at both room temperature and boiling temperature was studied previously by Pankaj et al. (17). However, they found that boiling temperature treated ginger extract (crude) lost its antimicrobial activity against *Klebsiella peumoniae, Escherichia coli* and *Staphylococcus aureus*. Onyeagba et al. (18) found the synergistic effect of ethanol extract of ginger and garlic against Bacillus spp. and Staphylococcus aureus. They also found the antimicrobial activity of the ethanol extract of ginger, lime and garlic against broad range of bacteria including *Bacillus spp., Staphylococcus aureus, Escherichia coli,* and *Salmonella spp*.

The synergistic antimicrobial effect of soybean and ginger at boiling temperature against food borne pathogens indicates the thermostable antibacterial property of ginger extracts. This thermostable property can be further investigated with different types of oils and extraction temperatures. Further studies have to be undertaken to prove the antimicrobial effects of ginger on gram negative anaerobic periodontal pathogens.

LIMITATIONS

1. Since ethanol by itself has antimicrobial properties and therefore the antimicrobial effect of ginger may not be established.
2. The current study proved the antimicrobial effect of ginger on aerobic bacteria. Further research has to be carried out to demonstrate the antimicrobial effect on gram negative anaerobic bacteria which are the major cause for periodontal disease.

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