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IN VITRO ANTIBACTERIAL ACTIVITY OF SOLVENT EXTRACTS OF *ZINGEBER OFFICINALE* *R*OSC.RHIZOME ON DIFFERENT MICRO ORGANISMS

Astha Jain[1], Vidya Chavan[2], Dinesh Labade[3], Minal Wani[4]

[1,4] Dr. D.Y. Patil Biotechnology and Bioinformatics Institute,
Dr. D. Y. Patil Vidyapeeth, Pune-411033, Maharashtra.(India)
[2,3]Rise n'Shine Biotech Pvt. Ltd, Theur, Pune, Maharashtra.(India)

ABSTRACT

Ginger (Zingiber officinale Rosc.) belongs to the family Zingiberaceae. Ginger rhizome contains several constituents which have antibacterial and anti-fungal effects.The present studies were carried out to evaluate the antimicrobial activitiesof different solvent extracts of ginger against Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Psuedomonas aerunginosa by employing antibiotic sensitivity test.Different solvents included ethyl acetate, methanol, ethanol, chloroform, dimethyl sulphoxide and soyabean oil. Streptomycin is used as a positive control.The antibacterial activity of extracts of ginger against all four microorganisms were tested positive.Soyabean oil extract indicated highest activity against all tested microbes compared to other extracts. Followed to which the zone inhibition activity was highest in methanolic extract and then by ethanolic extract whereas least effective in dimethyl sulphoxide extract used in experiments. In compared to all other microorganism, zone of inhibition for methanolic extracts was similar for most microbes and least effective in Pseudomonas aerunginosa. The growth inhibitory response varied with the type of solvents used and antibiotic used in experiments and with type of microorganisms. Although modern biomedicine to a significant degree employs synthetic drugs as therapeutic agents, plants still occupy a prominent place in contemporary pharmacy. Medicinal plants like ginger and its constituents play an important role in diseases management via modulation of biological activities.

Keywords: Ginger, Microorganisms, Streptomycin, Antimicrobial Activity.

I INTRODUCTION

There has been a great shift the prescription of antibiotics to the use of medicinal plants. It is estimated that there are 250,000 to 500,000 species of plant on the earth. Medicinal plants and its constituents such *curcumin*, black seed, olive fruits/leaves and dates shows a therapeutic role in disease controls via modulation of biological activities. For

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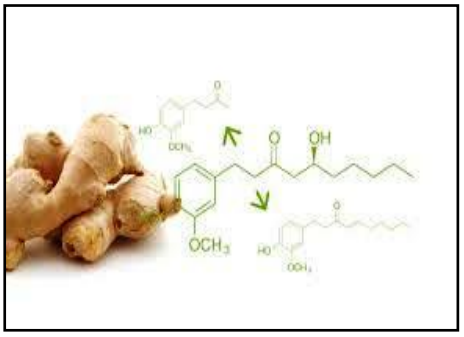
instance, water extracts of *Ociunun sanctum* and O*cimum gratissimum* and alcohol extracts of *Ocimum gratissimum* and *Ocimum sanctum* were highly toxic against fungi after 15 days culture. Furthermore, ethanol and aqueous extract of black pepper showed bactericidal activity.

Zingiber officinale Rosc. **(Ginger)** is an important medicinal plant of the family Zingiberaceae. The erect leafy aerial stem grows up to approximately 1 meter in height and has purple flowers (fig.1). It is a wild plant cultivated for its aromatic rhizome and leaves. Its roots are used as a spice in cooking throughout the world.

Fig:1 Ginger plant and Rhizome



The ginger plant has a long history of cultivation known to originate in China and then spread to India, South East Asia, West Africa and the Caribbean (Weiss, 1997; McGee, 2004). **Rhizome** of ginger has been used as a medicine in Chinese, Indian and Arabic herbal traditions since ancient times as carminative or anti- flatulent, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic and digestive aid, etc. (Kizhakkayil and Sasikumar, 2011). Ginger has been valued for its antibacterial properties for thousands of years in Asian cultures (Weil, 2005). Ginger contains up to 3% of an essential oil that causes the fragrance of the spice (O'Hara et al,1998). It has active ingredients of gingerol and shogaol within ginger. Since many studies indicated that the antimicrobial potency of ginger mainly caused by the presence of oxygenated mono- and sequel terpenes, phenolic compounds (shogaol, gingerol), which are lipid-soluble phenol compounds primarily isolated from the root of ginger. Microorganism prevalent in the patients' immediate hospital environment colonizes the patients' skin, mucous membrane, eye, ear and nostrils as well as the anterior urethra.

The development of new antibiotics and plant based antimicrobial compounds are effective against the resistant organisms. Ginger a common substance found increasingly in the diets of the global population, have known antibacterial effects and are commonly used together in teas. Many studies have implicated *Staphylococcus aureus*, and *Streptococcus pyogene* as leading causative agents of both community and hospital acquire infections

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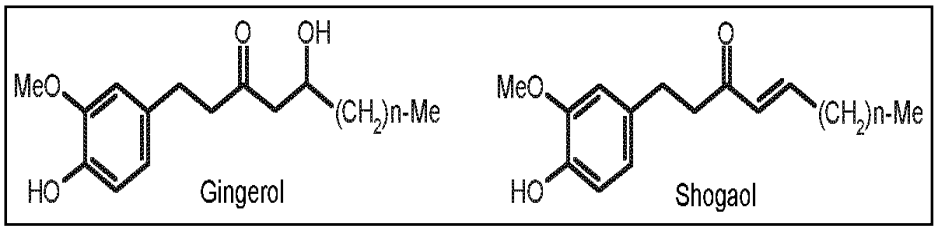
(Amita et al., 2003). Antibiotics are chemicals produced by micro-organisms. Microorganisms that produce antibiotics are mostly bacteria, but a few fungi also produce them. The aim of this research is to investigate the effect of plant extract (ginger) and three other antibiotics on two pathogenic bacteria.

Fig: 2 Chemical Structures of Gingerol and Shogaol



II MATERIAL AND METHOD

2.1 Collection and Preparation of Extracts

The ginger rhizome was collected from local market. The rhizome was cut small pieces and dried in oven for a week, till it form hard crush of small pieces of rhizomes. Final dried rhizome was ground into fine powder using a mortle and pestle. The powdered ginger obtained was stored in clean sterile bottles at room temperature and used for the extractions. A very simple solvent extraction process was used. A weighed 2 gm of powdered of ginger rhizomes was mixed with 10 ml different solvent (Ethanol, Methnol, Dimethyl Sulphoxide, Soyabean oil, Ethyl acetate, Chloroform) in conical flask and was kept on a rotary shaker for 2 hours. After vigorous shaking, extracts were filtered through multilayer muslin cloths using glass funnel. The filtrate was collected in sterile tubes and stored in refrigerator and same process was followed for other solvents too. The various extracts were used for the analysis of antibacterial activities and bacterial inhibition assay.

2.2 Preparation of The Nutrient Medium

The nutrient agar medium was prepared by dissolving Peptone: 1 gm, NaCl: 0.5 gm and Beef extract: 0.3 gm in 100ml distilled water. The solution was sterilized in an autoclave at 121°C (1.1N pressure) for 15 min. The suspension was cooled and poured into sterile Petri-dishes to solidify. The agar depth of the medium was 4.0 mm.

2.3 Preparation of Cultures and Inoculums:

Pure cultures of ***E. coli***, ***Bacillus subtilis***, ***Staphylococcus aureus*** and ***Pseudomonas aerunginosa*** were obtained from the Microbiology Laboratory in the Department of Microbiology, Dr. D. Y Patil, College of Biotechnology & Bioinformatics, Dr. D. Y. Patil Vidyapeeth, Pune, was separately used to inoculate the Petri-dishes. These strains were maintained separately on NA agar by repeating streaking on plates at intervals of 15 days and even glycerol

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stocks of each strain was maintained as back up. Strains were streaked on the surface of the plates in a four quadrant manner to get single colony. Actively growing single colony from the plate was inoculated in liquid nutrient agar broth and kept on a shaker at 37 °C for 24 hours.

2.4 Assay of Antibacterial Activity

The antibacterial activity was determined by the diffusion method of Kirby Bauer described by Duguid et al, (1989). Filter paper discs (6mm diameter) were prepared using a punch machine. Filter paper discs were sterilized in an autoclave at 121°C and kept in the oven at 40 °C to remove excess moisture and used for experimental purpose after 24 hours. Lawn of each bacterial isolate was prepared on Nutrient agar plates using a sterile spreader with 100 µl of liquid suspension. Filter paper discs were placed in 20 µl ginger extracts, allow soaking for 15 minutes and placed on NA agar lawn plates of different strains

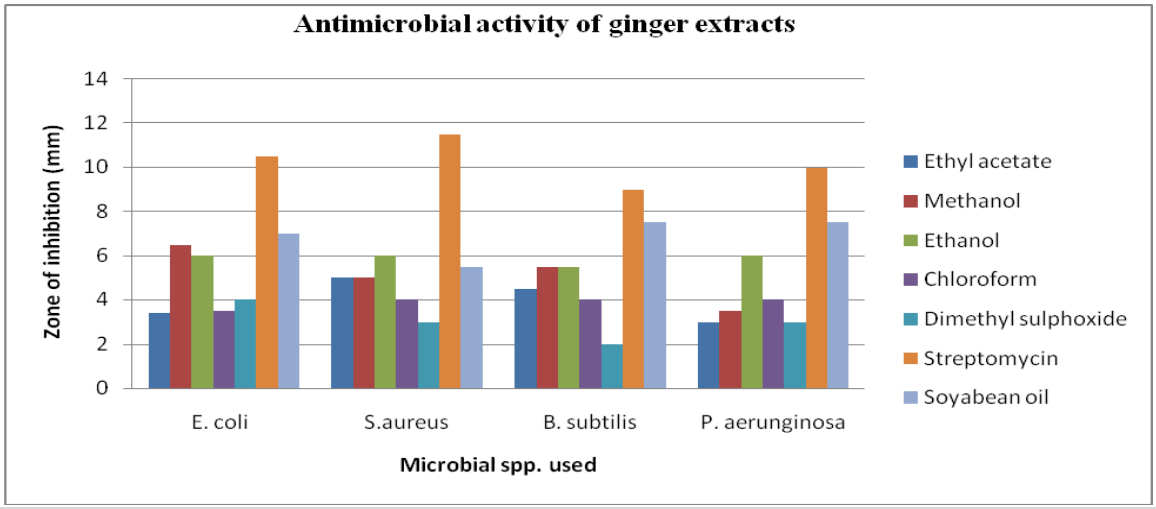
Soybean oil alone was used in order compare the antimicrobial activity with that of solvent extract. Commercially available **Streptomycin** was used as a control. All plates were incubated at 37 °C for 24 hours and the zones of inhibition (diameter in mm) were measured on the agar surface.

III RESULT AND DISCUSSION

3.1 Antibacterial Activity of Different Extracts

The study showed that ginger extracts have bactericidal activity, and it found that the ginger powder extract with different solvent have more or less bactericidal activity The sensitivity of different organism with different solvent extracts was studied using paper disc method shown in graph no. 1

Graph No: 1 Graphical representation susceptibility of bacteria spp.



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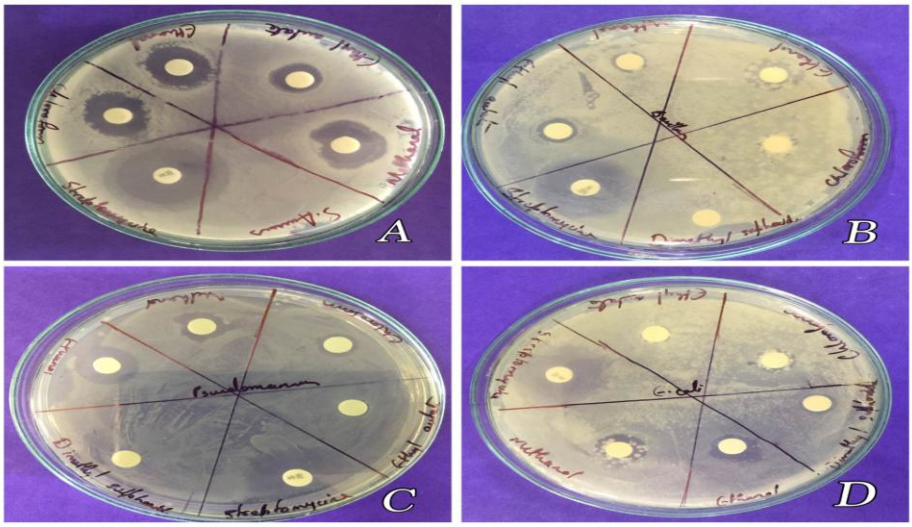
Methanolic extract of ginger was sensitive to all organisms used in experiment ***E. coli***, ***Bacillus subtilis***, ***Staphylococcus aureus*** and ***Pseudomonas aerunginosa.*** The widest zone of inhibition was observed in *E. coli* (6.5 mm), next it was observed in *Bacillus substilis* (5.5 mm). Even ethanolic extract of ginger showed bactericidal activity in the same trend as methanolic extract, it was widest 6.0 mm in three organism ***E. coli***, ***Staphylococcus aureus*** and ***Pseudomonas aerunginosa,*** least in ***Bacillus substilis*** (5.5 mm). Extracts with different solvents like ethyl acetate, chloroform and Dimethyl sulphoxide showed maximum zone inhibition for ***Staphylococcus aureus*** ranging from 3mm to 6 mm. In case of *Pseudomonas aerunginos,* ethanolic extract has a maximum zone of inhibition (6 mm), least in ethyl acetate (3 mm).

Fig: 3 Susceptibility of bacteria for different extract and controlled antibiotic



3.2 Antibacterial Based On Solvents

The above results show that the extraction of ginger has an antibacterial activity. This because of the presence of various compounds as active ingredient within ginger. Phytochemicals analysis has shown ethanol solubility include tannins, polyphenols, polyacetylenes, flavonol, sterols and alkaloids (Ivanovska, et al., 1996). Since many studies indicated that the antimicrobial potency of ginger mainly caused by the presence of oxygenated mono- and sequel terpenes, phenolic compounds (shogaol, gingerol), which are lipid-soluble phenol compounds primarily isolated from the root of ginger. Even on examination of various extracts for their ability to solubilize antibacterial compound from plants as well as other factors such as their relative ranking as biohazards and the ease of removal of solvent from the fraction and ranked them in the order: methylene dichloride > methanol > ethanol > water, Cowan, (1999). Simialr result was observed, when an ethanol extract of ginger showed the greatest effect on both *S. aureus* and *S. pyogene* compared to the leaf and root water extract and the leaf ethanol extract. This is an indication that ginger is effective against *S. aureus* and *S. pyogene* infections. The phytoconstitutuents of ginger have longed been known as its antibacterial properties have been widely reported (Roy et al., 2006). However, most

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reports on the activity of ginger have focused mainly on the commensal micro flora and community acquired infections, while information's on its activity against hospital based pathogens is scanty.

It also became clear in this research that the solvent of extraction affected the degree of antimicrobial activity of the extracts. The difference in the zone of inhibition directly related to the susceptibility of each test organism to the extracts. In the above experiment, widest zone of inhibition was observed in methanolic extract, as methanol has highest relative polarity 0.762 compared to ethanol 0.654, it means that methanol has one compound that is not soluble in ethanol. The least activity of bactericidal was observed in other solvent ethyl acetate, chloroform and dimethyl sulphoxide, it is clear that bactericidial compound only soluble in highly polar solvents. Ali, et al., (2007), who had reported antibacterial activity of water, petroleum ether, ethyl acetate, ethanolic and methanolic black pepper extracts against *B. megaterium, B. subtilis, S. aureus* and *E. coli*. According to Harold (2004), the antimicrobial activity of black pepper is due to the presence of essential oil (3%), whose aroma is dominated by monoterpenes hydrocarbons: sabinene, β-pinene and limonene. Furthermore, terpinene, α-pinene, myrcene, and monoterpene derivatives like borneol, carvone, carvacrol, 1, 8-cineol and linalool are also present.

3.3 Effect of Antibiotics

The bacteri**a** showed a high degree of inhibition to the antibiotics (streptomycin) used. All the bacteria tested were susceptible to streptomycin. Streptomycin is a protein synthesis inhibitor. It binds to the small 16S rRNA of the 30S subunit of the bacterial ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit (Sharma D *et al*., 2007). This leads to codon misreading, eventual inhibition of protein synthesis and ultimately death of microbial cells through mechanisms that are still not understood. Speculation on this mechanism indicates that the binding of the molecule to the 30S subunit interferes with 50S subunit association with the mRNA strand. This results in an unstable ribosomal-mRNA complex, leading to a frameshift mutationand defective protein synthesis; leading to cell death (*Raymon, Lionel P.2011).* The methanol extracts of ginger higher susceptibility of bacteria than the other extracts, but less compared to antibiotic and soyabean oil.

IV CONCLUSION

It may be concluded from the present studies that, *Zingiber officinale* (Ginger) produced marked inhibitory effect on tested microorganism. All the extracts can be used as a potential source of natural antimicrobial compounds, which if can be applied in Ayurved and for isolation of pure antibacterial compound observed in extract. Further research is required for the comparative and identification of bioactive molecule present in the different solvent.

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