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EVALUATION OF ANTIBACTERIAL ACTIVITY OF ZINGIBER OFFICINALE

Shiva Prasad Thoutu*

Vaagdevi College of Pharmacy (Affiliated by Kakatiya University), Hanamkonda, Warangal, Telangana, India.

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***Corresponding Author**
Shiva Prasad Thoutu
Vaagdevi College of Pharmacy (Affiliated by Kakatiya University), Hanamkonda, Warangal, Telangana, India.

ABSTRACT

This study was designed for evaluate the anti-bacterial activity found in association with bacterial diseases with Zingiber officinale. So many bacterial cultures available. I choose Escherichia coli. Inhibiting the bacterial activity by using Disc Diffusion Method the Kirby-Bauer method. Firstly, prepare the agar media with Peptone, Sodium chloride, Beef extract, Agar-agar and Water. After that prepare bacteria culture and swab in to the agar media. Using disc diffusion method after growing the E. coli it forms colonies in the Petri dish. Collect the organic Ginger powder and dissolve in DMSO extract dissolving in water and forming stock solution. Take the ginger solution apply it on the bacterial media. Few hours later slowly can observe inhibition of bacteria. Ginger extract had the overall widest inhibition E coli concentration. The results of different extracts were gained from extrapolation of zone diameter of inhibition of the concentration.

KEYWORDS: Zingiber officinale, Anti-bacterial activity, Disc diffusion method, Escherichia coli, Inhibition.

INTRODUCTION

Antibacterial is a community of microbial cells attached to the surface and is embedded in the extracellular polymeric substances. Antibacterial are sources of diverse problems in food industry, medicine and everyday life. The presence of Antibacterial in food processing environments is a potential source of contamination that may lead to food spoil and disease transmission. Bacteria included Antibacterial structure are generally more resistant to

antimicrobial agents than plank tonic cells. The effects of plant extracts to prevent formation and adherence have been showed in earlier studies.

Source: Ginger is a rhizome, obtained from the plant Zingiber officinale

The ginger (zinger officinal) is a flowering plant whose rhizome, ginger root or simply ginger, is idly used as a spice or a folk medicine. It is herbaceous perennial which grows annual stems about a mater tall bearing narrow green leaves and yellow flowers. Ginger is in the family Zingiberaceae, to which also belong turmeric (Curcuma longa), cardamom (Elettaria cardamom), and galangal. Ginger originated in the tropical rainforest in southern Asia. Although ginger no longer grows wild, it is thought to have originated no the Indian subcontinent because the ginger plants grown in India show the largest amount of genetic variation. Ginger was exported to Europe via India in the first century AD as a result of the lucrative spice trade and was used and was used extensively by the Roman.

In India Approximately, 25000 hectares of land is cultivated in India for the production of about 25000 tonnes of dry ginger annually. In almost all states of India, ginger is cultivated. Especially in, Kerala, Assam, Himachal Pradesh, Orissa, West Bengal and Karnataka. Ginger needs warm humid climate and is cultivated in areas with heavy rainfall. It is cultivated even at sea level, but still, it thrives best at an altitude of 1000-1500 meters.

Ginger (Zingiber officinale Roscoe, fam. Zingiberaceae) is a perennial herb, with leafy stem up to 60 cm. The rhizome is horizontal, branched, fleshy, aromatic, white or yellowish to brown. Leaves are narrowly or linear-lanceolate, up to 20 cm long and 1.5-2 cm wide. Flowers are produced in a dense spike, yellow green with purple endings. This plant is widely distributed in South-Eastern Asia. The rhizome is rich in the secondary metabolites such as phenolic compounds (Gingerol and Shogaol), volatile sesquiterpenes (Zingiberene and bisabolene) and monoterpenoids (Curcumin and Citral). Previous studies have demonstrated that plant extracts and isolated compounds from Zingiber officinale possess strong antioxidant130, antibacterial, antifungal, anticancer and anti-inflammatory effects. In food industry, both pathogenic and food spoilage bacteria can attach and form a Antibacterial on food contact surfaces and food product, on the other hand Zingiber officinale widely used as spice, so the aim of this study was ginger effectiveness in preventing this problem through the evaluation of antibacterial activity of methanolic extract of Zingiber officinale, as well as effect of this extract on Antibacterial formation against Proteus mirabilis.

Classification		
	Kingdom	Plantae – Planta, Vegetal, plants
	Sub kingdom	Viridiplantae
	Infra kingdom	Streptophyta – land plants
	Super division	Embryophyta
	Division	Tracheophyta – vascular plants, tracheophytes
	Subdivision	Spermatophytina – spermatophytes, seed plants, phanerogames
	Class	Magnoliopsida
	Super order	Lilianae – monocots, monocotyledons, monocotylédones
	Order	Zingiberales
	Family	Zingiberaceae – Ginger Family
	Genus	Zingiber mill – ginger
	Species	Zingiber officinale Roscoe

Properties of ginger

Ginger (Zingiber officinale) is a medicinal plant that has been widely used all over the world, since antiquity, 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. Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections. Ginger belongs to Zingiberaceae family. The Zingiber plants have strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes. Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. It has also Generally Recognized as Safe.

- ✓ Anti Inflammatory agent
- ✓ Emmenagogue
- ✓ Helps calm nausea and vomiting
- ✓ Digestive tract protection
- ✓ Brain health
- ✓ Migraine Relief
- ✓ Supports stable blood sugar
- ✓ Promotes healthy blood pressure
- ✓ Osteoarthritis
- ✓ Cardio vascular benefits

METHODOLOGY

Procedure

Required ingredients are weighed by simple balance and dissolve in 100ml of distilled water PH is adjusted to 1.4 and it is then distributed in test tubes or poured into conical flask, this will plug with cotton and wrapped with paper and sterilized by autoclaving at 15lbs pressure for 20min.

Preparation of agar media

Composition

- ✓ Peptone - 5gms
- ✓ Sodium chloride- 5gm
- ✓ Beef extract -3gms
- ✓ Agar-agar- 15gms
- ✓ Distilled Water- 100ml

Required quantity of ingredients weighed by electronic balance, mix thoroughly with distilled water and boil at 100°C for 10 min to dissolve agar completely. The prepared medium is poured into conical flask and autoclave after plugged them with cotton. Steam sterilization occur in autoclave at 121°C for about 20 min at 15lb pressure after medium is cooled for 45°C and poured into petri dish test tubes it as necessary.

Assay

Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of colonies from the stock culture to peptone water and incubated for 4hrs at 37°C. Anti-bacterial activity was determined by agar disc diffusion method standard suspension of bacteria was inoculated on the surface of Muller-Hinton agar plates. Dimethyl Sulfoxide and methanol were used to dissolve in the plant extract. Sterilized filter paper discs (5mm) containing 20ml of each extract (100mg/ml) was arranged on the surface of the inoculated plates and incubated at 37°C for 18-24hrs. Along with this 30µg disc (Himedia tetracycline andard) was studied for anti-microbial activity as a positive control whereas the solvent used for preparing extract was used as negative control.

Antibacterial activity was tested by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using microdilution method with resazurin.

Bacterial suspensions were prepared by direct colony method. The turbidity of initial suspension was adjusted by comparing with 0.5 McFarland's standard. Initial bacterial suspensions contain about 108 colony forming units (CFU)/ml and then 1:100 diluted in sterile 0.85% saline. Twofold serial dilutions of plant extract were made in a concentration range from 20 mg/ml to 0.0012 mg/ml in sterile 96-well plates containing Mueller–Hinton broth.

A 10 µl of diluted bacterial suspension was added to each well to give a final concentration of 5 x 105 CFU/ml. Finally, 10 µl of resazurin solution, as an indicator of microbial growth, was added to each well. The inoculated plates were incubated at 37 °C for 24 h. MIC was defined as the lowest concentration of tested compound that prevented resazurin colour change from blue to pink. MBC was determined by plating 10 µl of samples from wells, where no indicator colour change was recorded, on nutrient agar. At the end of the incubation period the lowest concentration with no growth (no colony) was defined as minimum bactericidal concentration. Tetracycline, dissolved in nutrient liquid medium was used as positive control. Solvent control test was performed to study an effect of 10% DMSO on the growth of bacteria. It was observed that 10% DMSO did not inhibit the growth of bacteria. Each test included growth control and sterility control. All tests were performed in duplicate and MICs were constant at the end of incubation, inhibition zones formed around the disc were measured with high media zone scale. The study was performed in triplicate and the mean values were presented.

Disc diffusion method

Principle of Disc Diffusion Method the Kirby-Bauer test for antibiotic susceptibility, called the disc diffusion test, is a standard that has been used for years. In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Solutions of known concentration (ug/ml) of the test sample are made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts placed on nutrient agar medium uniformly seeded with the test are microorganisms. Standard antibiotic (e.g. Kanamycin) discs and blank discs (impregnated with solvents) are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Barry, 1976). During this time dried discs absorb water from the surrounding media and then the test

materials are dissolved and diffused out of the sample disc. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel. As a result, there is a gradual change of test materials concentration in the media surrounding the discs. The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and there by yield a clear, distinct area defined as zone of inhibition.

The anti-microbial activity, of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimetre. This test must be rigorously standardized size is also dependent on inoculums size, medium composition, temperature of incubation, excess moisture and thickness of the agar. If these conditions are uniform, reproducible tests can be obtained and zone diameter is only a function of the susceptibility of the test organism. Zone diameter can be correlated with susceptibility as measured by the dilution method. Further correlations using zone diameter allow the designation of an organism as “susceptible”, “intermediate”, or “resistant” to concentrations of an antibiotic which can be attained in the blood or other body fluids of patients requiring chemotherapy. In the present study the crude extracts, fractions as well as some pure compounds were tested for antimicrobial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required.

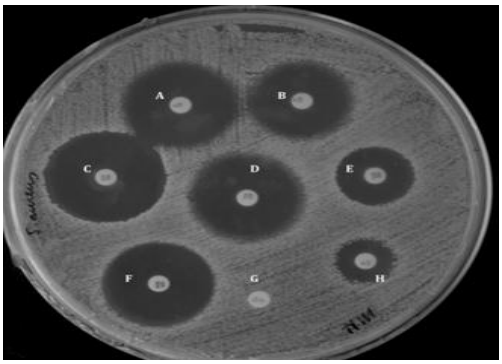

Fig. 1: Disk diffusion method.

Inoculum standardization

A loopful of the test isolate was picked using a sterile wire loop and emulsified in 3 – 4mls of sterile physiological saline. The turbidity of the suspension was matched with that of 0.5 McFarland Standard.

Sensitivity testing

Using sterile swab stick, standardized Inoculum of each isolate was swabbed onto the surface of Mueller Hinton Agar in separate Petri dishes. Disc of the extract and standard antibiotic (Augmentin 30µg) were placed onto the surface of the inoculated media. The plates were inverted and allowed to stand for for 18 hours. This was followed by measurement of 30mins for the extract diffuse into the agar after which the plates were incubated aerobically at 350C zone of inhibition formed by the test organisms around each of the extract and standard antibiotic discs.

RESULTS AND DISCUSSION

Image representation of media

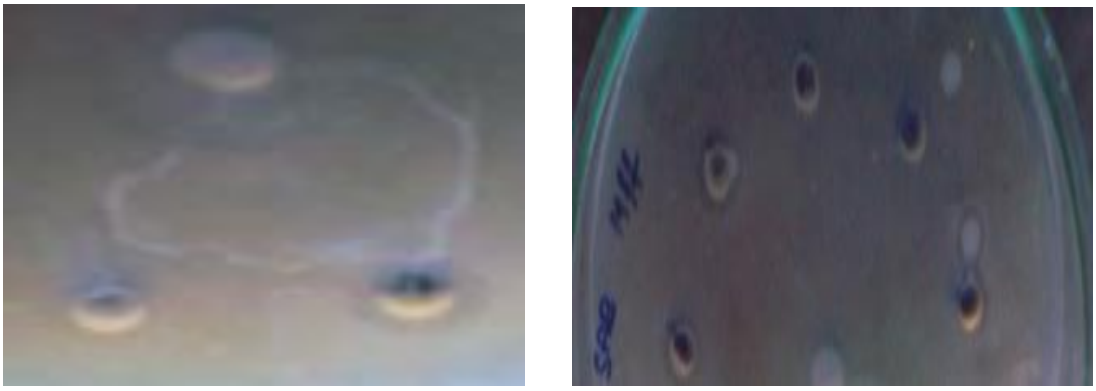

Fig. 2: Zone of inhibition.

Anti-bacterial activity of methanolic extract of Zingiber officinale

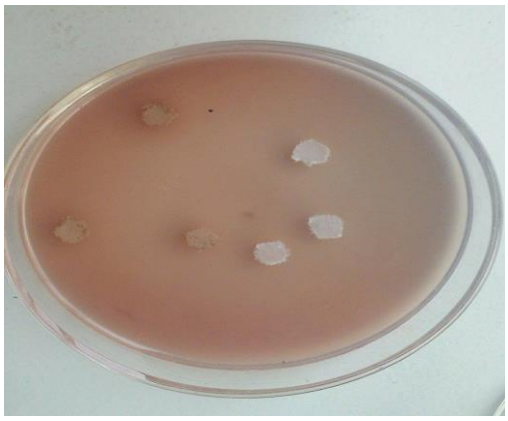

Fig. 3: Zone of inhibition test.

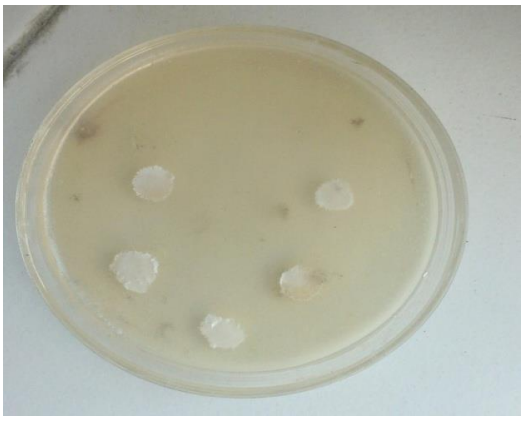

Fig. 3.1: Zone of standard inhibition.

Zone of inhibition

Table 1: Zone of Inhibition.

Concentration(µg/ml)	Zone of inhibition(mm)
Standard	22
Test	16

Ginger powder of organic extracts dissolve in DMSO extract dissolving in water and forming stock solution. Not all organic extract exhibited inhibition on the test microorganism, just methanol extract exhibited inhibition, another acetone and chloroform could not inhibit, aqueous extract could not exhibit inhibition anymore. Because methanol is an organic solvent and will dissolve organic compounds better, hence liberate active components required for antimicrobial activity.

During the introductory testing, it was observed that methanol, methanol extracts gave the widest diameter zone of inhibition (16.00,15.00,15.00) mm with E Coli when using concentration 100 microgram\ml, but weak inhibition or there is no inhibition with acetone, chloroform and aqueous extracts with same concentration. The antibacterial for those extract dependent on the concentration of the extract for example 100 microgram\ml of methanolic extract inhibition e. coli with 16 mm and inhibition E. coli 22 mm, while 50 microgram\ml of same extract inhibition E. coli 16 diameter inhibition zone. From these results, it becomes appropriate to detect the minimum inhibitory concentration (MIC) of different extracts. Methanol extract had the overall widest diameter zone of inhibition E. coli (22.00) mm with 100 microgram\ml and methanolic extract had the overall widest inhibition E coli concentration. The results of different extracts were gained from extrapolation of zone diameter of inhibition of the concentration

CONCLUSION

Natural plant sources are having medicinal properties. In the present study ginger powder were collected and extracted with methanol and aqueous extract to estimate the anti-bacterial activity. Ginger extract was selected as test substance for the estimation of anti-bacterial activity, Tetracycline was taken as standard substances. The extract of Ginger was showed anti-bacterial activity.

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