International Journal of Allied Practice, Research and Review
Website: www.ijaprr.com (ISSN 2350-1294)

Evaluation of Antimicrobial Activity and Phytochemical Analysis of *Zingiber officinale* (Ginger) Rhizome Extract

A .R. Tambe [1], S. B. Deokar [2] R.M. Pawar [3]
[1]Department of Zoology, Nowrosjee Wadia College, Pune, India
[2]Department of Biotechnology, Nowrosjee Wadia College, Pune, India
[3]Department of Zoology, Nowrosjee Wadia College, Pune, India

ABSTRACT – Drugs from natural sources are used for treating various diseases since ancient times. *Zingiber officinale* has long been used as naturopathy due to their potential antimicrobial activity against different microbial pathogens . The objective of study is to evaluate antibacterial and antifungal activity of *Zingiber officinale* (Ginger) rhizome extracts (Ethanol and Aqueous) against bacterial strain *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive) and fungal strain *Aspergillus niger*. Ethanol and aqueous extracts of varying concentrations such as 0% , 3% , 6% , 9% were prepared and tested against test microorganisms using agar well diffusion method. The values of Zone of inhibition were tabulated according to the concentration of the tested agent and data was statistically analyzed.. The Zone of inhibition showed efficie ncy of plant extract. The results showed that *S.aureus* showed highest antibacterial activity of 21mm Zone of inhibition at 9 % as compared with activity against *E.coli* of 19mm Zone of inhibition at 9%. Antifungal activity against *A.niger* showed highest Zone of inhibition of 19mm at 9% concentration of aqueous and extracts. The antibiotics such as gentamicin, penici llin and antifungicides such as streptomycin were tested against human pathogens as positive control. The antibacterial and antifungal activity of Ginger rhizomes and their utility in diseases have been confirmed experimentally. The results therefore confirm the traditional use of Ginger for its antimicrobial properties.

KEYWORDS– *Zingiber officinale ;* Maximum inhibitory concentration (MIC); Antibacterial activity ; Antifungal activity; Zone of inhibition (ZOI)

I. INTRODUCTION

The increased usage of antibiotics has induced microorganisms to acquire resistance factors which have bec ome a burning predicament. As a result there is an urgent need to find the alternative of chemotherapeutic drugs in d iseases treatment particularly those of plant origin which are easily available and have considerably less side effects. The antimicrobial activity of spices is due to certain phytochemicals or essential oils present in ginger. The rhizome is rich in secondary metabolites such as phenolic compounds. The essential components present in ginger are zingib erol, shogaols,gingerols, zingiberene,D-camphor,etc. The search for antifungal and antibacterial drugs has received

attention mainly as a result of considerable drawbacks in the use of major antibiotics. The aim of this study is to eva luate the antimicrobial activity of Ginger on different human pathogens and to carry out phytochemical screening of the extracts.

II. MATERIALS AND METHODS

SAMPLE COLLECTION-

The rhizomes of Ginger were collected from Botanical garden of Nowrosjee wadia college, Pune, In dia. The samples were washed thrice using tap water followed by distilled water and were dried under sha de in hygiene conditions for 10-12 days. All the materials was ground in an electric grinder to produce fin e powder. Powdered material was stored at 4°C in an air tight bottle.

COLLECTION OF TEST ORGANISMS

The test organisms used in this study consisted of E.coli NCIM 5010 (Gram negative) and *S.aureus* NCIM 2079 (Gram positive) bacteria and *A.niger* NCIM 501 fungal strain. Cultures were obtained from NCIM, Pune. Th e test organisms were cultured on nutrient agar (HiMedia) and Potato Dextrose agar (HiMedia) slants respectively and stored at 4°C in refrigerator.

PREPARATION OF WATER EXTRACTS

Fine grounded powder was measured with electronic weighing balance. Various concentrations were made such as 0%,3%,6% and 9%. For 0% ,3%,6%,9% solutions 0gm,3gm,6gm,9gm fine powder was suspended in 100ml each of distilled water .These were soaked for 72 hours ,kept on rotary shaker for constant stirring. The solution wa s carefully filtered with help of Whatmann filter paper no.1 into sterilized test tubes and filtrates were obtained. Filtr ates were covered with aluminum foil and stored in refrigerator at 4°C until required.

PREPARATION OF ETHANOL EXTRACTS

Fine grounded powder was measured with electronic weighing balance. Various concentrations were made such as 0%,3%,6% and 9%. For 0% ,3%,6%,9% solutions 0gm,3gm,6gm,9gm fine powder was suspended in 100ml each of ethanol solution .These were soaked for 72 hours,kept on rotary shaker for constant stirring.The solution was carefully filtered with help of Whatmann filter paper no.1 into a sterilized test tubes and filtrates were obtaine d. Filterates were covered with aluminum foil and stored in refrigerator at 4°C until required.

Inoculums preparation for bacteria-The loopfull of bacterial cultures were taken from slants and inoculated in Nu trient broth and incubated overnight at 37°C. The 50 µl of overnight culture of each bacterial strain was transferred i nto 5ml sterile nutrient broth (pH 7.4) and placed in shaking incubator at 37˚C for 16 hours. The bacterial cells were harvested at 3500 rpm for 10 mins at 4˚C, washed with phosphate buffer saline and resuspended in nutrient broth. T he 10^7 CFU/ml inoculum concentrations were adjusted.

Inoculum preparation for fungi- Fungal culture was grown on CzapekDox agar slants (sporulating medium). Sla nts were incubated at ambient temperature for 2-3 days. Spore suspension was prepared in sterile 0.01% Tween-20 and used as inoculum.The inoculum size was adjusted to 1.0 x10^6 spores/ml by microscopic enumeration with a cell counting Hematocytometer.

Agar diffusion method- The method is suitable for organisms that grows rapidly .The well of 6mm were pu nched in nutrient agar and potato dextrose agar media with sterile cork borer , after inoculation with bacterial cult

ure and spore suspension of bacteria and fungi respectively. When well is loaded with extract, it diffuses in the medi um and inhibits the growth of organism.. The zone of inhibition of bacterial growth around each well was measured and the susceptibility is determined.

Anti-bacterial activity assay- Using a micropippete added 30µl of bacterial suspension on nutrient agar plates. With help of sterile glass spreader, spreaded these suspension of *E.coli* and *S.aureus* respectively throughout t he plate. Wells were punched of 6mm diameter into plates. Loaded 20-30µl of the plant extract of various conce ntrations .Allowed to stand for 30mins for agar diffusion. Plates were incubated at 37°C for 24hrs. Observed th e bacterial activity by measuring the zone of inhibition against the test organism by measuring scale. Antibiot ics such as Penicillin, Gentamicin were used as positive control against Gram positive and Gram negative bacterial s train respectively. 0% extract was used as negative control, against bacterial strains.

Anti-fungal activity assay- Using a micropippete added 30µl of fungal spore suspension of *A.niger* on potato dextrose agar plates. With help of sterile glass spreader, spreaded the spore suspension throughout the plate. Wells were punched of 6mm diameter with sterile borer into plates. Loaded 20-30µl of the extract.Allowed to stand for 30 mins for agar diffusion. Plates were incubated at 22°C for 48-72 hrs. Observed the antifungal activity by measuri ng the zone of inhibition against the test organism by measuring scale. Antifungicide such as clotrimazole w as used as positive control against fungal strain .0% extract was used as negative control, against fungal strai ns.

PHYTOCHEMICAL SCREENING OF PLANT EXTRACT:

Chemical test were carried out using an aqueous extract to identify various components using standard methods.

Preparation of aqueous extract: 5gm fine ground powder of rhizome was suspended in 10ml of sterile distilled wa ter. kept overnight on rotary shaker.Extract was filtered with help of Whatmann filter paper no.1 and aqueous extrax t was used for further screening.

Test for reducing sugar: Benedict's test
To 1 ml of extract solution, 1 ml of water and 5 - 8 drops of Fehling's solution were added when hot and observed f ormation of brick red precipitate indicated presence of reducing sugars.

Test for phenols: Ferric chloride test
To 1ml of extract solution and few drops of ferric chloride solution were added and observed formation of bluish bl ack color indicated presence of phenols.

Tests for Flavonoids : Alkaline reagent test
To 1 ml of aqueous extract, 1 ml of 10% lead acetate solution was added and observed the formation of a yellow pre cipitate indicated positive test for flavonoids.

Test for Proteins: Xanthoproteic test: 1ml of rhizome extract was mixed with few drops of concentrated nitric aci d solution; formation of yellow colour indicates presence of proteins.

Tests for Tannins: 2 ml of the rhizome extract was mixed with 2 ml of distilled water and few drops of $FeCl_3$ Solut ion. Formation of green precipitate indicates presence of tannins.

Tests for Saponins: 2 ml of rhizome extract was added in 2 ml of distilled water in a test tube and warmed. The for mation of stable foam indicates the presence of saponins.

Test for steroids: when 2 ml of rhizome extract was added in 2 ml of chloroform and 2 ml concentrated sulphuric a cid , a red colour produced below the chloroform layer indicates the presence of steroids.

III. RESULTS AND DISCUSSION

In the present study,the ginger rhizomes extract (aqueous and ethanol) were tested for antimicrobial activity agains t selected human pathogens and phytochemical properties.

Figures indicate antibacterial activity of Ginger against *E.coli*

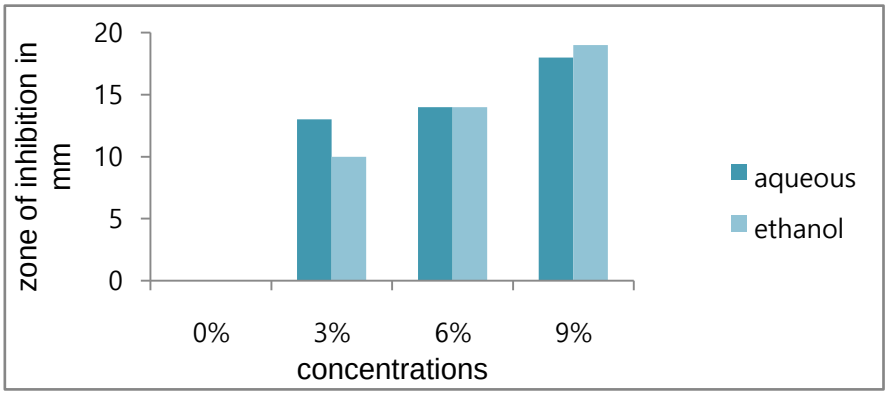
Figure.1- Effect of aqueous extract on *E.coli*

Zone of inhibition

Figure.2-Efffect of ethanol extract on *E.coli*

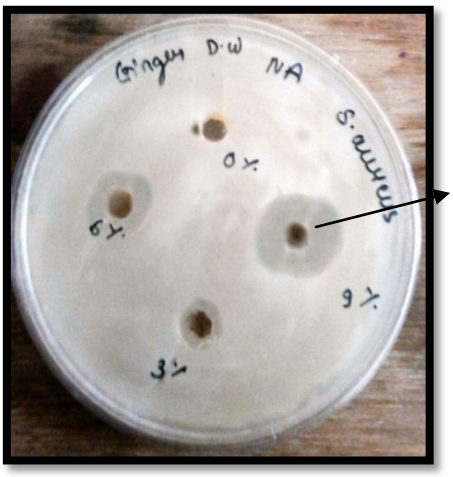
solvent	Concentration	*E.coli* (ZOI in mm)
Aqueous	0%	0
	3%	13
	6%	14
	9%	18
Ethanol	0%	0
	3%	10
	6%	14
	9%	19

Table.1- Antibacterial activity of Ginger extract against *E.coli*



Graph 1- Antibacterial activity of Ginger extract against *E.coli*

Figures indicate antibacterial activity of Ginger against *S.aureus*



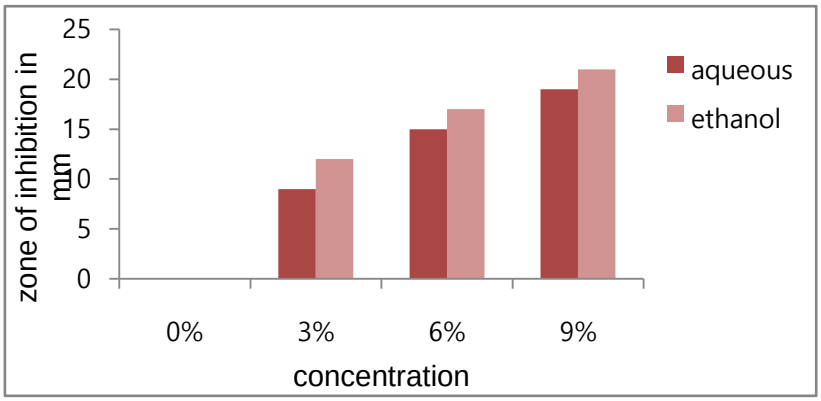
Zone of inhibition

Figure.3- Effect of aqueous extract on *S.aureus*

Figure.4- Effect of ethanol extract on *S.aureus*

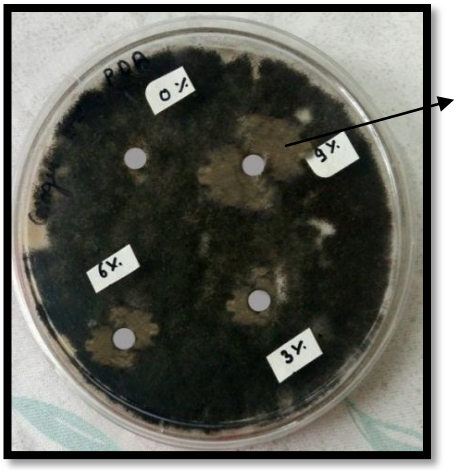
solvent	Concentration	*S.aureus*(ZOI in mm)
Aqueous	0%	0
	3%	9
	6%	15
	9%	19
Ethanol	0%	0
	3%	12
	6%	17
	9%	21

Table .2- Antibacterial activity of Ginger extract against *S.aureus*



Graph.2- Antibacterial activity of Ginger extract against *S.aureus*

Figures indicate antifungal activity of Ginger against *A.niger*



Zone of inhibition

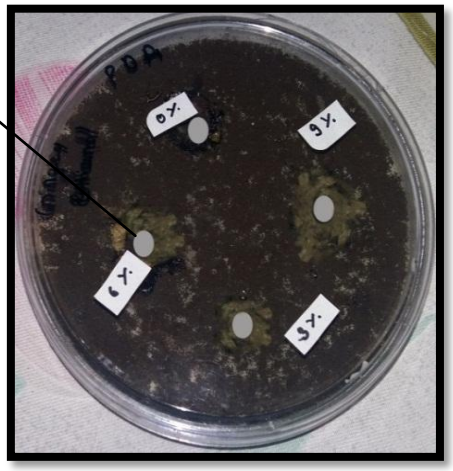
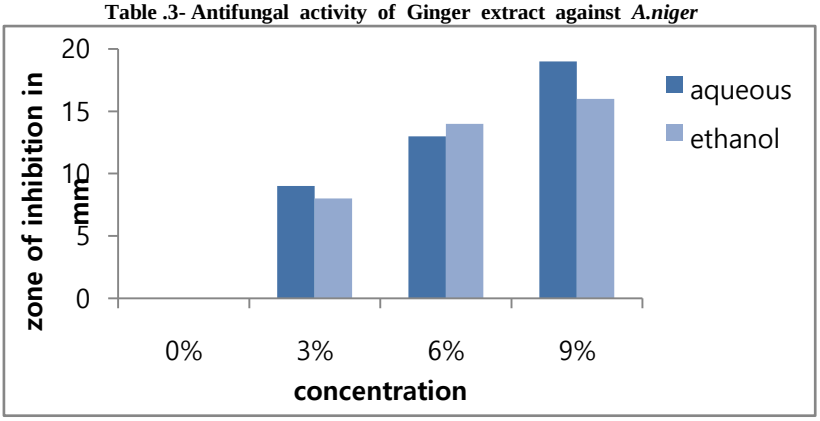


Figure.5- Effect of aqueous extract on *A.niger*

Figure.6- Effect of ethanol extract on *A.niger*

	Concentration	*A.niger* (ZOI in mm)
Aqueous	0%	0
	3%	9
	6%	13
	9%	19
Ethanol	0%	0
	3%	8
	6%	14
	9%	16

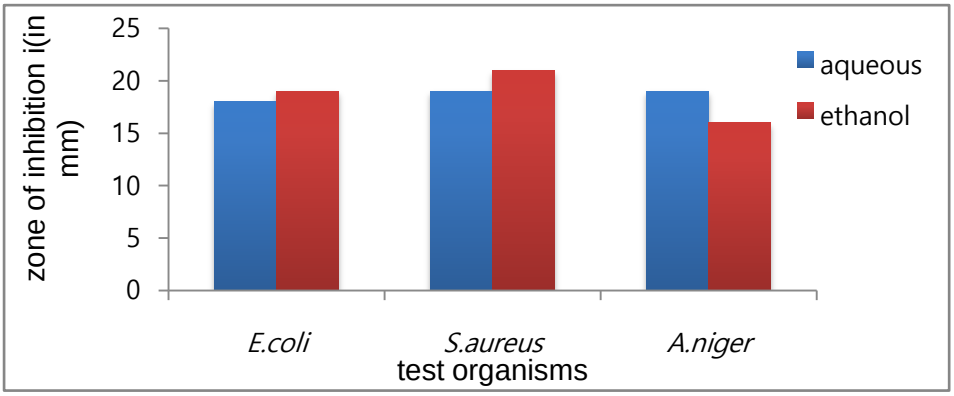
Table .3- Antifungal activity of Ginger extract against *A.niger*



Graph .3- Antifungal activity of Ginger extract against *A.niger*

Sr.no	Test Organism	Solvent (MIC) in mm	
		Aqueous	Ethanol
1.	*E.coli*	18	19
2.	*S.aureus*	19	21
3.	*A.niger*	19	16

Table 4. Antimicrobial activity showing maximum inhibitory concentration (MIC) against test organisms at 9% concentration



Graph 4. Antimicrobial activity showing maximum inhibitory concentration (MIC) against test organisms at 9% concentration

SrNo.	Test	Presence/Absence Of	Ginger Extract
1.	Benedicts Test	Reducing Sugars	+
2.	Ferric Chloride	Phenols	-
3.	Alkaline Reagent Test	Flavonoids	+
4.	XanthoproteicTest	Proteins	+
5.	Tannins	Tannins	+
6.	Saponins	Saponins	-
7.	Steroids	Steroids	-

Note: + : present - : absent

Table 5.phytochemical analysis of Ginger rhizomes extract

The results table 1. showed that the extract possessed antimicrobial activity against test organisms, depending upon their capacity for diffusion into agar medium. Aqueous extract showed maximum zone of inhibition of 18mm at 9% and minimum zone of inhibition of 13mm at 3% concentration. Ethanol extract showed maximum zone of inhibition of 19mm at 9% and minimum zone of inhibition of 10mm at 3%. Aqueous extract was found to be more efficient when compared with that of ethanol extract against *E.coli*. Figure1 and Figure 2 shows effect of Ginger extract against E.coli.

Table 2 showed effect of different concentrations of Ginger extract against *S.aureus*.Aqueous extract showed maximum ZOI of 19mm at 9% and minimum ZOI of 9mm at 3% which is comparatively less as compared with ZOI against *E.coli*. Ethanol extract showed maximum ZOI of 21mm at 9% and minimum ZOI of 12mm at 3% which is comparatively greater than ZOI of *E.coli*. Figure3 and Figure 4 shows effect of Ginger extract against *S.aureus*.

Table 3.Showed antifungal activity of Ginger against *A.niger*. Figure 5 and Figure 6 showed antifungal activity. Maximum ZOI was about 19mm at 9% and minimum ZOI was about 9mm at 3% by aqueous extract. Ethanol extract showed maximum ZOI of 16mm at 9% and minimum ZOI of 8mm at 3% which

is comparatively less as compared with antibacterial activity. Extract of Ginger rhizome showed highest zo ne of inhibition against each bacterial and fungal strain according to its concentrations. The higher the con centration higher efficiency was found against human pathogens.

Table 4.represent Antimicrobial activity showing maximum inhibitory concentration (MIC) against test org anisms at 9% concentration. It was interesting to know that all test organisms showed highest inhibitory concentrati on against plant extracts.

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiolog ical activities . The phytochemical characteristics of the rhizome extract of *Zingiber officinale* investigated are sum marized in table 5.The results reveal the presence of medicinally active constituents like Tannins, Alkaloid, and Fl avonoids, in the extract. While saponins and steroids were absent in this plants. The alkaloids contained in plants ar e used in medicine as anesthetic agents.

IV. CONCLUSION

The findings revealed that the knowledge of the antimicrobial activity of the extracts obtained from ginger c an be very useful and can be applied in different areas of research such as the pharmaceutical and food industries. P hytochemical constituents such as steroids, alkaloids, flavonoids, tannins, phenol and several other aromatic compo unds are secondary metabolites of plants that serve a defence mechanism against prediction by many microorganism s, insects and herbivores. These secondary metabolites exert antimicrobial activity through different mechanisms. H erbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea, colitis and dysentery etc. The alkaloids contain in plants are used in medicine as anesthetic agents. Ginger rhizome extract and their components can be used as alternative and effective novel therapeutic strategy.

V. ACKNOWLEDGEMENT

I am grateful to our Principal Dr. K. S .Venkataraghavan**,** and Head of the Department of Zoology Dr. V eena Rambal for giving me the opportunity and necessary infrastructure. I am thankful to the Department of Biotec hnology, Nowrosjee Wadia College, Pune, India and Department of Zoology ,Nowrosjee Wadia College, Pune, Indi a for providing me the laboratory facilities during the study work.

VI. REFERENCES

[1] Akintobi O A,Onoh C C,etal.,Antimicrobial activity of *Zingiber officinale* (ginger) extract against some selected pathogenic bacteria.Natur e and science 2013;11(1):7-15

[2] Cappucino .J. G,ShermanN.Microbiology A Laboratory Manual. Pearson. 7th edition.2005.13-16

[3] Daburet.al.,Antimicrobial activity of some Indian medicinal plants.Afr.J.Traditional.CAM 2007;4(3):313-318

[4] Deshmukh. A. M.Media,Stains and reagents in Microbiology.PAMA Publication.1997:39-139

[5] Gull iIram,SaeedM,etal;Inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria.Annals of Clinical Microbiology and Antimicrobials 2012,11(8):1-5

[6] Islam Kamrul,etal.,Antimicrobial activity of Ginger (*Zingiber officinale*) exracts against food-borne pathogenic bacteria.International Journal of Science ,Environment.2014,3:867-871

[7] Malhotra Samir and Singh AmritPal.Medicinal properties of Ginger (*Zingiber officinale* Rosc.)Natural Product Radiance.2003,2(6):296-301

[8] NikolicM.,etal.,Antibacterial and anti-biofilm activity of ginger (*Zingiber officinale*) ethanolic extract.Kragujeva Journal of Science.2014 , (36):129-136

[9] RahmaniAh.,etal.,Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities.International Journal of PhysiolPathophysiol Pharmacol.2014,6(2):125-136

[10] ReygaertW.C.Antimicrobial resistance mechanisms of *Staphylococcus aureus*.:297-305

[11] Silva NCC,FernandesJ.A.Biological properties of medicinal plants:a review of their antimicrobial activity.TheJornal of Venomus Animals and Toxins including Tropical Diseases.2010,16(3):402-413

[12] Stuart A.G.Ginger:1-13

[13] SuhadA.A,etal.,Study the antibacterial activity of *Zingiber officinale* roots against some of pathogenic bacteria.Al-Mustansiriya J.Sci.2012,23(3):63-70