INTERNATIONAL JOURNAL FOR SCIENCE AND RESEARCH IN TECHNOLOGY ISSN NO.2395-1052
www.ijsart.com Impact factor: 2.1
Volume 2 Issue 7-JULY 2016

Study of Antimicrobial potential and Phytochemical analysis of *Ocimum sanctum* (Tulsi) leaves extract

Ashwini .R. Tambe[1],Kshitija. S. Mutalik[2], Sulabha. B. Deokar[3]
[1]Department of Zoology
[2, 3]Department of Biotechnology
[1, 2, 3]Nowrosjee Wadia College, Affiliated to SavitribaiPhule Pune University, India

Abstract-*Tulsi is an medicinal plant also known as Oscimum sanctum. Ethanol and aqueous extract of Tulsi leaves were prepared and the objective of study was to evaluate antibacterial and antifungal activity against bacterial strains Escherichia coli (Gram negative), Staphylococcus aureus(Gram positive) and fungal strain Aspergillus niger. Ethanol and aqueous extracts of different concentrations were prepared and tested against test microorganisms using agar well diffusion method. Phytochemical analysis showed the presence of reducing sugars, saponins, tannins,proteins, flavonoids,phenols. The Zone of inhibition showed efficiency of plant extract.The results showed that E.coli showed highest antibacterial activity of 18mm Zone of inhibition at 9% concentration as compared with activity against S.aureus of 19mm Zone of inhibition at 9% concentration. Antifungal activity against A.niger showed highest Zone of inhibition of 17mm at 9% concentration of aqueous extracts.Antibiotics such as gentamicin, penicillin and antifungicides such as clotrimazole were tested against human pathogens as positive control. The antimicrobial activity of Tulsi leaves extract have been confirmed experimentally. The results therefore confirmed the traditional use of Tulsi for its antimicrobial properties and curing diseases.*

Keywords- Zone of inhibition (ZOI) , Maximum inhibitory concentration (MIC), Antibacterial activity , Antifungal activity, Colony forming unit (CFU)

I. INTRODUCTION

Tulsi is held sacred by Hindus and is used as medicinal plant. Tulsi is believed to promote longevity and life long happiness.Phytochemical compounds in leaf include eugenol, urosolic acid and rosmarinic acid and other active compounds includes caryphyllene and oleanolic acid. The Tulsi plant is even known to purify or de-pollute the atmosphere and also works as a repellent to mosquitoes, flies and other harmful insects. This study was designed to explore the antimicrobial activity of *Oscimum sanctum* on selected human pathogens and to evaluate its phytochemical properties.

Fig.1. Leaves of Tulsi

Plant kingdom has a rich source of organic compounds, many of which are in use as agents against several infectious and non-infectious diseases (Naik *et.al* 2015). In ethno-botanical literature of India, several hundreds of plants are known to have potential to treat many diseases and one of those popular ones is Tulsi, traditionally used for the treatment of diseases (Singh V *et al*.2011).Leaves possess antimicrobial activity. Infections with both Gram-positive and Gram-negative bacteria have clinically become intractable, due to the emergence of, multidrugresistant. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. Due to the development of resistance in pathogenic microorganisms to antibiotics used in modern medical science, there is a growing attention towards plant extracts as a source of new antimicrobial drug discoveries.

II. MATERIALS AND METHODS

SAMPLE COLLECTION- The leaves of Tulsi were collected from Botanical garden of Nowrosjee wadia college, Pune, India. The samples were washed thrice using tap water followed by distilled water and were

 corresponding author: sulabhadeokar69@ymail.com

dried under shade in hygiene conditions for 10-12 days. All the materials were ground in an electric grinder to form fine powder. Powdered material was stored at 4°C in an air tight bottle.

COLLECTION OF TEST ORGANISMS-

The test organisms used in this study included *E.coli* NCIM 5010 (Gram negative) and *S.aureus* NCIM 2079 (Gram positive) bacterial strains and *A.niger* NCIM 501 fungal strain and these cultures were obtained from NCIM, Pune.The test organisms were cultured on agar slants and stored at 4°C in refrigerator.

PREPARATION OF AQUEOUS EXTRACTS-

Fine grounded powder was measured with electronic balance.Various concentrations were made such as 0%,3%,6% and 9% using each 100ml distilled water.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 obtained. Filtrates were covered with aluminium foil and stored in refrigerator at 4°C until required.

PREPARATION OF ETHANOL EXTRACTS-

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

INOCULUM PREPARATION FOR BACTERIA-The loop full of bacterial cultures were taken from slants and inoculated in Nutrient broth and incubated overnight at 37°C. The 50 µl of overnight culture of each bacterial strain was transferred into 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 min at 4˚C, washed with phosphate buffer, saline and resuspended in nutrient broth. The 10^7 CFU/ml inoculum concentration was adjusted.

INOCULUM PREPARATION FOR FUNGI- Fungal culture was grown on CzapekDox agar slants (sporulating medium). Slants 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 Hemocytometer.

AGAR DIFFUSION METHOD- The method was suitable for organisms that grows rapidly .The well of 6mm were punched in nutrient agar and potato dextrose agar media with sterile cork borer , after inoculation with bacterial cultures and spore suspension of bacteria and fungi respectively. When well was loaded with extract, it diffused in the medium 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- Added 30µl of bacterial suspension on nutrient agar(HiMedia pvt.Ltd India) plates. With help of sterile glass spreader, suspension of *E.coli* and *S.aureus* respectively were spreaded throughout the plate. Wells were punched of 6mm diameter into plates. Loaded 20-30µl of the plant extract of different concentrations into medium .Allowed to stand for 30mins for agar diffusion. Plates were incubated at 37°C for 24hrs. Observed the bacterial activity by measuring the zone of inhibition against the test organism using measuring scale. Antibiotics such as Penicillin, Gentamicin were used as positive control against Gram positive and Gram negative bacterial strain respectively. 0% extract was used as negative control, against bacterial strains.

ANTI-FUNGAL ACTIVITY ASSAY- Added 30µl of fungal spore suspension of *A.niger* on potato dextrose agar (HiMedia pvt.Ltd India) 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 30mins for agar diffusion. Plates were incubated at 22°C for 48-72 hrs. Observed the antifungal activity by measuring the zone of inhibition against the test organism by measuring scale. Antifungicide such as clotrimazole was used as positive control against fungal strain .0% extract was used as negative control, against fungal strains.

corresponding author: sulabhadeokar69@ymail.com

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 tulsi leaves were suspended in 10ml of sterile distilled water. kept overnight on rotary shaker.Extract was filtered with help of Whatmann filter paper no.1 and aqueous extraxt was used for further screening.

Test for reducing sugar: Benedicts test

To 1 ml of extract solution, 1 ml of water and 5 - 8 drops of Benedict's solution were added when hot and observed formation 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 tbluish black color indicated presence of phenols.

Test 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 precipitate indicated positive test for flavonoids.

Test for Amino acids: Xanthoproteic test:

1ml of leaves extract was mixed with few drops of concentrated nitric acid solution,added few drops of 40% NaOH, formation of yellow colour indicated presence of proteins.

Test for Tannins:

2 ml of the leaves extract was mixed with 2 ml of distilled water and few drops of $FeCl_3$ Solution. Formation of green precipitate indicated presence of tannins.

Test for Saponins:

2 ml of leaves extract was added in 2 ml of distilled water in a test tube and warmed. The formation of stable foam indicated the presence of saponins.

Test for Steroids:

When 2 ml of leaves extract was added in 2 ml of chloroform and 2 ml concentrated sulphuric acid , a red color produced below the chloroform layer indicated the presence of steroids.

III. RESULTS

The current study showed that plant extract of Tulsi exerted antibacterial and antifungal activity against selected human pathogens. The results table 1. showed that the extract possesed antimicrobial activity against test organisms, depending upon their capacity for diffusion into agar medium. Aqueous extract showed maximum zone of inhibition of 16mm at 9% concentration and minimum zone of inhibition of 8mm at 3% concentration. Ethanol extract showed maximum zone of inhibition of 19mm at 9% concentration and minimum zone of inhibition of 10mm at 3% concentration. Aqueous extract was found to be more efficient when compared with that of ethanol extract against *E.coli*. Figure 2 and figure 3 shows effect of Tulsi extract against *E.coli*.
Table 2. showed effect of different concentrations of Tulsi extract against *S.aureus*. Aqueous extract showed maximum ZOI of 18mm at 9% concentration and minimum ZOI of 11mm at 3% concentration which is comparatively more as compared with ZOI against *E.coli*. Ethanol extract showed maximum ZOI of 18mm at 9% concentration and minimum ZOI of 10mm at 3% concentration which is comparatively less than ZOI of *E.coli*. Figure 4 and figure 5 shows effect of Tulsi extract against *S.aureus*.

Table 3.showed antifungal activity of Tulsi against *A.niger*. figure 6 and figure 7 showed antifungal activity which is tabulated in table 9.maximum ZOI was about 17mm at 9% concentration and minimum ZOI was about 8mm at 3% concentration by aqueous extract. Ethanol extract showed maximum ZOI of 16mm at 9% concentration and minimum ZOI of 7mm at 3% concentration which is comparatively less as compaired with antibacterial activity. Extract of Tulsi leaves showed

corresponding author: sulabhadeokar69@ymail.com

highest zone of inhibition against each bacterial and fungal strain according to its concentrations. The higher the concentration higher efficiency was found against human pathogens.

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

The results confirmed the presence of constituents which were known to exhibit medicinal as well as physiological activities . The phytochemical characteristics of the leaf extract of *Ocimum sanctum* investigated were summarized in table 5.The results reveal the presence of medicinally active constituents like tannins, alkaloid, steroids and flavonoids, phenols, in the leaves of *Ocimum sanctum*. While saponins and proteins were absent in these plants.

I. Figures indicate antibacterial activity of Tulsi extracts against *E.coli*

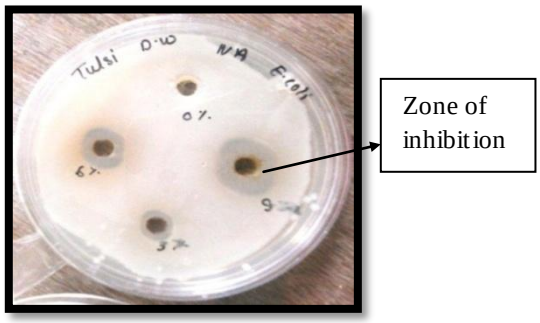


Fig.2-Effect of aqueous extract on *E.coli*

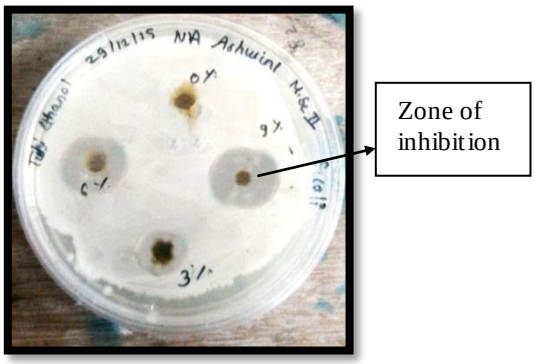
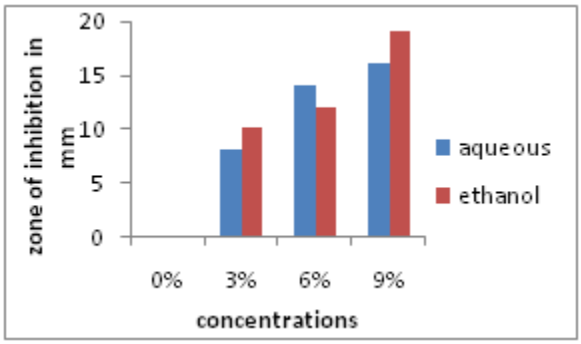


Fig.3 -Efffectof ethanol extract on *E.coli*

Solvent	Concentration	*E.coli* (ZOI in mm)
Aqueous	0%	0
	3%	8
	6%	14
	9%	16
Ethanol	0%	0
	3%	10
	6%	12
	9%	19

Table.1- Antibacterial activity of Tulsi extracts against *E.coli*



Graph.1- Antibacterial activity of Tulsi extracts against *E.coli*

II. Figures indicate antibacterial activity of Tulsi extracts against *S.aureus*

Fig.4- Effect of aqueous extract on *S.aureus*

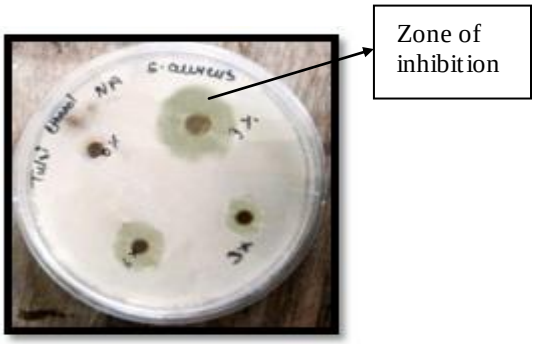
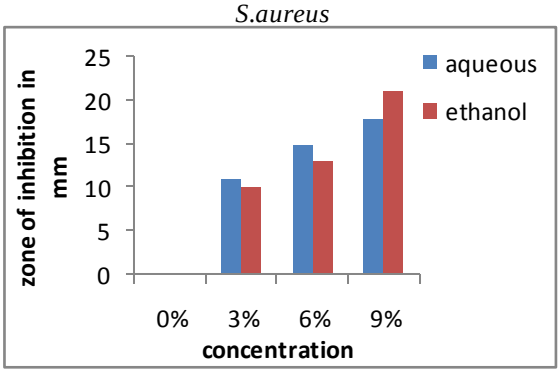


Fig.5 - Effect of ethanol extract on *S.aureus*

corresponding author: sulabhadeokar69@ymail.com

Solvent	Concentration	*S.aureus* (ZOI in mm)
Aqueous	0%	0
	3%	11
	6%	15
	9%	18
Ethanol	0%	0
	3%	10
	6%	13
	9%	18

Table .2- Antibacterial activity of Tulsi extracts against *S.aureus*



Graph .2- Antibacterial activity of Tulsi extracts against *S.aureus*

III. Figures indicate antifungal activity of Tulsi extracts against *A.niger*

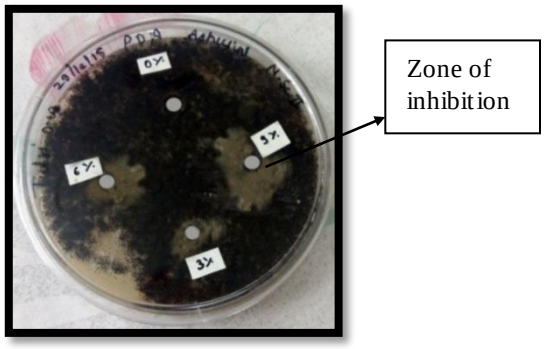


Fig.6- Effect of aqueous extract on *A.niger*

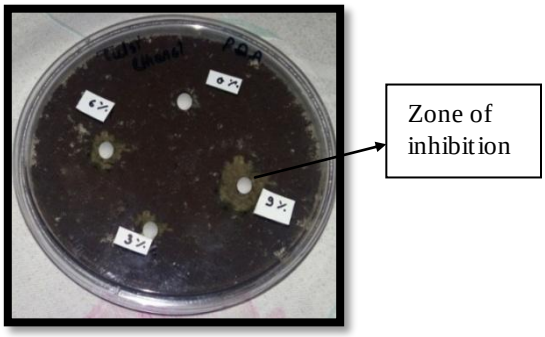
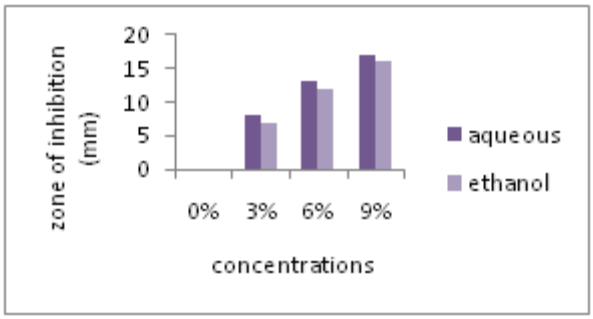


Fig.7- Effect of ethanol extract on *A.niger*

Solvent	Concentration	*A.niger* (ZOI in mm)
Aqueous	0%	0
	3%	8
	6%	13
	9%	17
Ethanol	0%	0
	3%	7
	6%	12
	9%	16

Table .3- Antifungal activity of Tulsi extracts against *A.niger*

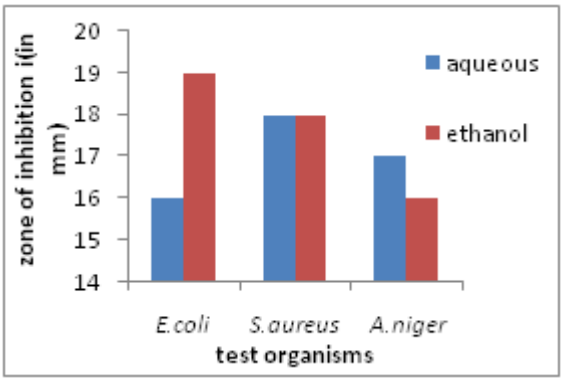


Graph .3- Antifungal activity of Tulsi extracts Against *A.niger*

Sr.no	Test Organism	Zone Of Inhibition in mm	
		9% Aqueous extract	9% Ethanol extract
1.	*E.coli*	16	19
2.	*S.aureus*	18	18
3.	*A.niger*	17	16

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

corresponding author: sulabhadeokar69@ymail.com



Graph 4. Antimicrobial activity showing Maximum Inhibitory Concentration (MIC) against testorganisms at 9% concentration

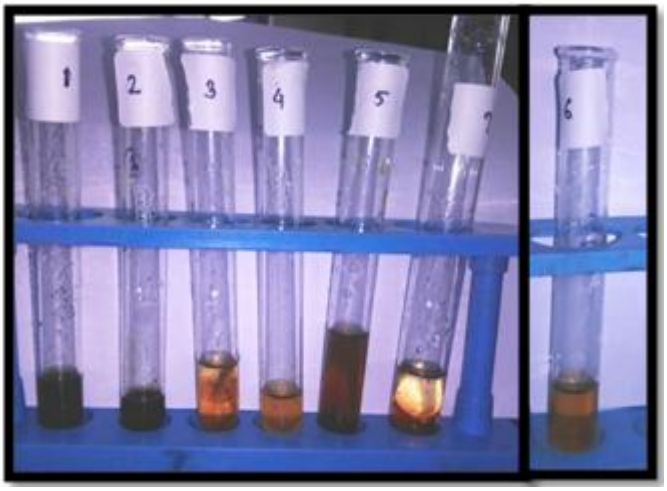


Fig.8- Phytochemical analysis of Tulsi aqueous extract

Sr no.	Test	Phytochemical constituents	Result
1.	Benedicts test	Reducing sugars	+
2.	Ferric chloride test	Phenols	+
3.	Alkaline reagent test	Flavonoids	+
4.	Xanthoproteic test	Amino acids	-
5.	Test for Tannins	Tannins	+
6.	Test for Saponins	Saponins	-
7.	Test for Steroids	steroids	-

Note: + : present - : absent

Table 5.Phytochemical analysis of Tulsi leaves aqueous extract

IV. DISCUSSION

Phytochemical constituents such as steroids, alkaloids, flavonoids, tannins, phenol and several other aromatic compounds were secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and herbivores. These secondary metabolites exert antimicrobial activity through different mechanisms. Herbs that have tannins as their main components were astringent in nature and were used for treating intestinal disorders such as diarrhea and dysentery. The alkaloids contain in plants were used in medicine as anesthetic agents.

ACKNOWLEDGMENT

We are grateful to our Principal Dr. K.S. Venkataraghavan, and Department of Biotechnology, Nowrosjee Wadia College, Pune-01. We would like to thank Department of Zoology, Nowrosjee Wadia College, Pune-01,Maharashtra,India.

REFERENCES

[1] Bhatt M.K, M.B.Shankar,etal.Evaluation of anti-microbial activity of *Ocimum sanctum* methanolic extract.Journal of pharmaceutical amd scientific innovation.2012;(4):39-41

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

[3] Chandra Rashmi,DwivediVinay,etal;Detection of antimicrobial activity of *Oscimum sanctum* (tulsi) & *Trigonella foenumgraecum* (methi) against some selected bacterial and fungal strains. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011;2(4):809-813

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

[5] Goswami P.Antibacterial effect of *Oscimum sanctum* Linn. (tulsi). International Journal of Allied Practice,Research and Review.42-46

[6] Gupta B, Kumar V N, Mallaiah S, Assessment of Antimicrobial Activity of various concentrations of commercially available tulsi (*Oscimum sanctum)*

corresponding author: sulabhadeokar69@ymail.com

powder against *Streptococcus* Mutans.Open Journal of Dentistry and Oral Medicine.2013, 1(2):19-24

[7] Gupta S.K.,JaiPrakash,SrivastavSushma,Validation of traditional claim of Tulsi *(Oscimum sanctum*) as a medicinal plant.Indian Journal of Experimental Biology.2002:765-773

[8] Jain.S.K. Medicinal plants.National book trust, India.1994.34-35

[9] Mistry K.S.et al.,The antimicrobial activity of *Azadirachta indica,Mimusop selengi,Tinospora cardifolia,Oscimum sanctum* and 2% chlorhexidinegluconate on common endodontic pathogens:An in vitro study.European Journal of Dentistry.2014;3:172-177

[10] Naik L.S et al,Antimicrobial activity and phytochemical analysis of *Oscimim tenuilforum* leaf extract.International journal of PharmTech Research.2015;8:88-95

[11] Nair L. N, Methods of microbial and plant biotechnology. New central book agency ltd. London.27-82

[12] RathnayakaR.M,Antibacterial activity of *Oscimum sanctum* extracts against four food-borne microbial pathogens.Scholars Journal of Applied Medical Sciences.2013,1(6):774-777

[13] Singh A.R,etal.,Phytochemical estimation and antimicrobial activity of aqueous and methanolic extract of *Oscimum sanctum*.J.Nat.Prod.Plant resource.2013,3(1):51-58

[14] Singh V.et al.,A review on ethnomedical uses of *Oscimum sanctum*(tulsi).International Research Journal of Pharmacy.2011,2(10):1-3

 corresponding author: sulabhadeokar69@ymail.com