

Preliminary phytochemical analysis and antimicrobial activity of extract of *Ocimum santum* L.

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I. Introduction:

Ocimum santum L. plant is serve as medicin in Ancient and today's medicine system. This plant is potential source of antimicrobial and bioactive molecule. *Ocimum santum* L is known for its antimicrobial activity against *E.coli* and *Staphylococcus aureus* (N.Pavithra et al 2012). *Ocimum santum* L producer of many industrial antimicrobial compounds. (Ashis Rajan et al 2013) This plant is commonly known as Tulsi belongs to family Lamiaceae. Tulsi is an incredible herb revered in Indian mythology for its medicinal properties. Tulsi is branched shrub 30-60 cm tall with hairy stem. This plant is used to prevent cough, cold, fever, asthma, hepatic disease and many skin disease. (Sunita Verma et al 2016) It is also known as aromatic plant. Plant derived drugs form an important segment of the modern medicine system. Tulsi is consider a nature treasures for biological research and its extract are useful for management of many infections and pathogen. Present paper were monitor of phytochemical analysis of plant parts extracts showed presence of secondary metabolites like alkaloids, phenols, glycoside and flavonoids. Methanolic extract was used to test antibacterial activity against human pathogenic bacteria. The development of drugs resistant to be a burning global issue (Pitout et al 2008) In this context plant extracts were alternative source of antimicrobial agents.

II. Material and Method:

- 1. Collection of plant material:** *Ocimum santum* L. were collected from in and around of Aurangabad.
- 2. Methanol extract:** 40 gm powder of fresh and shade dry Root, stem and leaves extracted by Soxhlet extraction process.
- 3. Test organism:** The authentic culture of human pathogenic bacteria viz. *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *E. coli* and *Staphylococcus aureus* were obtained from the department of Microbiology, Deogiri College, Aurangabad, Maharashtra. In vitro antibacterial assay of plant extract was carried out by using 96- well plate method.

4.96 – well plates method: About 100 µl sterile Mueller-Hinton broth medium was loaded into each well along with 2 µl serially diluted human pathogenic bacteria suspension, next 2, 4, 6, 8, and 10 µl concentrations of methanol extracted plant and ungal extract was added to each well of 96- well plate. Control was prepared by nutrient broth and bacterial suspension without adding extract. The prepared experimental 96- well plate was sealed with parafilm and incubated in incubator at 37°C for 24 hours. Finally optical density (OD) at 540 nm was measured on the spectrophotometer of each sample (Ataee, et al., 2012)

Detection test for Secondary Metabolites:

1. Glycoside: 1 ml of extract was taken and 0.5 ml of glacial acetic acid brown coloration at the junction of two layers and bluish green colour in the upper layers shows the presence of Glycoside.

2. Flavonoids: 5 ml extract was taken in test tube and hydrolyzed with 10% H₂SO₄ and allowed to cool then extracting with diethyl ether and divide into 3 equal portions in separated test tubes. 1 ml of 0.1N sodium hydroxide, 1 ml of dilute sodium carbonate and 1 ml of strong ammonia solution were added in test tubes respectively. Development of yellow colour indicates the presence of Flavonoids.

3. Alkaloids: Extract was taken in test tube and dilute HCl was added in it and filtered through Whatman filter paper, filtrate was treated with different alkaloids reagents.

a) Mayer's reagent: 1 ml of filtrate was treated with Mayer's reagent appearance of cream colour shows the presence of alkaloids.

b) Dragon draffs reagents: 1 ml of filtrate was treated with Dragon draffs reagent, reddish brown colour precipitation indicate the presence of alkaloids.

4.Phenols:ferric chloride test :2ml of extract was treated with 3-4 drops of ferric chloride formation of bluish black colour indicate the presence of phenols.(Shailaja 2015;Ibrahim and Kadhim 2015;Sridharetal 2016 Vasit 2017)

III. Results and Discussion :

Secondary metabolites and pathogenity test of plant was carried out by using conventional protocol for detection the presence of different phytochemical(Rajendra abhi D. 2017).Ocimumsanctum leaves and roots are showed Glycoside ,Flavanoids,Alkaloids and Phenols positive test.(Table I) stem were shows only phenols positive test(Table-I.)Minimum inhibitory concentration (MIC) of the methanol extract was evaluated by 96 well plate method followed by optical density at 450 nm was measured among the 5 serial dilution for each pathogenic bacteria were tested In which root, leaves and stem showed significant activity.8 µl concentration of root extract forSalmonella typhimurium, and Shigella flexneri was most significant.8,6µl concentration was effective for P. aeruginosa evaluating antibacterial activity also showed MIC at .6, 8µl concentration .(Table –II).The stem of Ocimum sanctumwere showed effective control at 2,6, and 8 µl respectively for , Shigella flexneri, Pseudomonas aeruginosa ,Staphylococcus aureus ,Salmonella typhimurium.E.coli(Table –III).MIC for leaf extract was 2, 6,8µl for ,Shigella Flexneri, Pseudomonas aeruginosa,E.coli and Salmonella typhimurium.(Table- III)Increase Concentration of root extract inhibits growth of bacteria. S.arueus shows more inhibition as compare to other bacteria. Increase and decrease Concentration of root extract inhibits growth of S. typhibacteria (Table-II, III) similar results was not observed in E.coli. Increase Concentration 6and 8µl of stem extract was most effective for Salmonella typhimurium and Shigella flexneri..(Table-III). Leaf extract 6µl Salmonella typhimurium and8 6,µl for Shigella flexneri. Similar results was observed in E. coli (Table IV).plants are animportant tool for pharmaceutical science. The review describes information of production of useful secondary metabolite and antibacterial activity of Tulsi .Ocimum sanctum contained all the chemicals except flavonoids and reducing sugar(Anjali Tiwari etal 2016) In present paper root and leaf are flavonoid positive while stem is negative . In this study Methanol extracted of plant was prepared. All extract of Root, Stem and, Leaves were used for antibacterial test by 96 well plate methods. (Ataee,etal 2012) Control was prepared by nutrient broth and bacterial suspension without adding extract .According to optical density at 540 nm. It was observed that methanolextracts of root and leaves of Ocimum sanctum against all pathogenic bacteria i.e. Escherichia coli, Proteus mirabilis, Staphylococcus aures was effective (Ashish Ranjan Singh etal (2013) It was observed that Root extract 6 µl was most effective forEcoli..(Table-I I) Antibacterial activity of the aqueous, alcoholic, chloroform extract and oil obtained from leaves of Ocimum sanctum were studied against E.coli, P. aeruginosa, S. typhimurium and S. aureus. Extract obtained from O. sanctum were observed equally effective against pathogenic gram-positive and gram- negative bacteria (Sunita Verma 2016) The leaves have shown the presence of all the phytoconstituents like carbohydrate, alkaloids, glycosides, phenolic compounds tannins, and flavanoids etc. (Rangita Tanwar etal 2015) In present study leaves were glycoside ,Flavanoids, Alkaloids and Phenols positive.

Table -I

Sr no	Name of plant O.sanctum	Glycoside	Flavanoids	Alkaloids	Phenols
1	Root	++	++	++	++
2	Stem	--	--	--	++
3	Leaf	++	++	++	++

++....Presence --.....Absence

Table II Antibacterial activity of Ocimum sanctumroot extract

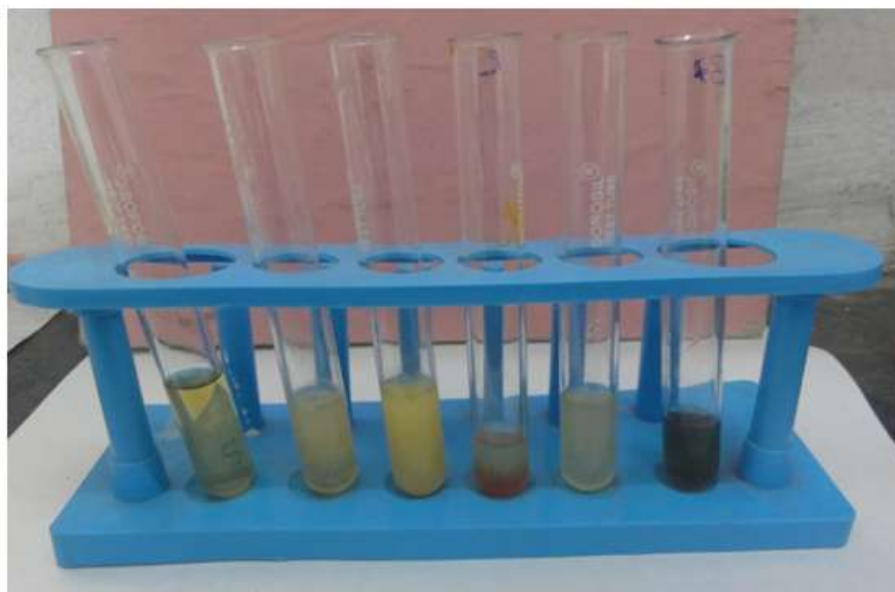
Sr.no	Ocimum root extract	Bacterial concentration(2µl)				
		S.typhi	S.flexineri	P.aurignosa	S.arueus	E.coli
1	2µl	0.06	0.05	0.08	0.08	0.05
2	4µl	0.03	0.02	0.04	0.02	0.03
3	6µl	0.04	0.01	0.03	0.02	0.01
4	8µl	0.02	0.03	0.03	0.04	0.06
5	10µl	0.05	0.04	0.06	0.05	0.05
	MIC	8µl	6µl	6,8 µl	4,6 µl	6,µl

Table II I Antibacterial activity of Ocimum sanctum stem extract

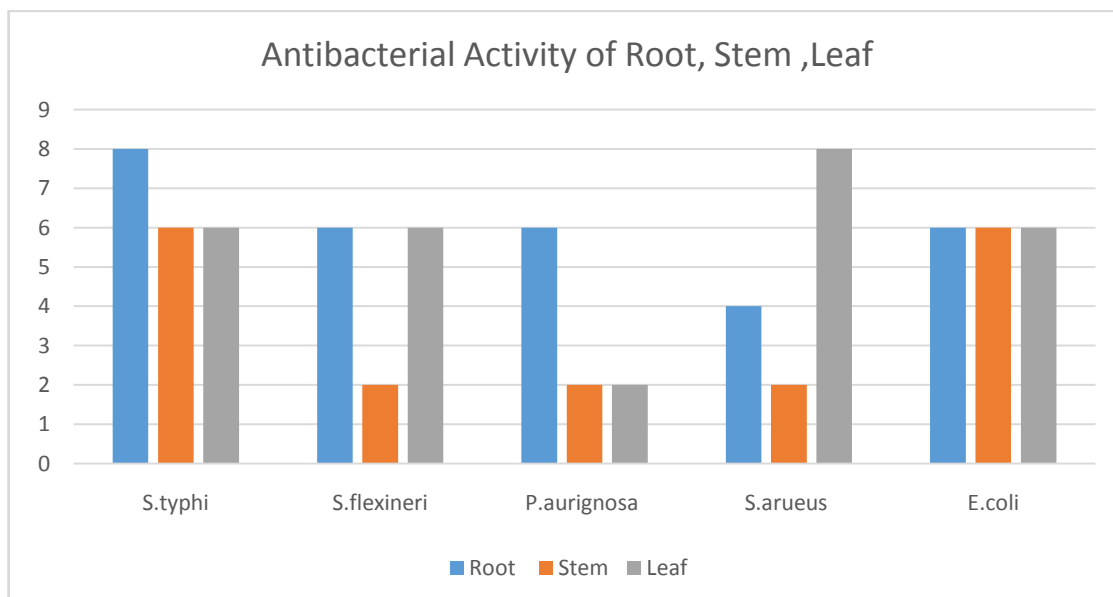
Sr.no	Ocimum stem extract	Bacterial concentration(2µl)				
		S.typhi	S.flexineri	P.aurignosa	S.arueus	E.coli
1	2µl	0.02	0.01	0.00	0.01	0.02
2	4µl	0.03	0.02	0.02	0.02	0.02
3	6µl	0.00	0.02	0.03	0.01	0.01
4	8µl	0.00	0.01	0.01	0.03	0.04
5	10µl	0.02	0.02	0.03	0.04	0.04
	MIC	6,8µl	2, 8µl	2µl	2,6 µl	6,µl

Table IV Antibacterial activity of Ocimum sanctum leaf extract

Sr.no	Ocimum leaf extract	Bacterial concentration(2µl)				
		S.typhi	S.flexineri	P.aurignosa	S.arueus	E.coli
1	2µl	0.16	0.14	0.24	0.21	0.22
2	4µl	0.22	0.19	0.20	0.23	0.22
3	6µl	0.15	0.12	0.18	0.13	0.13
4	8µl	0.20	0.12	0.16	0.14	0.14
5	10µl	0.19	0.14	0.17	0.18	0.08
	MIC	6,µl	6, 8µl	8µl	8 µl	6,µl



Reddish brown colour-Glycoside
 Yellow colour –flavonoids
 Cream colour –Alkaloide
 Bluish black colour –Phenols



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References:

- [1]. Shailaja J.R.(2015) Pharmacogenostic and Phytochemical Analysis of Gokshura w.s.r. to Cardoprotective Activity ,Anveshana Ayurveda Medical Journal ,Vol (5):pp.391-397.
- [2]. Ibrahim Nabaa M. and Kadhim Enas J.(2015) Phytochemical Investigation and Antioxidant Activity of Iraqi Tribulus,Iraqi J pharma Sci,Vol 24 (1):pp68-73.
- [3]. Sridhar,K.Rajesh ,B.Sangeeta K. (2016) Phytochemical screening and GC-MS Anaysis of Ethanolic Extract of Tribulus terrestris ,International Journal of Pharmacology Research Vol 6(1):pp 44-50.
- [4]. Anjali Tiwari ,Ankit Panday and O.P. Verma (2016)Phytochemical screening ofOcimum sanctum(Tulsi),Azadirachta indica (Neem) and Phyllanthus emblica (Amla) Volume 11 | Issue 1 | April, 2016 | ASIAN JOURNAL OF BIO SCIENCE
- [5]. Ashish Ranjan Singha, Vijay Kumar Bajaj, aPritam Singh Sekhawatb and Kapil Singhb (2013)Phytochemical estimation and Antimicrobial activity of Aqueous andMethanolic extract of Ocimum Sanctum L. Scholars Research LibraryJ. Nat. Prod. Plant Resour., 2013, 3 (1):51-58
- [6]. Sunita Verma (2016)Chemical constituents and pharmacological action of Ocimum sanctum (Indian holy basil-Tulsi) The Journal of Phytopharmacology 2016; 5(5): 205-207
- [7]. Rangita Tanwar, Prof. Amiya Pahare, Dr. S. M. A. Naqvi (2015) Ocimum sanctum (Tulsi) excellent Source of Phytochemical Indo American Journal of Pharmaceutical Research, 2015 ISSN NO: 2231-6876
- [8]. Srinivas Naik, Perka Shyam, K.Paul Marx, Srinivas Baskari,Prof.Ch.Venkata Ramana Devi(2015) Antimicrobial Activity and Phytochemical Analysis of Ocimum tenuiflorum Leaf Extract .International Journal of Pharm Tech Research CODEN (USA): IJPRIF, ISSN: 0974-4304 vol.8, No.1, pp 88-95, 2015
- [9]. P. Kalyan kumar *, M. Rupesh Kumar, K. Kavitha, Jagadeesh singh and Rawoof Khan2012Pharmacological Actions of Ocimum saantum– Review article (2012) IJAPBC – Vol. 1(3), Jul- Sep, 2012
- [10]. G. Devendran and U. Balasubramanian (2011)Qualitative phytochemical screening and GC-MS analysis ofOcimum sanctum L. leaves Asian Journal of Plant Science and Research, 2011, 1 (4):44-48
- [11]. Jyotsana Sharma Naveen Khurana Neha sharma Rakesh Garg.(2017) Phytochemical Evaluation and Antioxidant Screening studies of Ocimum tenuiflorumL. Seeds Asian Journal of Pharmaceutical and Clinical Research
- [12]. Abhijit Balasaheb Shinde1 and Yogini Ramkrishna Mulay2 (2015)Phytochemical Analysis and Antibacterial Properties ofSome Selected Indian Medicinal PlantsInt.J.Curr.Microbiol.App.Sci (2015) 4(3): 228-235
- [13]. Pranay Kumar Gupta, Siddarth Pulapalli, Srikanth (2015) Tulsi: An elixir for human life Research and Reviews: Journal of Medicinal ChemistryBritish Journal of Pharmaceutical Research3(2): 273-292, 2013 SCIENCEDOMAIN international
- [14]. Baby Joseph1 and Vrundha M. Nair (2013) Ethanopharmacological and Phytochemical Aspects of Ocimum sanctum Linn- The Elixir of life British Journal of Pharmaceutical Research 3(2): 273-292, 2013
- [15]. P. Kalyan kumar *, M. Rupesh Kumar, K. Kavitha, Jagadeesh singh and Rawoof Khan2012Pharmacological Actions of Ocimum saantum– Review article (2012) IJAPBC – Vol. 1(3), Jul- Sep, 2012

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