

A Comparative Study of *Ocimum* species (Tulsi) as a Potential Alternative Antimicrobial Agent

Kshama Murarkar

M.Sc., Ph.D, Assistant Professor, Department of Microbiology

Dr. R. G. Bhoyar Arts, Commerce, and Science College Seloo, Wardha, MS, India.

kmurarkr@gmail.com

Abstract: In recent years researchers have marked the importance of the plant-based antimicrobial agents due to the short life effectivity, and undesirable side-effects of chemical drugs. In this paper we discussed the antimicrobial effect of fresh leaf (FL) and dried leaf (DL) *Ocimum* species extracts against clinical pathogens. The phytochemicals were also analyzed qualitatively. The results shown that FL and DLO. *thyriflora* ethanol (95 %) and isopropanol extracts, and *O. tenuiflorum* FL ethanol (95 %) extract was shown remarkable antimicrobial effect on *C. albicans*, *S. aureus*, *E. coli*, and *S. aureus*, *K. pneumoniae*, *B. subtilis* respectively but not on *P. aeruginosa*. The *O. sanctum* FL isopropanol extract was effective on *C. albicans*, *S. aureus* and *K. pneumoniae*. The presence of tannins, and flavonoids may be responsible for this activity, and the difference in zone diameter may be related to phytochemical's quantity, and other factors present in the extract. The conclusion is that all the *O. thyriflora* and *O. tenuiflorum* FL and DL extracts, and *O. sanctum* FL isopropanol extract was antimicrobial potential against bacteria and yeast. According to published data *Ocimum* species is also useful against viral infections, hence can be used to treat viral infections in this pandemic situation.

Keywords: Antimicrobial effect, *O. thyriflora*, *O. tenuiflorum*, *O. sanctum*, clinical pathogens

I. INTRODUCTION

The problem of antibiotic resistivity is increasing in human pathogenic microorganisms. Apart from that during the treatment most of the drugs also shows various side effects which are harmful to human health [1-2]. Thus, one of the promising solutions for such issues is the use of medicinal plants. The remarkable benefits of the use of medicinal plants for therapeutic treatments is its low cost, no side effects, easily available, and effectiveness[3]. In recent years modern medical researchers are interested in searching alternative medicinal sources from plant origin for the therapeutics uses [4]. These natural medicines are the important source of potentially useful phytochemicals[5-6] which could provide new ideas for designing modern drug [7]. Among them 'aromatic therapeutic plants' are the great source of phytochemicals which may be used as medicine [8-10]. Tulsi (Holy Brasil) has provided the naturally safe solution for designing the useful drugs[10]. Tulsi is a Sanskrit word (means matchless) [11] is an aromatic therapeutic plant[12]. It comes under the family *Lamiaceae* and genus *Ocimum* [13]. This genus contains near about 150 species which are different from each other in their morphology, essential oil, and Phytochemical components [14]. Many researchers have reported the potential activity of basil leaf extract; as antioxidant, anti-aging, anticancer, antiviral, and antimicrobial activity[15-19]. The phytochemical rosmarinic acid present in sweet brasil is the biologically active content mainly responsible for various types of potent biological activities [20-22]. *Ocimum* species have great activity against bacteria, viruses, and fungi, its extracts also increase the activity of T helper cells and natural killer cells. [12,23]. It is also mentioned that an *Ocimum sanctam* plant had shown great antimicrobial effect against multi drug resistant strains of *Neisseria gonorrhea*[24]. The researchers have reported that aqueous and methanolic extracts of *Ocimum sanctum* was effective against *E. coli*, *S. aureus*, *A. niger*, and *Penicillium species* and these extracts also contain various types of phytochemicals[25]. Mittal *et al.* [21] have mentioned about the effectivity of *Ocimum sanctum*



leaves extracts prepared in ethanol, hexane and chloroform by the cold extraction method against *K. pneumonia* and *S. aureus*. It is published in one of the literatures that aqueous extract of *Ocimum kilimandscharicum*, *Ocimum tenuiflorum*, and *Ocimum gratissimum* had shown antimicrobial effect against *B. cereus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, and *A. niger* [26]. The scientists have reported antimicrobial effect of hot aqueous extract of *O. sanctum* leaves on the growth of New Castle Disease Virus in chicken embryo fibroblast monolayer culture [27]. Dixit *et al.* [28] published the antimicrobial effect of the methanolic, ethanolic, and aqueous extracts of *Ocimum thyrsoflora*, *Ocimum tenuiflorum*, and *Ocimum sanctum* on *Bacillus subtilis*.

The present study explains the antimicrobial effect of fresh leaf and dried leaf extracts (in 95 % ethanol and isopropanol) of three different varieties of *Ocimum* species in the Nagpur area; *O. thyrsoflora*, *O. tenuiflorum* and *O. sanctum* against clinical pathogens *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *K. pneumonia*, and *C. albicans*. The phytochemicals; tannins and flavonoids were also analyzed qualitatively. The results of all the *Ocimum* species were compared.

II. METHODS AND MATERIAL

2.1 Microorganisms

The pathogens; *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *K. pneumonia*, and *C. albicans* were isolated from clinical isolates of patients.

2.2 Collection of *Ocimum* species leaf for extraction:

The leaf of *O. thyrsoflora* (called Pandharpuri tulsi in Maharashtra), *O. tenuiflorum* (Krishna tulsi), and *O. sanctum* (Rama tulsi) has been collected from the home garden in the Nagpur area, dried in shed to make powder.

2.2.1 Preparation of extracts

The fresh leaf (FL) and dried leaf (DL) of *Ocimum* species have been macerated thoroughly with ethanol (95 %) and iso-propanol (1:5) separately, and kept for three days in tight containers at room temperature. After filtration filtrate was collected and stored in screw tight bottles for further use.

2.2.2 Antimicrobial effect of the extract

Antimicrobial effect of all the extracts was checked with Kirby-Bauer method. The microbial cultures (24 hours old, 0.1 ml) of *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *K. pneumoniae*, and *C. albicans* were spread on the separate Muller Hinton agar plate (MHA). Each plate was loaded with 0.1 ml *Ocimum* species extracts in individual 2 mm well on MHA plate. All the Petri plates were incubated at optimum temperatures. Depending upon the active ingredients and diffusion capacity into agar medium all the extracts has shown antimicrobial activity. The experiment has been repeated for three times.

2.3 Qualitative phytochemical test of fresh leaf and dried leaf extracts:

2.3.1 Ferric chloride test for tannins

Ocimum species leaf extract (1 ml) was mixed with few drops of ferric chloride (FeCl_3) solution. It gets developed into intense green, purple, blue in color which indicates the presence of tannins [29].

2.3.2 Ferric chloride test for flavonoid

Ocimum species leaf extract (0.5 ml) was boiled with distilled water, and then filtered. The filtrate (2 ml) was mixed with few drops of 10% ferric chloride (FeCl_3) solution. It gets developed into greenish blue, violet color which indicates the presence of a phenolic hydroxyl group [29].

III. RESULT AND DISCUSSION

O. thyrsoflora fresh leaf ethanol (95 %) extract was sensitive against *C. albicans* and *S. aureus* (23 mm, 23 mm) as compared to *O. sanctum* (14 mm and 13 mm). *O. tenuiflorum* had given the broad range of antimicrobial activity against selected microorganisms; *S. aureus* (25 mm), *K. pneumoniae* (20 mm), *B. subtilis* (18 mm), and *E. coli* (14 mm) [Figure 1 & 2]. Yamani *et al.* [2] reported that *Ocimum tenuiflorum* plant oil extract had shown microbicidal effect on



the growth of *S. aureus*, methicillin resistant *S. aureus*, and *E. coli*. Shokeen *et al.* [24] demonstrated the efficacy of *Ocimum sanctum* hexane extract against multidrug resistant and susceptible strains of *Neisseria gonorrhea*. Dried leaf (DL) *O. thyrsoiflora* ethanol extract also shown greater sensitivity against *C. albicans* (18 mm), *E. coli* (18 mm), and intermediate type of action against *S. aureus* (15 mm). *O. sanctum* DL extract not shown any effect on tested microorganisms [Figure 1 & 3]. Saurabh *et al.* [23] reported that dried leaf extract of *Ocimum sanctum* (green variety) in methanol was not effective against *E. coli* whereas black variety was effective against *E. coli* (16 mm). The fresh leaf isopropanol extract of *O. thyrsoiflora* and *O. sanctum* has shown greater antimicrobial effect on *C. albicans* and *S. aureus* (18 mm & 20 mm and 19 mm & 20 mm respectively) [Figure 1 & 4]. Dried leaf *O. thyrsoiflora* isopropanol extract has shown sensitive to intermediate type of antimicrobial effect against tested microorganisms except *K. pneumoniae*. *Ocimum tenuiflorum* DL extract was more effective on *C. albicans* (25 mm). Dried leaf *O. sanctum* has not shown any antimicrobial activity [Figure 5], and this may be related to the quantity of tannins and flavonoids, and other phytochemicals. The reported zone diameter of dried leaves *Ocimum sanctum* methanol extract by Saurabh *et al.* [23] for *Staphylococcus aureus* was 17 mm. This may be due to the solubility of phytochemicals in methanol. Dried leaf isopropanol and ethanol extracts, and fresh leaf isopropanol extract of *O. thyrsoiflora* has not shown any remarkable antimicrobial activity against *P. aeruginosa* (10 mm and 12 mm respectively). Yamani *et al.* [2] tested antimicrobial activity of oil extracted from *Ocimum tenuiflorum*, and it was less effective against *P. aeruginosa*. The phytochemical analysis of tannin and flavonoids has shown positive result for *Ocimum species* extracts. The phytochemical tannins inhibit the growth of many types of microorganisms [30, 31], and is toxic to many filamentous fungi, yeast and bacteria [32]. Dixit *et al.* [28] analyzed the qualitative presence of quinones, terpenoids, flavonoid of *O. thyrsoiflora*, *O. tenuiflorum* and *O. sanctum* ethanolic (absolute), methanolic and aqueous extracts. They reported the presence of quinone, terpenoids and flavonoids in all the three varieties, and the amount of flavonoids detected was more in *O. thyrsoiflora* followed by *O. tenuiflorum* and *O. sanctum* respectively [28]. It is reported that natural flavonoids have antimicrobial properties against the broad range of pathogens [33].

IV. CONCLUSION

All the fresh leaf and dried leaf *O. thyrsoiflora* extracts have shown the effect on *C. albicans*, *S. aureus* and *E. coli*. Fresh leaf *O. tenuiflorum* ethanol extract has shown greater antimicrobial activity on *K. pneumoniae* and *B. subtilis* than dried leaf extract. All the *Ocimum species* extracts were not effective on *P. aeruginosa*. Fresh leaf *O. sanctum* isopropanol extract was effective against *C. albicans*, *S. aureus* and *K. pneumoniae*, whereas dried leaf isopropanol extract was ineffective. The presence of tannins and flavonoids may be responsible for this activity. The type of antimicrobial activity, 'sensitive, Intermediate or resistant' may be depending on percent of the phytochemicals and other factors present in the extract of *Ocimum species*. From this we concluded that *Ocimum species* FL and DL extracts of *O. thyrsoiflora* and *O. tenuiflorum* in both the solvents, and FL *O. sanctum* isopropanol extract have shown broad spectrum antimicrobial activity against pathogenic yeast, gram positive and gram-negative microorganisms. So, it and can be used as a potential alternative natural source of drug to treat microbial infection (including drug-resistant microorganisms). Various researches have reported the antiviral, immune system boosting properties of *Ocimum species*. So, it can also be used to control viral infections in this pandemic situation apart from its other properties.

V. ACKNOWLEDGEMENT

Author is thankful to Dr.R.G.Bhoyar Arts, Commerce, and Science College, Seloo; district Wardha (MS), India, and Kamla Nehru Mahavidyalaya, Nagpur (MS), India for providing the facilities for this work.

REFERENCES

1. Russell AD. Antibiotic and biocide resistance in bacteria. Journal of Applied Microbiology. Symposium Supplement. 2002; 92:1S-3S.



2. Yamani HA, Pang EC, Mantri M, Deighton MA. Antimicrobial activity of tulsi (*Ocimum tenuiflorum*) essential oil and their major constituents against three species of bacteria. *Frontiers in Microbiology*.2016; 7. Article 681.doi.org/10.3389/fmicb.2016.00681.
3. Siddique HH. Safety of Herbal Drugs-An overview. *Drugs News Views*.1993; 1:7-10.
4. Bano N, Ahmed A, Tanveer M, Khan GM, Ansari MT. Pharmacological evaluation of *Ocimum sanctum*.J. *Bioequivalence Bioavailability*.2017; 9(3): 387-392.doi: 10.4172/jbb.1000330.
5. Georgiev MI (2014) Natural products utilization. *Phytochemistry Reviews*.13(2):339-341
6. Xiao JB, Muzashvili TS, Georgiev MI. Advances on biotechnology for glycosylation of high value flavonoids. *Biotechnology Advances*.2014; 32(6):1145-1156.
7. Newman DJ, Cragg GM. Natural products as source of new drug from 1981 to 2014.*Journal of Natural Products*.2016; 79(3): 629-661.doi: 10.1021/acs.jnatprod.5b01055.
8. Mathela CS. 'Allelochemicals in medicinal and aromatic plants', in *Allelopathy in Agriculture and Forestry*, Eds Narwaal SS and Tauro P (Jodhpur: Scientific Publishers); 1991, 213-228.
9. Cutler HG, Cutler SJ. Biologically active natural products: agrochemicals. 1st Ed.CRC Press: Boca Raton; 1999.doi: https://doi.org/10/ 9781420048629.ISBN9780429120626.
10. Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. *Journal of Natural Products*. 1997; 60(1): 52-60.doi:10.1021/np9604893.
11. Labh KB. Queen of herbs tulsi (*Ocimum sanctum*) removes impurities from water and plays disinfectant role. *Journal of Medicinal Plant Study*.2014; 2(2):1-8.
12. Patel RR. Tulsi: The Queen of medicinal herbs. *Journal of Bioequivalence & Biodiversity*.2020; 12(6):407.
13. Chowdhury Mandal A, Roy SC, Sarker DD. Diversity of the genus *Ocimum* (*Lamiaceae*) through morpho-molecular (RAPD) and chemical (GC-MS) analysis. *Journal of Genetic Engineering and Biotechnology*.2017; 15(1):275-286.
14. Danesi F, Elementi S, Neri R, Maranesi M, D'Antuono LF, Bordoni A. Effect of cultivar on the protection of cardiomyocytes from oxidative stress by essential oil and aqueous extracts of basil (*Ocimum basilicum* L.). *Journal of Agriculture and Food Chemistry*.2008; 56(21): 9911-9917.doi:10.1021/jf8018547.
15. Chiang LC, Ng LT, Cheng PW, Chiang W and Lin CC. Antiviral activities of extracts and selected pure constituents of *Odium bsilicum*. *Clinical and Experimental. Pharmacology and Physiology*.2005; 32 (10):811-816.
16. Bozin B, Mimic-Dukic N, Simin N and Anackov G.Characterization of the volatile composition of essential oils of some *Lamiaceae* spices and the antimicrobial and antioxidant activities of the entire oils. *Journal of Agriculture and Food Chemistry*.2006; 54(5):1822-1828.
17. Manosroi J, Dhumtanom P, Manosroi A. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Letters*.2006; 235(1):114-120.
18. de Almeida I, Alviano DS, Vieira DP. Antigardial activity of *Ocimum basilicum* essential oil. *ParasitologyResearch*.2007; 101(2)443-452.
19. Akujobi CO, Anyanwu BN, Onyeze GO, Igbekwe VI. Antibacterial activities and preliminary phytochemical screening of four medicinal plants. *Journal of Applied Science*.2004; 7(3):4328-4338.
20. Lee J, Scagel CF. Chicoric acid found in basil (*Ocimum basilicum* L) leaves. *Food Chemistry*. 2009;115(2): 650-656.
21. Mittal R, Kumar R, Chahal HS. Antimicrobial activity of *Ocimum sanctum* leaves extracts and oil. *Journal of Drug Delivery & Therapeutics*.2018;8(6):201-204.
22. Shiga Shoji K, Shimada H, Hashida SN, Goto F, Yoshihara T. Effect of light quality on rosmarinic acid content and antioxidant activity of sweet basil leaf extract decreases tumorigenicity and metastasis of aggressive human pancreatic cancer cells in vitro and vivo: potential role in therapy. *Cancer Letters*. 2013; 336(2):270-280.



23. Saurabh G, Komal S. Comparative Characterization for Antimicrobial Activity and Bioactive Compounds Present in Leaf Extract of *Ocimum sanctum*. Journal of Food Industrial Microbiology.2017; 3:1-5. doi:10.4172/2572-4134.1000120.
24. Shokeen P, Bala M, Singh M. Tondon V. In vitro activity of eugenol, an active component from *Ocimum sanctum*, against multidrug resistant and susceptible strains of *Neisseria gonorrhea*. International Journal Antimicrobial Agents.2008; 32:172-179.
25. Saranya T, Noorjahan CM and Siddique SA. Phytochemical screening and antimicrobial activity of tulsi plant. International Research Journal of Pharmacy.2019; 10(6): 52-57. Doi:10.7897/2230-8407.1006203.
26. Londhe AM. Kulkarni AS, Lawand RV. In-Vitro comparative study of antibacterial and antifungal Activities: A Case Study of *Ocimum kilimandscharicum*, *Ocimum tenuiflorum* and *Ocimum gratissimum*. International Journal of Pharmacognosy and Phytochemical Research. 2015; 7(1): 104-110.
27. Jayati, Bhatia AK, Kumar A, Goel A, Gupta S, Rahal A. In vitro antiviral potential of *Ocimum sanctum* leaves extract against New Castle Disease Virus of poultry. International Journal of Microbiology and Immunology Research.2013;2(7):051-055.<http://www.apexjournal.org>.
28. Dixit A, Gulathi B, Sharma G, Bhatia Priya R, Bhattacharya S. Evaluation of phytochemical and antimicrobial activity of *Ocimum spp*. Integrative Food Nutrition Metabolism.2021;8:8-14. doi: 10.15761/IFNM.1000299.
29. Evans CE, Evans D, Trease GE. Trease and Evans' Pharmacognosy.15th Ed. London: 42-44; 2002.
30. Velappan S, Thangaraj P. Phytochemical constituents and antiarthritic activity of *Ehretia Laevis Roxb*. 2014; 48: 487-491.
31. Ho PL,Yung RW,Tsang DN,Que TL,Ho M,Seto WH, *et al*. Increasing resistance of *Streptococcus pneumoniae* to fluoroquinolones: Results of a Hong Kong multicare study in 2000.Journal of Antimicrobial Chemotherapy.2001;48:659-665.
32. Scalbert A. Antimicrobial properties of tannins. Phytochemistry.1991; 30(12):3875-3883.
33. Xie Y,Yang W, Tang F, Chen X, Ren L. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Current Medicinal Chemistry.2015; 22(1):132-149. doi: [10.2174/0929867321666140916113443](https://doi.org/10.2174/0929867321666140916113443).

VII. FIGURES



(a) *C. albicans*

(b) *S. aureus*

(c) *E. coli*





(d) *B. subtilis* (e) *P. aeruginosa* (f) *K. pneumoniae*

Figure 1: Antimicrobial effect of ethanol (95 %) and iso-propanol extracts of *O. thyriflora*, *O. tenuiflorum*, and *O. sanctum* against pathogenic organisms.

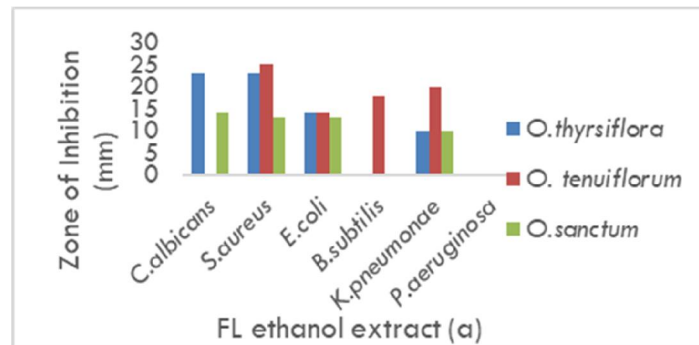


Figure 2: Antimicrobial activity of fresh leaf *Ocimum species* ethanol (95 %) extracts against clinical pathogens.

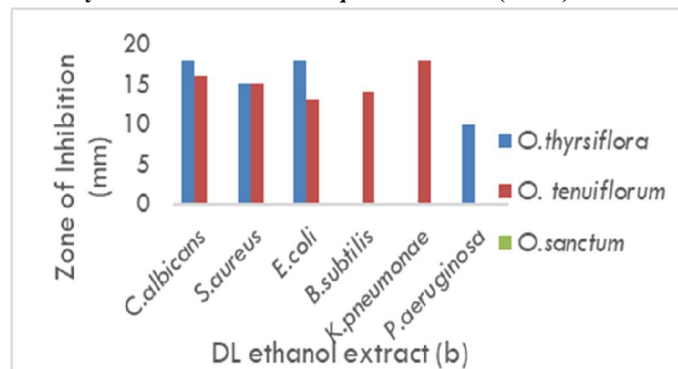


Figure 3: Antimicrobial activity of dried leaf *Ocimum species* ethanol (95 %) extracts against clinical pathogens



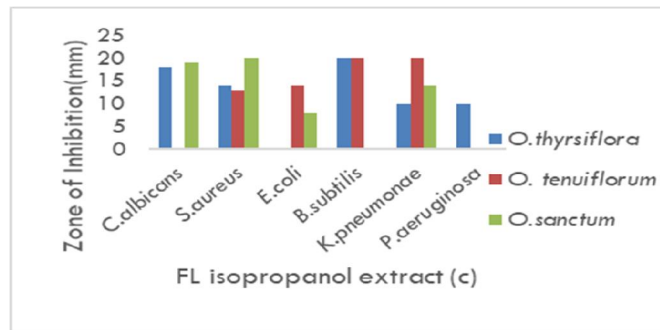


Figure 4: Antimicrobial activity of fresh leaf *Ocimum species* isopropanol extracts against clinical pathogens.

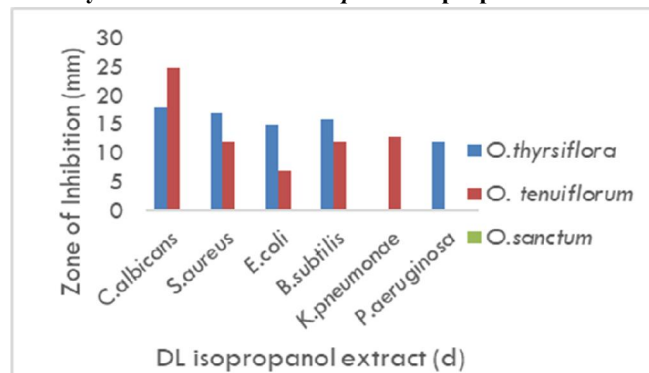


Figure 5: Antimicrobial activity of dried leaf *Ocimum species* isopropanol extracts against clinical pathogens.

VIII. ABBREVIATIONS

FL: Fresh leaf

DL: Dried leaf

