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Phytoconstituents and antibacterial activity of Tulsi (*Ocimum sanctum* Linn.): A review

AKM Mamun Ur Rashid[1], Md Abu Bin Nyeem[2], Razat Kishor Sinha[3], BM Rabiul Islam[4]
[1] Department of Public Health, American International University-Bangladesh
[2, 3] Department of Unani Medicine, Hamdard University Bangladesh, Hamdard City of Science, Education & Culture, Gazaria, Munshiganj, Bangladesh
[4] Department of Public Health, ASA University, Bangladesh

Abstract
Tulsi "Queen of herbs" is described as sacred and medicinal plant in ancient literature. It is an important symbol of the Hindu religious tradition. The name Tulsi is derived from "Sanskrit", which means "matchless one". Its other name, Vishnupriya means the one that pleases Lord Vishnu. This plant (*Ocimum sanctum*) belongs to the family Labiatae, characterized by square stem and specific aroma. This review has been conducted to pile up information that is available in different scientific literatures and this update compendium documentation has focused on some of the antibacterial aspects and phytochemical constituents. From the result it reveals that wide numbers of phytochemical constituents have been isolated from the plant as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugenol and is proved to have potential for medical effects as antimicrobial, anti-inflammatory, and antifungal activity. The antibacterial effects of *O. sanctum* may delay the development some life threating complications and this work stimulates the researchers for further research on the potential use of this medicinal plants having pharmaceutical potential.

Keywords: phytoconstituents, antibacterial activity, tulsi, *Ocimum sanctum*

Introduction
Ocimum sanctum has two varieties i.e. black (Krishna Tulsi) and green (Rama Tulsi), their chemical constituents are similar [1]. *Ocimum sanctum* is widely distributed covering the entire Indian sub continent, ascending up to 1800 m in the Himalayas and as far as the Andaman and Nicobar Island [2]. Tulsi is a Sanskrit word which means "the incomparable one" and has a very special place in the Hindu culture. Several medicinal properties have been attributed to the Tulsi plant not only in Unani, Ayurveda and Siddha but also in Greek and Roman systems of medicine [3].
O. sanctum L. (Tulsi) is an erect, much branched sub-shrub 3060cm tall, with simple opposite green or purple leaves that are strongly scented and hairy stems. Leaves have petiole and are ovate, up to 5cm long, usually somewhat toothed. Flowers are purplish in elongate racemes in close whorls [4].

Fig 1: *Ocimum sanctum*

Scientific classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Lamiales
Family	:	Lamiaceae
Genus	:	Ocimum
Species	:	*O.Tenuiflorum*
Binomial name	:	*Ocimum tenuiflorum* or *Ocimum sanctum* L.

Chemical composition
The chemical composition of Tulsi is highly complex, containing many nutrients and other biological active compounds. Fresh leaves and stem of *Ocimum sanctum*

extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugeno. The leaves of *Ocimum sanctum* contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene [5]. Two flavonoids orientin and andvicenin from aqueous leaf extract of *Ocimum sanctum* have been isolated [6].

Antibacterial activity

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 [7]. Fresh leaves essential oil had shown more antibacterial properties compared to dried leaves essential oil of Tulsi and in case of fungus the property is just the reverse [8].

Ocimum sanctum (OS) shows antimicrobial activity. Ethanolic, methanolic, and organic solvents extracts of *Ocimum sanctum* L. show wide zones of inhibition against Escherichia coli, Staphylococci sp., Shigella sp., Staphylococcus aureus and Enterobacteria sp [9]. *Ocimum sanctum* also acts against Pseudomonas aernginosa, Staphylococci sp., Salmonella typhi, Klebsiella pneumonia, Proteus, Candida albicans, Mycobacterium tuberculosis and Micrococcus pyogenes [10-11]. These results proves that OS can act as excellent antimicrobial agent against many microbes.

Essential oils extracted from the leaves of *Ocimum sanctum* L. has been found to inhibit growth of *E. coli*, *B. anthracis* and *P. aeruginosa in vitro*, showing its antibacterial activity. *Ocimum sanctum* also possesses antifungal activity against *Asperigillus niger* and aqueous extract of it was found to be effective in patients suffering from viral encephalitis [12]. In the treatment of ring worm infections, Tulsi leaves paste is indeed found to be very effective. Tulsi has significant natural antibacterial, antiviral and antifungal activities and is helpful in treating many serious systemic diseases, as well as localized infections.

Being the most commonly used medicinal plant in Indian house hold its antibacterial activities have been studied against common pathogens viz., *E. coli* and *Staph. Aureus* [13]; *Escherichia coli*, *Bacillus anthracis*, *Bacillus subtilis*, *Salmonella newport*, *Salmonella pullorum*, *Staphylococcus aureus*, *P. vulgaris* and *Pseudomonas aeruginosa* [14], *Mycobacterium tuberculsosis*, *Arthobacter globiformis*, *B. megatherium*, *E. coli* and Pseudomonas [15-18], *E. coli*, *Klebsiella aerogens*, *Proteus mirabilis*, *Salmonella typhimurium*, *Shigella dysentriae*, *Pseudomonas aeruginosa*, *Vibrio cholerae* and *Staphylococcus aureus* [19], *Pasturella multocida*, *E. coli*, *Bacillus subtilis* and *Staphlocuccus aureus* [20], *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella typhimurium* [21] and *E. coli*, *Klebsiella spp*., *Bacillus cerus*, *Bacillus subtilis*, *Staphylococcus aureus* [22]. Basically, *in vitro* studies are performed against specific pathogens under laboratory conditions and theses are correlated with the growth or the inhibition of the pathogen. In majority of cases dose dependent effects are observed for a specific period of time [23-24]. In this series essential oil from the leaves of Tulsi exhibited some inhibitory effect against *E. coli*, *B. anthracis*, *B. subtilis*, *Sal. newport*, *Sal. pullorum*, *Staph. aureus*, *P. vulgaris* and *P. aeruginosa* [25-26]. These essential oils include major constituents of leaves such as eugenol, methyl eugenol and caryophyllene. Among them all except caryophyllene were reported to inhibit the growth of *Arthobacter globiformis*, *B. megatherium*, *E. coli* and *Pseudomonas sp*. Eugenol was found most effective constituent [27-28]. The antibacterial effect of aqueous and alcoholic extracts of *O. sanctum* leaves are supposed to be more efficient as these are recommended in most of traditional medicinal systems. Similarly these were found effective against various enteric pathogens viz., (*E. coli*, *Klebsiella aerogens*, *Proteus mirabilis*, *Salmonella typhimurium*, *Shigella dysentriae*, *Pseudomonas aeruginosa*, *Vibrio cholera* and *Staphylococcus aureus*. The aqueous extract showed wider zones of inhibition for *Klebisella spp*., *E. coli*, *Proteus spp*. and *Staphylococcus aureus* whereas alcoholic extract showed wider zone for *Vibrio cholera* [29]. Other than leaves the seeds of Tulsi were also exploited for antibacterial activities and crude, supernatant, residue and dialyzed samples obtained from the seeds inhibited the growth of *P. multocida*, *E. coli*, *B. subtilis* and *Staph. aureus*. The minimum inhibitory concentration (MIC) values of extracts revealed that *Pasturella multocida* and *Bacillus subtilis* were most sensitive strains [30]. More specifically, *in vitro* anti salmonella activity of aqueous and methanolic extracts of *O. sanctum* leaves showed anti-salmonella activity against pathogenic Salmonella typhi, Salmonella paratyphi A and Salmonella typhimurium. The aqueous extracts were found more active than methanolic extracts [31-34].

Essential oil present in most of the *Ocimum species* is responsible for its antifungal, antibacterial and antiviral properties. Microorganisms develop resistance against various antibiotics and due to this an immense clinical problem develops in treatment of infectious diseases. Medicinal plants can be used to overcome this problem. Tulsi leaves have been reported to show strong antifungal activities against the *Aspiergillus species* [35]. *In vitro* antifungal activity was also observed against Candida species also when oil from *O. gratissimum* L. was used [36]. Ocimum shows strong antibacterial activity against Klebisella (*causes pneumonia* and urinary tract infections), *E. coli*, Proteus & Staphylococcus aureus and Vibrio cholerae. Studies have shown *O. basilicum* act as a strong antiviral agent against DNA viruses (herpes viruses (HSV), adenoviruses (ADV) and hepatitis B virus) and RNA viruses (coxsackievirus B1 (CVB1) and enterovirus71 (EV71) [37]. O. tenuiflorum also has been reported to be having antiviral activity against Bovine herpes virus -1. Essential oil from *Ocimum sp* which contain eugenol, carvacrol, methyl eugenol, caryophyllene are considered mainly to be responsible for various antimicrobial properties.

Based on the growth inhibition zone diameter obtained by 20 and 40 mg/ml Ocimum methanol extract concentration, bacterial strains were tested. This method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms. The antimicrobial activity of extracts of *O. sanctum* was used against four pathogenic

organisms, Escherichia coli, Staphylococcus aureus, Aeromonas hydrophila, and Enterococcus faecalis. The results shown that the Ocimum extracts at final concentrations of 40 mg/ml was active against the four types of micro-organism, while, at the concentration 20 mg/ml for the methanol extracts was not efficient to kill some types of treated microorganism. On the other hand, the final concentration of 40 mg/ml methanol extract of antioxidant for liver than muscle systems in oxidative stresses [38]. It was observed that there was an intimate relationship among the contents of natural antioxidant and recovery potential of plants from oxidative stress conditions in terms of antioxidant index [39-41].

AqE of OS showed growth inhibition for Klesbiella, *E. coli*, Proteus and Staphylococcus aureus; while AlE of OS showed growth inhibition for Vibrio cholerae. The AlE of OS was also found to be active against multidrug-resistant strains of S. aureus that are also resistant to common beta lactam antibiotics. Similarly, OS was found to be active against resistant Neisseria gonorrhea strains. OS fixed oil showed good antibacterial activity against *Bacillus pumilus*, *Pseudomonas aeruginosa* and *S. aureus*. Higher content of linolenic acid in OS fixed oil could contribute towards its antibacterial activity. Indian mythological book Padmottara Purana asserts that a house where a garden of Tulsi exists is itself a centre of pilgrimage; neither servants of Yama (The lord of death) nor disease can enter there and wherever fragrance of Tulsi goes, the air gets purified [42]. This statement seems to have some relevance because the essential oil, which forms the specific fragrance, is volatile in nature and can kill various types of microbes. The essential oil is reported to possess antibacterial and insecticidal properties. The oil has been shown to have inhibitory effects on growth of Mycobacterium tuberculosis and Micrococcus pyogenes var. aureus. It has one tenth anti-tubercular potency of streptomycin and one-fourth that of isoniazid [43]. Aqueous and acetone extracts of *Ocimum sanctum* were also found to be sensitive to many plant fungi, *Alternaria tenuis*, *Helminthosporium spp*, and *Curvularia penniseli*. Essential oil of Tulsi was tested on plant pathogenic fungi as well e.g. *Alternaria solani*, *Candida guillermondii*, *Colletotricum capsici*, *Curvularia spp*. *Fusarium solani*, *Helminthosporium oryzae* and the bacterial strains, *Anthrobacter globiformis*, *Bacillus megaterium*, *Escherichia coli*, *Pseudomonas spp*. *Staphylococcus aureus*, *Staphylococcus albus* and *Vibrio cholerae*. The essential oils of Tulsi have been effective against both Gram-positive and Gram-negative bacteria and the properties were comparable with the effectiveness of clove oil [44]. Antimicrobial activity of *Ocimum sanctum* was found to be higher as compared to commonly available other species of Ocimum (i.e. *O. canum*, *O. gratissimum*, *O. basilicum*) in India more so, aqueous extract, alcoholic extract and seed oil of Tulsi shown antimicrobial properties against enteric pathogens [45]. It also exhibited significant antimicrobial activities against some of the clinical isolates and multi-drug resistant Neisseria gonorrhoeae [46]. The ethanolic extracts have ability to inhibit clinical isolates of β-lactamase producing methicillin-resistant Staphylococcus aureus (MRSA) and methicillin–sensitive Staphylococcus aureus [MSSA]. Essential oil of *Ocimum sanctum* reported to have shown antimicrobial activity against Propionibacterium acnes in *in vitro* study and minimum inhibitory concentration (MIC) value found to be 3.0% v/v. Fresh leaves essential oil had shown more antibacterial properties compared to dried leaves essential oil of Tulsi and in case of fungus the property is just the reverse [47].

To study the antimicrobial activity of the different leaf extracts of Tulsi (*Ocimum tenuiflorum*), also known as *Ocimum sanctum*, against three human pathogens *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* different extracts (Ethanol, Methanol, Ethyl acetate and chloroform) of dried leaf of O. tensanctum were tested against three human pathogens strains such as Escherichia coli, Staphylococcus aureus and Candida albicans through the well diffusion and the poison plate method. The minimum inhibitory concentration (MIC) values of the crude extract of the tested plant leaves were determined. Both methods (well diffusion and poison plate) showed the strongest activity in methanol extract. Among four methanol extracts, they show more inhibition against in *S. aureus* than *E. coli* and *C. albicans*. The result showed methanol leaf extract inhibits *S. aureus* growth [48].

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 OS were observed equally effective against pathogenic gram positive and gram negative bacteria [49].

Conclusions

Plants have been used for the treatment of diseases throughout the world since the beginning of civilization. Several medicinal properties have been attributed to the plant not only in Unani, Ayurveda but also in Greek, Roman and Siddha. The vast survey of literature showed that *Ocimum sanctum* has a huge spectrum of pharmacological activities especially antibacterial activity. Future research on sacred basil should be emphasized for control of various diseases especially it should be explore as a significant remedy regarding antibacterial activity for the welfare & service of mankind.

References

1. Ghosh GR, Tulasi (N.O. Labiatae, Genus- Ocimum). New Approaches to Medicine and Health (NAMAH). 1995; 3:23-29.
2. Anonymous. Wealth of India. Publication and Information Directorate, CSIR, New Delhi. 1991; 7:p.79-89.
3. Das SK, Vasudevan DM. Tulsi: The Indian holy power plant. Natural Product Radiance. 2006; 5:279-83.
4. Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Phcog Rev. 2010; 4:95-105.
5. Uma Devi P. Radioprotective, anticarcinogenic and antioxidant properties of the Indian holy basil, *Ocimum sanctum* (Tulasi). Indian J Exp Biol. 2001; 39:185-190.
6. Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. Indian J Exp Biol. 2002; 5:765-773.
7. Mishra P, Mishra S. Study of antibacterial activity of *Ocimum sanctum* extract against gram positive and gram

negative bacteria. American J of Food Tech. 2011; 6:336-341.
8. Mondal S, Mahapatra SC, Mirdha BR, Naik SN. Antimicrobial activities of essential oils obtained from fresh and dried leaves of *Ocimum sanctum* (L) against enteric bacteria and yeast. Acta Hort. 2007; 756:267-269.
9. Rahman MS, Khan MMH, Jamal MAHM. Anti-bacterial evaluation and minimum inhibitory concentration analysis of Oxalis corniculata and *Ocimum sanctum* against bacterial pathogens. Biotechonology. 2010; 9:533-6.
10. Mishra P, Mishra S. Study of antibacterial activity of *Ocimum sanctum* extract against gram positive and gram negative bacteria. Am J Food Technol. 2011; 6:336-41.
11. Farivar TN, Fard AHM, Zahedani SS, Naderi M, Moud BS. Anti tuberculosis effect of *Ocimum sanctum* extracts in *in vitro* and macrophage culture. J Medicinal Sci. 2006; 6:348-51.
12. *Ocimum sanctum*. The Indian home remedy. In: Current Medical Scene, March-April 1992 (Edited and published by S. Rajeshwari, Cipla Ltd., Bombay Central, Bombay).
13. Joshi CG, Magar. Antibiotic activity of some Indian medicinal plants. J Sci. Ind. Res. 1952; 11b:261.
14. Gupta KC, Viswanathan R. A short note on antitubercular substances from *Ocimum sanctum*. Antibiotic Chemother, 1955; 5:22.
15. Grover GS, Rao JT. Investigations on the antimicrobial efficiency of essential oils from *Ocimum sanctum* and Ocimum gratissimum. Perfum Kosmet. 1977; 58:326.
16. Cowan MM. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 1999; 12:564-582.
17. Chopra P, Meena LS, Singh Y. New drug targets for Mycobacterium tuberculosis. Ind. J Med. Sci. 2003; 57:83-92.
18. Farivar TS. Antituberculosis effect of *Ocimum sanctum* extracts *in vitro* and macrophage culture. J Med. Sci. 2006; 6(3):348-351.
19. Geeta Vasudevan DM, Kedlaya R, Deepa S, Ballal M. Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against the enteric pathogens. Ind. J Med. Sci. 2001; 55(8):434-438.
20. Jabeen R, Shahid M, Jamil A, Ashraf M. Microscopic evaluation of the antimicrobial activity of seed extracts of Moringa oleifera. Pak. J Bot. 2008; 40(4):1349-1358.
21. Pasha C, Sayeed S, Al Md S, Khan MdZ. Antisalmonella Activity of Selected Medicinal Plants. Turk. J Biol. 2009; 33:59-64.
22. Sharma A. MVSc. Thesis. Department of Veterinary Microbiology and Immunology, DUVASU, Mathura. UP, India, 2010.
23. Kumar A, Rahal A, Dwivedi SK, Gupta MK. Prevalence and antibiotic profile of bacterial isolates from bovine mastitis in Mathura. Egyptian J of Dairy Sci. 2010; 38(1):31-34.
24. Kumar A, Verma AK, Parul, Singh VP. Microbial status of chicken meat sold in Western Utter Pradesh. Ind. J Pub. Health. 2011b; 9(2):111-114.
25. Gupta KC, Viswanathan R. A short note on antitubercular substances from *Ocimum sanctum*. Antibiotic Chemother, 1955; 5:22.
26. Sen P. Therapeutic potentials of tulsi: from experience to facts. Drugs News and Views. 1993; 2:15-21.
27. Grover GS, Rao JT. Investigations on the antimicrobial efficiency of essential oils from *Ocimum sanctum* and Ocimum gratissimum. Perfum Kosmet. 1977; 58:326.
28. Gislene GFN, Juliana L, Paulo CF, Giuliana LS. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian J Microbiol. 2000; 31:247-256.
29. Geeta Vasudevan DM, Kedlaya R, Deepa S, Ballal M. Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against the enteric pathogens. Ind. J Med. Sci. 2001; 55(8):434-438.
30. Jabeen R, Shahid M, Jamil A, Ashraf M. Microscopic evaluation of the Antimicrobial Activity of Seed Extracts of Moringa oleifera. Pak. J Bot. 2008; 40(4):1349-1358.
31. Williamson EM. Major herbs of Ayurveda. Londen: Churchill Livingstone, 2002.
32. Pasha C, Sayeed S, Al Md S, Khan MdZ. Antisalmonella Activity of Selected Medicinal Plants. Turk. J Biol. 2009; 33:59-64.
33. Sharma A. MVSc. Thesis. Department of Veterinary Microbiology and Immunology, DUVASU, Mathura. UP, India, 2010.
34. Joshi B, Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K, *et al*. Photochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), Eugenia caryophyllata (Clove), Achyranthes bidentata (Datiwan) and Azadirachta indica (Neem). J Microbiol. Antimicrobials. 2011; 3(1):1-7.
35. Joglekar GV, Chaudhary NY, Aiman R. Effect of indigenous plant extracts on glucose-absorption in mice. Indian Journal of Physiology and Pharmacology, 1959; 3:76.
36. Jyoti S, Satendra S, Sushma S, Anjana T, Shashi S. Antistressor activity of *Ocimum sanctum* (Tulsi) against experimentally induced oxidative stress in rabbits. Methods Find Exp Clin Pharmacol. 2007; 29(6):411-6.
37. Chiang LC, Ng LT, Cheng PW, Chiang W, Lin C. Antiviral activities of extracts and selected pure constituents of Ocimum basilicum. Clinical and Experimental Pharmacology and Physiology. 2005; 32(10):811-816.
38. Hussain A, Brahmbhatt K, Priyani A. Eugenol enhances the chemotherapeutic potential of gemcitabine and induce anticarcinogenic and anti-inflammatory activity in human cervical cancer cells, Cancer Biother Radiopharm. 2011; 26(5):519-27.
39. Rao SA, Vijay Y, Deepthi T, Lakshmi CS, Rani V, Rani S, *et al*. Antidiabetic effect of ethanolic extract of leaves of *Ocimum sanctum* in alloxan induced diabetes in rats, Int J Basic Clin Pharmacol, 2013; 2:613-6.
40. Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn Rev. 2010; 4(7):95-105.
41. Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant, Indian J Exp Biol., 2002; 40:765-73.
42. Ghosh GR. Tulasi (N.O. Labiatae, GenusOcimum). New

Approaches to Medicine and Health (NAMAH). 1995; 3:23-29.
43. Anonymous. Wealth of India, Publication and Information Directorate, CSIR, New Delhi. 1991; 7:79-89.
44. Phadke SA, Kulkurni SD. Screening of *in vitro* antibacterial activity of Teminalia chebula, Eclapta alba and *Ocimum sanctum*. Indian J Med Sci. 1989; 43:113-117.
45. Geeta, Vasudevan DM, Kedlaya R, Deepa S, Ballal M. Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against the enteric bacteria. Indian J Med Sci. 2001; 55:534-538.
46. Shokeen P, Bala M, Singh M, Tandon V. *In vitro* activity of eugenol, an active component from *Ocimum sanctum*, against multiresistant and susceptible strains of Neisseria gonorrhoeae. Int J Antimicrob Agent. s2008; 32:174-179.
47. Mondal S, Mirdha BR, Naik SN, Mahapatra SC. Antimicrobial activities of essential oils obtainedfrom fresh and dried leaves of *Ocimum sanctum* (L) against enteric bacteria and yeast. Eds, AK Yadav. Proceedings of International Symposium on Medicinal and Nutraceutical Plants. Acta Hort. 2007; 756:267-269.
48. Gomathinayagam Subramanian. Studies of Antimicrobial Properties of Different Leaf Extract of Tulsi (*Ocimum tenuiflorum*) against Human Pathogens. American International Journal of Contemporary Research, 2014; 4(8).
49. Mishra P, Mishra S. Study of Antibacterial Activity of *Ocimum sanctum* Extract against Gram Positive and Gram Negative Bacteria. American J of Food Tech, 2011; 6:336-341.