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Evaluation of phytochemical and antimicrobial activity of *Ocimum* spp.

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Abstract

Background: Tulsi (*Ocimum* spp.), an aromatic plant belongs to the family, Lamiaceae. Tulsi can be grouped to two broad categories, namely holy basil (*Ocimum sanctum*) and mediterranean basil (*Ocimum basilicum*). The present study was carried out to compare phytochemical and antimicrobial activity of three Tulsi species, namely Rama Tulsi (*Ocimum sanctum*), Krishna Tulsi (*Ocimum tenuiflorum*), and Thai Basil (*Ocimum thyrsiflora*). **Results:** The study revealed that all the three species possess varied amounts of phytochemicals qualitatively and the best antibacterial activity was shown against *Bacillus subtilis* by *Ocimum tenuiflorum*. **Conclusion:** The results confirm validity of the use of *Ocimum* spp. as medicine in ancient medicinal traditions and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

Abbreviations: CNS: Central nervous system, ZOI: Zone of inhibition, BSA: Bovine serum albumin, DCPIP: 2,6-dichlorophenolindophenol.

Introduction

Holy Basil also known as Tulsi (*Ocimum sanctum*), an aromatic herb has healing and curative properties. It is a sacred herb in India and is grown in all houses, temples, gardens, etc. It grows to a height of 1-1.5 meters and has quadrangular branches. Leaves grow opposite to each other with a length 2-4 cm, leaf margins are either entire or toothed, and possesses hair on both the surfaces. Tiny, purple flowers grow on Tulsi and inflorescence is 12-14 cm in length. The fruits which grow on Tulsi are small and smooth nuts [1]. Tulsi is used in treatment of a number of diseases like mental illness, cough and fever, gut diseases, bone and joint problems, eye diseases and other optic problems, ringworm, insect bite, snake bite and scorpion bite and malaria [2].

Tulsi has antimicrobial activities against many pathogens and can be used as mouth wash agent, for wound healing, and preservation of food stuff. Tulsi is antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, and can be used also for killing mosquitoes [3]. It has anti-oxidants and can be used as anti-cataract agent, anti-inflammatory agent, as well as protects from chemicals and radiations, good for the liver and nerves and heart, anticancerous agent, protects the immune system, central nervous system and memory its anti asthma and thyroid, and solves fertility issues [4].

Natural compounds like terpenoid, alkaloids, glycosides, tannins, flavonoids, etc and essential oils apart from carbohydrates, proteins, lipids are present in Tulsi. Herbal medicine imparts an integral role in the treatment and management of diseases [2].

Holy basil is one of the most worshiped and consumed herb in India. There are approximately 60 species identified under the *Ocimum* genus in plant family lamiaceae. Scientific studies show that it has anti-inflammatory, antioxidant, antibacterial, analgesic, antipyretic, cancer fighting, immune booster properties [2]. Tulsi can be divided mainly into two broad categories Holy basil (*Ocimum sanctum*) and mediterranean basil (*Ocimum basilicum*) [5].

The plant is sufficiently hard and can be grown on any type of soil except highly alkaline, saline or water clogged conditions and has a wide adaptability, also it can be grown in tropical and subtropical climates [6].

During the last few years, in modern science, a lot of researchers have considered the medicinal effects of certain areas of the tulsi on the overall human immune system, reproductive system, CNS, cardiovascular system etc. [7].

Scientists have also found that the medicinal importance of holy tulsi in the administrating of reassurance from various health issues, and they have assessed a scientific foundations for the medicinal abilities of tulsi [2].

Tulsi is known to help protect vital human organs and cells against various types of chemical strains from the prevalent industrial pollution and fossil fuels emissions and physical strains from extended physical exhaustion and restraint due to various physical problems and noise exposure to excessive and loud ones [7].

There are different varieties of Tulsi, and the common ones are Rama Tulsi, Krishna Tulsi, Thai Basil, Lemon Basil, and American Basil [5].

The present study involved comparative analysis of phytochemical and antimicrobial activity of three Tulsi species,

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namely Rama Tulsi (*Ocimum sanctum*), Krishna Tulsi (*Ocimum tenuiflorum*), and Thai Basil (*Ocimum thyrsiflora*).

Rama Tulsi has pure green leaves and better tolerance to winters, sun light than the other varieties. It is native to India, Malaysia and Sri Lanka, and is also known as 'The Queen of herbs'. In turn, Krishna Tulsi has purple fringed leaves and purple stems and has more medicinal properties than the other species. Additionally, Thai Basil is perennial, aromatic and culinary type species of basil, possess green colour pointed leaves, reddish purple colour stem. The herb used in Italian cuisines to make pesto sauce and for garnishing [5].

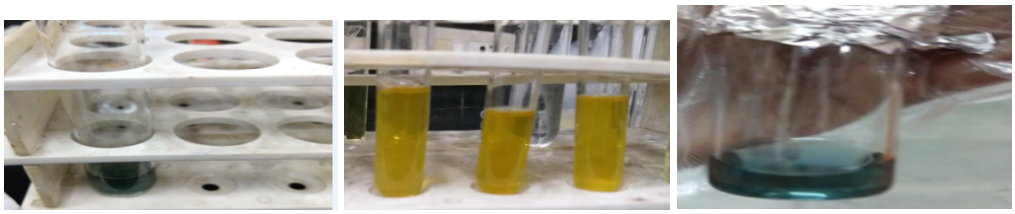
Material and methods

The different varieties of Tulsi were procured from local vendors at Noida, Sector 62, Uttar Pradesh, India. The spp. were analyzed for qualitative presence of quinones [8], terpenoids [8], flavanoids [8], phlobatannins [9], ascorbic acid estimation [10] as well as for quantitative estimation of proteins by Bradford's reagent [11] and study of antimicrobial properties by disc diffusion assay [12]. Ascorbic acid assay was studied by DCPIP method with usage of 1 mg/ml ascorbic acid as stock for preparation of standards. *Bacillus subtilis* used for antibacterial properties was used from the JIIT Biotechnology Laboratory culture collection and was cultured in Nutrient agar medium and broth. The spread plate grown culture of *Bacillus subtilis* was used for zone of inhibition (ZOI) studies in response to *Ocimum* extracts prepared in ethanolic (absolute ethanol), methanolic and aqeous extracts [13,14]. Whatmann Filter paper 42 discs was used for ZOI studies. For preparation of the extracts, 10 g of *Ocimum* leaves respectively was crushed in 200 ml of the solvents respectively and left for drying at 40 degrees C for the solvent extracts for 24 hours in incubator. The powders thus obtained from respective conditions were dissolved in respective solvents to obtain concentrations of 0.2, 0.3, 0.4, 0.5, 0.6 g/ml concentrations to be used for ZOI studies. All experiments were carried out in triplicates, and with regard to the initial studies of ZOI done with all the three *Ocimum* studies, further ZOI studies was carried out with Krishna Tulsi.

Results

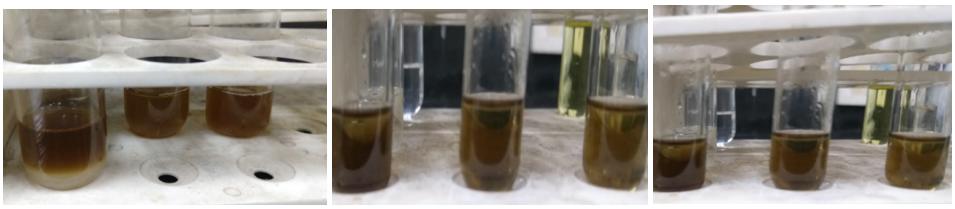
Quinone was detected in all the three spp. of Tulsi. Presence of terpenoids was also detected in all the three spp., though in Krishna Tulsi the observation gave indication of reduced amount in comparison to the other two spp. Flavanoids was also detected in all the three species, however its colourimetric presence was detected as more in Krishna Tulsi, followed by Thai Tulsi and Rama Tulsi. Phlobotannin was detected in all the three spp. and reduced amount was observed in case of Rama Tulsi. Varied amount of ascorbic acid was observed in all the three spp. with more amount of Krishna Tulsi. Further, Krishna Tulsi was observed to be most rich in protein content as from the absorbance values.

The spp. was studied for their antibacterial properties (against *Bacillus subtilis)* by disc diffusion assay and zone of inhibition, wherein it was observed that the methanolic extract gave more zone of inhibition than ethanolic and aqeous extracts (Figures 1-5) (Tables 1-3).



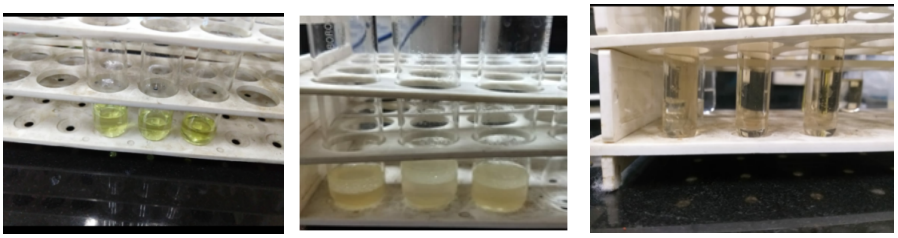
(a) Krishna Tulsi (b) Thai Basil (c) Rama Tulsi

Figure 1. Qualitative results of quinone experiment



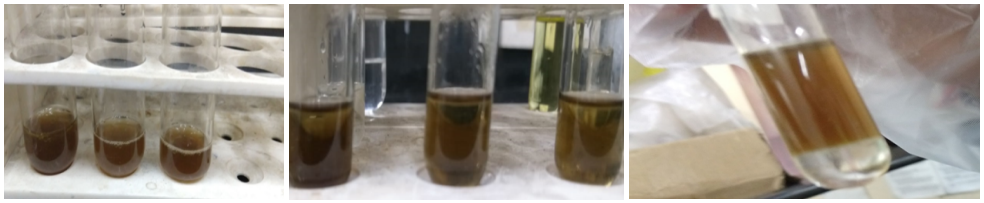
(a)Krishna Tulsi (b) Thai Basil (c) Rama Tulsi

Figure 2. Qualitative results of terpenoid experiment



(a)Krishna Tulsi (b) Thai Basil (c) Rama Tulsi

Figure 3. Qualitative results of flavanoid experiment



(a)Krishna Tulsi (b) Thai Basil (c) Rama Tulsi

Figure 4. Qualitative results of phlobotannin experiment

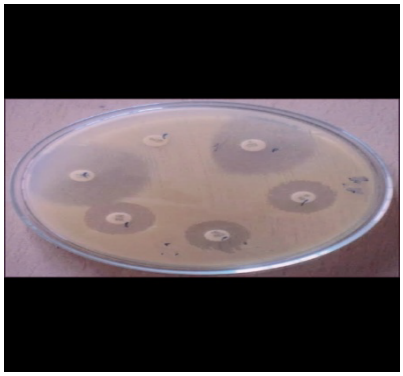


Figure 5. Zone of inhibition for different *Ocimum* concentrations prepared in methanolic extract

Table 1. Average volume of DCPIP used to titrate total ascorbic acid content in enzyme extracts of different varieties of Tulsi leaves

	Ram Tulsi	Thai Basil	Shyam Tulsi
Average Volume of DCPIP used (ml)	1.43	1.43	1.46

Table 2. Bradford assay for protein estimation

TEST TUBE NO.	BSA volume (µl)	H_2O volume (µl)	A (Absorbance)
1.(BLANK)	0	100	-
2.	20	80	0.444
3.	40	60	0.566
4.	60	40	0.620
5.	80	20	0.639
6.	100	0	0.669
	Sample volume (µl)		
7. Krishna Tulsi	100	-	0.171
8. Thai Basil	100	-	0.069
9. Rama Tulsi	100	-	0.074

Table 3. Results of zone of inhibition (ZOI) of *Bacillus subtilis* observed by varying degree of concentrations of *Ocimum tenuiflorum* extracts prepared in different solvents

Concentration in g/ml	ZOI (mm) (ethanolic extract)	ZOI (mm) (methanolic extract)	ZOI (mm) (aqeous extract)
0.2	0	2	0
0.3	0	5	0
0.4	4	8	6
0.5	12	12	8
0.6	15	16	11

Discussion

The presence of these phytochemicals indicates potential of the studied varieties of *Ocimum* for different purposes (Indian patent no. 220688, Indian patent no. 203986, European patent: EP2054349, US patent: US 8,697,429 B2) [15-18] and the antimicrobial potential of Krishna Tulsi suggests usage of ethanolic extract preparation against *Bacillus subtilis* and potential as novel source of antibiotic protypes. These plants may prove to be a rich source of compounds with possible antimicrobial activities, but more pharmacological investigations are necessary [19]. This study would be useful for the pharmacist for preparation of specific and more effective antimicrobial formulations by using Krishna tulsi species [5]. Further, the results confirm validity of the use of *Ocimum* spp. as medicine in ancient medicinal traditions and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new

drugs for the therapy of infectious diseases caused by pathogens [16]. It is quit safer to use as an herbal medicine as compare to chemically synthesized drug [14,20].

Declarations

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Conflicts of interest statement: The authors declare no conflict of interest.

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