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Research Article

In-Vitro Comparative Study of Antibacterial and Antifungal Activities: A Case Study of *Ocimum kilimandscharicum*, *Ocimum tenuiflorum and Ocimum gratissimum*

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ABSTRACT

Tulsi is known as queen of medicinal plants. Its numerous varieties are available. In this study comparison is done between three species of tulsi: Krishna tulsi (*Ocimum tenuiflorum*), Rama tulsi (*Ocimum gratissimum*) and Kapur tulsi (*Ocimum kilimandscharicum*). Method: The antimicrobial activity of leaf distillate of *O. tenuiflorum, O. gratissimum, O. kilimandscharicum* family Lamiaceae was studied against *Bacillus cereus, Bacilus subtilis, Escherichia coli, Pseudomonas aeruginosa and Aspergillus niger.* The inhibitory effect of leaf distillates was compared with the standard antibiotic penicillin and antifungal Fluconazole. Result: Among all the *ocimum* species *O. kilimandscharicum* is effective antibacterial agent against *B. cereus, E. coli, P. aeruginosa*, while leaf distillate of *O. gratissimum* is effective as antifungal against *A. niger*. Conclusion: This study would be useful to give guidelines to the pharmacist for preparation of specific and more effective antimicrobial formulations by using particular ocimum species.

Keywords: Tulsi, *Ocimum tenuiflorum*, *Ocimum gratissimum*, *Ocimum kilimandscharicum*, antibacterial activity, antifungal activity

INTRODUCTION

From ancient time tulsi is used as a traditional remedy for wound healing and microbial infections[1]. The difference in antimicrobial potency of different tulsi species depends on season, botanical source, storage condition, harvesting and processing, but mainly due to its chemical constituents. Constituents that may have biological activity including saponins, flavonoids, triterpenoids, and tannins[2]. Scientifically, tulsi is known as *Ocimum sanctum*, is a time-tested premier medicinal herb of Indian origin[3]. Tulsi is mother medicine of nature, termed as the queen of herbs. It has variety of pharmacological activities ranging from antiasthmatic[4], anticancer[5], antidiabetic[6], antiemetic[7], antifertility[8], antistress[9], analgesic[10], expectorant[11], diaphoretic[12], hepatoprotective[13], antispasmodic[14], stimulant[15] and insect repellent[16].

In India two forms of Tulsi are more common; Krishna Tulsi (*O. tenuiflorum)* and Rama Tulsi (*O. gratissimum*). The former possesses greater medicinal value and is commonly used for worship. *O. tenuiflorum* known as Krishna tulsi is purplish black in colour. The main chemical constituents in *O. tenuiflorum* are essential oils, carbohydrates, flavonoids and proline. It has also higher amount of linalool and moderate amounts of methyl chavicol, nerol , geraniol and citral and Ursolic acid [17]. *O. tenuiflorum* possesses antiinflammatory, antioxidant, antitumoral, antifertility, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and adaptogenic properties, and also effective in reducing the growth of a variety of cancer cell lines, in vitro [18].

O. gratissimum is a well-known plant used in the Indian herbal medicine. The flowers and the leaves of this plant are rich in essential oils, so it is used in the preparation of teas and infusions. The volatile oil of *O. gratissimum* contains mostly thymol and eugenol, those are probably responsible for its reported antimicrobial activity [19]. This plant is used in the treatment of epilepsy, headache, high fever and diarrhoea, abdominal pain, sore eyes, ear infections, coughs, upper respiratory tract infections ,convulsions, skin diseases, pneumonia, and also in the regulation of menstruation[20] [21]. Several activities of *O. gratissimum* has been reported in the literature such as antibacterial[22], anticonvulsant[23] , antidiarrheal[24], anti-nociceptive[25] , antiurolithiatic[26], anxiolytic[27] and larvicidal[28].

O. kilimandscharicum is known as Kapur tulsi. Aqueous extract of leaves contain camphor, 1,8-cineole, limonene, trans-caryophyllene, camphene, 4-terpeneol, myrtenol, α-terpineol, endo-borneol and linalool. It also contains flavonoids, tannins, saponins, sterols, carbohydrates, proteins and triterpenoids . Its essential oil contains oxygenated monoterpenes (95.8%), like camphor (64.9%), limonene (8.7%), camphene (6.4%) and (E)-ocimene (3.0%)[29]. *O. kilimandscharicum* attracted attention as a source of camphor[30]. Extracted oil from leaf is used as an insecticide and mosquito repellent. Kapur tulsi shows

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Table 1: Extractive values of *O. kilimandscharicum, O. tenuiflorum and O.gratissimum* in different solvents.

Plant	Alcohol	Benzene	Chloroform	Ether	Water
O. kilimandscharicum	3.5%	1.5%	0.5%	1.5%	3.0%
O. tenuiflorum	7.5%	1.5%	0.5%	1.0%	9.5%
O. gratissimum	5.5%	2.0%	1.0%	0.5%	4.0%

Table 2: Loss on drying values of three *Ocimum* species.

Plant	*O. kilimandscharicum*	*O. tenuiflorum*	*O. gratissimum*
Loss on drying	23%	47%	35%

Table 3: Shows the different types of ash values of *Ocimum* species.

Plant	*O. kilimandscharicum*	*O. tenuiflorum*	*O. gratissimum*
Total ash	18%	16%	0.15%
Acid insoluble ash	2%	3%	3%
Water soluble ash	6%	3%	5%
Sulphated ash	15%	11%	9%

Table 4: Phytochemicals present in the hydro distillates of *O. k*ilimandscharicum, *O. tenuiflorum* and *O.gratissimum.*

Organic constituents	*O. kilimandscharicum*	*O. tenuiflorum*	*O. gratissimum*
Reducing sugars	+	+	+
Monosaccharaides	+	+	+
Pentose sugar	-	-	-
Hexose sugar	+	+	+
Non reducing sugar	-	-	-
Starch	+	+	+
Proteins	-	-	-
Amino acids	+	+	+
Alkaloids	-	-	-
Tannins and phenolic compounds	+	+	+
Flavonoids	-	-	-
Citric acids	+	+	+
Tartaric acids	+	+	+
Malic acids	+	+	+

Table 5: Zone of inhibition for antibacterial activity

Tulsi plant	Bacterial strain (zone of inhibition in mm)			
	B. subtilis	*B. cereus*	*E. colli*	*P. aeruginosa*
O. kilimandscharicum	25	-	22	20
O. tenuiflorum	-	-	16	15
O. gratissimum	-	-	17	16
Penicillin	40	27	33	39

Table 6: Antifungal activity of *Ocimum* species.

Fungal strain Aspergillus niger	Tulsi species			Standard drug
	O. kilimandscharicum	*O. tenuiflorum*	*O. gratissimum*	Fluconazole
Zone of inhibition in mm	16	20	29	36

various activities such as antimalarial [31] anti-inflammatory[32], Anti-diarrhoeal[30], antimicrobial[33]. It also recommended in the management of various ailments including colds, coughs, abdominal pains, measles, bronchitis, anorexia and memory disorders[34].

Previous reports show that comparative studies have been performed on various species of tulsi. Aggarwal and co-worker showed that essential oil of *O. sanctum* has better antibacterial property than *O. kilimandscharicum* [35]. Previous comparative study between neem sticks, tulsi

Figure 1: Plant specimen of (A) *O. kilimandscharicum*, (B) *O. tenuiflorum and* (C) *O. gratissimum* prepared for authentication.

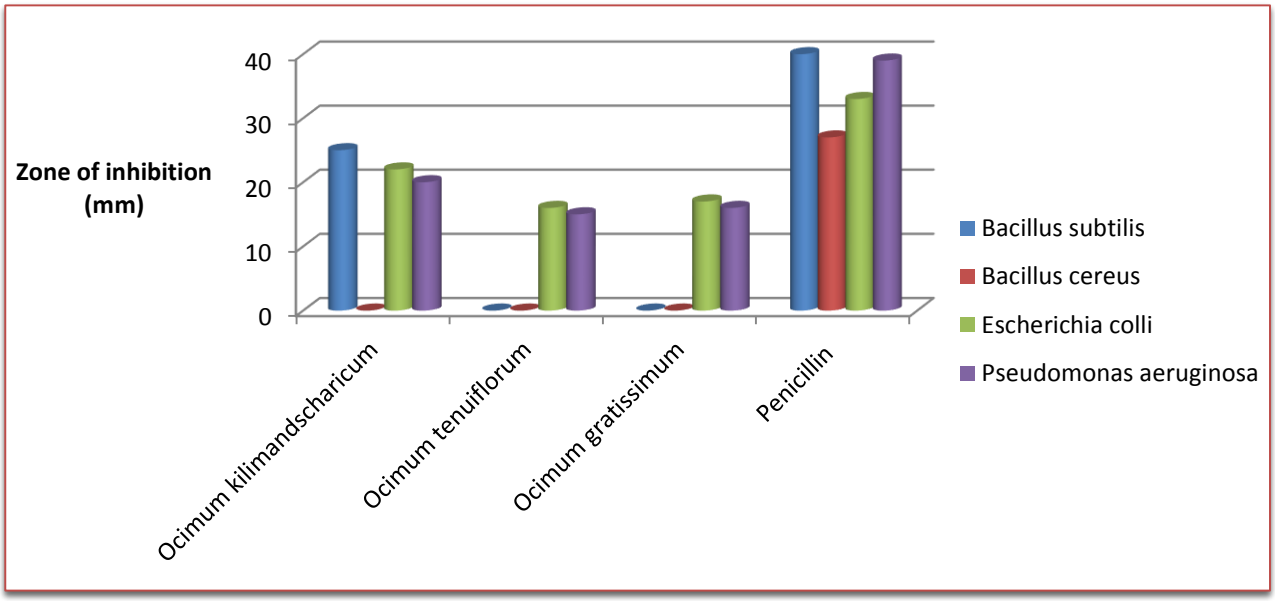

Figure 2: Graphical representation of antibacterial activity

leaves and triphala proved that neem and tulsi had a higher antimicrobial potential[36]. Essential oils from the different species of *Ocimum* (*O. basilicum, O. kilimandscharicum, O. gratissimum, O. sanctum, O. americanum*) were studied for the antifungal activity against pathogenic fungi *Rhizoctonia solani*, *O. basilicum* showed a maximum inhibitory effect [37]. Antimicrobial activities of the essential oils from *O. basilicum, O. gratissimum* and *O. tenuiflorum* have been investigated, it has been observed that the all essential oils showed concentration dependant antibacterial activity against *S. aureus* and *E. coli*. *O. basilicum* oil produced the strongest antibacterial effects on *S. aureus* and *E. coli*[38]. Antibacterial study of aqueous ethanolic extracts of *O. sanctum, Eugenia caryophyllata, Achyranthes bidentata* and *Azadirachta indica* against human pathogenic *E. coli, Salmonella typhi, Salmonella paratyphi and S. aureus* were carried out. It has been observed that among four plants tested *E. caryophyllata* was found to be the most effective[2,39].

Previously, antimicrobial activities of the three species of tulsi have been reported in the literature, but the comparative study of these three species has not performed yet. We have undertaken this study to investigate and compare the antimicrobial properties of *O. tenuiflorum, O. gratissimum and O. kilimandscharicum.* We have evaluated antimicrobial effects against *B. subtilis, B. cereus, E. coli, P. aeruginosa*, and *A. niger*. Individual *Ocimum* species were subjected to the physicochemical and phytochemical investigations.

MATERIAL AND METHOD

Collection and Authentication of plant material

Fresh leaves of Krishna Tulsi (*O. tenuiflorum*), Rama Tulsi (*O. gratissimum*), and Kapur Tulsi (*O. kilimandscharicum*) were collected from the local area of pune district from field (India) and authenticated from botanical survey of India, Pune (Ministry of environment and forest- No BSI/WRC/TECH/2010/871) (Figure 1). The collected leaves were shade dried under normal environmental

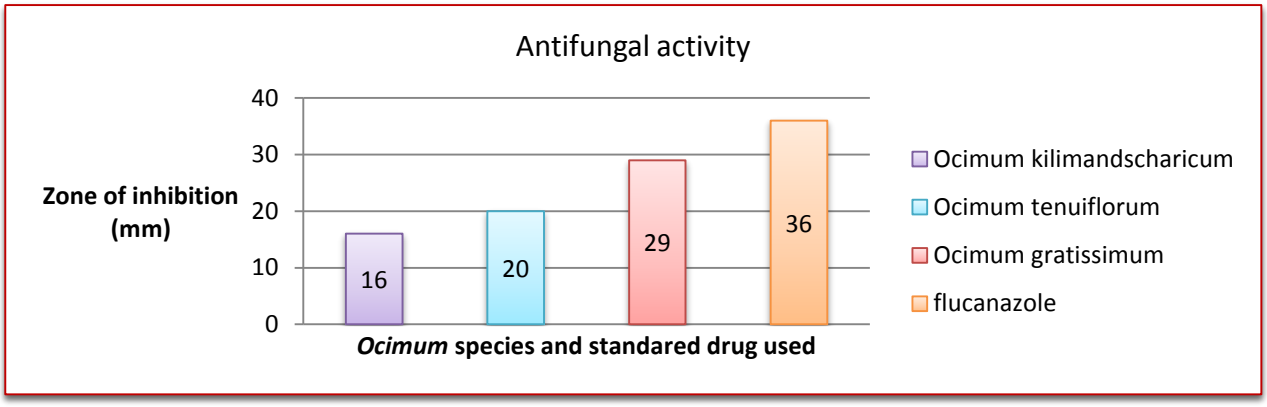

Figure 3: Graphical representation of antifungal activity.

condition, powdered, stored in a closed container for further use.

Physico-chemical evaluation of leaves

Extractive value-Extractive value determines the amount of active constituents present in the given plant material. Five solvents were used for determination of extractive values such as °shaking (maceration). After 24 hours, extract was filtered in weighted thin porcelain dish, solvent was evaporated and residue was weighed. Percentage of extractive value (w/w) was determined with reference to air dried tulsi leaves[40].

Loss on drying-LOD value was determined to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. 1gm of leaf powder was transferred to weighed thin porcelain dish and kept in oven at 100°C for 10 minutes. Dish was cooled in desiccator. The loss in weight is recorded as moisture[40].

Ash value- Ash contains inorganic radicals like phosphate, carbonates and silicates of sodium, potassium, magnesium, calcium, etc. It is useful to determine the quality and purity of herbal drugs. Different ash values such as total ash value, acid insoluble ash value, water soluble ash value, sulphated ash values were determined[40].

Total ash: 2 gm of powder leaf were taken in tarred crucible (previously ignited and weighed). Material was incinerated with the help of the burner, until vapors almost cease to evolve. Dish was heated until all carbon was burnt off. Dish was cooled, and percentage of total ash was calculated with the reference to the air-dried drug[41].

Acid insoluble ash: Ash was boiled with 25 ml dilute hydrochloric acid for 5 minutes. Insoluble matter was collected in the ash less filter paper. It was washed with the hot water, ignited, cooled in a dessicator and weighted. Percentage of acid insoluble ash was calculated with the reference to the air dried drug[41].

Water Soluble Ash: To determine the water soluble ash, it was boiled with 25 ml water for 5 minutes. Insoluble matter was collected in the ash less filter paper. It was washed with hot water, ignited, cooled in a dessicator and weighted. Percentage of acid insoluble ash was calculated with the reference to the air dried drug[41].

Sulphated ash value: To determine sulphated ash value, it was boiled with 25 ml 10% sulphuric acid for 5 minutes. Insoluble matter was collected in the ash less filter paper. It was washed with hot water, ignited, cooled in a dessicator and weighted. Percentage of acid insoluble ash was calculated with the reference to the air dried drug[41].

Hydro distillation

Fresh leaves of each tulsi species were subject to hydro distillation by means of clevenger apparatus. From graduated tube, distillates were collected and concentrated by using boiling-water bath[40].

Preliminary phytochemical screening

Leaf hydro distillates were used for the preliminary phytochemical screening. Tests were carried out for determination of organic chemical constituents like carbohydrates, reducing sugars, mono-saccharides, pentose sugar, hexose sugar, non-reducing sugars, starch, proteins, amino acids, alkaloids, tannins, citric acid, tartaric acid, malic acid[40].

Antibacterial activity

A screening assay using well diffusion method was carried out with four bacterial species: *B. subtilis, B. cereus, E. coli* and *P. aeruginosa.* Nutrient agar was used as medium. Bacterial suspension of each bacterial species was poured and spread over a plate. Wells of 10 mm size were prepared by means of sterile cork borer. 50 µl hydro distillates were filled inside the wells along with penicillin (0.2%) solution as reference drug[42].

Antifungal activity

Antifungal activity of hydro distillates of tulsi leaves was determined by cup diffusion method by using sabouraud medium. The reference drug used for the evaluation of antifungal study was fluconazole (0.2%). 50 µl hydro distillates were filled inside the 10 mm size cavity. Plates were incubated for 24 hours and zone of inhibition was measured by using scale[42].

RESULTS AND DISCUSSION

In the present study hydro distillates of *O. kilimandscharicum, O. tenuiflorum, and O. gratissimum* were used for the evaluation of antimicrobial activity. To evaluate its quality and purity, extractive value, loss on drying, ash values and phytochemical investigation were done.

Extractive value- The extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. Extractive values of *O. kilimandscharicum, O. tenuiflorum O.gratissimum,* is determined in alcohol, benzene, chloroform, ether and water, which is shown in Table 1.

Loss on drying- LOD value is one of the parameter for evaluation of crude drug. LOD value was determined for each tulsi species and it is enlisted in Table 2.

Ash value- Ash value represents the inorganic residues present in the crude herbal drug. Treatment of total ash with acid is comparatively better test to detect and limit excess of soil in the drug. Water soluble ash is specifically useful in detecting a sample which has been extracted from the water. Sulphated ash is mainly useful for evaluation of drugs obtained from natural origin. Therefore, ash value is an important parameter in the evaluation of crude materials. Ash values (total ash, acid insoluble ash, water soluble ash and sulphated ash) of *O. kilimandscharicum, O. tenuiflorum, O.gratissimum were* shown in Table 3.

Preliminary phytochemical analysis - Preliminary phytochemical analysis of hydro distillates reveals the presence of pharmacologically active water soluble constituents, which vaporizes at or below the temperature of distillation such as tannins, organic acids, amino acids, and mono-saccharide. Phytochemicals in hydro distillates of *O. kilimandscharicum, O. tenuiflorum, O.gratissimum* are shown in Table 4.

Antibacterial activity- Antibacterial potential was investigated by using panel of bacteria, including gram-positive *B. subtilis, B. cereus* and gram-negative *E. coli, P aeruginosa*. Among three plant species, *O. kilimandscharicum* showed more significant activity against gram-positive and gram-negative bacteria. It was observed that it shows maximum zone of inhibition of 25 mm on *B. subtilis* (gram-positive) and *E. coli* (gram-negative). Gram-negative bacteria were highly susceptible to all distillates of ocimum species. Gram-positive bacteria were shown resistant to leaf distillates except *O. kilimandscharicum*. *O. tenuiflorum and O. gratissimum* has shown approximately similar antibacterial potential against *E. colli* and *P. aeruginosa*. Results of antibacterial activity are shown in Table 5 and figure 2..

Antifungal activity- Antifungal activity was performed against *A. niger* species. Hydro distillates of all tulsi species have shown maximum antifungal potential. Largest zone of inhibition of 29 mm was obtained with *O. gratissimum* followed by *O. tenuiflorum* and *O. kilimandscharicum*. The summary of zone of inhibition produce by tulsi species is shown in Table 6 and Figure 3.

CONCLUSION

Tulsi is a tradition herb used in India, it has diverse healing properties and considered as adaptogenic. Many varieties of tulsi species are available, among them three species were selected for evaluation of antimicrobial activity. The present work was aimed to investigate and compared the antimicrobial activity of krishna (*O. tenuiflorum*) kapur (*O. kilimandscharicum*) and rama (*O. gratissimum*) tulsi. Our finding suggested that all tulsi species has potential to resist the growth of microorganism. Hydro distillates are produced at high temperature, which was slightly acidic so they tend to inhibit the growth of microorganism. This study indicates that *O. kilimandscharicum* (kapur tulsi) was found to possess more significant antibacterial activity than *O. tenuiflorum* (krishna tulsi) and *O. gratissimum* (rama tulsi). In case of antifungal activity, *O. gratissimum* has shown inhibition of *A. niger* as compared to other two species. Though all the *Ocimum* species shown less antimicrobial activity as compared to the standard drugs used, they have potential to inhibit bacterial growth. Overall, this study proves that *O. kilimandscharicum* (kapur tulsi) possess good antibacterial potential and *O. gratissimum* (rama tulsi) has the antifungal potential. Different tulsi species were investigated for their antimicrobial potential, and the results obtained from this study could be exploited to formulate the antimicrobial preparation using above mentioned individual or combined tulsi species for the ailments.

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