Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Suppl 1, 2012 ISSN - 0974-2441

Research Article

COMPARATIVE ANALYSIS OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *OCIMUM KILIMANDSCHARIUM*

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Received: 30 August 2011, Revised and Accepted: 11 January 2011

ABSTRACT

In this research work antimicrobial activity of essential oil of leaf of *O. Kilimandscharium* was studied, taking into consideration Gram postive and Gram negative bacteria. The fresh leaves of species was collected and hydrodistilled. The essential oil thus obtained was tested for its antibacterial activity. The antimicrobial activity of essential oil was determined using 100% essential oil as well as using dilution of essential oil. The results of antimicrobial activity of essential oil of this species were compared with essential oil (leaf) of *O. sanctum.* The study had showed that the antibacterial effect of essential oil of *Ocimum kilimandscharium* on the bacterial isolate, were observed interesting and promising when comparing with that of *O. sanctum*. So this species could also be used as an antimicrobial agent.

Key-words: Herbal plants, Tulsi, hydro distillation, antimicrobial activity, Clevenger apparatus, Minimum inhibition concentration

INTRODUCTION

Medicinal plants have contributed immensely to health care. An impressive number of modern drugs have been isolated from natural sources, notably of plant origin[1] (Cowan, 1999). The use of herbs as complementary and alternative medicine has increased dramatically in the last 20–25 years[2](Rios *et.al*, 2005). Among all families of the plant kingdom, members of the Lamiaceae have been used FOR centuries in folk medicine. *Ocimum gratissimum* L (Lamiaceae), commonly known as "alfavaca" is naturally used in the treatment of different diseases. *Ocimum* extracts are used in traditional medicine [3](Morales and Simon, 1996). The *Ocimum* oil is also active against several species of bacteria ([4]El-said *et al.*, 1969; [5]Begum *et al.*, 1993). The essential oil, leaves, seeds, flowers and roots of basils are used as medicine. African basil leaf is used to control dysentery, typhoid fever, headache and other domestic and acute illness [6] (Nargarajun *et.al,* 1989). Spice or sweet basil is also thought to be an antispasmodic, carminative stimulant and insect repellant.

Tulsi (*Ocimum sanctum)* is also called by names like Manjari/Krishna tulsi (Sanskrit), Trittavu (Malayalam), Tulshi (Marathi) and Thulsi (Tamil & Telegu). It is called Holy Basil in English. In addition to *Ocimum sanctum* proper, some other species on varieties of this plant also go by the same name viz Tulsi. These are *Ocimum canum* (Ram tulsi or Kali tulsi), *Ocimum basilicum* or bobai tulsi, *Ocimum kilmand* O. scharicum or camphor tulsi, etc. The medicinal effect of all these varieties is nearly similar, if not the same. The natural habitat of Tulsi varies from sea level to an altitude of 2000 m. These are aromatic because of the presence of a kind of scented oil in them [7] (Darrah,1980). A variety with green leaves is called Shri Tulsi and one with reddish leaves is called Krishna Tulsi. One variety with camphor smell is called camphor tulsi. Because of its medicinal virtues, Tulsi is used in Ayurvedic preparations for treating various ailments. Tulsi leaves contain a bright yellow volatile oil which is useful against insects and bacteria. The principal constituents of this oil are Eugenol. Eugenol serves as essential oil and reduced quantity of linalool which enhances the therapeutic and medical prescriptions of *O.gratissium,* [3](Morales,1996). According to Edeoga[8] (2006), *O.gratissimum* plants contain crude protein, crude fiber, ash and crude lipid. The oil is reported to possess anti-bacterial properties and acts as an insecticide. The oil is reported to possess anti-bacterial properties and acts as an insecticide. The best part of the matter is that certain Indian scientists are at the threshold of finalizing their discovery of a reliable medicine against cancer out of Tulsi plant. However a little works on antimicrobial activity of *O.kilimandscharium* has been reported. Antimicrobial activity of this variety is not very well exposed. Much research is required for the exposure of this species. Tulsi leaves, oil and extracts have a valuable large number of medicinal uses and can be used as an organic insecticide. For the same purposes, in the present study research on antimicrobial activity of *O. kilimandscharium* had been carried out and was compared with antimicrobial activity of *O. sanctum*.

BOTANICAL CLASSIFICATION

***Ocimum sanctum Linn* (Krishna Tulsi)**

Kingdom – Plantae

Division – Magnoliophyta

Order – Lamiales

Family – Lamiaceae

Genus – *Ocimum*

Species - *Sanctum*

***Ocimum kilimandscharicum Linn* (Camphor Tulsi):**

Kingdom – Plantae

Division – Magnoliophyta

Order – Lamiales

Family – Lamiaceae

Genus – *Ocimum*

Species - *kilimandscharium*

COLLECTIONS AND IDENTIFICATION

Leaves of *Ocimum kilimandscharium*, and *Ocimum sanctum* were collected from Nursery of Botany Division, Forest Research Institute DDun. The plant was identified by trained plant taxonomists of botany Division FRI. The sample was air dried in the shade and ground in a grinder to a fine powder and was stored in a plastic container.

PROCESSING OF PLANT SAMPLES

Extraction of Essential oil

The essential oil was extracted using Clevenger apparatus. Powdered samples were taken 100 gm (O.D basis) for hydro distillation. Oil yield of *O. kilimandscharium* were about 2.5 % and oil yield of *O.santum* were 2.1% on OD weight basis. The essential oils thus obtained were used for bioassays.

Microorganism Tested

A total of four bacterial strains (*B.cereus*, *E.coli*, *Pseudomonas sp*, *Klebsiella sp*) were tested for antimicrobial activity of essential oil. All strain of bacterial used were obtained from IMTECH, Chandigarh,

India. The bacteria was maintained by weekly transfers in tryptic soy broth (TSB) and distributed in 5ml volume in screw-capped tubes. Cells were grown at 37^{0}C for 48 hours and cultures were kept at 4^{0}C.

Testing of Antimicrobial Activity

Essential oils were used directly first and then were diluted in sterile distilled water containing 0.2%tween-20 to prepare different concentration. Filter paper discs were impregnated with different concentration of essential oil (5µl/disc). Filter paper disc treated with Vancomycin was used as control.

Disc Diffusion Method

Antimicrobial tests were done by disc diffusion Method[9] (Bauer et.al., 1966). The suspensions (60 µl each) of bacteria (0.5 Mc Farland Standard) were spread on Muller Hinton Agar (Himedia) and Potato Dextrose Agar (Himedia) plates respectively. The discs impregnated separately with different oil were placed on the inoculated media. The plates were incubated at 37^{0}C for 24 hr for bacterial. The zone of inhibition was measured using digital vernier caliper. Tests were repeated three times and the means of the results was reported.

RESULTS

In general, the zone of inhibition decreased with decrease in concentration of the Oil. The highest zone of growth of inhibition occurs at a concentration of 100m %, while the lowest zone of growth inhibition occurs at a concentration of 6.25 %.

Table 1: Antibacterial Activity of *Ocimum Kilimandscharium*

Microorganism	Inhibition zone (in mm)					Control
	Concentration					
	100 %	50%	25 %	12.5%	6.25%	
B.cereus	10.76	9.78	8.82	7.43	6.12	10.56
E.coli	10.23	9.45	8.42	6.23	NZ*	12.32
Klebsiella	9.34	8.78	7.95	NZ	NZ*	12.14
Pseudomonas	11.56	10.38	9.75	7.34	NZ*	11.35

*** No Significant zone**

Table 2: Antibacterial Activity of *Ocimum Sanctum.*

Microorganism	Inhibition zone (in mm)					Control
	Concentration					
	100 %	50%	25 %	12.5%	6.25%	
B.cereus	17.23	15.68	12.45	10.23	7.46	10.56
E.coli	12.45	10.12	8.96	6.78	NZ*	20.78
Klebsiella	11.43	10.23	8.75	6.24	NZ*	15.45
Pseudomonas	20.12	17.82	15.24	12.15	9.23	11.34

*** No Significant zone**

Table 3: Minima inhibitory concentration (mic %) of essential oil of *o. kilimandscharium* & *Ocimum sanctum.*

Bacteria	*O. Kilimandscharium* (MIC in%)	*Ocimum sanctum* (MIC in %)
B.cerus	25	12.5
E.coli	Resistance	resistance
Klebsiella	Resistance	resistance
Pseudomonas	25	12.5

DISCUSSION

The essential oil and their constituents from several plants are well-documented as potential antimicrobial agents [10] (Singh and Upadhyay,1993). Several species and varieties of plants of the genus Ocimum have been reported to yield oil of diverse nature, commonly known as basilic oils. It has been demonstrated that the oil isolated from *Ocimum gratissimum* presented antimicrobial activity [3](Morals and Simon,1996). The essential oil have carvicidal activity against house-flies and mosquitoes [11](Beckstrom-Sternberg, M.Stephen, J.A. Duke and K.K. Wain, 1994). In the present study, the antibacterial profile of essential oils of fresh leaf of *O.kilimandscharium* and *O. sanctum* were studied. Like essential oil of *O. sanctum* the essential oil of *O. kilimandscharium* was found to be effective against all the four bacteria *B.cereus, E.coli, klebsiella, Pseudomonas*. All the bacteria, tested were inhibited at 100%, 50%, 25 %, 12.5 & 6.25% concentration of essential oil along with control. The result of zone of inhibition after 24 hr was reported in (Table 1 & Table 2).

The essential oil of *O. kilimandscharium* was found to be sensitive against B.cereus. The minimum inhibitory concentration was found to be 25% while for essential oil of O. *sanctum*, minimum inhibitory concentration was found to be 12.5% (Table 3). The zone of inhibition of essential oil of *O. kilimandscharium* for E.coli with control was 12.32 mm. It didn't show zone at 6.25% concentration of oil% (Table 1). The essential oil was found to be resistant to bacterium. While for essential oil of *O. sanctum* the zone of inhibition for E.coli with control was 20.78 mm. The essential oil of *O. sanctum* was also found to be resistant to bacterium (Table 3). The zone of inhibition for *Klebsiella* with control was 12.14 mm% (Table 1). The essential oil was found to be resistant to *Klebsiella*. While for essential oil of *O. sanctum* the zone of inhibition for *Klebsiella* with control was 15.45 mm. The essential oil of *O. sanctum* was also found to be resistant to this bacterium (Table 2).

The zone of inhibition for *Pseudomonas* with control was 11.35 mm. The essential oil was found to be sensitive against Pseudomonas. The minimum inhibitory concentration was found to be 25 %. The minimalum inhibitory concentration (MIC) of the essential oil of *O. sanctum* was found to be 12.5% (Table 3).

COMPARISON BETWEEN ESSENTIAL OILS OF BOTH VARIETIES

The fragrance of essential oil of *O. kilimandscharium* was like that of camphor showed the presence of camphor as a major chemical constituent [12](Craveiro *et al*., 1981), while such fragrance was absent in the essential oil of *O.sanctum*. In comparision to *O. kilimandscharium*, the essential oil of *O.sanctum* showed better antibacterial properties. The minimum inhibitory concentration of essential oil of *O.sanctum* was slightly high. The oil yield of *O. kilimandscharium* was slightly higher in comparision of *O.sanctum*. So this species could also be used as an antimicrobial agent.

CONCLUSION

The study showed that the *Ocimum kilimandscharium* has a significant antibacterial activities. Essential oil of the leaves of this species had shown most promising effect on the bacterial isolate. So this species can also be used as antimicrobial agent if properly exposed.

ACKNOWLEDGEMENT

I am thankful to the principal Gaytari college of Biomedical Sciences for providing me lab facility.

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