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Biocidal activity of the essential oils of *Lantana camara*, *Ocimum sanctum* and *Tagetes patula*

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Essential oils of *Lantana camara, Ocimum sanctum* and *Tagetes patula* leaves were evaluated for antibacterial and antifungal activity. *L. camara* oil was also evaluated for larvicidal activity against different mosquito larvae. The growth of *Bacillus megatarim and Staphylococcus aureus* was inhibited at 1600 ppm. But, *L. camara* oil was able to inhibit the growth of *Kliebsella* sp only at higher concentrations compared to other two oils. *L. camara* oil was effective in inhibiting the growth of fungi, *Aspergillus niger*, and reducing the growth of other fungi. At a dosage of 200 ppm of oil, 100 % mortality of *Culex quinquefasciarus* larvae was obtained in 15 min. The bioactivity of *L. camara* oil is comparable or better than that of oils from *O. sanctum* and *T. patula*, against the strains tested.

Keywords: Antibacterial, Antifungal, Essential oil, *L*arvicidal activity, *Lantana camara, Ocimum sanctum, Tagetes patula*
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Introduction

Essential oils mainly contain monoterpenoids, sesquiterpenoids and other non-terpenoid compounds[1]. Essential oils of some plant species exhibit insecticidal, antibacterial and antifungal activities[2-8]. Disease preventive and non-nutritive secondary metabolites are produced by many plant species including weeds and floriculture plants. Floriculture plants are a good source of income for the farmers but due to non-uniform flowering and plunge in prices, sometimes farmers bear heavy losses. Better returns with value addition can be ensured if the crop is exploited for its multiple applications such as extraction of essential oils. Weeds can be exploited for essential oils, which can be evaluated for antimicrobial and insecticidal activities. Use of weeds will keep their proliferation under control, even as they serve as a source for insecticides/pesticides which are effective, environmentally safe, easily biodegradable, inexpensive and above all locally available.

Tagetes patula and *Ocimum sanctum* are commonly cultivated for floriculture and medical purposes respectively, while *Lantana camara* is one of the major weeds infesting several parts of India. The major essential oil components isolated from *T. patula* were (Z) and (E)-ocimenones, besides limonene, caryophllene, piperitone and piperitenone. Main components of essential oil of *L. camara* are β and α-caryophyllene (40%), besides L-α-phellandrene, geraniol, α and β-pinene, γ-terpinene, cadinene, elemene, gurguene, α-copaene, ε-murrolene farnesene and citral[9,10]. Extraction and analysis of *O. sanctum* oil showed eugenol, methyl eugenol, β-caryophyllene, cadinene and α-caryophyllene as the major constituents. Larvicidal properties of essential oil extracted from *T. erecta* leaves[11] have been reported. Several studies are available on antimicrobial and insecticidal[12-16] properties of essential oil of *O. sanctum*. However, work related to antimicrobial and larvicidal properties of *L. camara* are limited.

Present study pertains to the antibacterial and antifungal properties of the essential oils extracted from leaves of all the three plants. Quantitative analysis of their effectiveness against *Escherichia coli* was specifically conducted, as it is an indicator bacterium in water contamination. In addition, larvicidal activity was also evaluated for *L. camara* oil.

Methods

Oil Extraction

The leaves of *L. camara* and *O. sanctum* were collected from the campus of Indian Institute of Technology (IIT), Delhi, while the leaves of *T. patula* were collected from a village in Farrukhnagar, Haryana. The leaves were washed with water, dried in shade for 3 d and hydrodistilled for 3 h in a Clevenger's apparatus. The essential oil was extracted and dehydrated over anhydrous sodium sulfate and stored at 4°C. The oil yield (w/v) was: *L. camara*, 0.2; *T. patula,* 0.49; and *O. sanctum*, 1.2 %.

Antibacterial Activity

Bacterial cultures, *E. coli, Bacillus megatarium, Staphylococcus aurens,* and *Kliebsella sp*, were obtained from Department of Biotechnology and Bioengineering, IIT, Delhi and maintained in nutrient broth (NB). The antibacterial activity of the oils was evaluated by transferring essential oil aseptically to sterile nutrient agar (NA) media at 40°C, to obtain concentrations of 1600 and 2400 ppm. Bacteria from NB was swabbed on the surface of plates containing essential oil and incubated at 30 ±1°C for 2 d. The bacterial growth (zone of inhibition) was measured to the nearest millimeter. Five replicates of each test were carried out and control test was run simultaneously without using essential oil.

Quantitative analysis of *E. coli* was carried out by dilute plate count method. *E. coli* was cultured in NB (5 ml) and a stock was prepared. From this stock, 1 % inoculum (50 µl/5ml NB) of *E. coli* was transferred to the fresh broth. A mixture of 1 % SDS (sodium dodecyl sulphate), sterile water and essential oil in the ratio of 1**:**5**:**4 was prepared. The oil mixtures of 50, 100 and 200 µl corresponding to 4000, 8000, 16000 ppm, respectively, were added to the fresh broth containing 1 % inoculum and incubated for 2 d at 30°C. Controls of media without the oil and SDS were also taken. For counting the colonies, dilution of 10^{-2} was made by taking 50 µl inoculum in 5ml broth and from this 1 % inoculum i.e., 50 µl was plated on NA plate and incubated for a day at 30°C.

Antifungal Activity

Antifungal activity was assayed on *Aspergillus niger* NRRL-3*, Fusarium solani, Penicillium funiculosum, Rhizomucor auricus* and *Trichoderma reesi.* The strains were obtained from Department of Biotechnology and Bioengineering, IIT, Delhi.

All the fungal cultures were sub-cultured and maintained on Potato Dextrose Agar (PDA). The oil at 40 and 60µl corresponding to 1600 and 2400 ppm was poured in each petri dish followed by an exact volume of 25 ml of PDA at 40°C. The petri dishes were then rotated clockwise as well as anti-clockwise to completely mix the oil and the media leading to an identical internal atmosphere volume in all the dishes. These fungal cultures (0.5 cm diam) were placed upside down in the centre of the plate containing PDA and essential oil. The plates were incubated at 30 ± 1°C for 2 d. Thereafter, the colony diam was measured and per cent mycelial inhibition calculated. Five replicates of each treatment were carried out and control sets were run simultaneously without using the essential oil.

Larvicidal Activity

L. camara oil was assayed for larvicidal activity. For the laboratory trial, 4th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* were collected from storage tanks in the premises of Micro Model, IIT Delhi. The mosquito species were identified by an entomologist at Malaria Research Institute, New Delhi. The larvae were exposed to sub-lethal concentrations of 20, 40, 100, 200 and 400 ppm of oil in 250ml distilled water according to WHO procedure[17]. The essential oil was dissolved in ethanol (99.8 %) to make the stock solution of 10000 ppm (10µl/lml ethanol). This stock solution was further diluted in water to make different concentrations. The oil-ethanol-water solution was stirred for 30 sec with a glass rod. After about 15 min, 25 larvae taken on a strainer with fine mesh were transferred gently to the test medium by tapping. For each dose, 3 replicates were taken. Food (dry yeast) was sprinkled in each container. After 24 h, mortality count was observed. Simultaneous control sets (with 1ml ethanol in 249 ml water) were also set up.

Results and Discussion

The essential oils of all the plants were effective against all the bacterial strains when grown in nutrient agar

media (Table 1). The oils completely inhibited the bacterial growth at 1600 ppm except in the case of *Kliebsella* by *O. sanctum*, which was inhibited at 2400 ppm. Essential oil of *Tagetes* leaves (Table 2) completely inhibited the growth of *E. coli* even at lower concentrations while *L. camara* oil was unable to inhibit *E. coli* growth at these concentrations. Variation in the activity of oils may be attributed to different chemical composition of the oils. Essential oils containing carvacrol, eugenol or thymol have the highest antibacterial performances[18-20]. *O. sanctum* and *T. patula* oils contain eugenol and hence higher antibacterial activity compared to *L. camara* oil, in which eugenol has not been reported.

In contrast, *L. camara* oil, compared to *O. sanctum* and *T. patula*, completely inhibited the growth of *A. niger* (Table 3). Other fungal species also showed higher percentage of inhibition by *L. camara* oil. A positive correlation between the content of total monoterpernes other than limonene and antifungal activity has been verified for the oils[21]. Higher susceptibility of fungi to *L. camara* oil compared to other oils might be due to higher concentrations of active terpenoides.

Table 1 — Antibacterial activity of essential oils at two different concentrations

Name of the bacterium	Control	*Lantana camara* Oil concentration (ppm)		*Tagetes patula* Oil concentration (ppm)		*Ocimum sanctum* Oil concentration (ppm)	
		1600	2400	1600	2400	1600	2400
Bacillus megatarium	Lawn*	No growth	No growth	No growth	No growth	No growth	No growth
Staphylococcs aurens	Lawn	No growth	No growth	No growth	No growth	No growth	No growth
Kliebsela	Lawn	No growth	No growth	No growth	No growth	Growth present	No growth

*The plate was fully covered by the bacterial growth

Table 2 — Growth inhibition (percent) of *E. coli* at three different concentrations of essential oils.

	Concentration (ppm)	No. of colonies at 10-2 dilution	No. of colonies at 10-4 dilution	Percent inhibition
Control	-	Lawn*	3036	-
SDS control	-	Lawn	2934	3.36
L. camara	4000	12800	-	95.8
	8000	8960	-	97.0
	16000	3292	-	98.9
O. sanctum	4000	3440	-	98.9
	8000	348	-	99.9
	16000	118	-	100.0
T. patula	4000	0	-	100.0
	8000	0	-	100.0
	16000	0	-	100.0

*The plate was fully covered by the bacterial growth and the colonies are too numerous to count.

Table 3 — Growth inhibition (percent) of fungal strains at two different concentrations of essential oils

Name of fungi	Percent *Inhibition*					
	Lantana camara Oil concentration (ppm)		*Tagetes patula* Oil concentration (ppm)		*Ocimum sanctum* Oil concentration (ppm)	
	1600	2400	1600	2400	1600	2400
Control	Lawn*	Lawn	Lawn	Lawn	Lawn	Lawn
Aspergillus niger NRRL-3	100	100	10	12	50	78
Fusarium solani	47	53	8	15	Lawn	14
Penicillium funiculosum	53	55	17	18	Lawn	5
Rhizomucor auricus	35	41	7	8	50	65
Trichoderma reesi	32	41	1	10	Lawn	Lawn

C. quinquefasciatus was more sensitive to *L. camara* oil compared to *A. aegypti*. The mortality rate of both the mosquito larvae species increased with the concentration of the oil (Table 4). At higher concentrations of oil (200 and 400 ppm), 100 per cent mortality was recorded for *C. quinquefasciatus* within 15 min after the addition of the oil. Larvicidal activity of the oil can be attributed to the presence of triterpenes (~ 50%) in oil[9]. However, the larvicidal activity cannot be attributed to any single constituent; rather it may be due to synergistic effect of various constituents. Variation in the toxicity of essential oil against different mosquito species has also been reported[22].

Thus, it can be concluded that essential oil of *L. camara* can be effectively used for antibacterial, antifungal and larvicidal activity. However, its activity against microorganisms will have to be tested individually as it was unable to inhibit the growth of *E. coli* even at higher concentrations.

Table 4 — Per cent mortality of larvae of *Aedes aegypti* and *Culex quinquefasciatus* at different concentrations of essential oil of *L. camara*.

Dose	% Mortality after 24 h	
(ppm)	*Aedes aegypti*	*Culex quinquefasciatus*
400	55 ± 7.07	100 ± 0.00*
200	40 ± 0.00	100 ± 0.00*
100	35 ± 0.00	80 ± 0.00
40	0	22.5 ± 3.53
20	0	20 ± 0.00
0 (Control)	0	0

*100 % mortality in 15 min

*The plate was fully covered by the fungal growth and the colonies are too numerous to count.

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