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Research Paper

Chemical composition and antimicrobial activity of the essential oil of *Leea indica* (Burm. f.) Merr. flowers

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Abstract

The chemical compounds present in the essential oil obtained from the flowers of ***Leea indica* (Burm. f.) Merr.** were analyzed by GC-MS technique. More than 95% of the oil consisted of the esters of phthalic acid. Di-isobutylphthalate (>75%), di-n-butylphthalate (>7%), n-butylisobutylphthalate (>6%), butylisohexylphthalate (>3.5%) were identified as major constituents of the essential oil. Monobutyl carbonotrithioate, a sulphur compound was also identified in trace amount (0.01%). The essential oil showed moderate antibacterial activity against three Gram positive and two Gram negative bacteria and three pathogenic fungi.

Keywords: *Leea indica*, Leeaceae, Flower, Essential oil, GC-MS, Antibacterial, Antifungal.

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Introduction

The plant kingdom represents an enormous reservoir of antibiotic principles that are distributed widely, particularly among angiosperms[1, 2]. These compounds vary greatly in their potency and distribution within a plant[3]. In response to stimuli from microbial infections, plants produce antimicrobial phytochemicals known as phytoalexins for their defense[4]. Increasing emergence of resistance to the currently available antibiotics have necessitated continued search for new antimicrobial compounds having novel mechanisms of action. A wide spectrum of organisms is being screened in search of useful antimicrobials. In most of the studies, leaves, stems and root of the plants have been examined for antimicrobial activity[5].

***Leea indica* (Burm. f.) Merr.** (Family—Leeaceae) is widely spread in the forests of tropical and subtropical India from the Himalayas as far west as Kumaon and Southwards to the Peninsula. It is a perennial shrub with stout, soft wooded and glabrous stems. Its flowers are greenish -white in large, trichotomus, divaricated cymes on short peduncles which flower during June-August. The leaves are useful for the treatment of diabetes and the ointment prepared from roasted leaves relieves vertigo[6-8]. Its root is reported to possess sudorific, antidiarrhoeal, antidysenteric and antispasmodic activities. According to ethnobotanical information, the roots are used for the treatment of allergy, skin diseases and ear troubles while leaf is effective against snake bite[6, 9]. The traditional healers of Bilaspur region of Chattisgarh of Indian continent reported that a typical insect ingest this herb in wild. The healers use this insect as 'medicinal insect' in treatment of common fever. The insect is actually used in the form decoction[10]. Since there are no reports on the chemistry and antimicrobial activity of essential oil of its flowers the present study was taken up.

Materials and Methods

Plant material

The flowers of ***L. indica*** were collected from Dhoni Forest, 16Km from Palakkad District, Kerala, India (accession 1, A1) and from Calicut University Campus, Kerala, India (accession 2, A2) in August (325 and 200g, respectively) and duly identified by Dr. A. K. Pradeep, Department of Botany, University of Calicut.

Isolation of essential oil

The essential oil was obtained by hydrodistillation in a Clevenger apparatus for 3h. The essential oil was reextracted with diethyl ether and dried over anhydrous Na_2SO_4. The light yellow transparent oil (0.18% v/w from A1 and 0.13% v/w from A2) has a bitter odour and stored in a refrigerator at 4ºC until analyzed.

GC-MS analysis

GC-MS analysis was performed by the split injection (1:40) of 0.1µl of the

oil in hexane on a Shimadzu GC-MS-QP2010 (Japan) gas chromatograph fitted with cross banded 5% diphenyl 95% dimethyl polysiloxane RTX5 (MS) capillary column (30 m × 0.25 mm × 0.25 µm coating thickness), coupled with a mass detector. GC-MS operating conditions were as follows: injector temperature 250ºC, transfer line 250ºC, Detector temperature 220ºC, oven temperature programme: 60ºC hold for 5 min, 110ºC with ramping 5ºC/min, 200ºC with ramping 3ºC/min, 220ºC with ramping 5ºC/min, hold 220ºC for 5 min carrier gas: helium at 1.67ml/min, mass spectra: electron impact (EI^+) at 70eV, ion source temperature, 220ºC. Individual components were identified by Wiley139.LIB and NIST05.LIB database matching. The percentage composition was determined by area normalization.

Antimicrobial assay

Test microorganisms employed for *in vitro* antimicrobial assay were obtained from P. G. Centre, Department of Microbiology, Kuvempu University, Dhavangare, Karnataka, India. The antimicrobial assay of the essential oil was performed by standard filter paper disc diffusion technique[11, 12]. A total of three Gram positive, two Gram negative bacteria and five pathogenic fungi were used for this antimicrobial screening. The test solution was prepared by dissolving the essential oil from A1 in DMSO. For antibacterial studies, 0.5 and 1% of the test solution was used and 3 and 4% of the test solution was employed for the antifungal studies. To compare the antibacterial and antifungal activities, cephatoxime (0.1%) and bavistin carbendazim (0.1%) were used as standard antibiotics, respectively. As a negative control, a blank disc impregnated with DMSO followed by drying off was used.

Briefly, the test discs, standard discs and blank discs were placed in a petridish with a particular bacteria or fungi and then left in a refrigerator at 4ºC for 12-18 h in order to diffuse the material from the discs to the surrounding media. The petridishes were then incubated at 37ºC for overnight to allow the bacterial growth and 48-72 h for fungal growth. The antibacterial and antifungal activities of the essential oil were then determined by measuring the respective zones of inhibition (mm).

Results and Discussion

The compounds present in the essential oil of the flowers of ***L. indica*** collected from two different accessions were identified and compared by GC-MS analyses (Figs 1 and 2). The major constituents present in the flowers were identified to be phthalic acid esters (96.93% in A1 and 95.78% in A2). 3H-pyrazole and monobutyl carbonotrithioate were identified only in A1 while bis-(2-pentyl)phthalate and 5-decyne were detected only in A2. The percentage of other compounds differs slightly in the two accessions (Table 1). The percentage of di-isobutylphthalate, the major compound from the essential oil of both accessions was found to be more or less same (>75%).

Phthalates are used as plasticizers in the production of polyvinyl chloride and other plastics. It has been demonstrated that phthalates are widely distributed in the environment but that their levels are low because they are subject to relatively rapid photochemical and biological degradation[13].

There are only fewer reports of the occurrence of the phthalates from plants. Di-isooctyl phthalate has been reported from ***Limonium bicolor* Kuntze**[14] and ***Dracaena cochinensis* (Lour.) SC Chen**[15]. Butyl and isobutyl phthalates were also reported from ***D. cochinensis***[15]. Di-(2-ethyl) hexylphthalate has been isolated from the leaves of ***Cassia auriculata* Linn.**[16].

Bis(2-ethylhexyl)phthalate was reported from the roots of ***Euphorbia hylonoma* Hand.-Mazz**[17]. Bis(2-methylheptyl)phthalate has been isolated from the aerial parts of ***Hypericum hyssopifolium* Vill.**[18]. Diethyl phthalate has been reported from the essential oil from the skin of water caltrop[19]. Diethyl phthalate and derivatives of phthalic acids were isolated from the root exudates of barnyard grass ***Echinochloa crus-galli* Beauv.**[20].

Phthalates are reported to have antimicrobial and other pharmacological activities. So far phthalic acid has been known to be produced by *Gibberella fujikuroi*[21]. Bis-(ethylhexyl)phthalate reported from *Streptomyces bangladeshiensis* shows antimicrobial activity against Gram positive bacteria and some pathogenic fungi[22]. Bis-(2-methylheptyl)phthalate isolated from ***Pongamia pinnata* Pierre** leaves exhibit antiviral activity against White Spot Syndrome Virus of ***Penaeus monodon* Fabr**[23]. Di(2-ethylhexyl)phthalate isolated from ***Alchornea cordifolia*** reported to lower anti-inflammatory activity[24]. Di-isooctylphthalate isolated from ***Nigella glandulifera* Freyn**. was identified as inhibiting melanogenesis[25].

Phthalates are also reported to

affect the human beings. Di(-2-ethylhexyl)phthalate (DEHP), a commonly used plasticizer is harmful to human health[26]. Di-2-ethylhexylphthalate, di-n-hexylphthalate and di-n-octylphthalate were reportedly affect the functioning of liver, kidney and thyroid[27]. Di(2-ethylhexyl)phthalate, was also hepatocarcinogenic in rats[28].

The antibacterial and antifungal activities of the ***L. indica*** flower essential oil from accession 1 have been assayed at concentrations of 1 to 4% against strains of both Gram positive and Gram negative bacteria and pathogenic fungi (Plate 1). The susceptibility testing was carried out by measuring the inhibitory zone diameters on nutrient agar, with conventional paper disc method and the inhibitory zone diameters were read and rounded off to the nearest whole number (mm) for analysis. The inhibitory effects

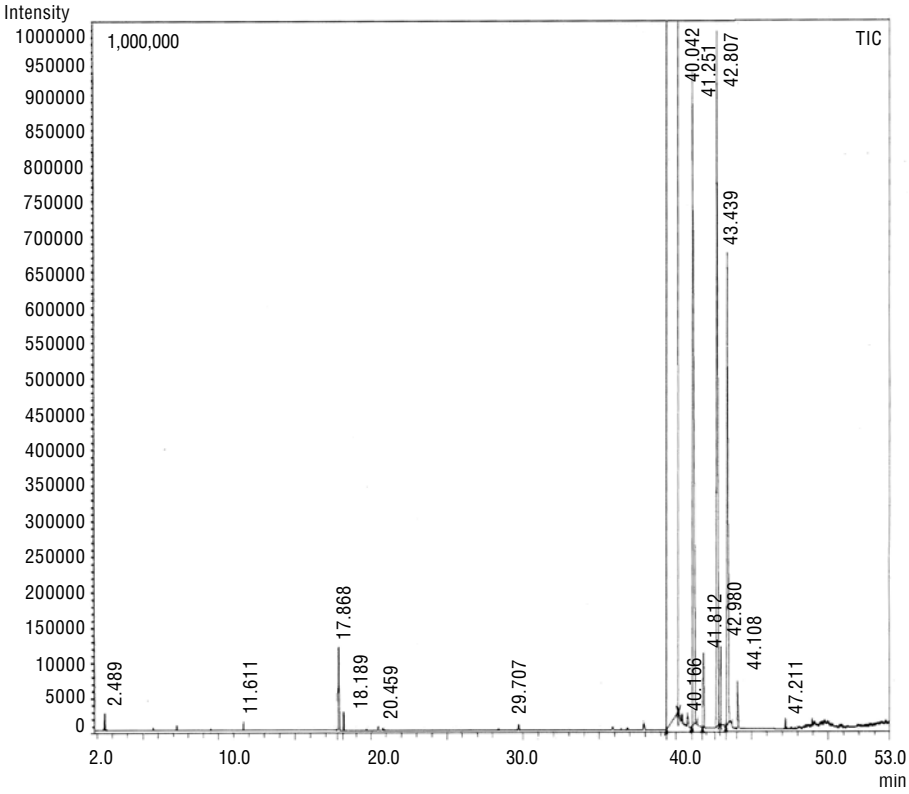

Fig. 1: Gas Chromatogram of the flower essential oil of ***Leea indica*** from accession 1

Table 1: Chemical composition of essential oil of *Leea indica* flower

S. No.	Retention time (min)	Compound identified		Concentration (%)	
		Accession 1	Accession 2	Accession 1	Accession 2
1	2.489	Homobenzvalene	Homobenzvalene	0.07	0.02
2	11.611	Guaiacol	Guaiacol	0.03	0.01
3	17.868	1,4-Benzenediol	1,4-Benzenediol	0.49	0.66
4	18.189	Anethole	Anethole	0.07	0.05
5	20.459	3,5-Heptadien-2-one	3,5-Heptadien-2-one	0.08	0.12
6	29.707	3H-pyrazole	–	0.03	–
7	40.042	Di-isobutylphthalate	Di-isobutylphthalate	79.00	75.64
8	40.166	Monobutyl carbonotrithioate	–	0.01	–
9	41.251	Di-n-butylphthalate	Di-n-butylphthalate	7.18	7.48
10	41.812	Isobutyl-2-pentylphthalate	Isobutyl-2-pentylphthalate	0.45	0.58
11	42.807	n-Butylisobutylphthalate	n-Butylisobutylphthalate	6.11	7.87
12	42.960	Butyl-2-ethylhexylphthalate	Butyl-2-ethylhexylphthalate	0.40	0.40
13	43.439	Butylisohexylphthalate	Butylisohexylphthalate	3.67	3.71
14	44.044	–	Bis(2-pentyl)phthalate	–	0.06
15	44.106	n-Pentylphthalate	n-Pentylphthalate	0.12	0.04
16	47.211	Phytol	Phytol	0.03	0.10
17	50.480	–	5-Decyne	–	0.04
–	–	Unidentified	Unidentified	2.26	3.22

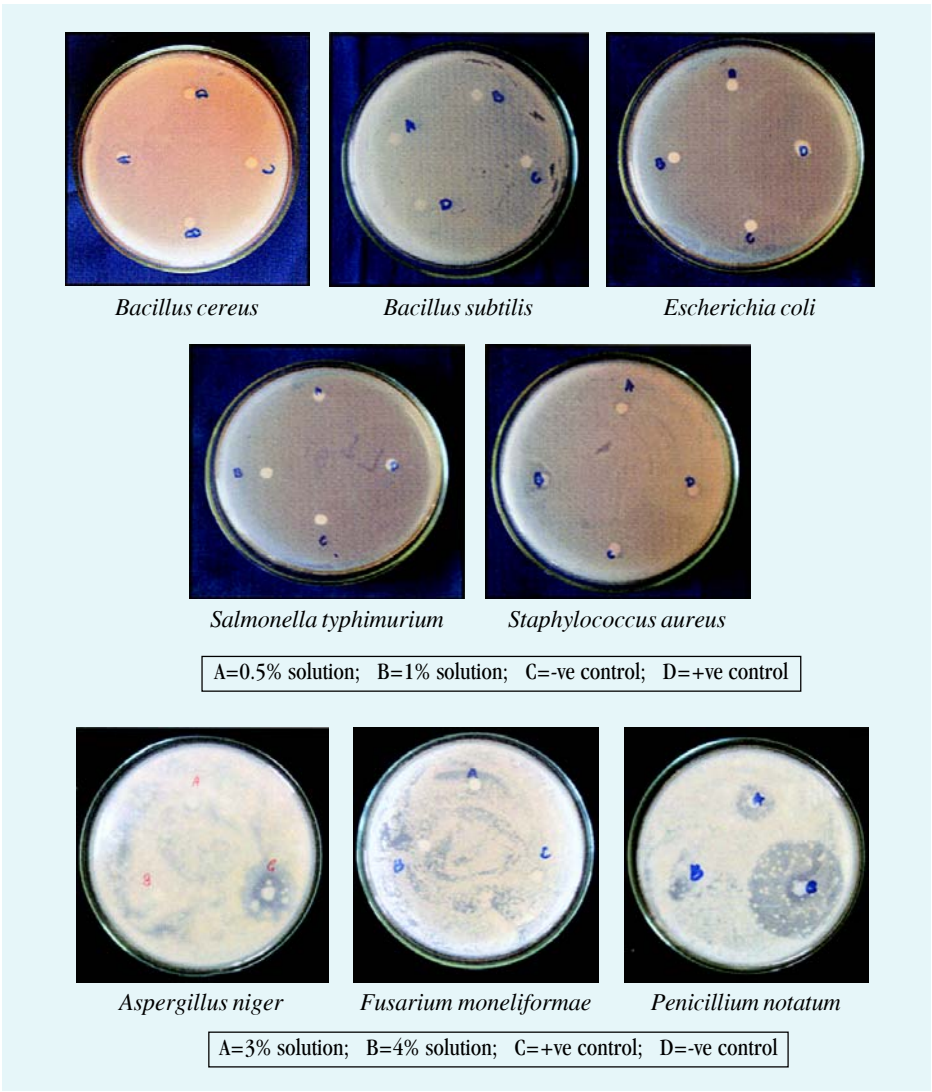

Plate 1: Antibacterial and antifungal activities of essential oil against different strains

Table 2: *In vitro* antibacterial activity of essential oil of *Leea indica* flower

Tested bacteria	Diameter of inhibition zone (mm)[a]		
	Essential oil		Cephatoxime
	0.5% (Acc. 1)	1% (Acc. 2)	0.1%
Bacillus cereus	6	7	8
Bacillus subtilis	-	6	9
Escherichia coli	10	10	25
Salmonella typhimurium	8	11	23
Staphylococcus aureus	5	6	10

[a] Values are the mean of three replicates

of the essential oil against these microorganisms are given in Tables 2 and 3.

The screening results indicate that the essential oil showed good antibacterial activity against Gram negative bacteria *Escherichia coli* and *Salmonella typhimurium* and moderate activity against Gram positive bacteria *Bacillus subtilis*, *B. cereus* and *Staphylococcus aureus*.

Similarly the screening results against some pathogenic fungi indicate that the essential oil showed good antifungal activity against *Penicillium notatum*, moderate activity against *Aspergillus niger* and *Fusarium monelliformae* and no activity against *Aspergillus flavus* and *Alternaria alternata* (Table 3).

In the present study, the antibacterial and antifungal activities of the essential oil of ***L. indica*** flowers is due to the higher percentage of phthalates in it. Guaiacol, anethole and 3H-pyrazole are reported to have antibacterial/ antifungal activity. The presence of these compounds increases the antimicrobial potential of the essential oil of ***L. indica*** flowers.

Conclusion

The results from the present study offer a scientific proof for the traditional use of the ***L. indica*** flowers. The essential oil from the flowers was found effective against some bacteria and fungi which are associated with fever, skin diseases, dysentery and respiratory problems. The antibacterial/ antifungal activity shown by the oil may be due to a synergetic effect of various components

present in it. It is usually found that such combined effect of various components is responsible for therapeutic activities of the plant extracts. However, this needs further investigation and the work is in progress.

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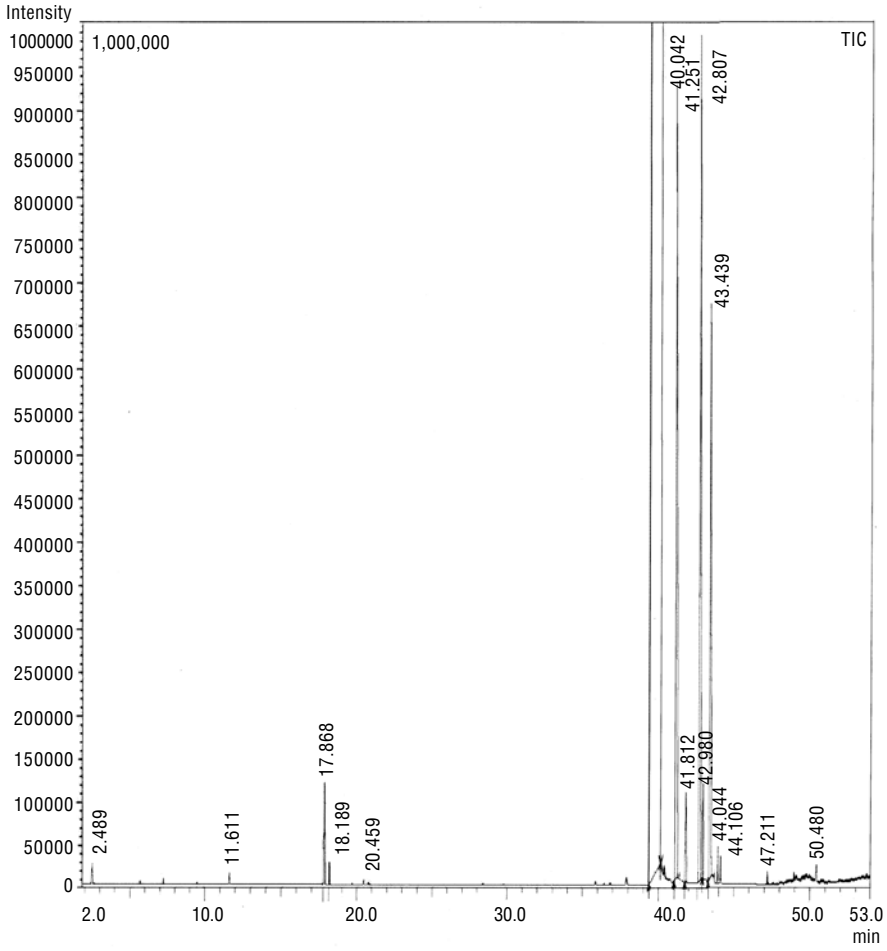

Fig. 2: Gas chromatogram of the flower essential oil of *Leea indica* from accession 2

Table 3: *In vitro* antifungal activity of essential oil of *Leea indica* flower

Tested pathogenic fungi	Diameter of inhibition zone (mm)[a]		
	Essential oil		Bavistin carbendazim
	3%	4%	0.1%
Alternaria alternata	-	-	16
Aspergillus flavus	-	-	9
Aspergillus niger	8	7	20
Fusarium monelliformae	6	5	7
Penicillium notatum	18	21	40

[a]Values are the mean of three replicates

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