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ORIGINAL ARTICLE

Chemical constituents and antibacterial activity of the leaf essential oil of *Feronia limonia*

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Abstract The essential oil from the leaves of *Feronia limonia* was extracted and the chemical constituents and antibacterial activity were studied. The GC and GC-MS analyses revealed that the leaf essential oil of *F. limonia* contained fourteen compounds representing about 98.4% of the total oil. The major chemical compounds identified were Eudesma-4 (14).11-dine (46.3%), carvacrol (29.6%) and 1,5-cyclodecandine (13.4%). The essential oil was screened for its antibacterial activity against different clinically isolated Gram positive and Gram negative bacterial strains by disc diffusion and minimum inhibitory concentration assay. The essential oil exhibited moderate antibacterial activity against all the tested bacterial strains with MIC values ranging from 125 to 500 μg/mL except *Proteus mirabilis*.

Keywords Antibacterial activity · Chemical constituents · *Feronia limonia* · Essential oil

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Introduction

Feronia limonia is a commonly occurring tropical plant species in the Indian sub-continent. In ancient literature of indigenous systems of medicine various medicinal properties viz., vasodilator, anti-microbial, luxative, purgative, astringent, anti-hypertensive have been attributed [1]. The fruits of the plant are edible and considered to be stomachic, astringent, diruetic, cardiotonic and tonic to liver and lungs and the leaves are aromatic and carminative, used for the treatment of indigestion and minor bowel infections of children [2]. Previous phytochemical work was mainly focused on the isolation of coumarins [3–5], steroids [6], pyrano-flavanone [7] and volatile compounds [8]. But there is no previous report on the antibacterial activity of the leaf essential oil. So, the objective of the work was to analyse the chemical constituents and its antibacterial activity of the leaf essential oil of *F. limonia.*

Materials and Methods

Plant material

The fresh leaves of *Feronia limonia* (Linn.) Swingle (syn. *F. elephantum*) were collected from a single healthy, well grown tree from Annamalainagar (11°23′17 N, 079°42′58 E) in the month of April, 2007. Voucher Specimen has been deposited at the herbarium of the Department of Botany, Annamalai University.

Extraction of the essential oil

Two hundred and fifty grams of fresh leaves of *F. limonia* were cut into small pieces and subjected to hydro distilla-

tion using Clevenger type of apparatus for 5 h. The essential oil was dried over anhydrous sodium sulphate for 4 h and the purified essential oil was stored in amber coloured vial at 4°C until further analysis and antibacterial assay.

GC and GC-MS analysis

GC analysis was carried out using Varian 3800 Gas Chromatography equipped with Mass Selective Detector (MSD) coupled to front injector type 1079. The chromatograph was fitted with VF 5 MS capillary column (Low bleed 5% phenyl, 95% dimethyl polysiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm). The injector temperature was set at 300°C, and the oven temperature was initially at 70°C then programmed to 200°C at the rate of 5°C/min, and held at 200°C for 10 min. Then the temperature was increased to 300°C at the rate of 20°C/min, finally held at 300°C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 mL/min. 1 μL of the sample (diluted with acetone 1:10) was injected in the split mode in the ratio of 1:100. The percentage of composition of the essential oil was calculated by the GC peak areas.

GC-MS analysis of essential oil was performed by using Varian 3800 Gas Chromatography equipped with Varian 1200 L single quadrupole Mass Spectrometer. GC conditions were same as reported for GC analysis and the same column was used. The Mass Spectrometer was operated in the Electron Impact (EI) mode at 70 eV. Ion source and transfer line temperature was kept at 300°C. The mass spectra were obtained by centroid scan of the mass range from 40 to 800 amu. Identification of constituents of the essential oil was made by matching their recorded spectra with the data bank mass spectra of WILEY library.

Antimicrobial activity

Microbial strains

The antibacterial activity of the essential oil of *F. limonia* was investigated against five Gram positive bacteria viz., *Bacillus subtilis, Bacillus pumilus, Micrococcus luteus, Staphylococcus aureus* and Beta Hemolytic *Streptococcus pyogenes* and six Gram negative bacteria viz., *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa* and *Salmonella typhimurium*. The clinical isolates were received from the Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamilnadu.

Disc diffusion assay

Antibacterial activity of *F. limonia* leaf essential oil was tested against the above Gram positive and Gram negative bacteria by disc diffusion method [9,10]. These bacteria were grown in Muller-Hinton Agar medium (pH 7.3). Twenty mL of Agar medium were poured into the plates to obtain uniform depth and allowed to solidify. The standard inoculum suspension (10^6 cfu/mL) were streaked over the surface of the media using sterile cotton swab to ensure the confluent growth of the organism. The 6 mm diameter discs were prepared with Whatmann No.1 paper and used for the study. Ten μL of essential oil was diluted with two volumes of 5% dimethyl sulfoxide (DMSO) and impregnated on the filter paper discs, and placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Ciprofloxacin (5 μg/disc) was used as positive reference standard to determine the sensitivity of the tested strains, and 5% DMSO was used as blind control. Finally the inoculated plates were incubated at 37°C for 24 h and observed the inhibition zones including the diameter of the disc (mm). All the experiments were done in triplicate.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration values were determined for the bacterial strains which were sensitive to the essential oil in disc diffusion assay. The MIC of the essential oil was tested in Mueller Hinton broth by broth macro dilution method [11]. The inoculation of the bacterial strains

Table 1 Chemical constituents of essential oil of *Feronia limonia*

Retention time (min)	Chemical constituents*	Composition (%)
7.594	α-Thujene	0.4
13.963	α-Pinene	0.2
14.124	Linalool	0.1
14.326	1,5-Cyclodecandine	13.4
15.206	Carvacrol	29.6
15.912	Caryophyllene	1.3
16.597	*cis*-Anethole	2.8
17.015	Eudesma-4(14).11-diene	46.3
17.576	Elemicin	0.9
18.547	Aromadendrene	0.1
19.136	Germacrene-D	1.2
20.506	3,4-Dimethyl cinnamic alcohol	0.1
20.753	Veratraldehyde	0.3
21.076	Caryophyllene oxide	1.7
	Total	98.4

* Compounds listed in the order of elution from VF 5 MS column.

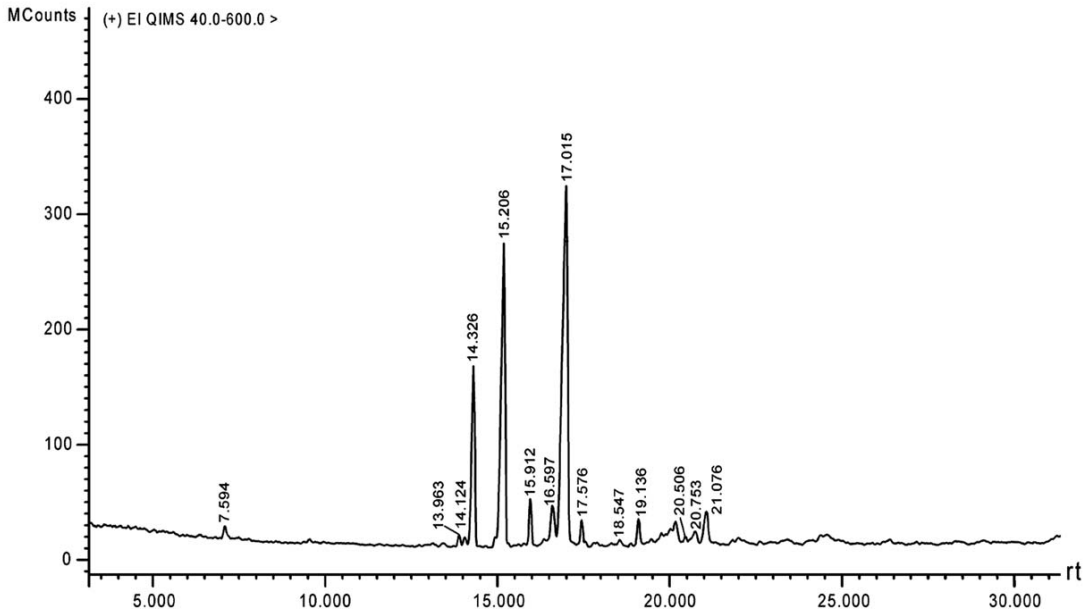

Fig. 1 Gas chromatogram of *Feronia limonia* essential oil.

Table 2 Antimicrobial activity of essential oil of *Feronia limonia*

Name of the microorganism	Mean zone of inhibition (mm)*		MIC of the essential oil (µg/mL)
	Essential oil (10 µL/disc)	Ciprofloxacin (5 µg/disc)	
Bacillus subtilis	11.2 ± 0.8	32.2 ± 1.8	125
Bacillus pumilus	7.5 ± 0.5	42.7 ± 2.5	500
Micrococcus luteus	8.3 ± 0.6	43.8 ± 2.4	500
Staphylococcus aureus	9.3 ± 0.6	22.2 ± 1.3	500
Beta Hemolytic *Streptococcus pyogenes*	12.0 ± 1.0	23.2 ±1.3	125
Escherichia coli	8.5 ± 0.5	14.0 ± 1.0	500
Klebsiella pneumoniae	8.6 ± 0.6	22.7 ± 1.5	500
Proteus mirabilis	NA	NT	NT
Proteus vulgaris	7.3 ± 0.6	15.0 ± 1.0	500
Pseudomonas aeruginosa	10.8 ± 0.6	13.0 ± 1.0	250
Salmonella typhimurium	10.2 ± 0.8	32.3 ± 2.1	250

*Mean zone of three assays; NA − No activity; NT − Not tested; MIC−Minimum inhibitory concentration.

were prepared from 12 h old broth cultures and suspensions were adjusted to standard turbidity (10^6 cfu/mL). The leaf essential oil of *F. limonia* was dissolved in 5% DMSO to obtain 1000 µg/mL stock solution. 0.5 mL of stock solution was incorporated into 0.5 mL of Muller Hinton broth to get a concentration of 500, 250,125 and 62.5 µg/mL. Fifty µL of standard suspension of the test organism was transferred onto each test tube. The control tube contained only organism and devoid of *F. limonia* essential oil. The culture tubes were incubated at 37°C for 24 h. The lowest concentrations which did not show any growth of tested organism after macroscopic evaluation was determined as MIC.

Results and Discussion

The fresh leaves of *F. limonia* yielded 0.45% (v/w) of colourless essential oil with pleasant smell. Gas Chromatogram of essential oil are presented in Fig. 1. Table 1 shows the chemical constituents identified in the leaf essential oil of *F. limonia* by GC, GC-MS analysis and listed in the order of elution from VF 5 MS column. Fourteen constituents were identified representing about 98.4% of the total oil. Eudesma-4(14).11-dine (46.3%), carvacrol (29.6%) followed by 1,5-cyclodecandine (13.4%) were identified as the major chemical constituents.

The oil was tested against different clinically isolated strains of both Gram positive and Gram negative bacteria and the results are presented in Table 2. Ten μL of the oil (1:2 dilution) with 5% DMSO produced mean zone of inhibition ranged from 7.3 to 12.0 mm against all the clinically isolated Gram positive and Gram negative bacteria tested. The oil showed no activity against *Proteus mirabilis.* The MIC values of the essential oil ranged between 125 and 500 μg/mL. The activity of the essential oil may be characterized by the presence of carvacrol, which has been tested previously for its antimicrobial activity [12, 13]. Some other authors have also reported the antimicrobial activity of some essential oil rich in phenolic compounds (carvacrol and thymol) [13–15]. In fact, it was also possible that the compounds in lower percentage might be involved in some type of synergism with the active compound [16].

Essential oil and extracts obtained from many plants have recently gained popularity and scientific interest. So the present study suggests that the leaf essential oil of *F. limonia* can be used as an antimicrobial agent because of its activity against clinically isolated bacterial strains. So far, many pathogenic microorganisms such as *E. coli, S. aureus, K. pneumoniae, Bacillus* sp., *Salmonella* sp., have been reported as the casual agent of food born diseases and/or food spoilage [17–20]. So, the essential oil can be used against these microorganisms.

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References

1. Kirtikar KR and Basu BD (1935) Indian Medicinal Plants, Lalit Mohan Basu Publishers, Allahabad, India, 496 p
2. Kirtikar KR, Basu BD and An ICS (1993) Indian Medicinal Plants, Vol. 1, Bishen Singh Mahendra Pal Singh, India, pp 496–498
3. Talapatra SK, Chudhuri MK and Talapatra B (1973) Coumarins from the root bark of *Feronia elephantum.* Phytochem 12:236–237
4. Gupta SR, Seshadri TR, Sharma CS and Sharma ND (1979) Chemical compounds of *Feronia limonia.* Planta Med 36: 95–96
5. Agarwal A, Siddique IR and Singh J (1989) Coumarins from the roots of *Feronia limonia,* Phytochemistry 28:1229–1232
6. Macleod AJ and Pieris NM (1981) Volatile flavour compounds of wood apple (*Feronia limonia*) and a processed product. J Agr Food Chem 29:49–53
7. Rahman MM and Gray AI (2002) Antimicrobial constituents from the stem bark of *Feronia limonia.* Phytochemistry 59: 73–77
8. Ahmad A, Misra LN and Thakur RS (1989) Composition of the volatile oil from *Feronia limonia* leaves. Planta Med 55: 199–200
9. Berghe DAV and Vlietinck AJ (1991) Screening methods for antimicrobial and antiviral agents from higher plants. In: Methods in Plant Biochemistry, Vol. 6 (Dey PM and Horbone JB eds). Academic Press, London, pp 47–69
10. Cappuccino G and Sherman N (1998) Microbiology: A Laboratory Manual. Benjamin/ Cumming Science Publishing, California, 254 p
11. Ericsson HM and Sherris JC (1971) Antibiotic sensitivity testing: Report of an international collaborative study. Acta Pathol Microbiol Immunol 217:1–90
12. Kim JM, Marshall MR, Cornell JA, Preston JF and Wei CI (1995) Antibacterial activity of carvacrol, citral and geraniol against *Salmonella typhimurium* in culture medium and on fish cubes. J Food Sci 60:1364–1368
13. Sirvopoulou A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T and Arsenakis M (1996) Antimicrobial and cytotoxic activities of *Origanum* essential oils. J Agric Food Chem 44: 1202–1205
14. Panizzi L, Flamini G, Cioni PL and Morelli I (1993) Composition and antimicrobial properties of essential oils of four Mediterranean lamiaceases. J Ethnopharmacol 39:167–170
15. Pellecuer J, Jacob M, Simeon M, de Buechberg and Allegrini J (1980) Therapeutic value of cultivated mountain savory (*Satureia montana* L.). Acta Hortic 96:35–39
16. Longaray Delamare AP, Moschen-Pistorello IT, Artico L, Atti-Serafini L and Echererrigaray S (2007) Antibacterial activity of the essential oil of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem 100: 603–608
17. Betts GD, Linton P and Betteridge RJ (1999) Food spoilage yeasts: Effects of pH, NaCl and temperature on growth. Food Control 10:27–33
18. Deak T and Beuchat LR (1996) Handbook of Food Spoilage, CRC Press, New York, USA
19. Pitt J and Hocking AD (1997) Fungi and food spoilage, 2nd edn, Blackie Academic and Professional, London, UK, p 596
20. Walker SJ (1988) Major spoilage microorganisms in milk and dairy products. J Soc Dairy Technol 41:91–92