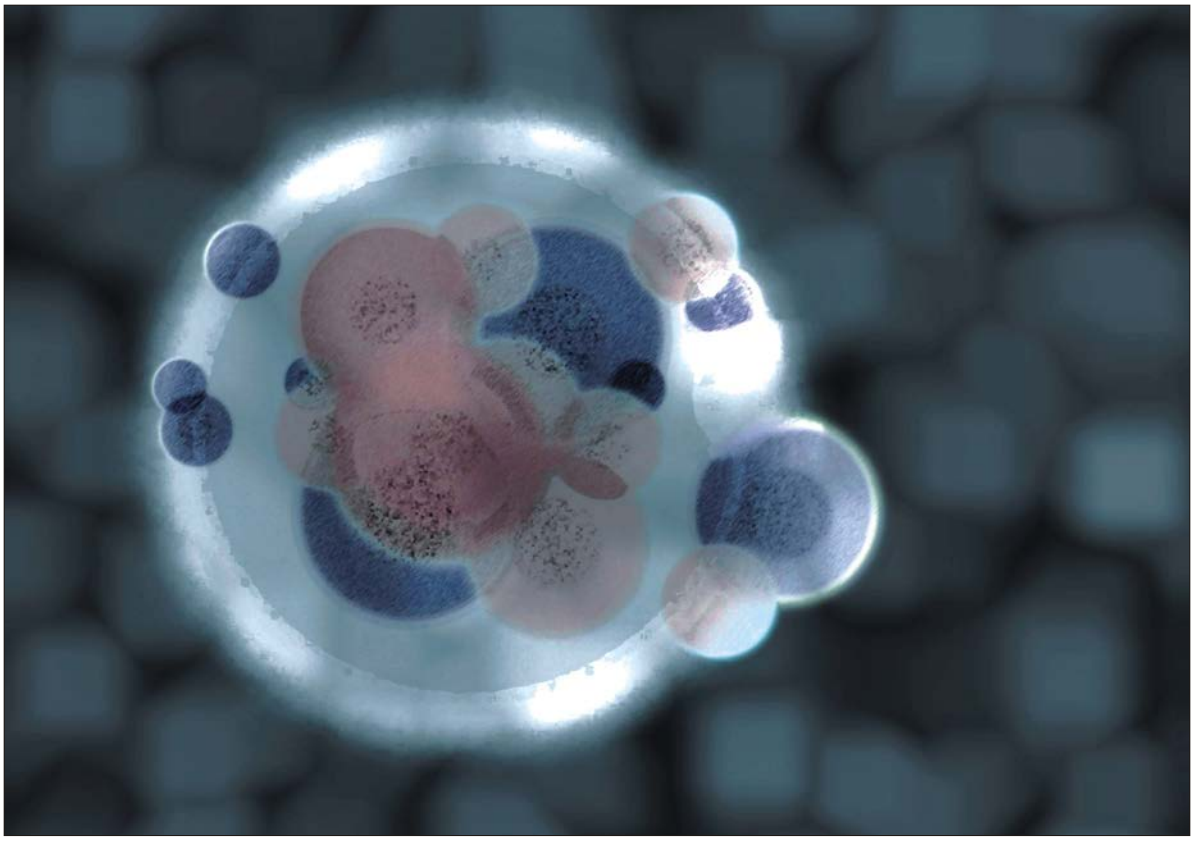

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Composition and Antibacterial Activity of Essential Oil from *Felicia muricata* Thunb. Leaves

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Abstract: The essential oil was extracted by hydrodistillation. A total of thirty-eight compounds were identified with α-pinene (9.1%) β-pinene (3.5%), myrcene (18.7%), limonene (26.5%), cis-ocimene (2.2%), trans-ocimene (4.8%) and terpineol (3.4%) as the major monoterpenes, while, cis-lachnophyllum ester (16.2%) was the major non-terpenoid polyacetylenic compound. The antibacterial activity of the oil was investigated against 16 bacterial strains using broth microdilution method. The oil inhibited all the test organisms with more pronounced activity on Gram-positive than the Gram-negative bacteria. The Minimum Inhibitory Concentration (MIC) for Gram-positive bacteria range from 0.08-2.50 mg mL^{-1}, whereas, it was 0.08-5.00 mg mL^{-1} for the Gram-negative bacteria. The ability of the oil from *F. muricata* to inhibit a range of nosocomial pathogenic bacterial strains at a concentration less than that of streptomycin makes the oil a candidate for possible development of antibiotic drug.

Key words: *Felicia muricata*, hydrodistillation, polyacetylenic, microdilution, nosocomial, antibiotic drug

INTRODUCTION

There is increasing interest in medicinal plants as a natural alternative to synthetic drugs (Fabio *et al*., 2007), particularly against microbial agents. There are over a hundred chemical substances that have been derived from plants for drugs and medicines. For example, antimalaria drug, artemisinin from *Artemisia annua*, anti-inflammatory drug, aescin from *Aesculus hippocastanum* and many others. This interest is due to increasing incidence of microbial infections in recent years, largely due to the increase in AIDS-related opportunistic bacterial pathogens and the emergence of resistance microbial species (Afolayan *et al*., 2002; Koduru *et al*., 2006). The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (Prabuseenivasan *et al*., 2006). Over the years, essential oils and other plant extracts have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of infectious diseases (Tepe *et al*., 2004; Kordali *et al*., 2005), food-borne diseases (Aureli *et al*., 1992; Fabio *et al*., 2003) and cancer cells (Sylvestre *et al*., 2006). Essential oils have also been reported to be useful in food preservation (Sandri *et al*., 2007) and fragrance industries. Production of essential oil by plants is believed to be predominantly a defense mechanism against pathogens and pests (Feng and Zheng, 2007).

Essential oils and their components are gaining increasing interest because of their relatively safe status, wide acceptance by consumers and their exploitation for potential multi-purpose functional use.

Felicia muricata Thunb (Asteraceae) is a small drought resistant perennial aromatic herb growing up to 0.2 m in height. The plant derived its name from muricate (rough, with sharp tubercles or protuberances). It plays a role as desertification indicator, becoming increasingly invasive in grassland regions (Jordaan and Kruger, 1993). The Zulu, Sotho and Xhosa traditional healers in the management of headaches, stomach catarrh, pains and inflammation have been reported (Hutchings, 1989a; Hutchings and Van Staden, 1994). Crude-extracted herb has been tested to exhibit 80-90% inhibition of cyclooxygenase, an important enzyme in the prostaglandin biosynthesis pathway (McGaw *et al*., 1997). Preliminary investigations on the local uses of the species revealed its medicinal importance for the treatment of stomach ache and cancer, for this purpose the aerial part is boiled and taking orally for the relief of pains. To the best of our knowledge, the chemical composition and the antibacterial activity of the essential oil from this herb has not been reported in literature. This investigation was to find out the chemical constituents and the antibacterial activity of essential oil from *F. muricata* by screening the oil against 16 selected bacterial strains. Further study is progressing on the activity of this oil on other infectious micro-organisms.

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MATERIALS AND METHODS

Plant material: Plants samples were collected in August, 2007 from a single population of *F. muricata* growing within the premises of Alice campus of the University of Fort Hare. The species was authenticated by Mr. Tony Dold, Selmar Schonland Herbarium, Rhodes University, South Africa. A voucher specimen (AshafaMed.2007/2) was prepared and deposited in the Griffen Herbarium of the University of Fort Hare.

Essential oil extraction: Two hundred and fifty grams of *F. muricata* fresh leaves were subjected to hydrodistillation for 4 h, using a Clevenger-type apparatus. Pale yellow oil (1.5 mL or 0.6%) was collected to storage in n-hexane.

GC-MS analysis: The oil was separated and analyzed using Hewlett Packard 6890 Gas Chromatograph linked with Hewlett Packard 5973 mass spectrometer system equipped with a HP5-MS capillary column (30 m×0.25 mm, film thickness 0.25 μm, Agilent Technologies Wilmington, DE, USA). The oven temperature was programmed from 70 to 240°C at a rate of 5°C min^{-1}. The ion source was set at 240°C with ionization voltage of 70 eV. Helium was used as a carrier gas. Spectra were analyzed using the Hewlett-Packard Enhanced Chem Station G1701 programme for Windows.

Chemical compound identification: The components of the oil were identified using their spectra and retention indices with the Wiley 275 library (Wiley, New York). Percentage composition was calculated using the summation of the peak areas of the total oil composition.

Bacterial strains: Four gram-positive bacteria strains viz; (*Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC29212), *Bacillus cereus* (ATCC 10702), *Bacillus pumilus* (ATCC 14884) and twelve gram-negative bacteria strains viz; *Escherichia coli* (ATCC 8739), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 19582), *P. aeruginosa* (ATCC 7700), *Klebsiella pneumoniae* (ATCC 10031), *K. pneumoniae* (ATCC 4352), *Serratia marcescens* (ATCC 9986), *Proteus vulgare* (ATCC 6830), *P. vulgare* (ATCC 0030), *Enterobacter cloacae* (ATCC 13047), *Acinetobacter calcaoceuticus* (UP) and *A. calcaoceuticus anitratus* (CSIR) used in this study were all reference isolates. They were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare. The bacteria strains were revived for bioassay by subculturing in fresh nutrient broth (Biolab, Johannesburg, South Africa) for 24 h before test.

Antibacterial activity assay: The Minimum Inhibitory Concentration (MIC) values of the oil on each organism were determined using microplate dilution method (Ellof, 1998), with slight modifications. Briefly, bacterial strains were cultured overnight at 37°C on Muller Hinton broth (MHB, BBL) and was adjusted to a final density of 10^6 cfu mL^{-1}. This was used to inoculate 96-well microtitre plates containing serial twofold dilutions of essential oil (10-0.08 mg mL^{-1}) under aseptic condition. The oil was dissolved in 10% aqueous dimethylsulfoxide (DMSO) in the ratio 1: 10. The plates were incubated under aerobic conditions at 37°C and examined after 24 h. As an indicator of bacterial growth, 40 μL of 0.2 mg mL^{-1} p-iodonitrotetrazolium (97% purity, Fluka Chemie) solution was added to each well and incubated for 30 min at 37°C. The colourless tetrazolium salt was reduced to a red-coloured product by the biological activity of the organisms. Each treatment was performed in triplicate and complete suppression of growth at a specific concentration of oil was required for it to be declared active (Ellof, 1998). Streptomycin was used as positive control in the experiment with pure solvent and sample free solutions as blank controls.

RESULTS AND DISCUSSION

Volatile constituents: A pale yellow oil was obtained from the leaves of *F. muricata* through hydrodistillation. The oil consisted of 38 compounds, constituting 97.5% of the total oil composition (Table 1). The oil was dominated by monoterpenoids, α-pinene (9.1%), β-pinene (3.5%), myrcene (18.7%), limonene (26.5%), cis-ocimene (2.2%), trans-ocimene (4.8%), 1,3,8-paramenthriene (2.7%), α-terpineol (1.1%) and a non-terpenoid polyacetylenic compound, *Cis*-lachnophyllum ester (16.2%). The high percentage of lachnophyllum ester in the essential oil is characteristic of the family Asteraceae (Avato and Tava, 1995; Hrutfiord *et al*., 1988).

Antibacterial activity: The results of the effect of the essential oil from *F. muricata* on tested bacterial strains are shown in Table 2. The oil inhibited all gram positive bacteria strains; *Staphylococcus aereus*, *Streptococcus faecalis*, *Bacillus cereus* and *Bacillus pumilus* at 0.63, 0.08, 1.25 and 2.5 mg mL^{-1}, respectively. All gram negative bacteria strains were also inhibited at concentration ranging from 0.08-5.0 mg mL^{-1}. It is worthy to note that

the essential oil from this plant exhibited greater activity than streptomycin against the bacterial strains at 0.08 mg mL^{-1} in *S. faecalis* and the two *P. aeruginosa* strains, while it was 2.5 and 5.0 mg mL^{-1} for *Klebsiella pneumoniae* (ATCC 10031) and *Enterobacter cloacae* (ATCC 13047), respectively. In general, the antimicrobial activity of *F. muricata* essential oil was more pronounced against gram-positive than those gram-negative bacteria strains, a fact previously observed with essential oils from other plant species (Nostro *et al*., 2000). The susceptibility of these nosocomial opportunistic pathogens to the essential oil from *F. muricata* is interesting, as many of these organisms have been implicated in cases of immuno-compromised hosts. They have been reported to cause urinary tract, respiratory system, dermatitis, soft tissue, bacteremia and gastrointestinal infections in hospitalized patients (Hoffman and Roggenkamp, 2003).

Table 1: Volatile compounds hydrodistilled from *F. muricata* leaves

Compounds	KI value	Composition (%)
Cis-3-hexenol	929	0.5
α-pinene	1025	9.1
β-pinene	1066	3.5
Myrcene	1106	18.7
Limonene	1159	26.5
Cis-ocimene	1169	2.2
Trans-ocimene	1174	4.8
γ-terpinene	1177	0.1
Cis-sabinene hydrate	1180	0.1
α-terpinolene	1198	1.1
Phenol, 2, 3, 5, 6- tetramethyl- (cas)	1202	0.1
Linalool	1206	0.5
Nonyl aldehyde	1209	0.1
D-fenchyl alcohol	1221	0.7
1, 4-cyclohexadiene	1229	1.0
1, 3, 8- para-menthtriene	1242	2.7
2, 4, 6-octatriene	1247	0.2
1-borneol	1273	0.3
Terpinen-4-ol	1285	0.4
α-terpineol	1309	3.4
Thymyl methyl ether	1344	0.3
2-cyclohexen-1-one	1354	tr
α-fenchyl acetate	1398	1
β-terpinene	1465	0.1
Eugenol	1474	0.1
Geranyl propionate	1480	tr
Cis-jasmone	1519	tr
Methyl eugenol	1525	0.4
β-caryophyllene	1541	0.2
Trans-farnesene	1583	1.7
Germacrene D	1609	0.4
Bicyclogermacrene	1625	0.4
Cis-lachnophyllum ester	1689	16.2
Farnesol	1709	0.1
α-cadinol	1798	tr
Mintsulfide	1885	0.2
Hexadecanoic acid	2104	0.2
9-octadecanoic acid	2261	0.2
Total		97.50%

Tr: Trace amount < 0.1; KI: Kovats index

Plant essential oils and extracts have been used for thousands of years in food preservation, pharmaceuticals, alternative medicine and natural therapies (Reynolds, 1996; Lis-Balchin and Deans, 1997). They are also potential sources of novel antimicrobial compounds (Meurer-Grimes *et al*., 1996; Rabe and Van Staden, 1997). The analysis of the essential oil from this plant showed that it has α-pinene, β-pinene, limonene, myrcene and lachnophyllum esters as the major components. These compounds possess strong antibacterial and antifungal activities (Sokmen *et al*., 2003; Deba *et al*., 2008; Magwa *et al*., 2006; Sandri *et al*., 2007). These chemical components exert their antimicrobial activity on microorganisms through the disruption of bacteria membrane integrity (Knobloch *et al*., 1989). Another important characteristic of essential oil and their components is their hydrophobicity. This enables them to penetrate the lipid components of the bacteria cell membrane and mitochondria, disturbing the cell structure and rendering them more permeable which cause leakages of critical molecules and eventual death of the bacteria cells (Sikkema *et al*., 1994; Denyer and Hugo, 1991).

Table 2: Antibacterial activity of essential oil from *Felicia muricata* Thunb

			Minimum inhibitory concentration	
Bacteria		Gram +/-	Oil (mg mL^{-1})	Streptomycin (mg mL^{-1})
Staphylococcus aureus	ATCC 6538	+	0.63	0.08
Streptococcus faecalis	ATCC 29212	+	0.08	0.08
Bacillus cereus	ATCC 10702	+	1.25	0.16
Bacillus pumilus	ATCC 14884	+	2.5	0.16
Escherichia coli	ATCC 8739	-	2.5	0.16
Escherichia coli	ATCC 25922	-	2.5	0.16
Pseudomonas aeruginosa	ATCC7700	-	0.08	0.16
Pseudomonas aeruginosa	ATCC 19582	-	0.08	0.16
Enterobacter cloacae	ATCC 13047	-	5	>10
Klebsiella pneumonia	ATCC 10031	-	2.5	5
Klebsiella pneumonia	ATCC 4352	-	0.16	0.08
Proteus vulgaris	ATCC 6830	-	5	0.08
Proteus vulgaris	CSIR 0030	-	5	0.63
Serratia marsceus	ATCC 9986	-	5	0.63
Acinetobacter calcaocenticus	UP	-	5	0.63
Acinetobacter calcaoceuticus anitratus	CSIR	-	5	0.63

+ = Positive, - = Negative

CONCLUSION

The biological activity of essential oils is due to the presence of a mixture of compounds and not to a single one (Bagamboula *et al*., 2004). The strong antibacterial activity of the essential oil from this herb against array of bacteria strains is an indication of the broad spectrum antimicrobial potential of the oil. This could make the oil a promising group of natural compounds for the development of 'safer' antibacterial agents.

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