Chiang Mai J. Sci. 2013; 40(2) : 214-223
http://it.science.cmu.ac.th/ejournal/
Contributed Paper

Antibacterial Activity and Chemical Composition of Essential Oil and Various Extracts of *Fagraea fragrans* Roxb. Flowers

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Received: 11 January 2012
Accepted: 2 April 2012

ABSTRACT

The constituents of essential oil and various extracts of *Fagraea fragrans* flowers were investigated by gas chromatography-mass spectrometry with 118 identified volatile constituents. Three-octadecyne, catalponone and elemicin dominated in the essential oil of the flowers. The dichloromethane extract of the flowers contained β-bisabolenol, occidol and eugenol as major constituents. Hexane extract showed 3-octadecyne, catalponone and sempervirol as the major components while grandiflorene, himachalol and occidol were evaluated as the dominant components in the methanol extract, respectively. The essential oil of *F. fragrans* flowers plays a major role as a remarkable bactericide. The extracts obtained from dichloromethane, hexane, and methanol showed minimum inhibitory concentration values ranging from 125 to 1000 μg/ml against gram-positive and gram-negative bacteria. The essential oil from *F. fragrans* flowers is also a superior antioxidant (IC_{50} value of 35.32 μg/ml) compared to all extracts (IC_{50} values ranging from 72.69 to 154.99 μg/ml) as indicated by 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.

Keywords: *Fagraea fragrans* Roxb., loganiaceae, essential oil, antibacterial activity, gas chromatography-mass spectrometry (GC-MS)

1. INTRODUCTION

Essential oils and extracts are natural mixtures of compounds with different functional groups which are obtained from many plants. These essential oils and extracts have recently gained popularity and scientific interest. Plant extracts and essential oils have been used as food preservatives preserving from oxidizing agents [1,2], for medical use, as well as in applications in the cosmetic, pharmaceutical, and food industries [3-5]. Extracts from plants are also used as alternative remedies for treatment of many infectious diseases, such as fungal and bacterial diseases. Most researchers have studied the biologically active compounds of these extracts for the elimination of pathogenic microorganisms because of the resistance those micro-organisms have built against antibiotics [6]. The antimicrobial properties of essential

oils and extracts obtained from various plants have been reported repeatedly [7,8]. *Fagraea fragrans* Roxb. belongs to the family of Loganiaceae, commonly known as Tembusu and Kankrao in Thai [9]. *F. fragrans* is a synonym of *Fagraea cochinchinensis*, *Cyrtophyllum giganteum* and *Cyrtophyllum peregrinum* [9]. The plant is widely distributed throughout Burma to Indo-Malaysia and Thailand. It is a large and tall tree. Leaves of *F. fragrans* are simple, opposite, elliptic with thin leathery blade. The inflorescences emerge at the twig terminals and at the leaf axils near the terminals. *F. fragrans* tree posses small flowers with white petals which gradually turn into yellow on fading. Its fruit is red and globular. *F. fragrans* is used in traditional medicine for many treatments in Thailand. The bark is used as a blood tonic and to treat vesicles. The heartwood is used to treat flatulence, fever, pain at joint, asthma, and act as a spleen tonic, blood tonic, synovial fluid tonic and promoting good health in general. In addition, antiplasmodial activity has been found for this plant as reported by Nguyen-Pouplin [10]. Jonville and co-workers [11] isolated sweroside from many parts of *F. fragrans* such as the stem bark, roots, fruits and stems which has moderate anti-HSV-1 (Herpes simples virus type 1) property. Most studies reported the chemical constituents from root and stem of this plant. However, this is no study reporting the biological activity of *F. fragrans* flowers. In order to develop stable and safe antimicrobial sources, the aim of our research is to investigate the chemical composition of essential oil and various extracts obtained from *F. fragrans* flowers. The antibacterial and antioxidant activities of essential oil and various extracts of *F. fragrans* flowers were then comparatively investigated.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh flowers of *F. fragrans* at the flowering stage were collected from Mae Fah Luang University, Chiang Rai Province located in northern Thailand in June 2010. Voucher herbarium specimens (QBG No.41404) of the plant were identified and deposited at the Queen Sirikit Botanical Garden, Mae Rim, Chiang Mai, Thailand.

2.2 Extraction of Essential Oil

The distillation of essential oil of fresh *F. fragrans* flowers was carried out in a modified Likens-Nickerson apparatus for 5 h with a yield of 0.37% (w/w). The distillate was collected in a conical flask which was then dried over anhydrous Na_2SO_4. The oil obtained was kept in a sealed vial prior stored at 4°C until required.

2.3 Preparation of the Crude Extracts

The fresh flowers of *F. fragrans* were treated using a blender until very small particles (almost a powder) were obtained. Seventy-five grams of this powder was macerated individually with 250 ml of dichloromethane, hexane and methanol. Each extraction was performed at room temperature for a week. All extracts were filtered through filter paper and concentrated under vacuum using a rotary evaporator. All extracts were stored at 4°C for further analysis. The yield obtained were 1.13%, 0.64% and 2.55% for dichloromethane, hexane and methanol extracts, respectively.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical composition of the essential oil and various extracts of *F. fragrans* flowers were analyzed using a Hewlett

Packard model HP6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5MS (5% phenyl-polymethylsiloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Agilent Technologies, USA) interfaced to an HP model 5973 mass-selective detector. The oven temperature was initially held at 60°C and then increased by 3°C/min to 230°C. The injector and detector temperatures were 250 and 280°C, respectively. Purified helium was used as the carrier gas at a flow rate 1 ml/min. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 29-300. The electron multiplier voltage was 1150 V. The ion source and quadrupole temperatures were set at 230°C and 150°C, respectively. Identification of volatile components was performed by comparison of their Kovat retention indices, relative to C_8-C_{23} *n*-alkanes, and using a comparison

Table 1. Key volatile components and their relative peak area percentage of the essential oil and various extracts obtained from *F. fragrans* flowers.

Compound	*LRI*[a]	Relative peak area (%)			
		ESS[b]	DCM[c]	HEX[d]	MET[e]
E-Linalool oxide (furanoid)	1081	0.37	-	-	-
3-methyl-1,2,4-Cyclopentanetrione	1090	-	-	-	1.87
Z-Linalool oxide (furanoid)	1098	0.61	0.17	0.73	-
Linalool	1110	1.97	0.27	1.05	-
E-Linalool oxide (pyranoid)	1183	0.52	0.90	1.72	-
α-Terpineol	1200	0.63	0.05	t	0.31
Methyl salicylate	1203	0.09	t	0.55	-
E-Ocimenone	1224	0.05	0.25	0.47	-
Nerol	1236	0.25	-	t	0.64
E-Ocimenone	1239	0.09	0.08	t	0.98
Geraniol	1263	0.64	2.05	t	2.12
cis-Myrtanol	1266	0.05	0.08	-	0.21
E-Cinnamaldehyde	1278	0.15	t	0.69	-
para-Anisyl alcohol	1282	t	0.16	t	0.18
Thymol	1298	t	t	-	2.22
E-Cinnamy alcohol	1315	0.34	0.90	1.73	1.85
para-Vinylguaiacol	1322	0.34	0.53	-	2.13
Hydroxy linalool	1355	-	0.13	0.45	0.18
Eugenol	1366	5.88	3.83	2.53	2.16
Cyperene	1399	-	0.28	0.48	-
Methyl eugenol	1413	1.52	0.52	0.65	-
E-Cinnamyl acetate	1452	t	0.11	0.45	3.66
β-Santalene	1467	t	0.13	0.96	0.22
Citronellol isobutanoate	1488	0.08	t	-	0.15
Bicyclogermacrene	1505	0.26	0.09	0.56	-
E,*E*-α-Farnesene	1515	1.13	0.15	0.96	-
E-γ-Bisabolene	1538	t	1.34	-	-

Table 1. (continued)

Compound	*LRI*[a]	Relative peak area (%)			
		ESS[b]	DCM[c]	HEX[d]	MET[e]
Liguloxide	1541	t	2.78	0.46	-
Elemol	1550	-	0.37	-	0.25
Elemicin	1566	10.37	0.96	2.21	-
E-Nerolidol	1571	4.28	0.34	1.00	0.09
E-Isoelemicin	1577	0.29	0.12	-	-
E-β-Elemenone	1605	0.09	0.07	-	-
Methoxy-eugenol	1612	t	0.97	0.81	-
Himachalol	1654	-	0.91	-	15.70
Patchouli alcohol	1666	0.07	0.07	-	0.08
Acorenone B	1701	0.74	0.25	-	-
β-Sinensal	1704	0.77	0.16	0.87	-
Z-β-Santalol	1720	1.88	0.75	-	-
2*Z*,6*Z*-Farnesol	1728	1.40	0.24	1.12	1.75
Aristolone	1787	-	-	1.06	0.80
Guaiazulene	1790	-	-	6.67	-
β-Bisabolenol	1794	0.05	42.25	-	-
iso-Acorone	1820	0.74	0.45	-	-
Occidol	1844	0.39	14.69	-	10.34
2*E*,6*E*-Farnesyl acetate	1854	0.54	0.60	-	-
Nerolidyl ethanol	1860	0.59	0.46	-	-
Benzyl salicylate	1870	0.13	0.11	-	-
E-β-Santalol acetate	1875	0.12	0.18	-	-
3-Octadecyne	1890	25.69	-	25.84	-
Catalponone	1897	17.94	0.46	15.65	0.39
Methyl hexadecanoate	1920	0.30	0.16	0.54	0.23
epi-Catalponol	1988	0.44	0.14	0.54	1.41
Manoyl oxide	1994	0.47	0.25	0.72	2.83
Methyl linoleate	2098	0.89	0.68	2.41	t
Grandiflorene	2165	-	-	-	19.62
Larixol	2265	0.41	0.42	1.73	t
Sempervirol	2285	2.65	3.32	9.83	-

[a]Linear temperature program retention indices on DB-5 column
[b]Essential oil; [c]Dichloromethane extract; [d]Hexane extract; [e]Methanol extract
[t]Trace amounts < 0.05%

of the mass spectra of individual components with the reference mass spectra in the Wiley 275 and NISTO5 databases. The key components obtained from various extracts were investigated by using a percent relative peak area as shown in Table 1.

2.5 Analysis of Antibacterial Activity of All Extracts

Essential oil and various extracts of *F. fragrans* flowers were screened for their antibacterial activity using the agar diffusion technique against 8 microorganisms of significant importance. The human pathogenic bacterial strains were obtained from the Ministry of Science and Technology, Bangkok, Thailand. These were four gram-negative strains: *Salmonella typhi*, *Serratia marcescens*, *Pseudomonas aeruginosa,* and *Escherichia coli,* and four gram-positive strains: *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus subtilis,* and *Listeria monocytogenes*. All strains were incubated in an oven at 37°C. Each strain was placed into the nutrient agar plate by using sterile cotton swab. Each extract was two-fold diluted with each individual solvent to obtain the final concentrations of 1000, 500, 250, 125, 62.50 and 31.25 μg/ml. Small amount of 10 μl of each extract was added into disc plate by using the sterile micropipette. Plates were incubated overnight at 37°C. The diameter of the clear zone around each disc plate was measured after incubation in millimeters with the value of mean ± standard deviation shown in Table 2. All experiments were performed in triplicate. In addition, minimal inhibitory concentration (MIC) values of each sample obtained are shown in Table 3. The positive control (standard antibiotic tetracycline) was examined by same procedure.

2.6 Antioxidant Activity (DPPH Radical Scavenging Assay)

The radical scavenging abilities of *F. fragrans* flowers oil and various crude extracts were analyzed compared to a

Table 2. Antibacterial activity of the essential oil, various extracts of *F. fragrans* flowers and tetracycline (10 μl corresponding to a concentration of 1000 μg/ml).

Bacteria	Diameter (mm ± SD)				
	tetracycline	ESS[a]	DCM[b]	HEX[c]	MET[d]
Gram-negative					
Salmonella typhi	31.9 ± 2.2	15.1 ± 0.2	14.0 ± 1.1	12.1 ± 2.3	10.2 ± 1.1
Serratia marcescens	20.5 ± 1.8	16.5 ± 0.3	13.5 ± 0.2	14.5 ± 0.4	9.4 ± 0.7
Pseudomonas aeruginosa	18.2 ± 0.8	12.3 ± 0.2	13.1 ± 0.4	8.5 ± 0.8	8.5 ± 1.2
Escherichia coli	22.4 ± 1.9	18.3 ± 0.6	16.3 ± 1.3	6.5 ± 1.8	7.5 ± 0.4
Gram-positive					
Micrococcus luteus	25.3 ± 3.1	9.5 ± 0.7	12.5 ± 2.2	-	-
Staphylococcus aureus	30.4 ± 2.3	10.5 ± 1.2	14.5 ± 0.7	8.5 ± 1.3	10.5 ± 2.1
Bacillus subtilis	23.1 ± 1.6	17.6 ± 0.4	12.2 ± 2.3	13.5 ± 1.1	-
Listeria monocytogenes	29.3 ± 1.2	12.4 ± 2.1	-	-	-

[a]Essential oil; [b]Dichloromethane extract; [c]Hexane extract; [d]Methanol extract
-Antibacterial activity not detected

Table 3. Antibacterial activity expressed as the minimum inhibitory concentration (MIC) of the essential oil, various extracts of *F. fragrans* flowers and tetracycline.

Bacteria	Minimum inhibitory concentration (μg/ml)				
	tetracycline	ESS[a]	DCM[b]	HEX[c]	MET[d]
Gram-negative					
Salmonella typhi	31.25	125	125	500	500
Serratia marcescens	62.50	125	250	125	1000
Pseudomonas aeruginosa	250	500	250	1000	1000
Escherichia coli	125	125	500	1000	500
Gram-positive					
Micrococcus luteus	31.25	500	250	-	-
Staphylococcus aureus	31.25	250	500	1000	500
Bacillus subtilis	62.50	125	125	500	-
Listeria monocytogenes	31.25	250	-	-	-

[a]Essential oil; [b]Dichloromethane extract; [c]Hexane extract; [d]Methanol extract
-Antibacterial activity not detected

standard butyl hydroxyl toluene (BHT) and α-tocopherol based on the reaction with 2,2-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$). This method was evaluated using a spectrophotometric method followed similar to the modified method of Blois [12]. One millilitre of various concentrations of each sample in methanol was added to 1 ml of a 0.003% methanol solution of DPPH and the reaction mixture was shaken vigorously. The tubes were allowed to stand at room temperature for 30 min. Each reaction mixture was then placed in the cuvette holder of a Perkin Elmer-Lamda 25 UV/Vis spectrophotometer and monitored at 517 nm against blank which used methanol as the baseline correction. The scavenging ability was calculated as follows: Scavenging ability (%) = 100 × [Absorbance of control - Absorbance of sample/Absorbance of control]. The antioxidant activity of all samples was expressed as IC_{50} which was defined as the concentration (in μg/ml) of oil required to inhibit the formation of DPPH radicals by 50%. The experiment was carried out in triplicate. The results of mean values and the standard deviation obtained are shown in Table 4.

Table 4. Antioxidant activity (IC_{50}) of the essential oil and various extracts of *F. fragrans* flowers. Values represent averages ± standard deviations for triplicate experiments. ESS; essential oil, HEX ext; hexane extract, DCM ext; dichloromethane extract, MET ext; methanol extract and BHT; butyl hydroxyl toluene.

Extracts	IC_{50} (μg/ml)
ESS	64.7 ± 0.9
DCM	72.7 ± 0.4
HEX	114.2 ± 0.9
MET	154.5 ± 0.8
BHT	30.4 ± 0.2
α-tocopherol	50.3 ± 0.2

3. RESULTS AND DISCUSSION

3.1 GC-MS Analysis of *F. fragrans* Extracts

Essential oil and various extracts of *F. fragrans* flowers appeared as viscous yellow liquids with a percentage yield of

0.24-2.55 (w/w), respectively. These extracts were subjected to detailed GC-MS analysis in order to investigate the volatile constituents. The key volatile components identified by GC-MS, their relative area percentages and their retention indices are summarized in Table 1. A total of 92 constituents representing 95.74% of the flower oil were established. The dominant components were 3-octadecyne, catalponone, elemicin, eugenol, *E*-nerolidol, sempervirol and hinasol acetate. In this study, 93 constituents representing 93.40% of the dichloromethane extract of *F. fragrans* flowers were identified. The major components were β-bisabolenol, occidol, eugenol, sempervirol, liguloxide and geraniol. Forty-nine volatiles were identified in the hexane extract of *F. fragrans* flowers representing 93.13% of the total peak area with the major components of 3-octadecyne followed by catalponone, sempervirol, guaiazulene, eugenol and methyl linoleate, respectively. Individually, *F. fragrans* flower extract from methanol solvent yielded 48 identified constituents representing 92.21% with dominant components consisting of grandiflorene followed by himachalol, occidol, *E*-cinnamyl acetate, dodecanol and manoyl oxide, respectively. All extracts contained of monoterpenes and sesquiterpenes in a similar profile; however, the differences in the quantitative chemical composition and yield of *F. fragrans* flower extracts may be correlated with the extraction method and efficiency of the solvent for extracting volatiles based on the "like dissolve like" concept. Basically, hexane, a non-polar solvent, is expected to be effective in extracting non-polar compounds, where as intermediate and polar solvents including dichloromethane and methanol are suitable for intermediate and polar component extraction. The steam distillation method is appropriate for lightly and medium volatile constituents.

3.2 Antibacterial Activity of Essential Oil and Crude Extracts of *F. fragrans* Flowers

The results of antibacterial activity of the essential oil and various extracts of *F. fragrans* flowers using the agar diffusion method at a concentration of 1,000 μg/ml are shown in Table 2 and their minimum inhibitory concentration (MIC) of various extracts of *F. fragrans* flowers is shown in Table 3. The essential oil of *F. fragrans* flowers exhibited greater antibacterial activity against all gram-positive bacteria as compared to their extracts. The dichloromethane extract of *F. fragrans* flowers was evaluated for antibacterial activity against all pathogenic strains of gram-negative and gram-positive (*M. luteus*, *S. aureus*, *B. subtilis*) bacteria. The hexane extract of *F. fragrans* flowers displayed an antibacterial effect against all gram-negative and gram-positive bacteria including of *S. aureus* and *B. subtilis* whereas an antibacterial effect was observed in the methanol extract of *F. fragrans* flowers with all strains of gram-negative and one gram-positive bacteria which is *S. aureus*. The antibacterial effect of the essential oil of *F. fragrans* flowers could be attributed to the occurrence of monoterpenes and sesquiterpenes in the essential oil, as indicated by the study of Cakir *et al.* [13] and Gudzic *et al.* [14], due to different chemical compositions in different classes. The antibacterial properties might be related to different contents of these compounds detected by GC-MS analysis. The antibacterial activity of all extracts of *F. fragrans* flowers could be due to terpenic components such as α-terpineol, *para-*

vinylguaiacol, elemicin, β-bisabolenol and thymol in which biologically active components have been reported by Carson and Riley [15]. More studies were found in the report of Aridogan *et al.* [16] that antimicrobial activity of geraniol and nerol was determined against both *S. aureus* and *E. coli* strains. Linalool could also contribute to the antimicrobial activity as reported by Knobloch *et al.* [17] and Osawa *et al.* [18] reported that sempervirol possessed antibacterial activity against oral pathogenic microorganisms with MIC values ranging from 3.1 to 25 ppm. *E*-nerolidol was evaluated to be the antibacterial properties as reported by Skaltsa *et al.* [19]. In addition, some components in lower content such as methyl eugenol and chavicol are known for their important antibacterial activity [2]. The antibacterial activity may also be involved to the aliphatic alcohols and aldehydes such as octen-3-ol, 2*E*-nonenal, dodecanol, tridecanol, tetradecanal and tetradecanol [20,21]. The differences in antibacterial activities among other extracts thus seem to be related to the presence of various types of compounds belonging to different classes. It is also possible that the components present at lower concentrations might be involved in some type of synergism with the other active compounds as reported by Marino *et al.* [22].

3.3 Antioxidant Activity of Essential Oil and Crude Extracts of *F. fragrans* Flowers

According to various extracts, the antioxidants properties are considered to be different. Antioxidant activities of the essential oil and various extracts of *F. fragrans* flowers were tested by the DPPH radical scavenging method. The violet color of the radical disappeared when mixed with the substances in the sample solution that donate a hydrogen atom. Antioxidant activities of all samples, standard BHT and α-tocopherol are presented in Table 4 in which lower IC_{50} values indicate higher antioxidant activity. In this study, the essential oil of *F. fragrans* flowers exhibited greater antioxidant activity than other extracts that is close to the synthetic antioxidant α-tocopherol. As seen, the essential oil contained high levels of monoterpenes and sesquiterpenes showing a moderate antioxidant activity. It was shown that these terpene hydrocarbons, whose antioxidant activity is close to that of phenolic compounds, break free-radical chain reactions, which could be accompanied by their irreversible oxidation into inert compounds as reported by Yanishlieva *et al.* [23]. In addition, Tepe *et al.* [24] reported that the essential oils which contain monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes have greater antioxidant properties. Antioxidant activity of essential oil and various extracts of *F. fragrans* flowers should be related to linalool, eugenol, farnesol, geraniol, isoborneol, chavicol and elemicin for which supporting studies were reported by Sato *et al.* [25].

4. CONCLUSION

The essential oils of *F. fragrans* flowers exhibited a wide spectrum of antibacterial activity. The antibacterial activity of *F. fragrans* flowers may be related to the presence of the terpene components such as linalool, nerol, α-terpineol, eugenol, β-bisabolenol, *para*-vinylguaiacol, elemicin, thymol and geraniol and other components identified in GC-MS analysis with minor percentages. It can be concluded that the essential oil of *F. fragrans* flowers could be considered

as alternative natural bactericide. In addition, essential oil of *F. fragrans* flowers was found to have high potential antioxidant activity.

ACKNOWLEDGEMENTS

Much appreciation is given to the Scientific and Technological Instrument Centre (STIC) of Mae Fah Luang University for their instrument support concerning the GC-MS. This research work was kindly supported by Office of the Higher Education Commission (OHEC). We are grateful to Queen Sirikit Botanical Garden for their help in the collection and identification of the *F. fragrans* plant.

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