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Antioxidant, Antibacterial, Anticancer Activities and Chemical Constituents of the Essential Oil from *Mesua ferrea* Leaves

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ABSTRACT

Essential oil from leaves of *Mesua ferrea* L. was isolated by hydrodistillation and analyzed by means of GC and GC-MS. The yield of obtained essential oil was at 0.064% and it was appearred as yellow liquid. Thirty-five constituents were identified, constituting 81.4% of the total volatile components. The major constituents were *trans*-caryophyllene (30.9%), β-caryophyllene oxide (17.9%), α-humulene (6.0%), δ-cadinene (4.1%), γ-muurolene (3.5%), γ-cadinene (2.3%), β-selinene (1.9%), germacrene D (1.8%) and β-bisabolene (1.6%). Antioxidant activity of the essential oil was evaluated by using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals scavenging assay. The essential oil showed antioxidant activity with the IC_{50} of 31.67 mg/mL. In addition, the essential oil exhibited significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* with the MIC values of 250 and 125 mg/mL, respectively. This oil also exhibited anticancer activities against KB, MCF-7 and NCI-H187 cell lines with the IC_{50} values of 24.02, 16.19, and 20.32 μg/mL, respectively, but it was non-cytotoxic to *Vero* cell line.

Keywords: *Mesua ferrea*, essential oil, antioxidant activity, antibacterial activity, anticancer activity

1. INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [1]. The pharmaceutical properties of the plants are partially attributed to essential oils. The essential oils have been shown to possess biological properties such as antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [2-4].

Mesua ferrea L. is a tree of tropical Asia and belongs to the family Guttiferae. Various parts of the plant are used medicinally in India, Pakistan, Indo-China, Malaysia and Thailand [5,6]. It bark is given in treatment of cough, dysentery, vomiting, sore throat and fever. Their flowers are astringent and stomachic. The leaves and flowers in combination with other drugs are used for

the treatment of snake bite and scorpion sting. The seed oil is used as an embrocation in rheumatism and found useful in the treatment of itch [7]. However, the antioxidant and biological activities of the essential oil from *M. ferrea* leaves are rarely reported. The aim of this study was to investigate the chemical constituents of essential oil from *M. ferrea* leaves and its antioxidant, antibacterial and anticancer activities.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Mesua ferrea* L. were collected from Payap University, Chiang Mai, Thailand in December 2010 and the specimen was identified by J. F. Maxwell, a botanist at the Herbarium of Biology Department, Faculty of Science, Chiang Mai University where a voucher specimen has been deposited under the code number Sukanya 02.

2.2 Isolation of the Essential Oil

Fresh leaves of *M. ferrea* (1 kg) were washed with distilled water, chopped into small pieces and subjected to hydrodistillation in a Clevenger-type apparatus for 8 h. The essential oil was collected, dehydrated over anhydrous sodium sulfate, and kept at 4°C for further analysis.

2.3 Analysis of the Essential Oil

The essential oil was analyzed on a Hewlett-Packard GC-6850 gas chroma tograph (FID) equipped with a HP-5 (HP 19091J-433E) fused silica capillary column, (30 m × 0.25 mm i.d., 0.25 μm film thickness) FID) and coupled with a HP 5973N mass selective detector. The thermal program was 40-275°C at the rate of 6°C/min and held isothermal for 12 min. The carrier gas was He at a flow rate of 20.0 mL/min. The injector and detector temperatures were 260°C and 280°C, respectively. The mass spectrometer was scanned over the 30-550 amu range with an ionizing voltage of 70 eV in the electron impact (EI) mode. The identification of the compounds in the essential oil was based on comparison of their retention index (RI), their retention time (RT), and mass spectra with those obtained from authentic samples and/or the Wiley, NIST libraries, and literatures [8-11]. The linear retention indices (RI) were determined in relation to homologous series of *n*-alkanes (C_7-C_{30}) under the same conditions.

2.4 Antioxidant Activity

The antioxidant activity of the essential oil of *M. ferrea* leaves was determined spectrophotometrically using the DPPH radicals scavenging assay [12] with some modification. The 20 mL of various concen trations (5-50 mg/mL) of the oil in ethanol was added to a 180 mL of DPPH radical solution in ethanol (0.004%). The mixture was incubated at room temperature for 30 min in dark and the measured absorbance was at 517 nm with a spectrophotometer (Multimode detecter, Beckman Coulter DTX880, U.S.A.). The percentage scavenging of the DPPH radical was calculated as $[(1\text{-}As/Ac)] \times 100$, where Ac is absorbance of control and As is absorbance of the oil/standard. The IC_{50} values denote the concentration of the sample, which is required to scavenge 50% of DPPH free radicals. Ascorbic acid and trolox were used as positive controls and the DPPH solution was used as negative control. Triplicate determinations were performed.

2.5 Antibacterial Activity

The determination of antibacterial activity was performed by using the microtiter broth microdilution method described by

Amsterdam [13] with some modifications. Two species of microorganisms, Gram-negative *Escherichia coli* (ATCC 25922) and Gram-positive *Staphylococcus aureus* (ATCC 25923) were employed. The oil was initially adjusted to 1000 mg/mL in 95% ethanol and then serially two-fold diluted with Mueller Hinton Broth (MHB) in microtiter plate. A positive control (without tested sample) and a negative control (without tested bacteria) were applied on each plate. After incubation for 24 h at 37°C, bacterial growth was determined by measuring the absorbance at 600 nm using the labsystems multiskan EX type 335 microplate reader (Helsinki, Finland). The MIC of the essential oil was defined as the lowest concentration causing the decrease of the absorbance when compared with the positive control. The same protocol was used to determine the MIC of Amoxicillin for the inhibition of all tested pathogenic bacteria. Triplicate determinations were performed.

2.6 Anticancer Activity

The anticancer activities of the essential oil against the three cancerous human-cell lines, KB (oral cancer, ATCC CCL-17), MCF-7 (breast cancer, ATCC HTB-22) and NCI-H 187 (small cell lung cancer, ATCC CRL-5804) cell lines were assayed employing the Resazurin microplate assay (REMA) as described by Brien *et al.* [14]. In brief, the oil was first diluted to 50 mg/mL in 0.5% DMSO and then serially three-fold diluted with 0.5% DMSO. Successively, 5 μL of each oil solutions and 45 μL of cell suspension were added to 384-well plates, incubated at 37°C in 5% CO_2 incubator. After the incubation period (3 days for KB and MCF-7 and 5 days for NCI-H187), 12.5 μL of resazurin solution (62.5 μg/mL) was added to each well, and the plates were then incubated at 37°C for 4 h. The fluorescence signal was measured using a SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) at excitation and emission wavelengths of 530 nm and 590 nm. Percent inhibition of cell growth (%) was calculated as $[1-(FU_T/FU_C)]*100$ whereas FU_T and FU_C are the mean fluorescent unit from treated and untreated conditions, respectively. The inhibitory concentration (IC_{50}) represented the concentration that caused 50% reduction in cancer cell line growth. Ellipticine and doxorubicin were used as positive controls and 0.5% DMSO was used as negative control. Triplicate determinations were performed.

2.7 Cytotoxic Activity

The cytotoxicity against primate cell line (*Vero*) of the essential oil was determined by using green fluorescent protein (GFP) detection described by Hunt *et. al.* [15]. The GFP-expressing *Vero* cell line was generated in-house by stably transfecting the African green monkey kidney cell line (ATCC CCL-81), with pEGFP-N1 plasmid (Clontech). The oil was first diluted to 50 mg/mL in 0.5% DMSO and then serially three-fold diluted with 0.5% DMSO. The assay was carried out by adding 45 μL of cell suspension at 3.3×10^4 cells/mL to each well of 384-well plates containing 5 μL of each oil solution and then incubating for 4 days in an incubator at 37°C with 5% CO_2. Fluorescence signals were measured by using SpectraMax M5 microplate reader (Molecular Devices, USA) in the bottom-reading mode with excitation and emission wavelengths of 485 and 535 nm. Triplicate determinations were performed. The percentage of cytotoxicity and the IC_{50} value of each sample has also been calculated. Ellipticine and 0.5% DMSO were used as positive and negative controls, respectively.

3. RESULTS AND DISCUSSION

3.1 Analysis of the Essential Oil

The yellowish oil isolated by hydro distillation from the leaves of *M. ferrea* was obtained in yield of 0.064% (w/w). The essential oil was analysed by means of GC and GC-MS. The components of the oil, the retention times (RT) the percentage constituent (%) and the retention indices (RI) are summarized in Table1. Thirty-five compounds were identified, constituting to 81.4% of the total oil components. The major components were *trans*-caryophyllene (30.9%), β-caryophyllene oxide (17.9%), α-humulene (6.0%), δ-cadinene (4.1%), γ-muurolene (3.5%), γ-cadinene (2.3%), β-selinene (1.9%), germacrene D (1.8%) and β-bisabolene (1.6%). The most representative compounds in the oil were sesquiterpene hydrocarbons (60.7%), oxygenated sesquiter penes (19.0%), carboxylic acid (0.5%), diter pene and triterpenes (0.4%), terpene related compounds (0.4%), saturated aliphatic hydrocarbons (0.3%) and others (0.2%). Sesquiterpene hydrocarbons were found in the major group of compounds in the essential oil of *M. ferrea* leaves. They might be responsible for biological properties of this oil.

There are a number of literature reports on the leaves oil analysis of *M. ferrea* [16-17]. One study showed that major components in the tender and mature leaves oils were α-copaene (19.3% and 9.9%) and *trans*-caryophyllene (18.8% and 26.0%) [16]. Another report showed that 2,6,10-dodecatrien-1-ol (36.65%), α-caryophyllene (32.66%) and squalene (6.74%) were the major components in the leaves oil obtained by microwave solvent extraction labstation, while caryophyllene (41.52%), naphthalene (13.28%) and germacrene (8.15%) were the main compounds in the oil obtained by hydrodistillation [17]. Our results were different from theirs. It may be attributed to different factors such as geographical environment, growth season and physiological age of the plant besides the method of oil isolation.

Table 1. Chemical compositions of the essential oil from *M. ferrea* leaves.

Compounds	RT (min)	Area (%)	RI (exp)	RI (lit)	ID	References
(Z) 3-Hexanol	4.41	0.1	866	859	RI,MS	[8]
Linalool	9.75	tr	1105	1097	RI,MS	[8]
Edulan I	13.40	tr	1263	1315	MS	[9]
α-Cubebene	15.40	0.3	1353	1351	RI,MS	[8]
α-Ylangene	15.89	0.3	1375	1375	RI,MS	[8]
α-Copaene	16.03	1.1	1381	1377	RI,MS	[8]
β-Bourbonene	16.21	0.8	1389	1388	RI,MS	[8]
β-Elemene	16.34	0.5	1395	1391	RI,MS	[8]
(*cis*)-Caryophyllene	16.67	0.4	1411	1409	RI,MS	[8]
(*trans*)-Caryophyllene	17.13	30.9	1434	1419	RI,MS	[8]
(+) Aromadendrene	17.40	0.7	1447	1441	RI,MS	[8]
α-Humulene	17.76	6.0	1465	1455	RI,MS	[8]
(-) Alloaromadendrene	17.85	1.1	1469	1460	RI,MS	[8]
γ-Muurolene	18.15	3.5	1483	1480	RI,MS	[8]
Germacrene D	18.27	1.8	1489	1485	RI,MS	[8]

Table 1. (continued)

Compounds	RT (min)	Area (%)	RI (exp)	RI (lit)	ID	References
β-Selinene	18.43	1.9	1496	1490	RI,MS	[8]
Valencene	18.50	1.0	1499	1496	RI,MS	[8]
α-Selinene	18.57	1.1	1503	1498	RI,MS	[8]
α-Muurolene	18.61	0.7	1505	1500	RI,MS	[8]
β-Bisabolene	18.78	1.6	1514	1506	RI,MS	[8]
γ-Cadinene	18.93	2.3	1522	1514	RI,MS	[8]
δ-Cadinene	19.04	4.1	1528	1523	RI,MS	[8]
(*cis*)-Calamenene	19.09	0.5	1530	1540	RI,MS	[8]
α-Calacorene	19.48	0.3	1551	1546	RI,MS	[8]
Caryophyllenyl alcohol	20.23	0.5	1588	1572	RI,MS	[8]
β-Caryophyllene oxide	20.42	17.9	1598	1583	RI,MS	[8]
T-muurolol	21.47	0.5	1655	1646	RI,MS	[8]
Hexahydrofarnesyl acetone	24.83	0.4	1846	1849	RI,MS	[8]
n-Hexadecanoic acid	26.92	0.5	1973	1989	RI,MS	[8]
Phytol	29.07	0.2	2114	2109	RI,MS	[8]
4,8,12,16-Tetramethyl heptadecan-4-olide	32.48	0.1	2356	2337	MS	[10]
Hexanedioic acid, bis (2ethylhexyl) ester	33.02	0.1	2396	-	MS	
Heptacosane	36.79	0.1	2697	2700	RI,MS	[8]
Squalene	38.19	0.2	2819	2829	MS	[11]
Nonacosane	39.07	0.2	2897	2900	RI,MS	[8]
Sesquiterpene hydrocarbons		60.7				
Oxygenated sesquiterpenes		19.0				
Diterpene and triterpenes		0.4				
Terpene related compounds		0.4				
Carboxylic acids		0.5				
Saturated hydrocarbons		0.3				
Others		0.2				
Total		**81.4**				

tr = trace (% area = 0.02); RT = retention time; RI (lit) = values from literature data;
RI (exp) = retention indices on HP-5 MS column; relative to n-alkane (C_7-C_{30});
ID = methods of identification: MS, comparison of the mass spectrum with MS libraries; RI of literature.

3.2 Antioxidant Activity

The antioxidant activity of this essential oil was performed by using the DPPH free radical scavenging assay. The DPPH is a stable free radical and often used for the evaluation of general radical scavenging abilities of antioxidants [12]. The odd electron in the DPPH is responsible for the absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant compounds, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The essential oil showed a moderately activity with the IC_{50} value of 31.67 mg/mL (Table 2). The standard compounds, vitamin C and trolox showed antioxidant activity with the IC_{50} values of 0.055 and 0.096 mg/mL, respectively.

The essential oil of this plant contains some active components that have been reported to exhibit antioxidant activity. *trans*-Caryophyllene, the most representative compound in this essential oil (30.91%) has reported antioxidant activity [18]. Moreover, T-muurolol, linalool, phytol and squalene have been reported to exhibit antioxidant activity [4, 19-21]. Results indicated that the activity of the essential oil may be due to the synergistic effects of these active compounds.

Table 2. Antioxidant, antibacterial, anticancer and cytotoxic activities of essential oil from *M. ferrea* leaves.

Activity	IC_{50} (mg/mL)	MIC (mg/mL)	IC_{50} (mg/mL)
Antioxidant	31.7	-	-
Antibacterial	-		-
E. coli		250	
S. aureus		125	
Anticancer			
KB-oral	-	-	24.0
MCF-7			16.2
NCI-H187			22.3
Cytotoxic	-	-	
Vero cell			NC*

*NC = non-cytotoxic

3.3 Antibacterial Activity

The antibacterial activity of the essential oil from the leaves of *M. ferrea* was evaluated *in vitro* using the microtiter broth method with the microorganisms as seen in Table 2. The essential oil was exhibited moderate antibacterial activity against *E. coli* and *S. aureus* with the MIC values of 250 and 125 mg/mL, respectively. Results implicated that the Gram-positive bacteria (*S. aureus*) was more sensitive to the essential oil than the Gram-negative bacteria (*E. coli*). Amoxicillin is one of the most common antibiotics of bactericidal activity against many Gram-positive and Gram-negative. It also showed activity against both *E. coli* and *S. aureus* with MIC value of 3.13 mg/mL.

Some sesquiterpene hydrocarbons present in this oil have been reported to exhibit antibacterial activity such as *trans*-caryophyllene, a-humulene, germacrene D, d-cadinene, a-cubebene, a-copaene, g-cadinene and b-elemene [22-24]. b-Caryophyllene

oxide is known to possess antibacterial properties against a wide range of bacteria [25] and *n*-hexadecanoic acid is found to be effective against *S. aureus* [26]. Our research findings revealed that medicinal plant *M. ferrea* can play a vital role in combating bacterial resistance and offers an option to the pharmaceutical industry of new natural medicine sources.

3.4 Anticancer and Cytotoxic Activities

The essential oil showed significantly anticancer activities against KB (oral cavity cancer), MCF7 (breast cancer) and NCI-H187 (small cell lung cancer) cell lines with IC_{50} values of 24.02, 16.19, and 22.32 μg/mL, respectively (Table 2). The essential oil was non-cytotoxic against *Vero* cells. These could be good candidates for further study. Ellipticine, standard drug showed anticancer activity against KB and NCI-H187 cell lines with IC_{50} values of 0.512, and 0.875 μg/mL, respectively. It also showed cytotoxicity with IC_{50} value of 1.335 μg/mL. Moreover, doxorubicin showed anticancer activity against KB, MCF-7 and NCI-H187 cell lines with IC_{50} values of 0.319, 0.858 and 0.050 μg/mL, respectively.

This study is the first report describes the anticancer activity of the essential oil from *M. ferrea* leaves against three human cell lines. The bioactivity of some compounds present in this oil have been reported to exhibit anticancer activity. *trans*-Caryophyllene has been reported to exhibit anticancer property [27], while a-humulene and T-muurolol have shown cytotoxicity in MCF-7 cell lines [28-29]. The anticancer activity of the essential oil of *M. ferrea* leaves may be synergistic effects of these active compounds or some compounds in the oil. Therefore, the anticancer activity of this medicinal plant may be used to the development of potent anticancer drug.

4. CONCLUSIONS

In conclusion, the chemical constituents of the essential oil from the leaves of *M. ferrea* was analysed by GC and GC/MS. Thirty-five compounds accounting for 81.4% of the oil were identified. The essential oil showed moderately antioxidant and antibacterial activity against *E. coli* and *S. aureus*. It also exhibited anticancer activity against three human cell lines (KB, MCF-7 and NCI-H187). Therefore, according to these results, we suggest that the essential oil of *M. ferrea* leaves could be another potential source for the new drug development.

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