

DEVELOPMENT OF ESSENTIAL OIL EXTRACTION BY MICROWAVE ASSISTED PROCESS FROM A LOCAL PLANT

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ABSTRACT

Microwave Assisted Process (MAP) is a high speed technique for selective extraction of target compounds of Essential Oil (EO) from selected raw materials using a microwave at 2.45GHz which is S-band applicator as the energy source during extraction leading to: fast processing time; improved yield and quality; direct extraction capability; lower energy consumption; reduced solvent levels and lower capital investment. Microwave Solvent Extraction Labstation (ETHOS SEL) and Hydro-Distillation (HD) were compared for the extraction of essential oil from *mesua ferrea* L. (Guittiferea) leaves. The results obtained revealed that the technique is efficient and used small amount of solvent, i.e. the first essential oil droplets came out in 5 min with ETHOS SEL and in 60 to 90 min for HD. In 40 min of extraction it is possible to collect a sufficient amount of essential oil for analytical determination by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC/MS). ETHOS SEL essential oil is found rich in major compounds compared to HD. This is a new technique that combines microwave heating and dry-distillation provides more valuable essential oils, reduces the extraction time, and allows a substantial saving energy.

INTRODUCTION

Microwaves are part of the electromagnetic spectrum and are located between 300MHz and 300GHz. The electromagnetic spectrum is characterized by wavelengths between 1mm to 1m. It is useful to consider the quantum energy of microwave in relation to other forms of electromagnetic energy. It is important to recognize that the energy delivered by microwaves is insufficient for breaking covalent chemical bonds. During microwave at high frequency heating, many materials properties affect the heating performance. Microwave heating is defined as the heating of a substance by electromagnetic energy operating in the frequency range. The frequency range, which also can be used for heating is very large. Only restricted microwave or high frequencies are freely allowed for heating in industrial, scientific, and medical applications (ISM) frequencies of these; only 2.45 GHz is commonly used in food processing in Europe, while 915 MHz dominates in American and 896 MHz in the UK. Thus, the fixed frequency for ETHOS SEL is 2,445 MHz (2.45GHz). Today microwave-heating base on microwave radiation is introduced. There is a fundamental difference in the nature of microwave heating when compared to conventional methods

of heating materials. Conventional heating relies on one or more of the heat transfers mechanisms of convection, conduction, or radiation to transfer thermal energy into the material.

Essential Oil (EO) also called Ethereal or Volatile Oils, are frequently referred to as the "life force" of plants. Unlike fatty oils, these "essential" oils are volatile, highly concentrated, substances extracted from leaves, flowers, stems, roots, seeds, barks, ruin or fruit rinds. Before such substances can be analyzed, they have to be extracted from the material. There are many extraction methods such as distillation, extraction and expression. But conventional method that the most common method usually used to extracted essential oil from plant is the distillation method like Steam-Distillation, Hydro-Distillation and Water-Steam-Distillation. EO consists of chemical compound, which have hydrogen, carbon, and oxygen as their building blocks. These can be subdivided into two groups: the hydrocarbons that are made up almost exclusively of *terpenes*; and the *oxygenated compounds*. The method of extraction that is employed depends on the quality of the material that is being used and the type of aromatic product there is required. Some plants materials, especially flowers, are subject to deterioration and should be processed as soon as possible after harvesting.

Mesua ferrea L. (Guttiferae), penaga lilin (Malay) is a moderate sized tree of tropical Asia. The tree bears flowers between April and July fruits between October and November. It has linear leaves, 3-6.5 inches in length and white flowers from the uppermost leaf axils (T.J. Dennis and K.Aksyhaya Kumar, 1998). The leaves are used in the form of poultice that is applied to the head in severe colds. *Mesua ferrea* spices are a well-known medical plant, widely used in indigenous system of medicine for the treatment of fever, dyspepsia, and renal diseases as enlisted by (Nadkarni A.K, 1954). Though still limited, pharmacological studies on the crude drug, some of its constituents and various extractives have revealed significant antibacterial, antifungal, anthelmintic, antispasmodic, diuretic, antiasthmatic, anti-inflammatory and anti-implantation activities providing some support to the ancient claims. Besides of that there is a function used for product as fodder: fuel: tannin or dyestuff: lipids: poison: from those parts of tree and the other product is the fragrant flowers are used to stuff pillows and cushions and in cosmetic products. A number of compounds, representing a variety of chemical structures isolated from the carious parts of this plant, may provide interesting leads for further pharmacological evaluation (J. Dennis and K.Aksyhaya Kumar. 1998).

The objective of this research is to compare Microwave Assisted Process (MAP) with Conventional Extraction; to identify and characterized extracted essential oil from the *mesua ferrea* leaves using GC and GC/MS and to developed an extraction prototype model for large scale treatment for scientific, industrial and technical applications.

MATERIAL AND METHOD

Plant Materials

The *mesua ferrea* leaves were gathered in Universiti Putra Malaysia Research Park, serdang, Malaysia during late October of 2000 (for experimental hydro-distillation) and 2003 (for experimental microwave extraction).

Apparatus

The apparatus used in this research were ETHOS SEL, heating mantle, 2L round bottom flask, Clevenger extractor according to *European Pharmacopoeia* (Sainte Ruffine, 1996), rotary vacuum evaporator used to remove solvents from reaction mixtures and desiccators. All analysis was performed by direct injection into Gas Chromatography Flame Ionization Detection (GC-FID) model SHIMADZU (GC-14B, GC-16A, GC-17A) and (GC/MS) model SHIMADZU QP5050A. The retention time will be showing the major or minor compound in the solution after using GC/MS. Other constituents were identified in oils of known composition. Identification of the components was achieved from their retention indices on both columns, determined with reference to a homologous series of alkanes and by a comparison of their mass spectral fragmentation patterns with those stored in the spectrometer data bank using the (WILEYL/NBS library) built-in libraries and the literature. Other materials such as anhydrous sodium sulphate a drying agent, distill-water and n-hexane as the solvent were also used in this research.

Conventional Extraction Procedure (Hydro-Distillation)

204.8 g of fresh *mesua ferrea* leaves were fully submerged in 1000 ml of water and were submitted to hydro-distillation with a Clevenger-type of apparatus consisting of a 2L round bottom flask set in a heating mantle and extracted for 8 hours at a temperature of 100°C and power 450 Watt. 240 ml of n-hexane were used throughout the process where by 30 ml of n-hexane were added to the Clevenger apparatus every 1 hour to enable the condensed essential oil to dissolve in the solvent. After 1 hour of extraction, two immiscible layers were formed; the oil plus solvent and the distilled water. The less dense oil and solvent will normally float on top of the distilled water component and were collected every 1 hour for an extraction of 8 hours, dried over anhydrous sodium sulphate, filtered, rotavaps and stored at 0°C until used.

Hydro-distillation GC/MS analysis

Samples were analyses using, a BPX5 non-polar column (30 m long; 0.53 mm i.d; 1.0µm film thickness) and GC-FID. Samples were analyzed by direct injection of 1µl into an injection port held at 330°C. The FID was held at 340°C. The GC oven temperature was held at 50°C for 7min and then ramped to 330°C at 10°C/min, where it was held for reminder of the run with a column head pressure of 15psi Helium gas. Calibration curves were created for *mesua ferrea* leaves essential oil using standard dilution.

Microwave Assisted Process (MAP) procedure (ETHOS SEL)

Dry-distillation was performed by giving 2.45 GHz microwave radiation from ETHOS SEL to extract essential oil from *mesua ferrea* leaves. 250 g of fresh *mesua ferrea* leaves were placed inside the microwave reactor with a Clevenger-type of apparatus consisting of a 2L round bottom flask without adding distill water and extracted for 40 min at a temperature of 100°C and microwave power controlled at 400-700 Watt. The microwave-generated heating of the water molecules within the plant material distends it causing the gland and olifeerous receptacles to burst. The essential oil is freed and is stripped away by azeotropic distillation with the evaporating “in-situ” water of the plant material. Cooling system out site the microwave oven cavity continuously condenses the distillates at 5°C. The condensates are guided to a bypass and the excess water is refluxed back to the sample in the reactor. Only 30 ml of n-hexane were added to the Clevenger apparatus to enable the condensed essential oil to dissolve in the solvent. Complete distillation is carried out in approximately 20-30 minutes for aromatic herbs and 40-60 minutes for spices. After 40 min of extraction, two immiscible layers were formed; the oil plus solvent and the distilled water. The less dense oil and solvent will normally float on top of the distilled water component and were collected, dried over anhydrous sodium sulphate, filtered, rotavaps and stored at 0°C until used.

ETHOS SEL GC-MS analysis

Samples were analyzed using mass spectrometry SHIMADZU QP5050A a BPX5 non-polar column (30 m long; 0.25 mm i.d; 0.25- μ m-film thickness) and Gas Chromatography with Flame Ionization Detection (GC-FID). Samples were analyzed by direct injection of 1 μ l into an injection port held at 330°C in the split mode (1:100). The FID was held at 340°C. The GC oven temperature was held at 60°C for 3 min and then ramped to 320°C at 5°C/min, where it was held for remainder of the run with a column head pressure of 4psi. The flow-rate of the carrier helium gas was 0.7ml/min. sampling rate 0.5 sec. Mass spectra were recorded in the ionization mode used was electronic impact at 70eV. Calibration curves were created for *mesua ferrea* leaves essential oil using standard dilution.

RESULTS AND DISCUSSION

The extraction of the first essential oil droplet begins only after 5 min by ETHOS SEL against 90 min for HD. Thus, an extraction time of 40 min. microwave extraction provides comparable yields to those obtained after 8 hours by means of HD and allows substantial saving of energy. More peak were detected in the essential oil extracted by microwave compounds compared to HD. 23 compounds have been identified in the *mesua ferrea* oil, extracted by ETHOS SEL whereas 17 compounds were detected in the oil extracted by HD (Table 1). The result was compared between both methods of extraction that shown in parameter profile (Table 2, 3 and Figure 1). A new major compound was detected by ETHOS SEL (Table 2). The yield of essential oil from *mesua ferrea* leaves obtained by HD were 0.003 g and those obtained by ETHOS SEL were 0.001 g. Regarding the yield ratios of the essential oil present in the treated leaves extracted by ETHOS SEL (40 min) and HD (8 h), ETHOS SEL allows extraction of

33%. It seems that ETHOS SEL is a more appropriate extraction method for small amount of sample. After a long extraction time (8 h), ETHOS SEL gives the same yield in essential oils.


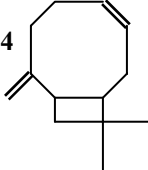
Table 1: Composition essential oil of *mesua ferrea* obtained by ETHOS SEL and HD

PN	ETHOS SEL				HD			
	RT	Compound	Intensity		RT	Compound	Intensity	
			Area %	Height %			Area %	Height %
1.	3.208	Toluenez	1.72	2.58	14.91 9	3-Hexen-1-ol	0.85	2.37
2.	9.114	3-Hexen-1-ol	1.13	1.50	17.12 9	Nonanal	2.11	5.37
3.	12.542	2-Nonen-1-ol	0.75	0.97	23.60 7	Copane	3.85	6.30
4.	21.040	Copaene	1.80	2.08	25.51 3	Caryophyllene	41.52	20.88
5.	22.449	2,6,10-Dodecatrien-1-ol*	36.65	35.14	26.58 5	α -Caryophyllene	6.22	7.64
6.	22.606	1,3,6,10-Dodecateraene	1.35	1.46	27.01 9	Naphthalene	8.21	9.24
7.	23.509	α-Caryophyllene	32.66	30.49	27.50 0	Germacrene	8.15	1.00
8.	24.733	1,6,10-Dodecatriene	0.38	0.44	27.71 9	4,11,11-trimethyl-8-methylene-Bicyclo[7.2.0]undec-4-ene	4.41	4.61
9.	25.946	E-12-Tetradecnal	0.42	0.48	28.52 0	Naphthalene	13.28	15.59
10.	27.099	Trans-Z- α -Bisabolene epoxide	1.19	1.22	30.99 1	Caryophyllene oxide	2.55	4.30
11.	27.862	1,5,5,8-tetramethyl-dodeca-3,7-diene	1.06	1.03	31.83 9	3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	0.97	1.96
12.	32.846	E-11- Tetradecenyl acetate	4.98	4.65	32.40 8	1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-1-Naphthalenol	2.09	2.52
13.	32.942	2,6-Dimethyl-6-trifluoroacetatoxyoc tane	0.42	0.46	32.73 1	α -Cadinol	1.79	2.91
14.	33.167	2-Tetradecanone	0.21	0.16	32.88 2	4a-trimethyl-8-methylene-2-Naphthalenemethanol	1.16	1.94
15.	33.572	3-Eicosyne	0.73	0.64	34.48 3	Aromadendrene oxide	1.69	1.82
16.	34.137	3-Eicosyne	1.86	1.48	34.76 7	1,1,2-trimethyl-3,5-bis(1-methylethenyl)-Cycohexane	0.59	1.52
17.	46.321	Alloaromadendrene	0.39	0.43	39.71	1-Docosene	0.55	1.08

		oxide			7
18.	46.877	Alloaromadendrene	1.07	1.35	
19.	47.322	4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylidene-2-one	0.56	0.65	
20.	48.708	Di-n-octyl phthalate	2.00	2.30	
21.	52.092	Squalene	6.74	8.34	
22.	52.984	Octacosane	0.63	0.91	
23.	55.381	Eicosane	1.28	1.24	

Compound listed in order of elution; PN, peak no.; RT, retention time on BPX5 column; HD, Hydro-Distillation (8 h); ETHOS SEL MAP, microwave assisted process (40 min);

Table 2: Results of extraction process obtained by ETHOS SEL and HD

Specification	ETHOS SEL (Dry-D)	Conventional Extraction (Hydro-D)
1. Time of extraction (min)	40	480
2. 1 st drop EO (min)	5	90
3. Solvent used (ml)	30	240
4. Sample weight (g)	250	204.8
5. Water boiling point 100°C (min)	5	90
6. Possibility to collect sufficient EO for analytical determination (min)	40	90
7. Power used (watt)	400-700	450
8. Yield of essential oil (g) [#]	0.001	0.003
	Compound name: 2,6,10-Dodecatrien-1-ol SI:90 Formula: C ₁₅ H ₂₆ O CAS: 4602-84-0 MolWeight: 222	Compound name: Caryophyllene SI: 94 Formula: C ₁₅ H ₂₄ CAS: 87-44-5 MolWeight: 204
9. Major compound rate	 New major compound*	

[#]yield expressed in g essential oil/kg.

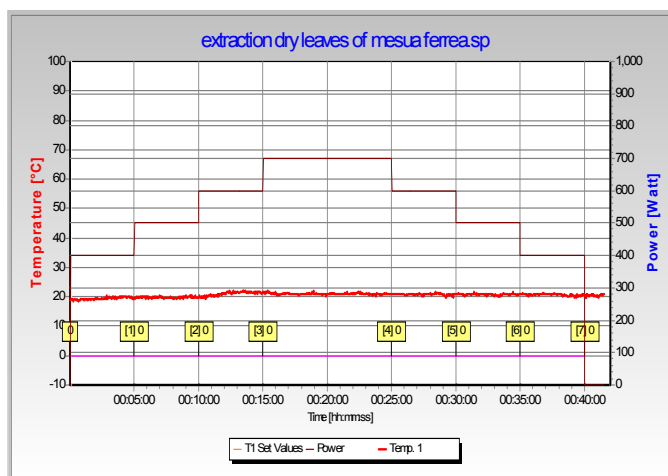


Figure 1: Power parameter profile of ETHOS SEL and Energy calculation from: Time = 00:00:00-00:40:00, Integral (KJ)=1319.30, AVG (Watt)=548.73

Table 3: Power standing and time profile [400/5min-500/5min-600/5min-700/10min-600/5min-500/5min-400/5min-0/2minWatt]- 2minVentilation

Step	Power setting (watt)	Time (min)	Integral (KJ)	AVG (Watt)
1	400	0-5	120.00	397.92
2	500	5-10	149.00	503.60
3	600	10-15	179.80	605.04
4	700	15-25	419.80	702.18
5	600	25-30	180.80	604.38
6	500	30-35	149.50	502.96
7	400	35-40	120.40	400.00
8	0	40-42	000.00	000.00

CONCLUSION

This ETHOS-SEL method combining microwave heating and dry-distillation provides more valuable essential oils, reduces the extraction time, and allows a substantial saving energy. After 40 min of ETHOS SEL, it is possible to collect sufficient essential oil for analytical determinations, whereas HD provides the first essential oil droplet only after 90 min. All these advantages make ETHOS-SEL microwave extraction a good alternative for the extraction of essential oil for analytical determinations.

ACKNOWLEDGMENT

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