International Food Research Journal 24(4): 1525-1528 (August 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

GC-MS analysis of essential oil of *Pogostemon cablin* growing in Indonesia extracted by microwave-assisted hydrodistillation

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Article history

Received: 15 June 2016
Received in revised form: 19 July 2016
Accepted: 22 July 2016

Keywords

Pogostemon cablin
GC-MS
Indonesian patchouli oil
Patchoulol
Microwave-assisted hydrodistillation

Abstract

The essential oil from the leaf of *Pogostemon cablin,* grown in Indonesia was extracted by microwave-assisted hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Nineteen compounds were identified representing approximately 97.97% of the oil. The main compound of the oil was the oxygenated terpene patchoulol (26.32%). Other major compounds of the essential oil were δ-guaiene (14.69%), α-guaiene (12.18%), α-Gurjunene (11.13%), seychellene (8.42%), viridiflorol (5.93%), β-caryophyllene (4.63%) and β-patchoulene (2.87%). Four new compounds were found in this oil: 2(1H)-naphthalenone, octahydro-1-methyl-1-(2-propenyl)-, (1a,4ab,8aa)- (2.64%); iso-.aplha.-cedren-15-al (0.35%); 7-oxabicyclo[4.1.0]heptane, 1,3,3-trimethyl-2-(3-methyl-1,3-butadienyl)-, [1a,2b(Z),6a]- (0.12%) and hexahydrothunbergol (0.11%).

Introduction

Patchouli or *Pogostemon cablin* (Blanco) Benth is native to the Philippines and grows wild and cultivated in Indonesia, Malaysia, China, Brazil, and India. Patchouli is used in perfumery, cosmetics and processed food industry. The chemical constituents of the essential oil were found to be twenty-two sesquiterpenoids, in which patchouli alcohol became major constituent (Buranthep *et al.,* 2006). Patchouli (Pogostemon cablin) oil is one of the important natural essential oils used to give a base and lasting character to a fragrance perfumery industry (Singh *et al.,* 2002). The oil produced during extraction is called patchouli oil.

Patchouli oil is an aromatic material of plant origin, which is widely used in perfumery, antibacterial agent and flavourings. They represent the "essential aroma" of the plant from which they are obtained. Patchouli is a perennial bushy herb or shrubs with strong large stems and aromatic furry leaves. Its aromatic oils can be found in various parts of the plant, including leaves, stalks, branches, and roots. Patchouli is best grown in area up to 400 meters above the sea level and requires constant quantity of rainfall, 2500 to 3500 mm every year. The ideal temperature is 22°C to 28°C, with plenty of sunlight, and with humidity greater than 70% (Yahya and Yunus, 2013).

Organic patchouli essential oil is widely used in medical, perfumery, rituals, cosmetics and to scent clothes and textiles. Along with scenting textiles, patchouli oil also acts as a deterrent to insects. Indeed, patchouli oil is used extensively in the flavoring industry, and serves as an ingredient in many foods and beverages. It is also on the FDA's (Food and Drug Administration) list of substances approved for human consumption, in section 172.510, as a natural additive for food flavoring (FDA, 2002).

In aromatherapy, patchouli essential oil is used as a topical remedy for skin problems such as acne, eczema, inflamed, cracked, chapped and irritated skin. It is known as a cell rejuvenator and helpful in healing wounds and scars. As an antifungal, patchouli oil has been used to treat athlete's foot. For the nerve system, patchouli essential oil helps to reduce tension, insomnia and anxiety. Furthermore, patchouli oil is use as a fixative in many natural perfume formulations, soaps and cosmetic products (Betts, 1994; Deguerry *et al.,* 2006).

Patchouli alcohol in patchouli could also be responsible for the high repellency activity of the essential oils. Jantan and Zaki (1998) discovered significant repellency effect exhibited by the patchouli leaf oils towards Aedes aegypti and excellent protection from mosquitoes (Jantan and Zaki, 1998). Moreover, the plant (Pogostemon cablin) is widely used in traditional Chinese medicine as it offers various types of pharmacological activity according

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to the composition of the oil (Wu *et al.,* 2004; Hu *et al.,* 2006).

The composition of patchouli oil is unique and complex because it consists of over 24 different sesquiterpenes, rather than a blend of different mono-, sesqui- and di-terpene compounds (Deguerry *et al.,* 2006). The sesquiterpene patchoulol is the major constituent and is the primary component responsible for the typical patchouli aroma. This essential oil is also characterized by a large number of other sesquiterpene hydrocarbons such as α-/β-/γ- patchoulenes, α-guaiene, seychellene, and α-himachalene. Although α-patchoulene is found in small amounts, it is an important constituent of patchouli oil because, together with patchoulol, it also determines the aroma of the oil. Also, it is believed that the antifungal activity of the essential oil is closely related with these two compounds (Singh *et al.*, 2002; Wu *et al.*, 2004; Deguerry *et al.,* 2006). Thus, the greater the concentration of these compounds in the essential oil, the better the quality and the higher the commercial value (Sonwa, 2000).

The present study was undertaken in order to improve the knowledge of the chemical composition of patchouli oil. In this paper, we report the qualitative analysis of the volatile components of Indonesian patchouli oil extracted by microwave-assisted hydrodistillation.

Materials and Methods

Material and chemicals

Dried leaf of *Pogostemon cablin* was collected from Tulungagung, East Java, Indonesia. The leaves then were chop to a size around 5-10 mm using a commercial grade blender (Arte Blender, BL-001) and stored at room temperature until required. Aquadest and anhydrous sodium sulphate used in the experimental work were all of analytical grade.

Microwave-assisted hydrodistillation

A domestic microwave oven (EMM-2007X, Electrolux, 20 l, maximum delivered power of 800 W, 2.45 GHz) was modified for microwave-assisted hydrodistillation operation. The dimensions of the PTFE-coated cavity of the microwave oven were 46.1 cm x 28.0 cm x 37.3 cm. Eighty grams of patchouli leaves were placed in a 1 l flask containing aquadest (400 ml). The flask was setup within the microwave oven cavity and a condenser was used (outside the oven) to collect the extracted essential oils. The microwave oven was operated at 600 W power level for a period 1 h. To remove water, the extracted essential oils were then dried over anhydrous sodium sulfate and then stored in amber vials at 4°C until they were used for analysis.

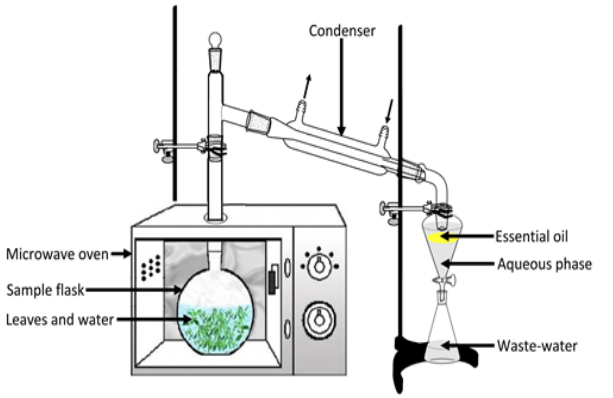

Figure 1. Schematic representation of the microwave-assisted hydrodistillation apparatus used in this study (Kusuma and Mahfud, 2016)

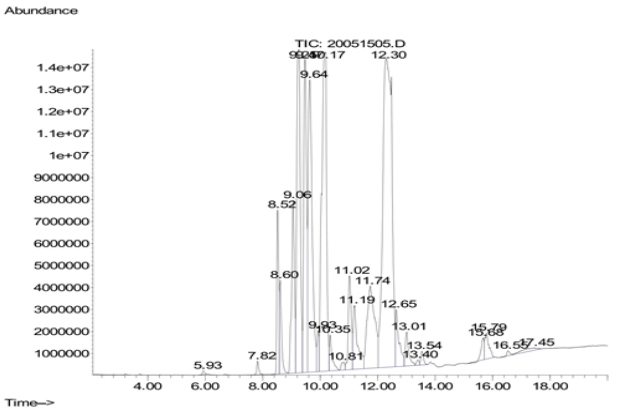

Figure 2. Total ion chromatograms from GC-MS for essential oils (EOs) obtained from Pogostemon cablin leaves by MAHD

Chemical analysis of essential oil constituents

Essential oils composition was determined by gas chromatography coupled to mass spectrometry (GC–MS) analysis on a Hewlett–Packard 6890 gas chromatograph coupled to a 5973A mass spectrometer, using two fused-silica-capillary columns with different stationary phases. The non-polar column was HP5MS™ (30 m length, 0.25 diameter and 0.25 μm film thickness) and the polar one was a Stabilwax ™ consisting of Carbowax™-PEG (60 m length, 0.25 mm diameter and 0.25 μm film thickness). GC–MS spectra were obtained using the following conditions: carrier gas He; flow rate 1.0 ml min-1; split 1:50; injection volume 1.0 μL; injection temperature 300°C; oven temperature progress from 100 to 250°C at 10°C min-1; the ionisation mode used was electronic impact at 70 eV. Identification was confirmed by comparison of their mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology and Wiley libraries) and with mass spectra literature data (Adams, 1995; Arctander,

Table 1. Chemical compositions of essential oils (EO) obtained from Pogostemon cablin leaves by MAHD using GC-MS

No.	Compounds[1]	CAS Number[2]	Molecular Formula	Molecular Weight (g/mol)	RT[3] (min)	Area [%]
Sesquiterpenes						
1	δ-Elemene	20307-84-0	$C_{15}H_{24}$	204.35	7.82	0.25
2	β-Patchoulene	514-51-2	$C_{15}H_{24}$	204.35	8.52	2.87
3	Valencene	4630-07-3	$C_{15}H_{24}$	204.35	8.61	1.86
4	β-caryophellene	87-44-5	$C_{15}H_{24}$	204.35	9.06	4.63
5	α-Guaiene	3691-12-1	$C_{15}H_{24}$	204.35	9.25 13.01	12.18
6	Seychellene	20085-93-2	$C_{15}H_{24}$	204.35	9.47	8.42
7	α-Gurjunene	489-40-7	$C_{15}H_{24}$	204.35	9.63	11.13
8	β-Maaliene	489-29-2	$C_{15}H_{24}$	204.35	9.93	0.73
9	δ-Guaiene	3691-11-0	$C_{15}H_{24}$	204.35	10.17	14.69
10	Selina-3,7(11)-diene	6813-21-4	$C_{15}H_{24}$	204.35	10.35	0.90
11	Aromandendrene	489-39-4	$C_{15}H_{24}$	204.35	10.81	0.25
12	Valencene	4630-07-3	$C_{15}H_{24}$	204.35	12.65	1.91
Oxygenated terpenes						
13	2(1H)-Naphthalenone, octahydro-1-methyl-1-(2-propenyl)-, (1a,4ab,8aa)-	97571-39-6	$C_{14}H_{22}O$	206.32	11.02	2.64
14	Aromadendrene oxide	1000156-12-8	$C_{15}H_{24}O$	220.35	11.19	2.68
15	Viridiflorol	552-02-3	$C_{15}H_{26}O$	222.37	11.74	5.93
16	Patchoulol	5986-55-0	$C_{15}H_{26}O$	222.37	12.30	26.32
17	7-Oxabicyclo[4.1.0]heptane, 1,3,3-trimethyl-2-(3-methyl-1,3-butadienyl)-, [1a,2b(Z),6a]-	97550-03-3	$C_{14}H_{22}O$	206.32	13.40	0.12
18	Iso-.aplha.-cedren-15-al	69993-59-5	$C_{15}H_{22}O$	218.33	13.54	0.35
19	Hexahydrothunbergol	20489-83-2	$C_{20}H_{40}O$	296.53	16.54	0.11
Extraction time (min)						
Total non-oxygenated compounds (%)						60
Total oxygenated compounds (%)						59.82 38.15
Total identified (%)						97.97

MAHD, Microwave-assisted hydrodistillation; GC-MS, Gas chromatography-mass spectrometry; nd, not detected. [1]Essential oil compounds sorted by chemical families. [2]Abstract Service number. [3]Retention time.

1994). For each compound on the chromatogram, the percentage of peak area relative to the total peak areas from all compounds was determined and reported as relative amount of that compound.

Results and Discussion

The essential oil from the leaf of Pogostemon cablin, grown in Indonesia was extracted by microwave-assisted hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Nineteen compounds were identified representing approximately 97.97% of the oil (Table 1). It can be observed in Table 1 that patchouli essential oil is comprised of a mixture of chemical compounds, most of them in small quantities. Four new compounds were characterized from this patchouli essential oil; namely, 2(1H)-naphthalenone, octahydro-1-methyl-1-(2-propenyl)-, (1a,4ab,8aa)-; iso-.aplha.-cedren-15-al; 7-oxabicyclo[4.1.0]heptane, 1,3,3-trimethyl-2-(3-methyl-1,3-butadienyl)-, [1a,2b(Z),6a]- and hexahydrothunbergol.

The compounds present in higher quantities are the same in all patchouli essential oils: patchoulol, δ-guaiene, α-guaiene, α-patchoulene and β-caryophyllene. The major volatile compound of leaf were patchoulol (26.32%), δ-guaiene (14.69%), α-guaiene (12.18%), α-gurjunene (11.13%), seychellene (8.42%), viridiflorol (5.93%), β-caryophyllene (4.63%) and β-patchoulene (2.87%). The non-oxygenated compounds predominated with 59.82% followed by oxygenated compounds (38.15%). These compounds are the same as those found by Betts (1994) in the analysis of a commercial sample of patchouli essential oil (Rivendell, Bunbury, Western Australia). The commercial sample had 28.5% of patchoulol, 12.0% of δ-guaiene, 10.4% of α-guaiene, 6.9% of seychellene, 6.4% of α-patchoulene, 4.7% of caryophyllene and 1.7% of β-patchoulene.

Conclusion

The result revealed that essential oil obtained from the leaves of Pogostemon cablin growing in Indonesia extracted by microwave-assisted hydrodistillation contains nineteen compounds in various concentration. The major compound of leaves is patchoulol (26.32%). Since this species is widespread and a common species in the genus Pogostemon in Indonesia, elite varieties may be developed through plant breeding in order to increase the essential oil content and quality. Future studies will be aimed at defining the optimum operating conditions for the microwave-assisted hydrodistillation for the extraction of patchouli essential oil.

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