CHEMICAL COMPOSITION OF ESSENTIAL OIL EXTRACTED FROM LEAVES OF *VITEX NEGUNDO* LINN. FROM BINH THUAN PROVINCE BY HYDRODISTILLATION AND MICROWAVE HYDRODISTILLATION

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Abstract - Essential oils from fresh and dry leaves of *Vitex negundo* (HD-Fresh, HD-Dry) were obtained by traditional hydrodistillation (HD) and microwave-assisted hydrodistillation (MHD) (MHD-Fresh, MHD-Dry). The chemical constituents of essential oil of leaves are analyzed by GC/MS technique. The results indicate that the major compound of four essential oil contains the same dominant components β-caryophyllen (23.5%, 16.3%, 16.4% and 16.8%), eremophilene (18.9%, 15.1%, 14.4% and 14.2%), eucalyptol (16.2%, 16.3%, 13.6% and 19.6%), α-terpinyl acetate (10.8%, 7.6%, 9.2% and 8.8%), and sabinene (7.3%, 8.6%, 8.5% and 10.3%), respectively in oils obtained by MHD, HD from fresh leaves, MHD and HD from dry leaves. The total amount of sesquiterpenoid hydrocarbons (51.5% and 45.3%) is higher than monoterpenoids (44.8% and 43.5%) in essential oil obtained by MHD, respectively in oils from fresh and dry leaves. In contrast, the essential oil obtained by HD shows the greater concentration of monoterpenoids (45.3% and 53.6%) than sesquiterpenoids (44.3% and 41.0%), respectively in oils from fresh and dry leaves. By using MHD method, it is superior in terms of saving energy and extraction time although the total composition decreases with this method.

Key words - *Vitex negundo*; essential oil; microwave-assisted hydrodistillation; GC/MS; hydrodistillation.

1. Introduction

Essential oils are composed of a wide range of bioactive chemical compounds. They traditionally found application as flavour, fragrances and medicinal aroma. *Vitex negundo* Linn. belonging to Verbenaceae family is an important herb with a broad spectrum of pharmacological activities, medicinal properties and applications. Its essentional oil extract has been analyzed elsewhere [1].

All parts of the *Vitex negundo* are used as medicine, however, the leaves are specially considered to be the most potent for the isolation of medicinal constituents. It has been used for the treatment of eye-disease, inflammation, leucoderma, and toothache, skin-ulcers, in catarrhal fever, rheumatoid arthritis, gonorrhea, sinuses and bronchitis [2].

The main techniques to obtain essential oils from the medicinal herbs are hydrodistillation (HD), steam distillation, steam and water distillation, maceration, expression. Among these techniques, HD has been the most common method to extract the essential oils from plants. The HD method has several drawbacks such as long extraction time, high energy use and so on. Hence, in order to increase the extraction yield, save energy and time extraction, new approaches are improving. In recent years, the use of microwave-assisted hydrodistillation (MHD) method has been increasing, especially for extraction [3], [4]. By using microwave energy, the materials reach their boiling point rapidly, leading to short extraction or distillation time and saving energy.

Based on using *Vitex negundo* as flavor and medicinal products, the aim of this study is to compare and evaluate HD and MHD for their effectiveness in the extraction of essential oils leaves, and to determine and compare the composition of the essential oil obtained by HD and MHD.

2. Experiment

2.1. Plant materials

The fresh plants of *Vitex negundo* were collected in Binh Thuan province, Vietnam. One part of the healthy matured leaves of *V. negundo* is thoroughly washed with distilled water, dried by centrifuge, preserved in low temperature fridge of 5^0C and finally cut into small pieces of 3 mm before hydrodistillation. The second part is also thoroughly washed with distilled water, shade dried in dust free condition for 2 weeks and finally cut into small pieces of 3 mm before hydrodistillation. Moisture content of the sun-dried samples is measured by a moisture analyzer.

2.2. Hydrodistillation (HD)

The *Vitex negundo* leaves (300 g fresh sample versus 300 g dried sample) are placed in a 1L round bottom flask and connected to a Clevenger-type apparatus. The evaporation is condensed by a condenser combined with a chiller at 10°C. Hydrodistillation is completed for 2hs. after boiling. The *Vitex negundo* hydrodistillation oil is collected after water separation, stored in a culture tube.

2.3. Microwave-assisted hydrodistillation (MHD)

First MHD of 300 g fresh *Vitex negundo* leave sample is carried out without adding water in a 1L glass tube flask put in a special MHD equipment (Milestone ETHOS X, Italia). Second experiment used 300 g dried sample with adding 250 ml distilled water in the same glass tube flask and MHD equipment. Parameter setting is microwave energy of 1800W, running time of 20 mins, condensation temperature at 10°C. The *Vitex negundo* oil is collected using separation funnel and stored in culture tube.

2.4. GC/MS analysis

GC/MS data is obtained on the Gas Chromatography-Mass Spectrometry (GC/MS: SCION 456 equipped SQ mass spectrometer) using RXi5-ms (30 m×0.25 mm, film

thickness 0.25 µm). The mass range is 50 to 500 amu. Carrier gas is nitrogen at a linear flow rate of 1.5 ml/min; injector volume for all samples is 0.1µl. Temperature programming is from 50ºC to 280ºC. Column oven temperature is held isothermal at 50ºC for 3 minutes then heated at 35ºC/min to 100ºC, again it is heated at 7ºC/min to 220ºC, continue heat at 50C/min to 2800C and is held isothermal for 3 minutes. The total program time of the instrument is 34.57 min. The injector and detector temperatures are 270ºC and 280ºC respectively. The oil is injected neat with split injection mode having split ratio of 1:50. Quantitative results are mean data derived from GC analysis. The final confirmation of constituents is made by computer matching of the mass spectra of peaks with the Wiley and NIST libraries mass spectral databases. Relative amounts of individual components are based on GC peak areas [5].

3. Results and discussion

Sensory properties and yield of *Vitex negundo* leaf oils: The moisture content of all dried *Vitex negundo* leaves after sun drying is around 13.8 %. The essential oils from the *Vitex negundo* leaf obtained by (MHD) and classical hydrodistillation (HD) are compared in terms of yield and chemical composition. All the essential oils are pale yellow liquids with strong characteristic odor and dried over anhydrous Na_2SO_4. The HD dry leaves essential oil has strongest odor. The essential oils obtained by HD give a yield of 0.05 % and 0.35 % (w/w) on a dry weight basis from fresh and dry leaves, respectively. When extracted by MHD 0.04 % and 0.30 % (w/w) on a dry weight basis are obtained for fresh and dry leaves, respectively. Each of the extract is stored in a sealed glass bottle in a refrigerator until analysis. The data shows that MHD technique produces lower oil yield in comparison to HD.

Chemical analysis of *Vitex negundo* leaf oils

The chemical composition of the essential oils achieved from leaves *Vitex negundo* collected from two methods (microwave-assisted hydrodistillation and hydrodistillation) are represented together with the retention time in Table 1. The GC–MS analyses of four samples reveal the presence of a total of 30 components including monoterpenoids (44.78%, 43.50%, 45.25% and 53.62), sesquiterpenoids (51.50%, 44.40%, 43.46% and 40.48) and diterpenoids (3.72 %, 10.45%, 8.22% and 5.00) from MHD (fresh and dry leaves) and HD (fresh and dry leaves), respectively. This result shows the major component of essential oil obtained by MHD is sequiterpenes, but monoterpenoids is the main compounds in essential oil obtained by HD method.

From Table 1, it is clearly found that there are significant differences in the essential oils composition isolated by two methods (HD and MHD). The essential oil using MHD for dry leaves detects 30 compounds and then 22 compounds for dry and fresh leaves, while 27 and 29 compounds are detected in HD method for dry and fresh leaves, respectively.

According to results in current study, the major compounds are found to be *β*-caryophyllen (23.5%, 16.3 %, 16.4%, and 16.8%), eremophilene (18.9%, 15.1%, 14.4%, and 14.2%), eucalyptol (16.2%, 16.3%, 13.6% and 19.6%), α-terpinyl acetate (10.8%, 7.6%, 9.2% and 8.8%), and sabinene (7.3%, 8.6%, 8.5%, and 10.3%) in oils obtained by MHD, HD from fresh leaves, MHD and HD from dry leaves, respectively. The major compound in essential oil obtained by MHD from fresh leaves and HD from fresh leaves is *β*-caryophyllen, but eucalyptol is the major compound in essential oil obtained by HD from dry leaves. Moreover, *β*-caryophyllen is the highest (23.5%) in oils extract from fresh leaves by MHD method. It has shown anti-bacterial activities by similar chemical composition [1] and anti-inflammatory [6] and anesthetic [7] effects.

While the total number of compounds in fresh leaves essential oil achieved from MHD is less than that from HD and the oil yield is lower than HD method, the total number of compounds in dry leaves essential oil from MHD is more than from HD method. MHD method is important in terms of saving energy and extraction time (20 min compared to 120 min with HD method) and the essential oil with higher content of monoterpenes exhibits better antibacterial activities [8].

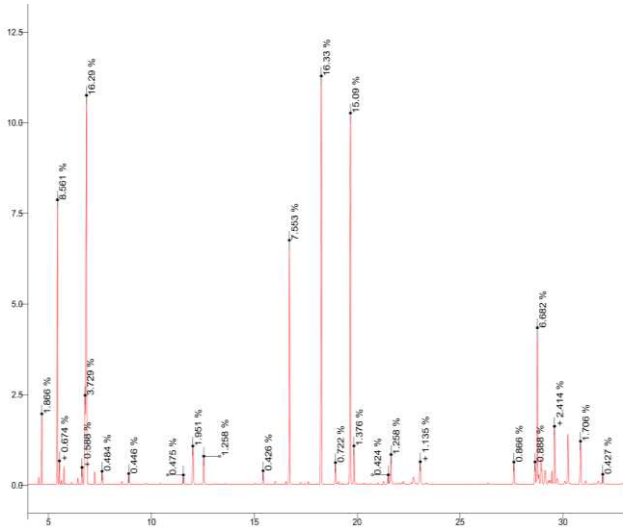

Figure 1. *Total ion chromatogram (obtained by GC-MS analysis) of the Vitex negundo from fresh leave essential oil extracted by HD method*

Table 1. *The retention times and chemical composition of essential oils of Vitex negundo*

No	RT*	Compound	%			
			MHD Fresh	HD Fresh	MHD Dry	HD Dry
1	4.68	3- carene	0.88 ±0.21	1.87 ±0.20	1.64 ±0.20	2.32 ±0.20
2	5.44	sabinene	7.34 ±0.89	8.56 ±0.75	8.53 ±0.91	10.34 ±0.85
3	5.53	(-)-β-pinene	-	0.67 ±0.20	0.70 ±0.29	0.84 ±0.31
4	5.75	*β*-myrcene	-	0.51 ±0.12	0.57 ±0.19	0.57 ±0.05
5	6.43	*α*-terpinene	-	-	0.35 ±0.11	0.52 ±0.17
6	6.79	*β*-phellandrene	3.74 ±0.37	3.73 ±0.32	3.88 ±0.43	4.33 ±0.35

7	6.86	eucalyptol	16.21± 0.56	16.29	13.57 ±0.44	19.57 ±0.63
8	7.25	*β*-ocimene	0.63 ±0.11	0.44 ±0.23	0.69 ±0.20	0.62 ±0.32
9	7.61	*γ*-terpinene	0.71 ±0.73	0.48 ±0.94	0.73 ±0.76	0.98 ±0.84
10	8.90	*β*-linalool	0.70 ±0.39	0.45 ±0.32	0.62 ±0.28	0.72 ±0.34
11	11.56	*δ*-terpineol	0.57 ±0.17	0.48 ±0.20	0.36 ±0.05	0.53 ±0.12
12	12.02	(-)-terpinen-4-ol	2.04 ±0.20	1.95 ±0.07	1.26 ±0.17	2.07 ±0.12
13	12.55	terpineol	1.16± 0.78	1.26 ±0.67	0.88 ±0.52	1.37 ±0.72
14	15.43	lavandulol acetate	-	0.43 ±0.03	0.46 ±0.10	-
15	16.73	*α*-terpinyl acetate	10.81 ±1.01	7.55 ±0.85	9.24 ±0.92	8.83 ±0.83
16	18.28	*β*-caryophyllen	23.50 ±0.86	16.33 ±0.55	16.42 ±0.34	16.79 ±0.65
17	18.95	*α*-caryophyllen	1.16 ±0.52	0.72 ±0.47	0.97 ±0.77	0.73 ±0.44
18	19.70	eremophilene	18.92 ±0.39	15.09 ±0.20	14.37 ±0.25	14.20 ±0.18
19	19.86	(+)-bicyclogemacrene	2.34 ±0.20	1.38 ±0.12	2.05 ±0.09	1.50 ±0.21
20	21.65	caryophyllene oxide	0.54 ±0.19	1.26 ±0.16	0.75 ±0.34	0.50 ±0.42
21	23.07	*α*-cadinol	0.55 ±0.42	1.14 ±0.54	1.24 ±0.39	0.90 ±0.57
22	27.62	cambrene	0.69 ±0.77	0.87 ±0.64	1.41 ±0.86	0.56 ±0.43
23	28.78	widdrol	4.50 ±0.20	6.68 ±0.89	8.59 ±0.75	5.86 ±0.38
24	28.94	geranyl-*α*-terpinene	0.66 ±0.23	1.11 ±0.20	1.73 ±0.15	0.88 ±0.26
25	29.14	*α*-guainene	-	0.88 ±0.03	0.91 ±0.12	0.50 ±0.03
26	29.47	epimanool	-	0.55 ±0.01	0.48 ±0.02	-
27	29.60	cis-3,14-clerodadien-13-ol	0.53 ±0.54	2.41 ±0.81	2.10 ±0.72	1.20 ±0.71
28	30.26	kaur-15-ene	1.85 ±0.98	1.97 ±1.20	4.33 ±1.01	2.37 ±1.00
29	30.86	phenanthrene	-	1.71 ±0.54	0.73 ±0.51	0.40 ±0.47
30	31.94	kolavelool	-	0.43 ±0.30	0.40 ±0.41	-
Monoterpenoids			44.78	44.66	43.50	53.62
Sesquiterpenoids			51.50	42.59	44.40	40.48
Diterpenoids			3.72	7.33	10.45	5.00
Total percentage			94.44	83.37	85.74	90.28
Yeild (%) (w/w)			0.04	0.05	0.3	0.35
Extraction time (mins)			20	120	20	120

*RT: Retention time

4. Conclusion

With MHD method, time extraction is significantly shorter than with HD method. MHD results in a reduced extraction time and a substantial energy saving compared to the conventional HD technique. After 20 minutes of MHD extraction, it is possible to collect almost all the existing essential oils of the *Vitex negundo* leaves.

However, the essential oils achieved from the two methods have a strong characteristic odor and the essential oil obtained by HD from dry leaves is the strongest. This probably causes headache when we smell it for a long time. In the future, the study on the composition contributing to this strong characteristic odor will continue.

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