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Full Length Research Paper

Study on the chemical composition and extraction technology optimization of essential oil from *Wedelia trilobata* (L.) Hitchc.

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The chemical composition of hydro-distilled oil from the ground aerial parts of *Wedelia trilobata* (L.) Hitchc. was analysed by gas chromatography/gas chromatography–mass spectrometry (GC/GC–MS). 18 compounds representing all the oils were identified. The main components were demonstrated to be α-phellandrene (28.84%), germacrene D (15.79%), D-limonene (14.22%), 1,5,5-trimethyl-6-methylene-cyclohexene (10.11%) and caryophyllene (9.08%). In addition, some pharmaceutical components such as β-pinene, D-limonene, germacrene D and phytol were discovered. Orthogonal methodology $L_9(3^4)$ were applied to optimize extraction technology and evaluate the extraction yield, and the effects of soaking time, extraction time and solid to liquid ratio on oil extraction efficiency were studied. Experimental results showed that extraction time had significant effect on the yield of oil, the influence order of different factors: extraction time > soaking time > solid to liquid ratio. The optimum operating parameters were determined as follows: soaking time, extraction time of 3 h and solid to liquid ratio of 1:10. Under the optimized conditions, the maximal yield of oil was enhanced to 0.1778%. Antioxidant activity of the oil was also assessed by the free radical scavenging, 2,2'-diphenyl-b-picrylhydrazyl (DPPH). The study offers theoretic basis for utilization of the medicinal herb *W. trilobata*.

Key words: *Wedelia trilobata*, essential oil, chemical composition, orthogonal methodology, extraction yield.

INTRODUCTION

The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of consumers in ingredients from natural sources and also because of the increasing concern about potentially harmful synthetic additives (Reische et al., 1998). In fact, there are many reports concerning the antimicrobial, antifungal and antioxidant radical-scavenging properties by spices and essential oils in some cases (Burt, 2004; Bamoniri et al., 2010). These findings will most likely trigger further studies of the chemical composition of the essential oil in the coming years. The genus *Wedelia* of the family *Asteraceae* is a flowering plant genera commonly called "creeping-oxeyes" and mostly distributed in the tropics and subtropics (Bottenberg et al., 1999).

The species *Wedelia trilobata* (L.) Hitchc is a creeping evergreen perennial herb, spreading widely and preventing regeneration of other species. It has a very wide ecological tolerance range and can tolerate inundation and high levels of salinity (Wu et al., 2010). The plant is native to the tropics of Central America and has naturalized in many wet tropical areas of the world. This invasive species could also be found in China

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intertidal estuarine zones; especially the Hainan island coast which has the richest wild sources. It has been historically used as traditional folk medicinal plant for the treatment of various ailments, which has been demonstrated to be an important and well-known traditional herbal: crushed leaves are used as a poultice; tea is given to alleviate symptoms of colds and flu. In South America, local people use it to treat hepatitis, inflammations, infections, fevers and clear the placenta after birth. It is also used for reproductive problems, amenorrhea and dysmenorrhea (Tsai et al., 2009; Govindappa et al., 2011; Meena et al., 2011). Moreover, several studies has already been conducted and it was reported that numerous potential bioactive molecules such as sesquiterpenes, diterpenes, triterpenes and lactones with antioxidant, anti-inflammatory, antimicrobial, hepato-protective activity, insecticidal activity and anticancer activity, have been isolated from various parts of the plant (Huang, 2006; Maldini et al., 2009; That et al., 2007; Wu and Zhang, 2008; Zhang et al., 2004; Brito et al., 2006; Taddei and Rosas-Romero, 1999).

The literature survey indicates that several reports are available regarding the chemical compositions and antimicrobial activity of essential oil (EO) from *W. trilobata* species (Koheil, 2000; Yang et al., 2010), but no attempt has been made previously to explore the chemical compositions of EO from *W. trilobata* collected in Hainan island and its extraction technology optimization. The present study was therefore designed to analyze the chemical composition and optimize extraction conditions of EO obtained by hydrodistillation of *W. trilobata*. Orthogonal methodology $L_9(3^4)$ were applied to evaluate the extraction yield, and the effects of soaking time, extraction time and solid to liquid ratio on oil extraction efficiency were also studied. Antioxidant activity of the oil was assessed by the free radical scavenging, 2,2'-diphenyl-b-picrylhydrazyl (DPPH). The findings from this work may add to the overall value of the medicinal potential of the plant.

MATERIALS AND METHODS

Solvents and chemicals

These include absolute ethanol (≥99.7% purity, Tianjin) and anhydrous sodium sulphate (≥96.0% purity, Guangzhou). All other reagents were of analytical grade.

Plant materials

W. trilobata plants were collected from the lakeside of Hainan University. The voucher specimen of the plant was deposited in the herbarium of College of Landscape and Horticulture, Hainan University, China.

Extraction of the essential oil

The plant aerial parts were submitted for 3 h to water-distillation using an all-glass Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored in an amber vial at +4°C until tested and analyzed.

Gas chromatography (GC) analysis

Oil obtained from aerial parts of *W. trilobata* was analyzed using Hewlett Packard 6890 GC equipped with a flame-ionization-detector (FID) and HP-FFAP ms capillary column (30 m × 0.25 mm, film thickness 0.25 µM). GC oven temperature was kept at 60°C for 3 min initially, and then raised at the rate of 3°C/min to 250°C. Helium was the carrier gas, at a flow rate of 1 ml/min. Diluted samples 1/1000 in n-pentane, v/v) of 1.0 µL were injected manually and in the splitless mode. Peaks area percents were used for obtaining quantitative data.

Gas chromatography-mass spectrometry (GC-MS) analysis

The analysis of the essential oil was performed under the same conditions with GC, using a Hewlett Packard 6890 gas chromatograph equipped with a Hewlett Packard 5973 mass selective detector in the electron impact mode (70 eV). Identification of the components was based on comparisons of their relative retention times and mass spectra with those obtained from standards and/or the NIST98 and Wiley275 library data.

Free radical-scavenging activity

The radical scavenging ability (RSA) of the essential oil of *W. trilobata* was estimated by using DPPH method adapted from Sharififar et al. (2009). Thus, an aliquot of EO solution (1 ml) was added to 3 ml of ethanolic DPPH (60 µM). The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark and absorbance was measured at 517 nm. The free radical scavenging activity was calculated as follows:

$$\%RSA = [(A_{blank} - A_{sample} / A_{blank}] \times 100\%$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

RESULTS AND DISCUSSION

GC-MS results

The analysis of the oil from the ground aerial parts of *W. trilobata* was simultaneously performed using gas chromatography-mass spectrometry (GC–MS). The detected components of the essential oil of the plant and its relative percentages according to their relative retention indices (RI) are given in Table 1. 18 components were identified representing all the oils. α-Phellandrene (28.84%), germacrene D (15.79%), D-limonene (14.22%) and 1,5,5-trimethyl-6-methylene-cyclohexene (10.11%) were the main constituents and totally comprised 58.85% of the oil. In addition, high amounts of monoterpenes were found to compose a major chemotype of the EO, such as β-pinene, bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl), α-phellandrene, D-limonene, cyclohexene, 4-methylene-1-(1-methylethyl), 3-carene

Table 1. Chemical composition of the essential oil from twig of *Wedelia trilobata* L.

No.	R.I.[a]	MF[b]	Component	Composition (%)
1	3.43	$C_{10}H_{16}$	β-Pinene	2.79
2	3.60	$C_{10}H_{16}$	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)	0.58
3	4.36	$C_{10}H_{16}$	α-Phellandrene	28.84
4	4.94	$C_{10}H_{16}$	D-Limonene	14.22
5	5.67	$C_{10}H_{16}$	Cyclohexene, 4-methylene-1-(1-methylethyl)	1.96
6	6.07	$C_{10}H_{16}$	3-Carene	1.67
7	6.40	$C_{10}H_{14}$	Benzene, tert-butyl-	2.71
8	15.23	$C_{15}H_{24}$	Caryophyllene	9.08
9	17.13	$C_{15}H_{24}$	α-Caryophyllene	6.04
10	18.24	$C_{15}H_{24}$	Germacrene D	15.79
11	18.83	$C_{10}H_{16}$	1,5,5-Trimethyl-6-methylene-cyclohexene	10.11
12	26.64	$C_{12}H_{20}O_2$	Fenchyl acetate	0.34
13	28.00	$C_{14}H_{20}O_2$	Ethanone, 1-(1,3a,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)-	0.56
14	31.57	$C_{10}H_{12}O$	3-(Cyclohex-1-enyl)-furan	0.53
15	34.32	$C_{20}H_{40}O$	Phytol	3.70
16	36.89	$C_{16}H_{32}O_2$	*n*-Hexadecanoic acid	0.29
17	37.50	$C_{20}H_{28}O_2$	1-Phenanthrenecarboxylicacid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R-(1.α.,4a.β.,10a.α.)]	0.23
18	37.81	$C_{15}H_{24}$	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	0.55

[a]R.I., Relative retention indices on HP-5 ms column in reference to n-alkanes; [b]MF, molecular formula of the component.

and 1,5,5-trimethyl-6-methylene-cyclohexene, attaining 2.79, 0.58, 28.84, 14.22, 1.96, 1.67 and 10.11%, independently.

The reports on the chemical composition of the essential oils of *W. trilobata* are few in the literature. Some of the compounds never reported previously in the oil of same species, were found in considerable quantity in this plant, such as germacrene D (15.79%) (Koheil, 2000; Yang et al., 2010). It is noteworthy to point out that the constituents of the plants essential oils are normally influenced by several factors such as geographical, climatic, seasonal and experimental conditions. Simultaneously, some pharmaceutical components were discovered. Research has indicated that β-pinene has intense antimicrobial potential, which could significantly inhibit the growth and cell viability of potential infectious endocarditis-causing Gram positive bacteria (Leite et al., 2007). D-Limonene is considered to have fairly low toxicity and is successful marketed to manage the symptoms of gastroesophageal reflux disease and chronic heartburn as an extremely promising natural remedy (Sun, 2007). Germacrene D has been reported to have insecticidal activity against mosquitoes (Kiran and Devi, 2007), as well as repellent properties against aphids (Bruce et al., 2005) and ticks (Birkett et al., 2008). As a cholesterol lowering agent, phytol can be administered to patients with disease conditions related to increased levels of cholesterol or triglycerides such as type II diabetes, obesity or other patients in risk of cardiovascular diseases due to elevated cholesterol levels. On the other hand, phytol was proposed to maintain normal levels of serum cholesterol of healthy individuals (Olofsson et al., 2011).

Optimization of the experimental conditions

The extraction technology of EO obtained by hydrodistillation of *W. trilobata* was optimized by orthogonal experiments $L_9(3^4)$, in which the extraction yield, soaking time, extraction time and solid to liquid ratio were considered as orthogonal layout factors for the oil extraction. As shown in Table 1, the experimental condition of the highest yield of EO (0.1776%) were namely the fourth group, whose levels of factors involved soaking time (2 h), extraction time (3 h) and solid to liquid ratio (1 g: 10 ml), respectively. Fifth group showed the lowest yield of EO with 0.1404%, with corresponding factors and levels including soaking time (3 h), extraction time (4 h) and solid to liquid ratio (1 g:6 ml), respectively. According to the largest donating rule, as far as each investigated factor, the largest value which affects the extraction yield of oil should be the selected value. In view of orthogonal analysis, statistical software MS Excel software was used to calculate values of K and R. Analysis

Table 2. Extraction of essential oils from *Wedelia trilobata (L.)* Hitchc. $L_9(3^4)$.

Level	Factor			Yield of EO (%)
	A	B	C	
	Soaking time (h)	Extraction time h)	Solid to liquid ratio (g /ml)	
1	1	3	1:6	0.1606
2	1	4	1:8	0.1565
3	1	5	1:10	0.1539
4	2	3	1:10	0.1776
5	2	4	1:6	0.1404
6	2	5	1:8	0.1534
7	3	3	1:8	0.1754
8	3	4	1:10	0.1565
9	3	5	1:6	0.1741
K_1	0.4710	0.5136	0.4751	
K_2	0.4714	0.4534	0.4853	Y=1.4484
K_3	0.5060	0.4814	0.4880	
R	0.0350	0.0602	0.0129	
$\underline{K_1}$	0.1570	0.1712	0.1584	
$\underline{K_2}$	0.1571	0.1511	0.1618	$\underline{Y}$=0.1609
$\underline{K_3}$	0.1687	0.1605	0.1627	
$\underline{R}$	0.0117	0.0201	0.0043	

K: The total amount of each yield of essential oil at every level for a certain factor.
R: The extreme difference equals the maximum minus the minimum for a certain factor.

of variance was also performed by ANOVA procedure. The results exhibit the influence order of different factors on the oil yield: extraction time > soaking time > solid to liquid ratio, according to magnitude order of R value (maximum difference) (Table 2). According to R value among four factors and result of analysis of variance, extraction time was found to be markedly correlative with extraction yield of oil. The optimum technological operating parameters were determined as following, soaking time of 3 h, extraction time of 3 h, and solid to liquid ratio of 1:10. Under the optimized conditions, the maximal yield of oil was enhanced to 0.1778%. This indicated that the extraction yield of EO could be enhanced using a combination of those factors at different levels in the preparation process.

Antioxidant activity

All the EO samples from the orthogonal experiments were subjected to radical scavenging ability assay by using DPPH method. All samples, however, proved to be devoid of significant activity at 500 µg/ml in the bioassays used.

Conclusion

In this study, 18 components identified by GC-MS representing all of the oils and monoterpenes were found to compose a major chemotype of the chemical composition. Additionally, some pharmaceutical com- ponents were discovered, such as β-pinene, D-limonene, germacrene D and phytol. Meanwhile, orthogonal methodology $L_9(3^4)$ were applied to evaluate the extraction yield of EO obtained by hydrodistillation of *W. trilobata*. Optimal operating parameters were determined as following, soaking time of 3 h, extraction time of 3 h, and solid to liquid ratio of 1:10. Under the optimized conditions, the maximal yield of oil was enhanced to 0.1778%.

Moreover, the ethanol extract of *W. trilobata* (L.) Hitchc. had been described to be antioxidant of DPPH radical assay, and this also prompted us to investigate the antioxidant activity of our EO against DPPH radical. However, in contrast to the previous report, none of the compounds investigated showed any significant activity when tested at an initial concentration of 500 µg/ml in the bioassays used. These results therefore encourage complementary and more in-depth studies on the chemical composition of the plant extracts with the aim of separation and structure elucidation of their active components and evaluation of biological activity of each compound separately.

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