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Composition, Antimicrobial and Antioxidant Activities of the Essential Oil of *Murraya exotica* from Hainan of China

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The objective of the works reported here was to make a thorough inquiry of composition, antimicrobial and antioxidant activities of the essential oil of *Murraya exotica* from Hainan Province, China. The essential oil of *Murraya exotica* were extracted by hydrodistillation and analyzed by GC and GC/MS. The relative content of each peak were detected by the normalization method of peak area. Thrity four components of *Murraya exotica* essential oil were identified which accounted for 97.76 % of total essential oil and the content of caryophyllene was up to 45.51 %, particularly. The results of the antioxidant activity test showed that the volatile oil had significant free radical scavenging capacity (IC_{50} = 0.1511). The antibacterial test results showed that the volatile oil of *Murraya exotica* had broad-spectrum antimicrobial activity on all bacteria other than *Pseudomonas aeruginosa*. Of which, the inhibition to *Bacillus subtilis* was about four times of the positive control and had a certain antifungal capabilities. Volatile oil of *Murraya exotica* had a certain antioxidant and antibacterial activity. The results can provide reference for further research on the chemical components of *Murraya exotica* volatile oil as well as for the exploration and utilization of it.

Key Words: *Murraya exotica*, GC-GC/MS, Antioxidant, Antimicrobial.

INTRODUCTION

The genus of *Murraya* L. belonging to the family Rutaceae of the Rutales is a kind of ornamental bush or small macrophanerophytes which contains 12 species and is widely distributed in southern Asia and northeastern Australia[1]. *Murraya exotica* (*M. exotica*) is the model of the family and the leaves of this plant called curry leaf are known as a kind of condiment which has been used popularly in Indian cooking[2]. It is reported that *M. exotica* has also played an important role in folk medicine in eastern Asia and Australia, where it is used to cure indigestion, vomit and relieve nausea[3]. According to Li *et al.*[4], essential oil of *M. exotica* show definite toxicity against *S. zeamais*, *T. castaneum* adults and *Calletotrichum musae*. However, the study on its anti-pathogenic bacteria was absolute a gap. For this reason, this work reported here was urgently needed for searching natural germifuga and antioxidant.

EXPERIMENTAL

The fresh leaves of *M. exotica* were collected from Hainan province, China. The leaves were washed gently and dried in the sky. The dried samples were powdered with a blender and passed through an 80-mesh sieve and placed in bags and immediately stored in a desicator. Standard solution of the *n*-alkanes C_8-C_{20} and C_{21}-C_{40} mixture, DPPH and Trolox were all purchased from the Sigma Chemical Co, USA. The test bacterial strain used in this study were *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* (freeze-dried powder) obtained from the Beijing Lianchuang Biotechnologies Institute. *Fusarium graminearum*, *Botrytis cinerea*, *Exerohilum turcicum* and *Lecannosticta acicola* were provided by the microbiology laboratory in Zhejiang Agriculture and Forestry University. The purity of the standards were all above 99.5 %. The main instruments used in this work were volatile oil extraction device and GC-MS analysis of the oils were carried out on a Trace 2000 instrument (Finnigan MS Co., USA), equipped with FID and DB-WAX elastic quartz capillary-tube chromatographic column (30 m × 0. 25 mm, 0. 25 mm). Infinite M 200 Enzyme Microplate Reader (Switzerland Tecan) was used in determination of antioxidation activity.

Extraction of essential oil: Dried leaves powder (*ca.* 50 g) were swollen with 500 mL distilled water in a standard extractor for 12 h, then were extracted by hydrodistillation in a Clevenger-type apparatus for 4 h procedure described in the

Chinese Pharmacopoeia (2005)[5]. Collect the steam liquid and extract it with ether, then recycle the ether and the oil was obtained after dehydration with anhydrous sodium sulfate. The essential oil samples were stored at 4 ºC in dark before analysis. The yield of the essential oil was 1.32 % (v/w of dried sample).

GC-MS analysis: A FID and DB-WAX elastic quartz capillary-tube chromatographic column (30 m × 0.25 mm i.d., 0.25 μm): Agilent J & W scientific, Folsom, CA, USA) was used to separate the essential oil components. The initial temperature of the oven was held at 45 ºC for 3 min, increased to 100 ºC with the rate of 10 ºC min^{-1}, then increased to 170 ºC at 4 ºC min^{-1}, of which the final temperature was increased to 240 ºC at the rate of 10 ºC min^{-1} and held for 7 min. The ion source and injector temperatures were 200 and 250 ºC, respectively. The spectrometers were operated in electron-impact (EI) mode; the ionization energy was 70 eV with a mass range of 40-300 m z^{-1} and a scan interval of 0.5 s. The detector potential was 350 V and the solvent delay time was 3 min. Helium was used as carrier gas at a constant flow rate of 0.8 mL min^{-1} flow-rate.

Calculation of retention indices: This quasi-linear equation proposed by Van den Dool and Kratz[6] was used to calculate retention indices in this work:

$$IT = 100 \times n + 100 \times \frac{(tx - tn)}{(tn + 1 - tn)}$$

where IT is the temperature-programmed retention index of the interesting compound and tn, tn + 1 and tx are the retention times of the two standard n-alkanes containing n and n + 1 carbons and the compound of interest, respectively.

Determination of antioxidation activity: The free radical scavenging activity of essential oils were determined based on their ability to react with the DPPH (2,2'-diphenyl-1-picrylhydrazyl) free radical with micro-plate quantification method[7]. 14.00 mg of Trolox was dissolved in ethanol and diluted to 50 mL, then diluted to 0.056, 0.112, 0.168, 0.196, 0.224, 0.224 and 0.280 mg mL^{-1}, respectively. Essential oil samples were dissolved to 0.01, 0.02, 0.04, 0.06, 0.08 and 0.10 mg mL^{-1} by ethanol. 15.56 mg DPPH was dissolved in ethanol and diluted to 50 mL (0.3112 mg mL^{-1}). 100 μL essential oil samples of all the concentrations in which 200 μL DPPH were added to micro-plate incubated at 25 ºC for 0.5 h, then the ultraviolet absorption (As) was determined at 517 nm. The antioxidant activity (%) of radical-scavengers was calculated as followed:

$$\text{Scavenging } (\%) = \frac{1 - (A_s - A_r)}{A_0} \times 100\ \%$$

where A_s is the absorbance of 50 μL sample mixed with 200 μL DPPH solution, while A_r is the absorbance of blank sample without DPPH and A_0 is the absorbance of 50 μL absolute ethyl alcohol mixed with 200 μL DPPH solution, parallel tests were carried out 3 times in every experiments at 517 nm and IC_{50} values were reported as mean value of triplicates.

Determination of antimicrobial activity: By oxford cup method[8], inhibition zone were used to estimate the antibacterial ability of the essential oil[8]. 50 μL of essential oil was dissolved diethyl ether and diluted to 2 mL. Four species of fungi were spread on the potato culture medium and four species of bacteria (10^7-10^8 cfu L^{-1}) were spread on the agar culture medium. Then the oxford cup was put on the middle of the plates which contained 200 μL oil samples (10 μL mL^{-1}) as well as positive and negative controls. After the plates were incubated at 37 ºC for 24 h, the Inhibition zone was observed. The positive controls used gentamicin (80 U mL^{-1}) and the negative controls used diethyl ether for each strain assay and the diameters of the inhibition zones were measured in mm. All the assays were performed in triplicate.

RESULTS AND DISCUSSION

GC-MS Analysis: GC-MS total ion current chromatogram of the essential oil from the *Murraya exotica* is given in Fig. 1. The relative content of each peak was detected by the normalization method of peak area. By searching in NIST2008 standard mass spectrometry library and calculating with the normalization method of peak area, 34 compounds were identified (Table-1).

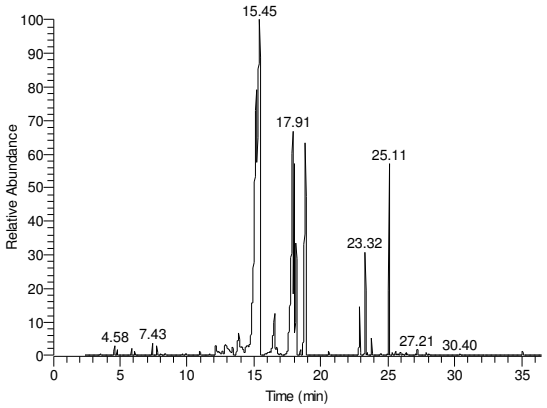

Fig. 1. GC-MS total ion current chromatogram of the essential oil from the *Murraya exotica*

As listed in Table-1, 48 peaks are separated in the essential oil of *M. exotica*, of which 34 compounds are identified and most of that belonged to sesquiterpenoids, such as carypohyllene, cedrene (15.12 %) and 1-(1,5-dimethyl-4-hexenyl)-4-methyl-benzene (11.12 %) and the relative content of carypohyllene was up to 45.51 %.

Antioxidant activity analysis: DPPH method was used to determine the free radical scavenging capacity of volatile oil from *M. exotica*. Trolox in different concentrations were determined and the results were calculated by regression analysis. According to the regression equation, IC_{50} were obtained and listed in Table-2.

Table-2 shows that there is favourable linear relationship between the free radical scavenging capacity of essential oil and its concentration and its IC_{50} (0.1511) is less then Trolox (0.1879).

Antibacterial activity analysis: Oxford cup method was used to analyze the antibacterial activity of the essential oil of *M. exotica* with gentamycin as positive control and ether as negative control. According to the inhibition zone, the antibacterial abilities were evaluated and the results could be seen in Table-3.

TABLE-1
CHEMICAL COMPOSITION OF VOLATILE OIL IN THE *Murraya exotica*

No.	Name of components	RT (min)	m.f.	RI	Relative concent (%)
1	1R-α-Pinene	4.58	$C_{10}H_{16}$	1018	0.29
2	R(-)3,7-Dimethyl-1,6-octadiene	4.80	$C_{10}H_{18}$	1031	0.10
3	Eucalyptol	7.75	$C_{10}H_{18}O$	1201	0.17
4	D-Limonene	7.43	$C_{10}H_{16}$	1222	0.23
5	Benzaldehyde	13.40	C_7H_6O	1338	0.19
6	α-Cubebene	12.14	$C_{15}H_{24}$	1454	0.43
7	Copaene	12.30	$C_{15}H_{24}$	1462	0.22
8	Nonanal	10.97	$C_{15}H_{24}$	1475	0.08
9	β-Elemene	12.65	$C_{15}H_{24}$	1479	0.08
10	Octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1*H*-cyclopenta[1,3] cyclopropa[1,2]benzene	13.79	$C_{15}H_{24}$	1535	2.01
11	2,6-Dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene	14.41	$C_{15}H_{24}$	1566	0.61
12	Caryophyllene	15.20	$C_{15}H_{24}$	1604	45.51
13	(1S-Endo)-2-methyl-3-methylene-2-(4-methyl-3-pentenyl)-bicyclo[2.2.1] heptane	15.96	$C_{15}H_{24}$	1642	0.07
14	(E)-7,11-Dimethyl-3-methylene-1,6,10-Dodecatriene	16.46	$C_{15}H_{24}$	1667	0.82
15	(Z)- 7,11-Dimethyl-3-methylene-1,6,10-Dodecatriene	16.55	$C_{15}H_{24}$	1671	1.71
16	α-Caryophyllene	16.70	$C_{15}H_{24}$	1678	0.33
17	Cedrene	17.91	$C_{15}H_{24}$	1738	15.12
18	2,4α,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-1*H*-benzocyclo-heptene	18.01	$C_{15}H_{24}$	1743	5.35
19	1-Ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane	18.15	$C_{15}H_{24}$	1750	4.19
20	3,3,6,6,9,9-Hexamethyl-Z,Z,E-(1-methylethylidene)-cyclohexanetetracyclo [6.1.0.0(2,4).0(5,7)]nonane	18.51	$C_{15}H_{24}$	1767	0.23
21	1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene	18.83	$C_{15}H_{22}$	1783	11.12
22	(Z)- 5-(1-Propenyl)-1,3-benzodioxole	20.58	$C_{10}H_{10}O_2$	1943	0.10
23	Caryophyllene oxide	22.91	$C_{15}H_{24}O$	1984	1.31
24	Z-α-*Trans*-bergamotol	23.41	$C_{15}H_{24}O$	2009	0.09
25	3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	23.79	$C_{15}H_{26}O$	2028	0.31
26	(E,E)-12-Methyl-1,5,9,11-tridecatetraene	23.86	$C_{14}H_{22}$	2056	0.05
27	(-)-Spathulenol	25.11	$C_{15}H_{24}O$	2093	4.44
28	*Trans*-Z-α-bisabolene epoxide	25.34	$C_{15}H_{24}O$	2104	0.05
29	1,2-Dimethoxy-4-(2-propenyl)-benzene	23.32	$C_{11}H_{14}$	2113	2.05
30	*cis*-Lanceol	25.79	$C_{15}H_{24}O$	2126	0.05
31	Z-α-*Trans*-bergamotol	25.95	$C_{15}H_{24}O$	2134	0.06
32	Decahydro-1,1,7-trimethyl-4-methylene-1*H*-cycloprop[e]azulen-7-ol	26.37	$C_{15}H_{24}O$	2155	0.05
33	Octahydro-1,4,9,9-tetramethyl-1*H*-3α,7-Methanoazulene	27.21	$C_{15}H_{26}$	2196	0.10
34	*n*-Hexadecanoic acid	35.07	$C_{16}H_{32}O_2$	2555	0.15
Total					97.67

TABLE-2
ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OIL FROM THE *MURRAYA EXOTICA* GIVEN AS IC_{50} AND REGRESSION EQUATIONS

Samples	Regression equation	R^2	IC_{50}
M. exotica	y = 0.1309x + 0.0093	0.9919	0.1511
Trolox	y = 1.844x + 0.1535	0.9901	0.1879

As listed in Table-3, the essential oil of *M. exotica* displayed abroad spectrum antibacterial activity to the bacteria apart from *Pseudomonas aeruginosa*. Particularly, *Bacillus subtilis* was highly controlled, as it was almost 4 times of the antibacterial activity of positive control, which corresponded to *Escherichia coli* and *Staphylococcus aureus*, respectively. Oppositely, the fungus were not inhibited greatly by the essential oil, as the antibacterial activity of *Lecannosticta acicola* was nearly equivalent to the positive control and the antibacterial activity of *Botrytis cinerea* was obviously less than the positive control.

TABLE-3
INHIBITION ZONE OF ESSENTIAL OIL IN *M. exotica* In MICROBIOLOGICAL ASSAYS BY CUP OXFORD METHOD

Microorganism	Inhibition zone (mm)		
	M. exotica	Positive control	Negative control
Escherichia coli	39.60	29.73	+
Staphylococcus aureus	12.07	14.51	+
Bacillus subtilis	43.13	12.73	+
Pseudomonas aeruginosa	–	13.65	+
Botrytis cinerea	12.00	13.03	+
Fusarium graminearum	–	15.80	+
Exerohilum turcicum	–	14.60	+
Lecannosticta acicola	11.86	13.36	+

Inhibition zone (mm) = average from three determinations, + = inhibition zone has not been seen, ++ = full of microorganism in the cups.

Conclusion

Here, we had identified 34 essential chemical compounds in *M. exotica*, of which caryophyllene was the highest content

and accounted for almost half of the essential oil. One of the most common content of plant essential oil is caryophyllene, a kind of bicyclic sesquiterpenes and had always been used as spice, however, its antioxidant activity had not been comprehensively illustrated yet. Donatelli and Buffon[9] had demonstrated that β-caryophyllene had anaesthetic effects using procaine as control. But further researches aiming at its antimicrobial had never been performed. Moreover, in the current studies, we hadn't found such a high content of caryophyllene in other aromatic plants as that in *M. exotica*. Hence, we proposed that caryophyllene should play a key role in the antioxidant activity and antimicrobial effect of *M. exotica*.

In this study, we showed that *M. exotica* greatly inhibited *Bacillus subtilis* and its inhibitory capability was about 4 times higher than the positive control. Until now, natural *antibacillus subtilis* chemical medicine hasn't been found while those harmful traditional pesticide residues, such as bordeaux mixture and carbendazim require continuous use, which might cause serious pesticide residue in woods and soil and result in the drug resistance of germs. Therefore, the anitfungal activity of *M. exotica* volatile oil has wide application value.

M. exotica is one of the most common shrubs in Hainan Province with abundant resource, has not been efficiently utilized yet. Researches on the antioxidant activity of volatile oil in Indian *M. exotica* , which is much more worthy than being exploited, have already drawn certain attentions. The results of this study have provided basis for further usage of Hainan *M. exotica* resources.

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