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Chemical Composition, Antioxidant, and Cytotoxic Properties of the Essential Oil from the Leaves of *Murraya alata* Drake

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Abstract: The essential oil from the leaves of *Murraya alata* Drake was extracted by hydrodistillation and analyzed by GC-MS. A total of 70 components were identified, accounting for 93.7% of the oil composition. The major compound is α-cadinol (18.9%), followed by α-muurolol (10.0%), *trans*-prenyl limonene (7.7%), germacra-4(15),5,10(14)-trien-1-α-ol (6.0%), and cedryl acetate (5.4%). *M. alata* oil was characterized by a high content of sesquiterpenes (91.2%), with cadinene-type being the most representative compounds. The structural diversity of sesquiterpenes as well as their chemotaxonomic significance was discussed. In addition, *M. alata* oil showed moderate *in vitro* antioxidant and cytotoxic effects.

Keywords: *Murraya alata*; essential oil; GC-MS; antioxidant activity; cytotoxic activity.

1. Introduction

Among sorts of natural products, the essential oils, mostly derived from medicinal herbs and spices, have gained extensive attention and wide usages. It has been widely accepted that these plant-origin aromatic substances are the most potential candidates for developing natural antioxidants for food preservation and flavoring agents. Various spice essential oils have been reported to possess strong antioxidant properties [1-4]. Meanwhile, they could avoid the toxicity problems or carcinogenic effects on health arising from the long term usage of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone [5, 6]. Some other biological activities of essential oils, such as cytotoxic, anti-inflammation, and antibacterial properties, have also attracted particular attention of the phytochemists and flavor specialists [7, 8].

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Murraya is a small genus of the plant family Rutaceae, mainly distributed in tropical and sub-tropical regions of Southeast Asia, China, and northeast Australia [9]. There are nine species and one variety distributed in China and most of which are well known for their aromatic and medicinal characters. Among this genus plants, *M. koenigii*, also known as the "Indian curry tree", is the most extensively studied plant [10-14]. In addition, chemists and flavor specialists have also paid attention to the essential oils of *M. exotica* [15, 16] and *M. paniculata* [17, 18], due to their aromatic characteristics and traditionally medicinal applications. As for the other *Murraya* species, there are few reports for their volatile oils [19, 20].

M. alata is a shrub, distributed in thickets in sandy areas or near sea level. From the plant taxonomic point of view, it is the closest species to the medical plants of *M. exotica* and *M. paniculata* [9]. However, our current knowledge and understanding on its essential oil are limited [21-23]. Thus, the aim of the present study is to carry out a more detailed analysis of the essential oil of *M. alata*, as well as its antioxidant and cytotoxic potentials.

2. Materials and Methods

2.1. Plant material

The leaves of *M. alata* were collected from Sanya, Hainan Province, China in August, 2010. A voucher specimen of the plant (No. MA201008) is deposited in the Modern Research Center for Traditional Chinese Medicine, Peking University, Beijing, China.

2.2. Solvents and chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, *β*-carotene, ascorbic acid, and tocopherol were purchased from the Sigma-Aldrich Chemical Company (USA). Analytical grade methanol, ethanol, and dimethylsulfoxide (DMSO) were procured from Beijing Chemical Works (Beijing, China).

2.3. Isolation of essential oils

The air-dried plant material (100 g) was subjected to hydro-distillation for 6 h by using a Clevenger-type apparatus. The obtained essential oil was dried over sodium sulphate and the amount was measured. It was then stored in sealed vials at 4-6 ^{o}C until GC-MS analysis and the bioactivity screening.

2.4. GC-MS analysis

The GC-MS analysis was performed on a Thermo/Finnigan Trace GC-MS system equipped with a HP-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm, Crossbond 5% phenyl-95% dimethylpolysiloxane, Sigma-Aldrich). Analytical conditions: injector temperature at 220 ºC; injection volume of 0.5 μL with a split ratio of 1:50; the temperature was programmed from 80 ºC, at a rate of 10 ºC/min to 130 ºC, kept for 2 min, and then rising to 190 ºC at a rate of 3 ºC/min, and to 250 ºC at a rate of 8 ºC/min. Helium was used as the carrier gas at a flow rate of 1 mL/min. MS operating parameters: electron impact ionization at 70 eV with mass scan range of 40–500 *m*/*z* and transfer line temperature at 240 ºC. Identification of the constituents was based on comparison of the mass spectral pattern and retention indice (RI) with those given in the literature [24]. The retention indice (RI) was calculated in relation to the series of *n*-alkanes [25].

2.5. Gas chromatography (GC-FID)

Quantitative analysis of the chemical constituents was performed by flame ionization detector (FID), using Agilent 6890N Network GC System. The column and the analytical conditions are same as those of GC–MS. Quantification of each constituent was determined by the normalization of the peak areas detected by FID.

2.6. DPPH radical assay

The hydrogen atom or electron donation abilities of the essential oil and the positive control were determined from bleaching of the purple colored methanol solution of DPPH. The scavenging activities of the plant essential oil on DPPH radical were estimated as per Sarker, S.D. [26] with minor modifications. Briefly, stock solutions (1 mg/mL each) of the essential oil and the antioxidant ascorbic acid were prepared in methanol and DMSO, respectively. Dilutions are made to obtain various concentrations ranging from 2 to 1000 μg/mL and 1 mL of which was added to 1 mL of freshly prepared DPPH radical methanol solution (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed to stand for 30 min in the dark at room temperature. Absorbances of the resulting solutions, as well as a blank (without sample) and a positive control (ascorbic acid), were measured at 517 nm by a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Each sample assay was carried out in triplicate and the data were presented as a mean of three values. The values were calculated as a percentage using the following formula:

$$I\% = [(A' - A) / A'] \times 100$$

Where A′ is the absorbance value of the blank and A is the absorbance value of the test sample.

2.7. β-Carotene/linoleic acid assay

In this assay, the antioxidant capacity of the samples and the positive control was evaluated in a *β*-carotene/linoleic acid system, which could undergo a rapid color fading reaction in the absence of an antioxidant, according to the method described by Hsouna *et al* [27], with minor modifications. Briefly, 1 mL of a solution of *β*-carotene in chloroform (0.5 mg/mL) was added into a flash containing 25 mg of linoleic acid and 200 mg of Tween 20. The chloroform was completely removed by flushing with nitrogen to give a residue, which was then fully dissolved in 50 mL of distilled water to produce a stable emulsion.

50 μL of the samples prepared at different concentrations as described above were dispensed into test tubes, and 100 μL of reaction mixture was added. The emulsion system was incubated for 3 h at 50 °C. The same procedure was repeated with BHT as a positive control, and a blank as a negative control. After the incubation period, the absorbances of the resulting solutions were measured at 470 nm. Each sample assay was carried out in triplicate. The antioxidant activity in this model was calculated as a percentage (A %) using the following formula:

$$A\% = [1 - (A_0 - A_t) / (A'_0 - A'_t)] \times 100$$

Where A_0 and A'_0 are the absorbance values of the test samples and the blank, respectively, measured at 0 h, and A_t and A'_t of the test samples and the blank, respectively, measured after 3 h.

2.8. Cytotoxic activity

Human liver cancer cells (HepG2, BEL7402, and BEL7403) and human uterine cervical carcinoma cell (Hela) were obtained from the Cell Bank of the Chinese Academy of Medical Sciences. The cytotoxicity activity of the *M. alata* oil was conducted by using MTT method [28]. Briefly, the cells were cultured in RPMI1640 medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (Hyclone). Cancer cells were seeded into 96-well plates at a density of 1.0×10^4 cells per well. After incubation with different drugs for a designated period of time (24 h), the medium was removed and the MTT (0.5 mg/mL in culture medium) was added and incubated for 4 h at 37 ºC. Then, the medium was removed, and the cells were dissolved in DMSO for 10 min. Absorbance was recorded at 550 nm using a microplate reader. Each experiment was performed in triplicate. The cell viability was expressed as the percentage of viable cells relative to untreated control cells. Doxorubicin was used as a positive control.

3. Results and Discussion

3.1. Chemical analysis of the essential oil

The leaves of *M. alata* afforded a greenish colored essential oil with a percentage yield of 0.6% (w/w) (calculated on a dry weight basis). The GC-MS analysis of the essential oil led to the identification of 70 constituents representing 93.7% of the total oil. The most prominent compound is α-cadinol (18.9%), followed by α-muurolol (10.0%), *trans*-prenyl limonene (7.7%), germacra-4(15),5,10(14)-trien-1-α-ol (6.0%), and cedryl acetate (5.4%). The quantitative and qualitative results are presented in detail in Table 1, and the structural types of compounds was given in Supporting Information (Table S1).

Table 1. Chemical components (%) identified in the essential oil of *M. alata.*

Constituents	Ex. RI [a]	Lit. RI [b]	Area [%]
Allyl hexanoate	1074	1079	1.0
Tridecane	1300	1300	t [c]
δ-Elemene	1337	1338	0.3
α-Cubebene	1350	1350	0.2
Silphiperfol-4,7(14)-diene	1360	1360	0.7
2-Methyl-undecanal	1368	1368	t
Iso-dihydro carveol acetate	1327	1327	t
α-Ylangene	1375	1375	0.5
β-Panasinsene	1381	1381	t
β-Bourbonene	1392	1392	t
β-Elemene	1397	1390	1.1
α-Santalene	1412	1417	0.2
β-Funebrene	1415	1414	t
(*E*)-caryophyllene	1419	1419	t
cis-Thujopsene	1429	1431	1.6
γ-Elemene	1434	1434	t
β-Gurjunene	1436	1433	1.2
α-Guaiene	1439	1439	t
4,9-Guaiadiene	1446	1444	1.9
cis-Muurola-3,5-diene	1449	1450	1.2
α-Neoclovene	1453	1454	1.2
trans-Prenyl limonene	1462	1459	7.7
α-Acoradiene	1466	1466	t
cis-Muurola-4(14),5-diene	1467	1466	t
Cumacrene	1474	1472	t
γ-Muurolene	1478	1479	0.1
α-Amorphene	1483	1484	t
Germacrene D	1486	1485	t
δ-Selinene	1491	1492	t
γ-Amorphene	1495	1495	t
Bicyclogermacrene	1501	1500	1.5
δ-Amorphene	1513	1512	3.0

Constituents	Ex. RI [a]	Lit. RI [b]	Area [%]
γ-Cadinene	1518	1513	1.1
δ-Cadinene	1521	1523	t
Zonarene	1524	1529	t
trans-Cadina-1,4-diene	1532	1534	1.0
α-Calacorene	1544	1545	2.1
Elenol	1551	1549	t
β-Calacorene	1559	1565	t
(*E*)-nerolidol	1562	1563	t
β-Vetivenene	1568	1565	t
1*α*,10*α*-Epoxy-amorph-4-ene	1572	1527	t
Spathulenol	1582	1578	0.2
Caryophyllene oxide	1586	1583	2.1
β-Copaen-4-*α*-ol	1590	1590	2.1
Rosifoliol	1598	1600	t
Guaiol	1600	1600	1.3
9,11-Epoxy-guaia-3,10(14)-diene	1606	1601	0.5
Humulene epoxide II	1613	1608	1.4
1,10-Di-epi-cubenol	1618	1619	0.2
cis-Cadin-4-en-7-ol	1632	1636	0.3
Caryophylla-4(12),8(13)-dien-5*α*/*β*-ol	1641	1640	0.4
α-Muurolol	1645	1646	10.0
α-Cadinol	1652	1654	18.9
Neo-intermedeol	1661	1660	t
Bulnesol	1667	1671	t
Epi-Zizanone	1669	1670	0.1
14-Hydroxy-9-epi-(*E*)-caryophyllene	1671	1669	2.1
Mustakone	1673	1677	1.2
Germacra-4(15),5,10(14)-trien-1-α-ol	1686	1686	6.0
Cyperotundone	1693	1695	t
Eudesm-7(11)-en-4-ol	1700	1700	4.4
Nootkatol	1709	1715	4.3
Cedryl acetate	1769	1767	5.4
Guaiazulene	1773	1779	1.5
β-Vetivone	1823	1822	2.2
(5*E*,9*E*)-farnesyl acetone	1908	1913	t
Sandara copimara-8(14),15-diene	1962	1968	0.5
n-Hexadecanoic acid	1973	1973	0.7
Eicosane	2300	2300	0.3
***Total identified* (%)**			**93.7**

[a] Experimental RI, [b] Literature RI, [c] t: trace (<0.05%)

The chemical constitution of *M. alata* oil showed a structural diversity with four chemotypes existed (monoterpenes, sesquiterpenes, diterpenes, and others) (Table S1), among which, the sesquiterpenes occupied the largest proportion of the oil (91.2% of the total). Even in the

sesquiterpene fraction, a high chemotypical difference was observed. A total of 27 kinds of sesquiterpenoid skeletons were detected, and the cadinene-type occupied the predominance (Table S1). The diverse sesquiterpenoid profile concerning the contents and compound numbers of each sesquiterpenoid skeleton is illustrated in Figure 1. As shown in this histogram, cadinene sesquiterpenes presented an absolutely dominant position in the oil composition either in content or in compound numbers. Within this group, as well as in the total oil of *M. alata* leaves, α-cadinol (18.9%) was found to be the most prominent compound. However, this major component found in the present case was undetectable in a previous report [21], in which α-gurjunene (29.6%), guaiol (7.7%), (*E*)-caryophyllene (7.6%), and β-eudesmol (6.2%) were the main constituents. These data revealed that the chemical profile of the essential oil of *M. alata* has the individual difference, which could be related with the different harvest time or ecophysiological conditions, as well as the storage of the medicinal materials.

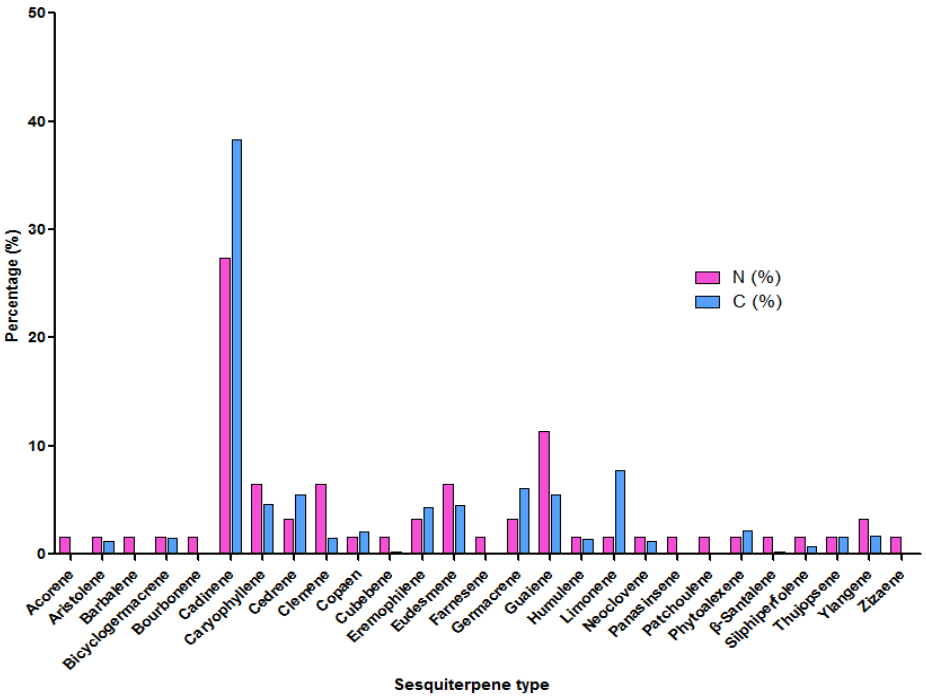

Figure 1. The numbers (N) and the contents (C) of sesquiterpenes with various chemotypes from *M. alata* oil.

From the chemotaxonomic point of view, *Murraya* has been divided into two distinct sections, namely sects. *Murraya* and *Bergera*, on the basis of the distribution of the alkaloids and coumarins [9]. Furthermore, the different chemical composition of volatile oil also supported such a division, for sesquiterpenes rich in plant oils of the sect. *Murraya* and monoterpenes rich in the sect. *Bergera*, respectively [21]. Our current results agreed well with the above proposed chemotaxonomy-based classification by disclosing the predominant sesquiterpenoids existed in the oil of *M. alata*, which belongs to the sect. *Murraya*.

3.2. Antioxidant and cytotoxicity activities

The essential oil of *M. koenigii* has been reported to be cytotoxic against three cancer cell lines [14], and the *M. paniculata* essential oil exhibited strong antioxidant activity in a complementary antioxidant assay [18]. Considering the close relationship of *M. alata* with the two above species, the

antioxidant and cytotoxic activities were screened to explore the potential biological properties of *M. alata* essential oil.

The antioxidant evaluation of essential oil with a single test mode could not give comprehensive results, due to the chemical complexity of oil. Thus, in the present study, the *in vitro* antioxidant activity of the *M. alata* oil was evaluated by combined use of two extensively employed assays, namely the DPPH free radical scavenging method and the *β*-carotene/linoleic acid model. The DPPH free radical scavenging method could reflect the ability of hydrogen-donating in scavenging the DPPH free radical, and the *β*-carotene/linoleic acid model was used to describe the inhibitory effect of volatile organic compounds arising from linoleic acid oxidation.

The results of DPPH radical scavenging capacity and the antioxidant activity of preventing the linoleic acid oxidation by the oil from *M. alata*, along with their respective positive controls, are presented in Table 2. The essential oil exhibited a moderate DPPH radical scavenging capability with an IC_{50} value of 57.2 ± 6.9 μg/mL, and a better ability for preventing the linoleic acid oxidation with an IC_{50} value of 12.7 ± 2.3 μg/mL. Research [2] reported that sesquiterpene hydrocarbons and their derivatives possessed very low antioxidant effect, whereas the present results showed that the sesquiterpene-rich *M. alata* oil had a moderate protective action in both radical-mediated oxidation (DPPH free radical scavenging essay) and lipid oxidation (*β*-carotene/linoleic acid essay) systems. This difference could be related to a high level of cadinene-type sesquiterpenes occurring in this tested *M. alata* oil, which has been reported to possess undeniable antioxidant properties [29, 30]. Thus, the cadinene-type sesquiterpenes might be the major constituents responsible for the antioxidant activity of *M. alata* oil.

The cytotoxic effect of *M. alata* oil was evaluated using three human liver cancer cell lines, HepG2, BEL7402, and BEL7403, and one adenocarcinoma cell line Hela by MTT assay. The oil was found to be active against all these four tested cancer cell lines, with IC_{50} values of 33.9–51.6 μg/ml (Table 2). The cytotoxicity is likely due to the high concentrations of sesquiterpene hydrocarbons contained in the oil of *M. alata* [31].

Table 2. The antioxidant and cytotoxic activities of *M. alata* oil.

IC_{50} (μg/mL)	Antioxidant activity		Cell line			
	DPPH radical assay	*β*-Carotene/linoleic acid assay	HepG2	BEL7402	BEL7403	Hela
M. alata oil	57.6 ± 2.9	12.7 ± 2.3	40.4 ± 8.4	51.6 ± 3.7	40.4 ± 7.2	33.9 ± 5.6
Ascorbic acid	6.1 ± 1.2	-	-	-	-	-
BHT	-	2.9 ± 0.5	-	-	-	
Doxorubicin	-	-	7.6 ± 0.7	5.8 ± 1.0	0.5 ± 0.2	6.3 ± 0.9

In the present study, the chemical composition of the essential oil of the title plant is described in detail. The oil was found to contain a high content of sesquiterpenoids, which further supports the chemical division of *Murraya* into two sections, *Murraya* and *Bergera* according to their contents of sesquiterpenes and monoterpenes [21]. In addition, the biological study on *M. alata* oil for its antioxidant and cytotoxic activities also provided scientific data valuable for the further investigation and development of *M. alata* and its related species for the perfuming, antioxidant and anti-cancer agents.

Supporting Information

Supporting Information accompanied with this paper on http://www.acgpubs.org/RNP

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