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GC-MS Analysis of Petroleum Ether Extracts in Resource Plant of *Mussaenda esquirolli* Lévl. Leaves

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Mussaenda esquirolli Lévl., belonging to the family Rubiaceae, is an important resource plant in China with medicinal, healthy, landscaping and ornamental value. In the present study, both no decolorization and decolorization petroleum ether extracts in leaves of *Mussaenda esquirolli* Lévl., were subjected to GC-MS analysis, respectively. The results showed that the major chemical constituents are phytol (37.49%, 42.86%), squalene (5.59%, 47.15%), linolenic acid (9.79%) and β-sitosterol acetate (15.49%), all of which have high use value. The study provided the medicinal and health function for *Mussaenda esquirolli* Lévl. on the basis of the chemical compositions.

Keywords: Resource plant, *Mussaenda esquirolli* Lévl., Gas chromatography-mass spectrometry, Phytol, Squalene.

INTRODUCTION

Mussaenda is a paleotropical genus of 132 species[1], with 30 species occurring in China[2]. Species in *Mussaenda* are characterized by having enlarged petaloid calyx lobes, valvate-reduplicate aestivation of the corolla lobes and indehiscent, berry-like fruits and the woody, scandent or liana habit[3]. At present, *Mussaenda* (Rubiaceae) from China were studied rarely. The research contents of *Mussaenda* (Rubiaceae) were about made to heathy tea for clearing away heat, species identification[3,4] and tissue culture[5]. There are less studies on the extraction component analysis and function research of bioactive constituent from *Mussaenda* (Rubiaceae). Only some bioactive constituents of *Mussaenda frondosa* Linn were identified with the aid of GC-MS technique[6].

Mussaenda esquirolli Lévl. (Fig. 1) belongs to *Mus-saenda*, Rubiaceae, Gentianales in botany classification. *Mussaenda esquirolli* Lévl. is an important resource plant in China with ornamental, medicinal, healthy, landscaping value, *etc.*[7]. The current study was carried out to GC-MS analysis of the active phytochemical constituents by petroleum ether in the leaves of *Mussaenda esquirolli* Lévl. The objective is to provide the medicinal and health function for *Mussaenda esquirolli* Lévl. on the basis of the chemical compositions.

EXPERIMENTAL

Mussaenda esquirolli Lévl. was collected in Anhui Qingliang Mountain National Nature Reserve, which was identified and authenticated by Prof. Hui-Chong Zhang, College of Life and Environment Sciences, Huangshan University, Huangshan, Anhui, China.

Fig. 1. Photographic images of *Mussaenda esquirolli* Lévl.

Sample preparation: The fresh leaves of *Mussaenda esquirolli* Lévl. were obtained in June, 2012. The leaves were washed thoroughly with distilled water, dried in the thermostat at 60 °C and then pulverized to powder.

Petroleum ether extraction: *Mussaenda esquirolli* Lévl. leaf powder (2.5 g) was extracted with petroleum ether (125 mL) in Soxhlet extractor at 90 °C for 2 h to get no decolorization extraction. This extraction process was repeated once, followed

by decolorization with activated carbon to get decolorization extraction. The two extract samples were concentrated under reduced pressure in a rotary evaporator at 45 °C respectively and stored in a refrigerator at 4 °C until to use.

Gas chromatography-mass spectroscopy: The no decolorization and decolorization petroleum ether extraction were subjected to GC-MS analysis (Agilent 6890N GC and 5973 inert MSD) using a pulsed pressure injection of 1 mL onto a HP-5 MS capillary column (30 m × 250 μm × 0.25 μm) with electron ionization mode (EI). The initial temperature was programmed from 60 to 290 °C (at 5 °C /min) and maintained at 290 °C for 20 min and then to 325 °C. Helium (99.999 %) was used as carrier gas at a constant flow of 1 mL/min. The injection volume was 0.5 μL and the split ratio was 10:1. The ionization mass spectroscopic analysis was done with 70 eV and the ion-source temperature was 230 °C.

Identification of components: The identification of compounds was based on the comparisons of their mass spectra with National Institute Standard and Technology (NIST) Library 2008 WILEY8, FAME[8].

RESULTS AND DISCUSSION

GC-MS analysis of the no decolorization petroleum ether extract: GC-MS chromatogram of the no decolorization petro-leum ether extract of *Mussaenda esquirolli* Lévl. (Fig. 2) showed many peaks indicating the presence of many phyto-chemical constituents. Comparison of the mass spectra of the constituents with the NIST library, the thirty-five phyto-constituents were characterized and identified (Table-1), accounting for 85.9 % of the no decolorization petroleum ether extract. The no decolorization petroleum ether extract mainly comprised of alcohols accounted for 39.07 % (phytol accounted for 37.49 %), ester compounds accounted for 18.15 % (β-sitosterol acetate accounted for 15.49 %), carboxy-lic acid compounds accounted for 10.27 % (linolenic acid accounted for 9.79 %), olefin(e) compounds accounted for 6.60 % (squalane accounted for 5.59 %), alkane compounds accounted for 6.23 % and heterocyclic compounds accounted for 4.42 %.

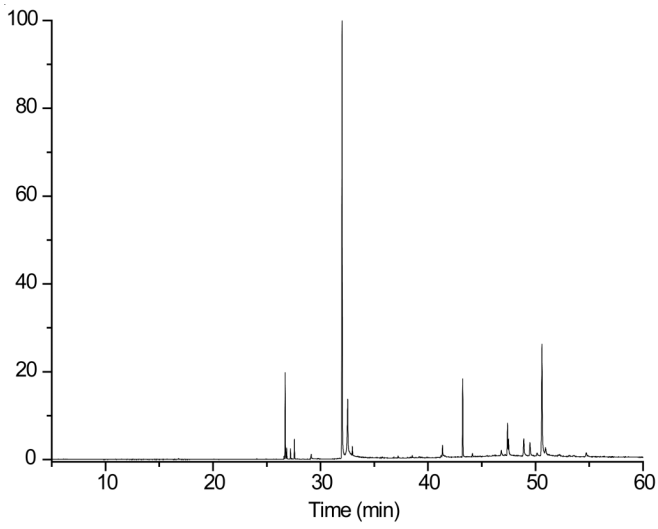

Fig. 2. GC-MS chromatogram of the no decolorization petroleum ether extract of the leaf of *Mussaenda esquirolli* Lévl.

GC-MS analysis of the decolorization petroleum ether extract: GC-MS chromatogram of the decolorization petro-leum ether extract of *Mussaenda esquirolli* Lévl. (Fig. 3) showed 6 peaks. Comparison of the mass spectra of the consti-tuents with the NIST library, the four phytoconstituents were characterized and identified (Table 2), accounting for 97.75 % of the decolorization petroleum ether extract. Compared with Table 1, with the activated carbon decolorization, phytol concen-tration increased to 42.86 % and squalene content swelled to 47.15 %.

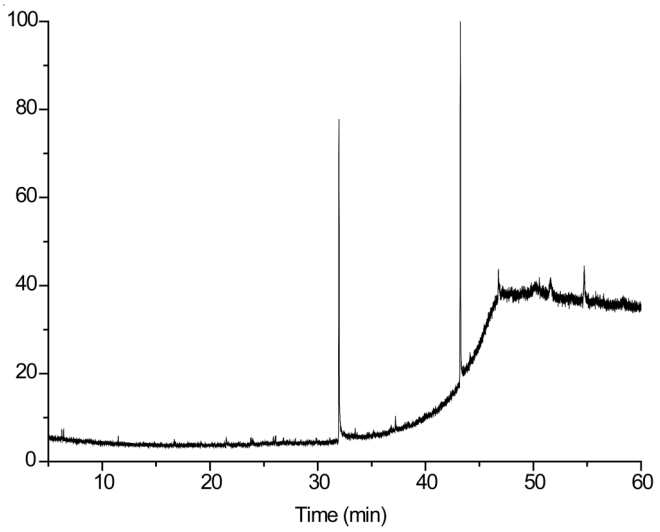

Fig. 3. GC-MS chromatogram of the decolorization petroleum ether extract of the leaves of *Mussaenda esquirolli* Lévl.

By GC-MS analysis of the no decolorization and de-colorization petroleum ether extracts, there were many bioactive substances, such as phytol, squalene, linolenic acid, β-sitosterol acetate, linolenic acid ethyl ester, palmitic acid, pinane *etc*.. The presence of such a variety of phytochemicals may be attributed to the medicinal function and health care function of *Mussaenda esquirolli* Lévl.. Especially, there were four phytocomponents accounted for a higher proportion, which were linolenic acid, phytol, squalene and β-sitosterol acetate.

Linolenic acid: Linolenic acid is unsaturated essential fatty acid which cannot be synthesized in the human body and is a major component of human tissues and cells. Linolenic acid can promote energy production, lower blood fat and cholesterol, defer senescence, anti-allergic and inhibit the development and metastasis of cancer[9], so linolenic acid have important significance to the health of human body. Linolenic acid was rich in *Mussaenda esquirolli* Lévl., which may contri-bute to some health functions of *Mussaenda esquirolli* Lévl..

Phytol: Phytol, the side chain of chlorophyll, is called leaf alcohol for short. It belongs to fatty alcohol and is the raw material to synthesize vitamin K and vitamin E, which could prevent teratogenesis[10]. Phytol is not only an important source of energy in the body metabolism, but also can be used as signal molecules with special biological functions in glucose and lipid metabolism[11]. Phytol can significantly reduce the fatty acids in the blood plasma, increase free carnitine levels

TABLE-1
PHYTOCOMPONENTS IDENTIFIED IN THE NO DECOLORIZATION PETROLEUM ETHER EXTRACT OF THE LEAVES OF *Mussaenda esquirolli* Lévl.

No.	RT (min)	Phytoconstituents	m.w.	m.f.	Peak area (%)
1	2.82	Methylbenzene	92	C_7H_8	0.11
2	2.90	1,3-Two methyl cyclohexane	112	C_8H_{16}	0.08
3	4.04	*p*-Xylene	106	C_8H_{10}	0.08
4	16.79	2-Methyl pyrrolidone	81	C_5H_7N	0.07
5	26.72	Pinane	138	$C_{10}H_{18}$	5.32
6	26.85	2-Methyl-2-cyclohexyl butane	154	$C_{11}H_{22}$	0.91
7	27.22	9-Eighteen carbyne	250	$C_{18}H_{34}$	0.69
8	27.35	Diisobutyl phthalate	278	$C_{16}H_{22}O_4$	0.07
9	29.14	Palmitic acid	256	$C_{16}H_{32}O_2$	0.48
10	32.01	Phytol	297	$C_{20}H_{40}O$	37.49
11	32.53	Linolenic acid	278	$C_{18}H_{30}O_2$	9.79
12	32.84	Ethyl linoleate	308	$C_{20}H_{36}O_2$	0.57
13	35.26	2-Methyl-5-(1-methyl vinyl)-2-cyclohexene-1-ketone	150	$C_{10}H_{14}O$	0.27
14	37.22	2,2'-Methylene *bis*-4-methyl-6- tert-butyl phenol	340	$C_{23}H_{32}O_2$	0.16
15	38.38	Tetracosane	339	$C_{24}H_{50}$	0.09
16	38.53	Glyceryl fatty esters	331	$C_{19}H_{38}O_4$	0.27
17	39.18	Phthalic acid mono-2-ethylhexyl ester	278	$C_{16}H_{22}O_4$	0.18
18	41.22	Isopropyl linoleate	322	$C_{21}H_{38}O_2$	0.20
19	41.35	Linolenic acid ethyl ester	306	$C_{20}H_{34}O_2$	1.24
20	41.50	3,5-Dichloro-2-pyridone	164	$C_5H_3Cl_2NO$	0.13
21	41.61	Dimethyl hexadecanedioate	314	$C_{18}H_{34}O_4$	0.13
22	43.23	Squalane	411	$C_{30}H_{50}$	5.59
23	44.13	1-Nonadecene	266	$C_{19}H_{38}$	0.35
24	46.03	Aminocarb	208	$C_{11}H_{16}N_2O_2$	0.14
25	46.82	Tetracosanol	355	$C_{24}H_{50}O$	0.91
26	47.17	Cholesterol	387	$C_{27}H_{46}O$	0.19
27	47.39	Methyl-6-formyl-3-pyridinecarboxylate	165	$C_8H_7NO_3$	2.73
28	47.49	9-(Methylaminomethyl)anthracene	221	$C_{16}H_{15}N$	1.67
29	50.15	1-Heptacosanol	397	$C_{27}H_{56}O$	0.48
30	50.60	β-Sitosterol acetate	457	$C_{31}H_{52}O_2$	15.49
31	50.94	5-Bromo-2-fluorocinnamic acid	245	$BrC_6H_3(F)CH=CHCO_2H$	1.69
32	52.24	Pyridate	379	$C_{19}H_{23}ClN_2O_2S$	0.17
33	53.15	2-Amine-6-methoxy purin	165	$C_6H_7N_5O$	0.12
34	53.57	Benalaxyl	325	$C_{20}H_{23}NO_3$	0.11
35	54.72	β-humulene	204	$C_{15}H_{24}$	0.66

TABLE-2
PHYTOCOMPONENTS IDENTIFIED IN THE DECOLORIZATION PETROLEUM ETHER EXTRACT OF THE LEAVES OF *Mussaenda esquirolli* Lévl.

No.	RT (min)	Phytoconstituents	m.w.	m.f.	Peak Area (%)
1	2.73	2,5-Dimethyl hexane	114	C_8H_{18}	3.65
2	2.90	*trans*-1,3-Dimethyl cyclohexane	112	C_8H_{16}	4.09
3	31.97	Phytol	297	$C_{20}H_{40}O$	42.86
4	43.23	Squalene	411	$C_{30}H_{50}$	47.15

in liver and promote fatty acid β-oxidation. Therefore, phytol has some effects on the treatment of obesity and lipid me-tabolism disorders induced by insulin sensitivity[12,13].

Squalene: Unique medicinal value of squalene has been in the extensive and indepth attention. Squalene can amelio-rate immune system and improve the body's stress ability and immunity as an antioxidant[14]. In marine fish oil products, squalene is only identified to control tumors and widely used for medicine in cancer patients.

Many species of plants contain squalene, but the content is not high. Only in a few species, there are higher content of squalene. The experimental materials, *Mussaenda esquirolli* Lévl. can be considered as resource plant to extract squalene.

β-Sitosterol acetate: Recently, lowering the cholesterol level through dieting such as functional foods has been attracted great attention. If the phytosterol or phytostanol are sufficient in the daily diet, the gastrointestinal assimilation of cholesterol can effectively reduce and thus it can lower serum cholesterol concentration[15]. β-sitosterol acetate belongs to the plant sterols and it has some functions beneficial to health such as lowering blood lipid and cholesterol[16], antioxidant activity[17] and antitumor effects[18]. Therefore, *Mussaenda*

esquirolli Lévl. can be considered as resources to develop commercial food ingredients and health food or beverage based on plant sterols to lower cholesterol.

Conclusion

In the present study, many phytoconstituents, belonging to essential fatty acids esters, alcohols, ethers, *etc*., have been identified from petroleum ether extract of the leaves of *Mussaenda esquirolli* Lévl. by GC-MS analysis. It could be concluded that *Mussaenda esquirolli* Lévl. contains various bioactive compounds. Linolenic acid, phytol, squalene and β-sitosterol acetate which were present in *Mussaenda esquirolli* Lévl. may be responsible for the medicinal and health care activity. Consequently, *Mussaenda esquirolli* Lévl. is recommended as important plant resources with great phytopharmaceutical and health function. Isolation and bioactivity study of individual phytochemical constituents will be presented in the further work.

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