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Original Research Article

Chromatographic-mass spectrometric analysis of ethanol extract of *Maesa perlaria* var *formosana*

Jyh-Ferng Yang[1], Ming-Tsai Liang[1], Cheng-Hong Yang[2], Zi-jie Gao[1], Yuh-Wern Wu[1]and Li-Yeh Chuang[1]

[1]Department of Chemical Engineering and Institute of Biotechnology and Chemical Engineering, I-Shou University, [2]Department of Electronic Engineering, National Kaohsiung University of Applied Sciences, Kaohsiung, 807, Taiwan

For correspondence: **Email: ywwu@isu.edu.tw, chuang@isu.edu.tw; **Tel:** +886-07-657-7711; **Fax:** +886-07-657-8945*

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Abstract

Purpose: *This study analyzes the chemical composition of ethanol root extracts of Maesa perlaria var. formosana by gas chromatography-mass spectrometry (GC-MS).*
Methods: *The dried root of Maesa perlaria var. formosana was extracted with 95 % ethanol for composition analysis under the following optimum GC-MS conditions: 250 °C inlet temperature, 250 °C MSD detector temperature, and GC oven temperature programmed as follows: initial temperature held at 70 °C for 15 min, then increased at a rate of 2.5 °C/min and held at 170 °C for 15 min; then raised at a rate of 2 °C/min and kept at 180 °C for 20 min; then raised at 2 °C/min and kept at 250 °C for 20 min. Finally, it was raised at 3 °C/min and kept at 280 °C for 15 min.*
Results: *A total of 59 chemical compounds were identified, representing 88.82 % of the composition of the ethanol extracts. The three major components, include 2,4-di-tert-butylphenol (16.76 %), stigmasterol (15.86 %) and campesterol (7.33 %)*
Conclusion: *The results show that a total of 59 components were identified in the ethanol extract of Maesa perlaria var. formosana. The major component, 2,4-Di-tert-butylphenol, exhibits various biological activities.*

Keywords: *Maesa perlaria var. formosana, 2,4-Di-tert-butylphenol, Stigmasterol, Campesterol, Gas chromatography-mass spectrometry*

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INTRODUCTION

Plants have been long been known to be rich source of biologically active substances with low side effects, high stability, and low toxicity. Medicines derived from natural herbs have made a great contribution to human health. *Maesa perlaria* var. *formosana*, is a common shrub indigenous to Taiwan, often found growing along the roadside. The plant of *Maesa perlaria* var. *formosana* is smooth, and stands about 1.0 - 3.5 m tall with elliptic or oval leaves. Its flowers are white or purplish-white, wide and bell-shaped, measuring about 2.5 ~ 3.0 mm in length. Its fruits are globose, purple berries, measuring 3.5 ~ 4.0 mm. It is distributed widely in Taiwan, China and Japan.

Maesa perlaria has been used as a folk medicine to relieve internal heat or fever and to reduce swelling, improve spleen and stomach function, and to heal stomach problems, headaches and low back pain [1]. Gas chromatography mass spectroscopy (GC-MS) has been widely used to analyze chemical compositions from natural products for the identification and quantification

purposes. The chemical compositions of solvent plant extracts can be determined by interpreting mass spectra based on matching the peak distribution against records in the NIST MS database [2]. To the best of our knowledge, the chemical composition of *Maesa perlaria* has not yet been reported. This study seeks to analyze and characterize the chemical constituents of the ethanol extract of *Maesa perlaria* by GC-MS. The aim is to identify the bioactive phytochemical compounds of *Maesa perlaria* var. *formosana*, an herbal medicine indigenous to Taiwan [3-7].

EXPERIMENTAL

Instruments

Experiments were conducted using an Agilent 7890B gas chromatography instrument, combined with an Agilent-5977A mass spectrometer equipped with electron ionization (EI) and quadrupole analyzer, and an Agilent Chem Station data system. GC separation was performed on a 30 m HP-5 ms Ultra Inert capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 µm (Agilent 19091S-433UI, USA).

Materials and chemicals

Maesa perlaria var. *formosana* was purchased from a local folk medicine dealer in Kaohsiung and verified by Department of Traditional Chinese Medicine at Kaohsiung Medical University by Dr Hsueh-Wei Chang. A voucher specimen (no. isu10237005M) was deposited at Department of Chemical Engineering, I-Shou University. All organic solvents were of analytical-reagent grade and purchased from Merck.

Sample preparation

The dried herb (roots of *Maesa perlaria* var. *formosana*) was extracted with 95 % ethanol overnight by shaking in an incubator set at 200 rpm and 28 °C. The extraction procedure was repeated twice and then filtered using Whatman filter paper No. 1 to remove insoluble debris. After filtration, the ethanol extracts were dried by vacuum dryer at 40 °C. The dried extracts were stored at 4 °C until analysis.

Analysis of phytochemical composition of the extract

Qualitative analysis was performed using an Agilent system consisting of a model 7890B gas chromatographer, a model 5977A mass selective detector (MSD, electron energy, 70 eV). The carrier gas was helium (99.99 %) with a flow rate of 0.8 mL/min. The injector and detector temperatures were set at 250 °C. Spectra were obtained over a scanning range of 50 to 550 amu at 2 scans/s. The GC program proceeded as follows: the initial oven temperature was held at 70 °C for 15 min and then increased at 2.5 °C/min to 170 °C where it was held for 15 min; it was then raised at 2 °C/min and kept at 180 °C for 20 min, then raised at 2 °C/min and kept at to 250 °C for 20 min, then finally raised at 3 °C/min and kept at 280°C for 15 min. The extract (0.06 g) was dissolved in 1 mL of ethyl acetate and vortex-mixed for 3 min, and 0.8 µL of the extract solution was injected with a split mode (0.1: 1). Interpretation of the mass spectrum was made by comparing their peak distribution against the database of the National Institute Standard and Technology (NIST MS 14.0, Gaithersburg, MD, USA). Relative percentages of the chemical compositions were calculated based on the GC peak areas without using correction factors.

RESULTS

Figure 1 shows the total ion chromatograms (TIC) of each component. The constituents were identified by comparing their mass spectra against the NIST MS 14.0 database with matches greater than or equal to 90 %. As listed in Table 1, 59 different components were identified from ethanol extracts, accounting for 88.82 % of the total area. Among the 59 different compounds, three major components (Figure 2) of the ethanol extract were 2,4-Di-tert-butylphenol (16.76 %), stigmasterol (15.86 %), and campesterol (7.33 %). The minor components were endo-borneol (5.23 %), decanedioic acid, bis(2-ethylhexyl) ester (4.53 %), (E)-2-tetradecene (3.34 %), ɣ-Sitosterol (3.25 %), 1-Octadecene (2.94 %) and 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (2.19 %). Other components constituted less than 2 % of the total yield.

DISCUSSION

Phytochemicals are natural bioactive compounds widely distributed in herbal medicines. In the present study, the preliminary phytochemical screening of the ethanol extracts from the roots of *Maesa perlaria* reveals that the plant contained appreciable amounts of bioactive constituents. The results show that 2,4-Di-tert-butylphenol, stigmasterol, and campesterol are the major components in the extracts. In many reports, plant extracts containing 2,4-Di-tert-butylphenol were shown to possess antioxidant and neuronal protective effects [8-10].

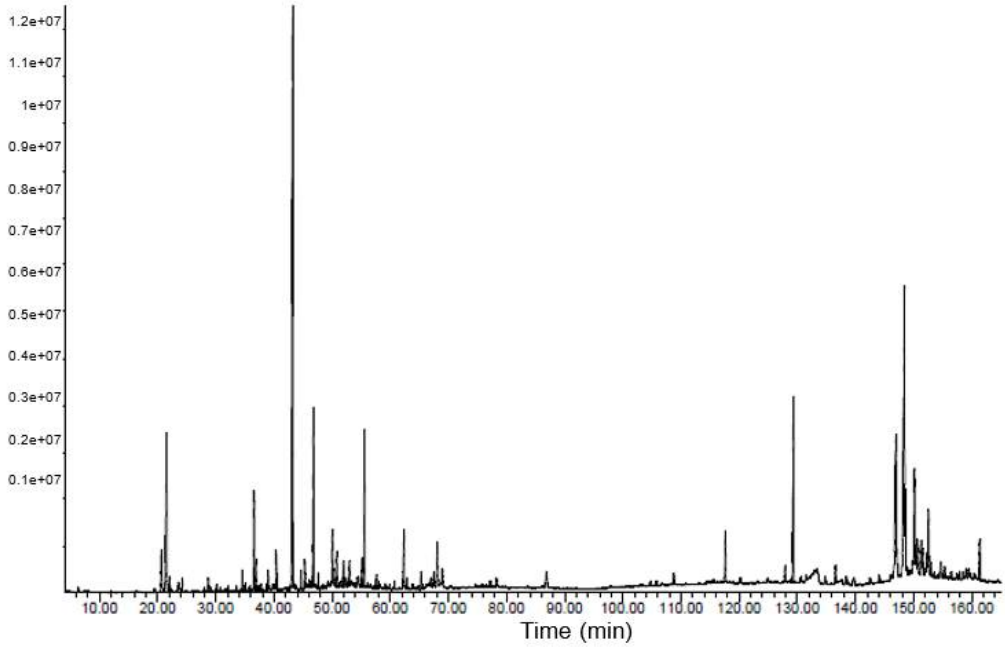


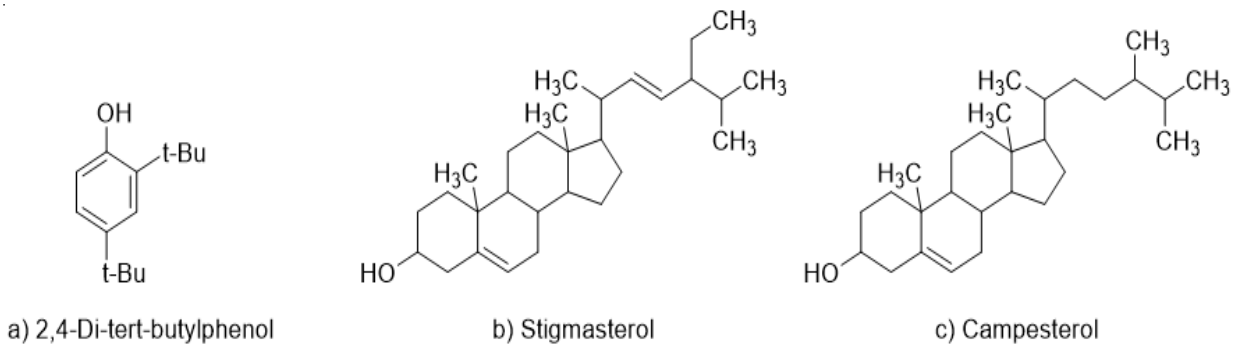
Figure 1: GC-MS total ion chromatograms (TIC) of chemical constituents of the ethanol root extracts of *Maesa perlaria* var. *formosana*

Table 1: Chemical constituents of *Maesa perlaria* ethanol extract

Compd no.	RT (min)	Name of the compound	Molecular Formula	Molecular Weight	% Area
1	20.64	Isoborneol	$C_{10}H_{18}O$	154.14	1.44
2	21.55	endo-Borneol	$C_{10}H_{18}O$	154.14	5.23
3	22.12	5-methyl-2-(1-methylethyl)-cyclohexanol	$C_{10}H_{20}O$	156.15	0.34
4	24.23	*tert*-Butyl-p-benzoquinone	$C_{10}H_{12}O_2$	164.08	0.28
5	30.12	Anethole	$C_{10}H_{12}O$	148.09	0.21
6	34.55	Eugenol	$C_{10}H_{12}O_2$	164.08	0.40
7	36.62	(E)-2-Tetradecene	$C_{14}H_{28}$	196.22	1.74
8	38.71	Coumarin	$C_9H_6O_2$	146.04	0.16
9	39.04	1-(2-hydroxy-4-methoxyphenyl)- Ethanone	$C_9H_{10}O_3$	166.06	0.35
10	40.01	(-)-3,7,7-trimethyl-11-methylene-spiro[5.5]undec-2-ene	$C_{15}H_{24}$	204.19	0.08
11	40.42	2,6-bis(1,1-dimethylethyl)-2,5-Cyclohexadiene-1,4-dione	$C_{14}H_{20}O_2$	220.15	0.65
12	43.20	2,4-Di-*tert*-butylphenol	$C_{14}H_{22}O$	206.17	16.76
13	43.36	4-hydroxy-3-methoxy- Benzoic acid, methyl ester	$C_9H_{10}O_4$	182.06	0.09
14	45.31	*t*-Butylhydroquinone	$C_{10}H_{14}O_2$	166.10	0.76
15	46.78	(E)-2-Tetradecene	$C_{14}H_{28}$	196.22	3.34
16	49.36	3-Butylisobenzofuran-1(3H)-one	$C_{12}H_{14}O_2$	190.10	0.03
17	50.04	aR-Turmerone	$C_{15}H_{20}O$	216.15	0.99
18	50.24	Z-Butylidenephthalide	$C_{12}H_{12}O_2$	188.08	0.44
19	50.80	2,4-di-*tert*-butyl-6-nitrophenol	$C_{14}H_{21}NO_3$	251.15	0.55
20	52.30	3,5-bis(1,1-dimethylethyl)-1,2-Benzenediol	$C_{14}H_{22}O_2$	222.16	0.20
21	52.99	(E)-3-Butylidene-4,5-dihydroisobenzofuran-1(3H)-one	$C_{12}H_{14}O_2$	190.10	0.51
22	53.25	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.08	0.17
23	54.56	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.21	0.20
24	55.13	Pentaerythritol tetraacetate	$C_{13}H_{20}O_8$	304.12	0.58
25	55.24	(E)-5-Octadecene	$C_{18}H_{36}$	252.28	0.14
26	55.58	1-Octadecene	$C_{18}H_{36}$	252.28	2.94
27	57.64	Neophytadiene	$C_{20}H_{38}$	278.30	0.26
28	57.98	6,10,14-trimethyl-2-Pentadecanone	$C_{18}H_{36}O$	268.28	0.13
29	62.38	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.17	2.19
30	62.84	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.26	0.29
31	65.30	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	278.15	0.51
32	66.95	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.24	0.31
33	68.11	n-1-Nonadecanol	$C_{19}H_{40}O$	284.31	1.69
34	77.23	(E)-9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.27	0.25
35	86.88	1-Docosene	$C_{22}H_{44}$	308.34	0.80

Table 1: *Chemical constituents of Maesa perlaria ethanol extract (Continued)*

Compd no.	RT (min)	Name of the compound	Molecular Formula	Molecular Weight	% Area
36	108.77	1-Tetracosene	$C_{24}H_{48}$	336.38	0.33
37	117.63	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.28	1.34
38	120.24	1-Nonadecene	$C_{19}H_{38}$	266.30	0.13
39	127.93	(Z)-13-Docosenamide	$C_{22}H_{43}NO$	337.33	0.39
40	129.36	Decanedioic acid, bis(2-ethylhexyl) ester	$C_{26}H_{50}O_4$	426.37	4.53
41	130.61	α-Tocospiro A	$C_{29}H_{50}O_4$	462.37	0.10
42	131.49	α-Tocospiro B	$C_{29}H_{50}O_4$	462.37	0.12
43	142.53	(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-methanol	$C_{11}H_{20}O$	168.15	0.13
44	144.14	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.38	0.26
45	146.10	β-Amyrin	$C_{30}H_{50}O$	426.39	0.13
46	146.98	Campesterol	$C_{28}H_{48}O$	400.37	7.33
47	147.15	Cholestanol	$C_{27}H_{48}O$	388.37	0.93
48	148.47	Stigmasterol	$C_{29}H_{48}O$	412.37	15.86
49	148.63	N,N'-Bis(2-hydroxyethyl)-1,2,4,5-benzenetetracarboxylic diimide	$C_{14}H_{12}N_2O_6$	304.07	1.52
50	149.89	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a, 9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	424.37	0.41
51	150.18	γ-Sitosterol	$C_{29}H_{50}O$	414.39	3.25
52	150.41	Stigmastanol	$C_{29}H_{52}O$	416.40	0.76
53	150.69	β-Amyrin	$C_{30}H_{50}O$	426.39	0.86
54	151.32	4-Campestene-3-one	$C_{28}H_{46}O$	398.36	1.09
55	151.57	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424.37	0.77
56	152.31	Lupeol	$C_{30}H_{50}O$	426.39	0.67
57	152.58	4,22-Stigmastadiene-3-one	$C_{29}H_{46}O$	410.36	1.89
58	154.71	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412.37	0.65
59	155.38	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.38	0.40



a) 2,4-Di-tert-butylphenol b) Stigmasterol c) Campesterol

Figure 2: Three major components of the ethanol extract from the roots of *Maesa perlaria* var. *formosana*

Research has indicated that stigmasterol may be useful in the prevention of certain cancers and possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties [11]. In addition, many vegetables, fruits, nuts and seeds have been found to contain campesterol, which is thought to have anti-inflammatory effects and inhibit several pro-inflammatory and matrix degradation mediators typically involved in osteoarthritis-induced cartilage degradation [12]. In our previous study, the antioxidant capacity of ethanol extracts from *Maesa perlaria* was confirmed based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, total antioxidant capacity (Trolox Equivalent Antioxidant Capacity), and reducing ability [13]. The results obtained in this study indicated that the three major components, 2,4-di-tert-butylphenol, stigmasterol, and campestero may be the most important contributors to the antioxidant activity and some of the pharmacological effects of *Maesa perlaria* var. *formosana*.

CONCLUSION

The results reveal that the chemical components of the ethanol extract from the roots of *Maesa perlaria* contains fifty-nine compounds in various concentrations, with the major component being 2,4-Di-tert-butylphenol. The natural compound and extract might be suitable for use as natural antioxidants to reduce oxidant stress in human beings or for applications in food, cosmetic and medicinal products.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

CONTRIBUTION OF AUTHORS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. In this study, LYC and CHY conceived and designed the study, MTL and YWW coordinated it, JFY and ZJG collected and analyzed the data while LYC and YWW prepared the manuscript. All authors read and approved the final manuscript.

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