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GC-MS Analysis of Phytoconstituents in 12 Ma-dan Extracts (*Garcinia schomburgkiana*)

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Abstract

Garcinia schomburgkiana (locally known as Ma-dan) was studied to determine their phytochemical constituents by using gas chromatography-mass spectrometry. To obtain 12 extracts with a variety of nutrients and bioactive compounds, 4 parts (leaves, twigs, branches and roots) of Ma-dan were extracted with 3 different polarity solvents (CH_2Cl_2, Acetone and MeOH). According to the GC-MS analysis with both original and TMS-derivatized extracts, 51 known compounds (23 volatile compounds from original extracts and 28 non-volatile compounds from TMS-derivatized extracts) were detected. In addition, many beneficial compounds for the food and pharmaceutical industry such as benzoic acid, vanillin, citric acid, linoleic acid, oleic acid, protocatechuic acid, catechol, and phloroglucinol were found.

Keywords: *Garcinia schomburgkiana*, GC-MS analysis, TMS-derivatized extract

Introduction

Garcinia is a rainforest plant in the Guttiferae family, which is widely known as a rich source of bioactive secondary metabolites. The phytochemical diversity of this plant genus has been extensively reported [1]. *Garcinia schomburgkiana*, a small tree, is commonly found growing in Thailand. Its fruits and leaves can be cooked. Additionally, they, together with roots, have been used as a laxative and for a treatment for coughs and diabetes [2]. Compounds from *Garcinia* plants have previously been isolated and identified through several techniques such as high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and classical column chromatography (CC) [3-5]. These traditional techniques required a standard compound and a large amount of a sample. In contrast, the combination of the gas chromatography with the mass spectrometry (GC-MS) is a beneficial technique in the natural product field. GC-MS provides a full spectrum data which is used for the comparison with mass spectral libraries. Furthermore, the amount of sample needed for this technique is less than 5 µL [4,6].

In recent years, natural products, like phenolic compounds, flavonoids, xanthones, benzophenones and others, have been isolated from the bark, stem and wood of Ma-dan with the use of column chromatography. Although the chemical constituents and cytotoxicity against cancer cell lines of *G. schomburgkiana* have been widely reported [7-10], only the bark and stem of Ma-dan were studied on their chemical constituents through column chromatography. This study, therefore, aims to report the chemical components of the 12 *G. schomburgkiana* extracts from leaves, twigs, branches and roots with 3 different polarity solvents through the GC-MS technique.

Materials and methods

Plant material

Leaves, roots, twigs and branches of *Garcinia schomburgkia* were collected in Trang province, southern Thailand, in August 2012. The samples were cut into small sizes and dried at room temperature for 5 - 7 days. Each part was macerated thrice with dichloromethane, subsequently with acetone and finally with methanol, for 3 days at room temperature. The solutions were filtered and evaporated under vacuum to obtain 12 crude extracts, shown in **Table 1**.

Table 1 Parts of Ma-dan extract and their abbreviation.

Abbreviations	Solvent extractions	Parts
LD	Dichloromethane	
LA	Acetone	Leaves
LM	Methanol	
RD	Dichloromethane	
RA	Acetone	Roots
RM	Methanol	
TwD	Dichloromethane	
TwA	Acetone	Twigs
TwM	Methanol	
BD	Dichloromethane	
BA	Acetone	Branches
BM	Methanol	

GC-MS analysis

Twelve Ma-dan extracts were analyzed on their phytochemicals through GC-MS as 2 types: original extract and trimethylsilyl (TMS)-derivatized extract. For the original extract, one mg of the sample was dissolved in 500 µL methanol and for the TMS-derivatized extract, one mg of the extract was dissolved in 200 µL of BSTFA, 300 µL of acetonitrile and the mixture was heated at 50 °C for 90 min.

This investigation was carried out on a gas chromatography mass spectrometry (Hewlett-Packard Model 7890A, USA) equipped with a DB-5ms column (J&W Scientific, USA) at the dimensions of 30 cm × 0.25 mm ID and 0.25 µm film thicknesses. The injector temperature was set at 250 °C and the split mode in ratio 1:25 was used. Regarding the oven temperature, the initial temperature ranging from 60 to 200 °C was first set at 10°C/min (However, for the derivatized extract, the initial temperature was held at 80 °C for 3 min.) and the temperature from 200 to 270 °C at 5 °C/min and held at 270 °C for 5 min. The ion source and transfer line temperatures were 230 and 250 °C, respectively. The electron energy was 70 eV and the mass spectrum ranging from 35 to 650 was recorded. The flow rate of carrier gas (Helium) was maintained at 1 mL/min in a constant flow mode. The compositions were elucidated based on the NIST 2008 library.

Results and discussions

*Garcinia schomburgkian*a, extracted with 3 different polar solvents, yielded 12 extracts. All extracts were subjected to GC-MS analysis. Due to the limitation of this technique, all extracts were derivatized with BSTFA in order to obtain the volatile TMS-derivatized extract [4]. Both the original and TMS-derivatized extracts were elucidated, herein, for the first time with the use of GC-MS technique.

According to a comparison with the NIST 2008 library, although, there was no significant effect of solvent polarity on the observed compounds, a higher variety of non-volatile compounds could be generally extracted with the use of Acetone and MeOH, rather than CH_2Cl_2. However, the 51 compounds, shown in **Tables 2** and **3**, which have been reported as essential nutrients [11], food additives [12,13] and biological active compounds [14-17], were discovered in this study.

The 23 compounds, which were volatile components in the original extract fell into the following 3 categories:

1) 11 fatty acids: myristic acid, methyl palmitate, palmitic acid, methyl linolate, methyl 11, 14-octadecadienoate, methyl *α*-linolenate, methyl stearate, linoleic acid, oleic acid, *α*-linolenic acid and stearic acid,
2) 2 Phenolic compounds: catechol and phloroglucinol, and
3) 10 miscellaneous compounds: maleic anhydride, acetophenone, 3-hydroxy-2,3-dihydromaltol, 5-hydroxymethyl-2-furfural, α-gurjunene, 3,4-dihydro-8-hydroxy-3-methylisocoumarin, phytol, n-hexadecanol, 3,5,11,15-tetramethyl-1-hyhexadecen-3-ol and *E*-10-pentadecenol.

The other 28 non-volatile compounds, which were detected in the form of volatile TMS derivative, included the following 4 categories:

1) 16 sugars: D-arabinose, L-arabitol, D-arabitol, D-fructose, sorbopyranose, methyl glucofuranoside, dulcitol, D-glucose, methyl lyxopyranoside, 2-deoxy-galactopyranose, D-xylose, D-xylopyranoside, glucopyranose, mannose, α–D-mannopyranose and talose,
2) 3 Phenolic compounds: vanillin, isovanillic acid and protocatechuic acid,
3) 6 organic acid: benzoic acid, succinic acid, propanoic acid, malic acid, citric acid and methyl citric acid, and
4) 3 miscellaneous compounds: L-alanine, 2,3,4,5-tetrahydroxypentanoic acid-1,4-lactone and glycerol.

In the leaves of Ma-dan, *α*-linolenic acid, linoleic acid, L-Alanine, D-(-)-Fructose, D-Arabinose and D-Glucose were observed; these detected compounds have been previously reported as important nutrients for human body [11]. Regarding the roots, branches and twigs of Ma-dan, these parts yielded bioactive compounds, consisting of protocatechuic acid, catechol, phloroglucinol and oleic acid, with potent antioxidant and anti-inflammatory activities [14-17]. Moreover, food additives [12,13] such as benzoic acid, vanillin, citric acid, succinic acid and malic acid were isolated from every part of the Ma-dan.

From the herbal traditional use, drinking juice from salted Ma-dan leaves helps relieve coughs and the roots have been used for the treatment of diabetes and the improvement of menstrual blood quality [2]. In addition, the results from this study provide convincing supporting data for Ma-dan consumption and the promotion of its use as an alternative source in the nutrition and pharmaceutical industries.

Table 2 Chemical components in the original extracts.

RT (min)	Chemical component	Formula	LD	LA	LM	TwD	TwA	TwM	RD	RA	RM	BD	BA	BM
5.312	Maleic anhydride	$C_6H_6O_3$	-	-	-	-	-	✓	-	-	-	-	-	-
6.510	Acetophenone	$C_9H_{10}O_3$	-	-	-	-	-	-	✓	-	-	-	-	-
6.916	3-Hydroxy-2,3-dihydromaltol	$C_6H_6O_4$	-	-	-	-	-	-	-	-	✓	-	-	-
7.492	Catechol	$C_6H_6O_2$	-	-	-	✓	-	-	✓	-	-	✓	-	-
8.005	5-Hydroxymethyl-2-furfural	$C_6H_6O_3$	-	-	✓	-	✓	✓	-	-	✓	-	✓	✓
10.671	α-Gurjunene	$C_{15}H_{24}$	✓	-	-	-	-	-	✓	-	-	-	-	-
12.398	3,4-Dihydro-8-hydroxy-3-methylisocoumarin	$C_{10}H_{10}O_3$	-	-	-	✓	-	-	-	-	-	-	-	-
12.652	Phloroglucinol	$C_6H_6O_3$	-	-	-	-	-	-	-	✓	-	-	✓	✓
14.689	Myristic acid	$C_{14}H_{28}O_2$	-	✓	-	-	-	-	-	-	-	-	-	-
15.678	Phytol	$C_{20}H_{40}O$	✓	✓	-	-	-	-	-	-	-	-	-	-
16.243	n-Hexadecanol	$C_{16}H_{34}O$	-	-	-	-	-	-	✓	-	-	-	-	-
16.804	Methyl palmitate	$C_{17}H_{34}O_2$	✓[b]	✓	✓[b]	✓[b]	✓[b]	-	-	-	-	✓	✓[b]	-
17.086	3,5,11,15-Tetramethyl-1-hexadecen-3-ol	$C_{20}H_{40}O$	-	✓	-	-	-	-	-	-	-	-	-	-
17.211	Palmitic acid	$C_{16}H_{32}O_2$	✓[a]	✓[a]	✓[a]	✓[a]	✓[a]	-	✓[a]	✓[a]	-	✓[a]	✓[a]	-
18.655	*E*-10-Pentadecenol	$C_{15}H_{30}O$	-	-	✓	-	-	-	-	-	-	-		-
19.120	Methyl linolate	$C_{19}H_{34}O_2$	✓	-	✓[b]	-	-	-	-	-	-	-	✓	-
19.125	Methyl 11,14-octadecadienoate	$C_{19}H_{34}O_2$	-	✓		-	-	-	-	-	-	-	✓	-
19.212	Methyl *α*-linolenate	$C_{19}H_{32}O_2$	✓	✓	✓[b]	-	-	-	-	-	-	-	✓	-
19.594	Methyl stearate	$C_{19}H_{34}O_2$	-	-	✓	-	-	-	-	-	-	-	-	-
19.622	Linoleic acid	$C_{18}H_{32}O_2$	✓[a]	✓[a]	-[a]	-	✓[a]	-	-	-	-	-[a]	-[a]	-
19.709	Oleic acid	$C_{18}H_{34}O_2$	-	-	-	✓[a]	✓[a]	-	✓[a]	✓	-	✓[a]	✓[a]	-
19.728	*α*–linolenic acid	$C_{18}H_{30}O_2$	-[a]	✓[a]	✓[a]	-	-	-	-	-	-	-	-	-
20.053	Stearic acid	$C_{18}H_{36}O_2$	-	✓[a]	-	-	-	-	-	-	-	✓[a]	-	-

RT = Retention time, ✓ = observed, - = not observed

[a] = Found in TMS-derivatized extracts in the form of a TMS derivative

[b] = Found in TMS-derivatized extracts in the form of the native compound

Table 3 Chemical components in the TMS-derivatized extracts.

RT (min)	Chemical component	Formula	LD	LA	LM	TwA	TwM	RD	RA	RM	BA	BM
5.988	L-Alanine	$C_3H_7NO_2$	-	-	✓	-	-	-	-	-	-	-
8.245	Benzoic acid	$C_7H_6O_2$	✓	✓	-	-	-	-	-	-	-	-
8.513	Glycerol	$C_3H_8O_3$	-	✓	-	✓	✓	-	✓	✓	✓	✓
9.142	Succinic acid	$C_4H_6O_4$	-	-	-	✓	-	✓	✓	-	✓	-
9.314	Propanoic acid	$C_3H_6O_4$	-	-	-	-	-	-	-	-	-	✓
11.424	Malic acid	$C_4H_6O_5$	-	✓	✓	✓	✓	-	✓	✓	✓	✓
12.120	Vanillin	$C_8H_8O_3$	✓	-	-	-	-	-	-	-	-	-
13.220	2,3,4,5-Tetrahydroxypentanoic acid-1,4-lactone	$C_5H_5O_5$	-	-	✓	-	-	-	-	-	-	-
13.295	D-Arabinose	$C_5H_{10}O_5$	-	-	✓	-	-	-	-	-	-	-
14.118	D-(+)-Arabitol	$C_5H_{10}O_5$	-	-	-	✓	✓	-	-	-	✓	-
14.118	L-(-)-Arabitol	$C_5H_{10}O_5$	-	-	-	-	-	-	-	-	-	✓
14.761	Isovanillic acid	$C_8H_8O_3$	-	-	-	-	-	-	-	-	✓	-
15.137	D-(-)-Fructose	$C_6H_{12}O_6$	-	-	✓	-	✓	-	✓	-	✓	✓
15.314	Sorbopyranose	$C_6H_{12}O_6$	-	-	✓	-	-	-	-	✓	✓	-
15.380	Citric acid	$C_6H_6O_7$	-	✓	✓	-	-	-	-	-	-	-
15.449	Protocatechuic acid	$C_{16}H_{33}O_4$	-	-	-	✓	-	✓	-	-	✓	-
15.607	Methyl citric acid	$C_7H_8O_7$	-	✓	✓	✓	✓	-	-	-	-	-
15.711	Methyl glucofuranoside	$C_7H_{14}O_6$	-	-	-	✓	✓	-	✓	✓	✓	✓
16.310	Dulcitol	$C_6H_{12}O_6$	-	-	-	-	-	-	-	-	-	✓
16.397	D-Glucose	$C_6H_{12}O_6$	-	-	✓	-	✓	-	-	✓	-	✓
16.467	Methyl lyxopyranoside	$C_6H_{12}O_5$	-	-	-	-	-	-	-	-	✓	-
16.519	2-Deoxy-galactopyranose	$C_6H_{12}O_5$	-	-	-	-	-	-	-	-	✓	-
16.631	D-Xylose	$C_5H_{10}O_5$	-	-	-	-	-	-	✓	-	-	-
16.644	D-Xylopyranose	$C_5H_{10}O_5$	-	-	-	-	-	-	-	✓	-	-
16.645	Glucopyranose	$C_6H_{12}O_6$	-	-	-	-	-	-	-	-	-	✓
17.792	Mannose	$C_6H_{12}O_6$	-	-	✓	-	-	-	-	-	✓	-
17.793	α–D-Mannopyranose	$C_6H_{12}O_6$	-	-	-	-	-	-	✓	-	-	-
17.799	Talose	$C_6H_{12}O_6$	-	-	-	-	-	-	-	✓	-	✓

RT = Retention time, ✓ = observed, - = not observed

Conclusions

The use of the GC-MS analysis, which was more effective than other traditional techniques, led to the identification of the 51 compounds in *Garcinia schomburgkiana* extracts; 23 volatile compounds from original extracts and 28 non-volatile compounds detected from TMS-derivatized extracts. These compounds could be highly beneficial for development by the pharmaceutical and food industries.

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