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Gas chromatography-mass spectrometry analysis of various organic extracts of *Merremia borneensis* from Sabah

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ABSTRACT

Objective: To analyse the chemical composition of different extracts of *Merremia borneensis* (*M. borneensis*) by gas chromatography-mass spectrometry (GC-MS). **Methods:** The dried leaves powder was extracted with methanol at room temperature by using Soxhlet extractor. Methanol crude extracts of *M. borneensis* were extrastel with hexane, chloroform, ethyl acetate and butanol. **Results:** Qualitative analyses of various organic crude extracts showed that majority of these are flavonoids, terpeniods, alkaloids and glycosides. Most of the identified compounds by GC-MS are biologically important. Further the *M. borneensis* leaf possesses certain characteristics that can be ascribed to cultivation on a domestic plantation. **Conclusions:** The suitable extracts for respective compounds can be chosen on the basis of above GC-MS analysis. All the major compounds from different extracts are biologically active molecules. Thus the identification of a good number of compounds from various extracts *M. borneensis* might have some ecological significance.

1. Introduction

Medicinal plants have occupied an important position in the socio-cultural, development of rural people of Malaysia. Crude drugs are usually dried parts of the medicinal plants that form an essential raw material for the production of traditional remedies of Ayurveda, Siddha, Unani, Homeopathy *etc*. It has been estimated by WHO that 80% of the people living in the developing countries rely upon the traditional health practices for their primary health care needs[1]. Chemical constituents found in low concentrations in other plant parts are highly concentrated in bark.

Plants have great potential sources for producing new drugs of benefit to mankind. There are many approaches in the search for new biologically active principles in higher plants. Many efforts have been scientifically expended to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One such resource is folk medicine and systematic screening of these traditional herbs may result in the discovery of novel effective compounds[2].

Antibacterial properties of different parts of plant like roots, stems, leaves, flowers, fruit and seeds have been well documented for some of the medicinal plants for the past two decades[2]. Most of the medicinal and aromatic plants and essences are rich sources in antibacterial compounds which can be an alternative to combat bacterial diseases [3]. In recent years antimicrobial properties of Bangladeshi medicinal plants have been increasingly reported[3].

Chemical bactericides are known to be highly effective to control the postharvest diseases in various vegetables and fruits. However, they are not able to consider as long-term solutions due to the health concerns associated with exposure risks, health and environmental hazards, residue persistence, and development of tolerance[4]. The increasing recognition and importance of bacterial infections and the difficulties encountered in their treatment have stimulated the search for synthetic chemical bactericides alternatives. In recent years, researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that they have developed to antibiotics[5]. Plant crude extracts and essential oils are made up of many different types of volatile compounds and have been shown to possess antimicrobial and anti-bacterial properties[3].

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Essential oils and organic plant extracts are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional uses[6]. So, essential oils and organic plant extracts are one of the most promising groups of natural compounds for the development of safer anti-bacterial agents.

Merremia borneensis (*M. borneensis*) is applied as a medicinal plant because of the diuretic, anti-fungal and bacteriostatic properties of its leaves[7]. Most of the scientific and academic papers dealing with this subject refer these effects to the content of potassium, inositol and lipophilic flavones in *M. borneensis* leaves[8]. In addition to the above mentioned components, saponins, sterols, polyphenols, rosmarinic acid and ursolic acid and essential oil have been also detected[9]. The leaves are suitable to be used as wrapper to the famous fermented rice or fermented tapioca known in Malaysia as 'Tapai'. The medicinal plant creeps well and is very productive in shady areas as well as open areas and are known to blanket a wholesome tree or on any objects that it chooses to make its habitat. The stem contains latex that is highly sticky and the flowers are white in colour. The Bilaran leaves, according to natives in Sarawak, Malaysia, are used to relieve breast cancer[7]. In some cases, the essential oil, is the reason for diuretic effects of plant drugs, has not yet been described in detail. Based on preliminary analyses, however, there is no report available in the literature on the detailed analyses of plant organic extracts of *M. borneensis* by GC-MS. Therefore, the aim of this present study is to examine the chemical composition of different organic extracts isolated from the leaves of *M. borneensis* by GC-MS.

2. Materials and methods

2.1. Plant material

The leaves of *M. borneensis* was collected from the campus area at University Malaysia Sabah in Malaysia, in February 2011 and initially identified by morphological features and data base present in the library, School of Biology, University Malaysia Sabah.

2.2. Sample collection

The fresh green leaves of *M. borneensis* were collected from the campus of University Malaysia Sabah, Malaysia. The leaves of this plant were harvested during the month of January, 2011. The leaves sample were collected at 2:00 pm-3:00 pm on February 14, 2011 and packed in polyethylene bags and stored at 4 ℃ until required. The plant samples initially identified by morphological features and data base present in the library, School of Biology, University Malaysia Sabah, Malaysia. About 1 kg of leaves was ground using a grinder (Blender 80115) for 20 s. The unfermented *M. borneensis* leaves was kept in the oven at 40 ℃ and put in a desiccator for at least 24 h prior to analysis.

2.3. Preparation of crude extracts

The small pieces of leaves were homogenised in a grinder for 3 min to 30-40 mesh size. The air-dried leaves were pulverized into powdered form. The dried leaves powder (250 g) was extracted with methanol at room temperature by using Soxhlet extractor. The crude methanol extracts were evaporated by a vacuum rotary evaporator (Buchi Labortech AG, model 1, R-215) under reduce pressure. The crude extract was (20 g) diluted by water (100 mL) and extracted successively with hexane, chloroform, ethyl acetate and butanol to give hexane (3.24 g), chloroform (2.45 g), ethyl acetate (1.92 g) and butanol (0.841 g) and residual methanol fractions (8.09 g), respectively. The extracts were filtered using Whatman No. 41 filter paper to obtain particle free extract. The residue was reextracted twice by solvent and filtered. The extracts were pooled and then concentrated and dried under vacuum pressure. Solvents (analytical grade) for extraction were obtained from E-Merck.

The dried powder of *M. borneensis* (50 g for each) was subjected to extract separately and exhaustively in Soxhlet apparatus with *n*-hexane, ethyl acetate, chloroform and butanol. All extracts were filtered followed by evaporation to desire volume by a rotatory evaporator.

2.4. GC-MS analysis

The GC-MS analysis of various crude extracts from leaves was performed using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 500, USA) equipped with a VF-5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). For gas chromatography- mass spectroscopic detection, an electron ionization system with ionization energy of 70 eV was used. Inert helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Mass transfer line and Injector temperature were set at 220 and 300 ℃, respectively. The oven temperature was programmed from 50 to 150 ℃ at 3 ℃/min, then held isothermal for 10 min and finally raised to 250 ℃ at 10 ℃/min. Diluted samples (1/100, v/v, in methanol) of 1 μL was manually injected in the splitless mode. The relative percentage of the crude extracts constituents was expressed as percentage by peak area normalization.

Identification of chemical compounds of various extracts were based on GC retention time on VF-5 capillary column, computer matching of mass spectra with those of standards (Mainlab, Replib and Tutorial data of GC-MS systems) and, whenever possible, by co-injection with authentic compounds.

3. Results

3.1. Physical properties

The different extracts have different in colours. The hexane extract was brown in colour, ethyl acetate was orange in colour, chloroform extract was orange and the butanol extract was gray in colour.

3.2. Chemical composition of different extracts

The hexane crude extract was analysis by using GC-MS had led to the identification of 33 different organic compounds, representing 7.12% of the total extract from leaves samples. The identified chemical compounds are listed in Table 1 according to their elution order on a VF-5 capillary column. The major chemical compounds that were found in hexane extract (shown in Figure 1 and Table 1) are oxalic acid

(3.11%), 2-Hexyl-1-octanol (21.09%), 2,6,10,15-tetramethyl heptadecane (13.35%), butanolic acid (11.32%), orthosiphol A (2.91%), orthosiphol U (1.67%), tetratetracontane (3.27%), nonadecane (0.99%), 7-hexyleicosane (2.54%), octadecanoic acid (19.03%), 1-hexacosanol (1.06%), pentafluoropropionic acid (4.69%) and hexatriacontane (1.32%).

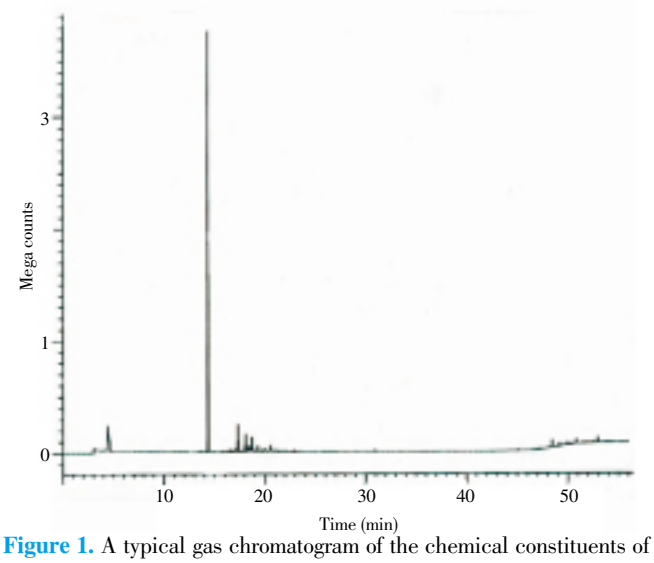

Figure 1. A typical gas chromatogram of the chemical constituents of hexane extract.

The ethyl acetate extract was analysis by using GC-MS had led to the identification of total 58 different organic compounds using the same capillary column and conditions, representing 13.98% of the total extract from leaves samples. The major chemical constituents that were found in ethyl acetate extract (Figure 2 and Table 1) are 1,2-dimethoxy-4-(2-propenyl)benzene (53.06%), 2-pentanone (18.06%), 4-methyl-2-pentyl acetate (3.11%), aromadendrene oxide-(2) (1.16%), caryophyllene oxide-1(1.44%), 1,2-benzenedicarboxylic acid (0.44 %) and 10-heneicosene (0.30%).

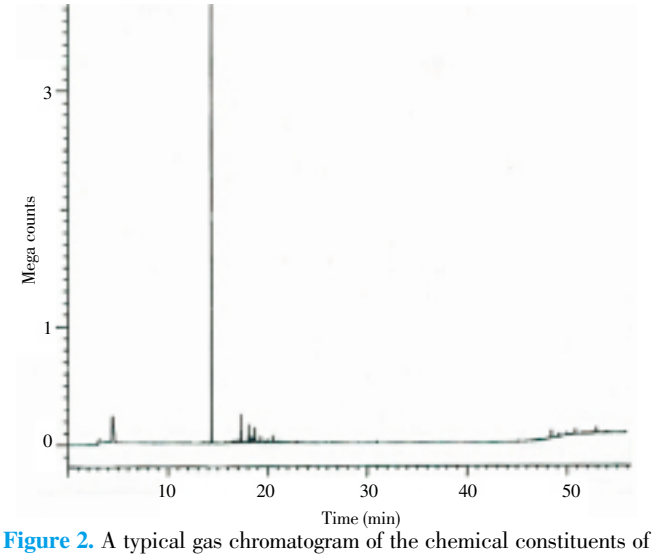

Figure 2. A typical gas chromatogram of the chemical constituents of ethyl acetate extract.

The chloroform extract was analysis by using GC-MS had led to the identification of 65 different organic compounds using the same capillary column and conditions, representing 29.12% of the total extract from leaves samples. The major chemical constituents that were found in chloroform extract (Figure 3 and Table 1) are 1,2-dimethoxy-4-(2-propenyl)benzene (29.69%), 4-methyl-2-pentyl acetate (7.48%), ledene oxide (1.12%), longipinocarvone (0.65%), phthalic acid, butyl hexyl ester (0.62%), sinensetin (1.01%), 2,4-dihydroxychalcone (1.22%) and 2-pentanone (0.44%).

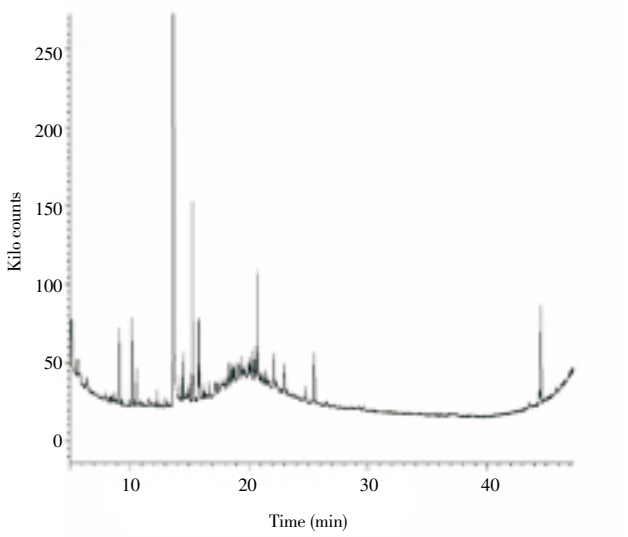

Figure 3. A typical gas chromatogram of the chemical constituents of ethyl acetate extract.

Finally the butanol extract was analysis by using GC-MS had led to the identification of 49 different organic compounds, representing 19.56% of the total extract from leaves samples. The major chemical constituents that were found in butanol extract (Figure 4 and Table 1) are butanamide (12.26%), 2-pentadecyl-1,3-dioxane (3.55%), cyclobutanethiol (1.12%), propionic acid (2.65%), phthalic acid, butyl hexyl ester (0.62%), N-aminomorpholine, glyoxal imine (1.01%), butanoic acid (0.22%), cyclopropanecarboxylic acid (4.07%), Chimilether (5.12%) and 2-pentanone (0.44%).

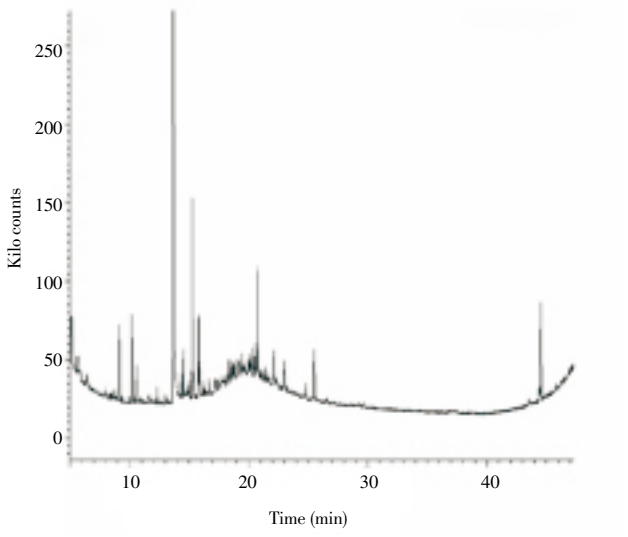

Figure 4. A typical gas chromatogram of the chemical constituents of butanol extract.

Table 1
Chemical composition of different extracts of *M. borneensis*.

Extract	Sl No	Name of compounds	Retention time (min)	Leave (%)
Hexane extract	1	Oxalic acid	6.12	3.11
	2	2-Hexyl-1-octanol	7.99	21.09
	3	2,6,10,15-Tetramethyl heptadecane	10.32	13.35
	4	Butanolic acid	18.11	11.32
	5	Orthosiphol A	19.02	2.91
	6	Orthosiphol U	23.97	1.67
	7	Tetratetracontane	25.28	3.27
	8	Nonadecane	28.94	0.99
	9	7-Hexyleicosane	31.54	2.54
	10	Octadecanoic acid	44.18	19.03
	11	1-Hexacosanol	48.09	1.06
	13	Pentafluoropropionic acid	51.11	4.69
	14	Hexatriacontane	55.89	1.32
Ethyl acetate extract	1	1,2-Dimethoxy-4-(2-propenyl)benzene	9.08	53.06
	2	2-Pentanone	14.56	18.88
	3	4-Methyl-2-pentyl acetate	18.24	3.11
	4	Aromadendrene oxide-(2)	22.15	1.16
	5	Caryophyllene oxide-1	25.91	1.44
	6	1,2-Benzenedicarboxylic acid	31.45	0.44
	7	10-Heneicosene	39.67	0.30
Chloroform extract	1	1,2-Dimethoxy-4-(2-propenyl)benzene	9.08	29.69
	2	Orthoformic acid	11.03	0.92
	3	Phenyl-piperidin-3-yl-methanone	12.89	0.48
	4	4-Methyl-2-pentyl acetate	18.24	7.48
	5	6-Methyl-4-undecene	20.40	1.22
	6	Ledene oxide	21.73	1.12
	7	3-Methyl-2-(2-oxopropyl)furan	23.88	0.09
	8	4-Methyl-dodec-3-en-1-ol	25.07	0.08
	9	Longipinocarvone	28.95	0.65
	10	Phthalic acid	29.56	0.04
	11	Phthalic acid, butyl hexyl ester	31.34	0.62
	12	Sinensetin	34.29	1.01
	13	2,4-Dihydroxychalcone	39.44	1.22
	14	2-Pentanone	43.67	0.44
	15	Dodecane, 1-fluoro-	48.08	0.09
Butanol extract	1	2-propenoic acid, chloromethyl ester	5.89	0.07
	2	Orthoformic acid, tri-2-butenyl ester	6.09	0.09
	3	2-N-Butylthiolane, SS-dioxide	7.11	0.05
	4	1,3-Propylene glycol, O,O-di(pivaloyl)-	9.01	0.03
	5	Butanamide	11.09	12.26
	6	2-Pentadecyl-1,3-dioxane	15.45	3.55
	7	Cyclobutanethiol	18.78	1.12
	8	Propionic acid	23.76	2.65
	9	Phthalic acid, butyl hexyl ester	26.39	0.62
	10	N-aminomorpholine, glyoxal imine	29.56	1.01
	11	Butanoic acid	35.02	0.22
	12	Cyclopropanecarboxylic acid	48.26	4.07
	13	Chimilether	52.55	5.12
	14	2-Pentanone	56.87	0.44

4. Discussion

The suitable extracts for respective compounds can be chosen on the basis of above GC-MS analysis. 2-Hexyl-1-octanol was found to be the most abundant in the hexane extract. Ethyl acetate extract contains 1,2-dimethoxy-4-(2-propenyl)benzene and 2-pentanone; chloroform extract contains 1,2-dimethoxy-4-(2-propenyl)benzene and 4-methyl-2-pentyl acetate and butanol extract contains butanamide and cyclopropanecarboxylic acid. All the major compounds from different extracts are biologically active molecules. They are considered to be a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants named 'phytoanticipins' or 'phytoprotectants'[7-9]. Thus, the identification of a good number of compounds from various extracts *M. borneensis* might have some ecological significance. All the compounds have previously been reported from a number of other plant species.

Conflict of interest statement

We declare that we have no conflict of interest.

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