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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.08.015

GC–MS analysis of bioactive compounds present in different extracts of an endemic plant *Broussonetia luzonica* (Blanco) (Moraceae) leaves

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ARTICLE INFO

Article history:
Received 18 Jan 2016
Received in revised form 13 Jul 2016
Accepted 22 Aug 2016
Available online 20 Sep 2016

Keywords:
Broussonetia luzonica (Blanco)
Selective sequential extraction
GC–MS analysis
Endemic
Percolation

ABSTRACT

Objective: To investigate and characterize the chemical composition of the different crude extracts from the leaves of *Broussonetia luzonica* (Blanco) (Moraceae) (*B. luzonica*), an endemic plant in the Philippines.

Methods: The air dried leaves were powdered and subjected to selective sequential extraction using solvents of increasing polarity through percolation, namely, *n*-hexane, ethyl acetate and methanol to obtain three different extracts. Then, each of the extracts was further subjected to gas chromatography–mass spectrometry.

Results: Qualitative determination of the different biologically active compounds from crude extracts of *B. luzonica* using gas chromatography–mass spectrometry revealed different types of high and low molecular weight chemical entities with varying quantities present in each of the extracts. These chemical compounds are considered biologically and pharmacologically important. Furthermore, the three different extracts possess unique physicochemical characteristics which may be attributed to the compounds naturally present in significant quantities in the leaves of *B. luzonica*.

Conclusions: The three extracts possess major bioactive compounds that were identified and characterized spectroscopically. Thus, identification of different biologically active compounds in the extracts of *B. luzonica* leaves warrants further biological and pharmacological studies.

1. Introduction

Plants play a significant role in the prevention and treatment of diseases and can even prevent and reduce the adverse effects of conventional treatments [1]. They can be a source of chemical compounds of biological and pharmacological importance. History reveals that plants are sources of successful drugs, and will continuously be important for screening of new lead compounds [2]. An essential part in the investigation of plant is the identification of the biologically active compounds present in plant leading to further biological and pharmacological studies [3–5]. *Broussonetia luzonica* (Blanco) (Moraceae) (*B. luzonica*), a tree endemic in the Philippines, is commonly found in thickets and forests at low and medium altitudes. For Filipinos especially those from the northern part of Luzon, the leaves and flowers are edible and used in vegetable recipes such as pinakbet and bulanglang [6,7]. Compounds present in other species of *Broussonetia* exhibited remarkable biological activities. *Broussonetia papyrifera* exhibited potent antiproliferative effects on estrogen receptor-positive MCF-7 breast cancer cells *in vitro* [5]; furthermore, its methanolic extract exhibited cytotoxicity against HeLa cell lines and HepG2 cell lines [8]. Plants under the same genus might also exhibit the same biological activities because of similar active principles present in them [9]. Identification of bioactive compounds present in the leaves of *B. luzonica* is conducted for further reference of future studies on one of the edible and endemic plants of the Philippines. There are no published literatures that determine the bioactive compounds present in the different extracts of *B. luzonica* leaves by gas chromatography–mass spectrometry (GC–MS) analysis.

This study aimed to investigate and characterize the bioactive compounds in the different crude extracts of *B. luzonica* leaves.

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Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

2. Materials and methods

2.1. Plant sample

Fresh leaves of the endemic plant *B. luzonica* were collected from Solano, Nueva Vizcaya, Philippines in June 9–10, 2015. Sample of plant was identified by a botanist and herbarium curator of Research Center of Natural and Applied Sciences, University of Santo Tomas, Manila and assigned with herbarium accession number of 012978.

2.2. Extraction of crude extracts

The leaves of the plant were air dried at room temperature for about 3 days. The dried leaves were ground using Wiley Mill Model No. 2 (Arthur H. Thomas Co., Philadelphia, USA). Dried and powdered samples were subjected to selective sequential extraction using solvents of increasing polarity, namely, *n*-hexane, ethyl acetate and methanol [10]. Then the extract was evaporated to dryness using Heidolph Rotavapor (Germany).

2.3. The GC–MS analysis

The GC–MS analysis of bioactive compounds from the different extracts of the leaves of *B. luzonica* was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 –150 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in an splitless mode. Relative quantity of the chemical compounds present in each of the extracts of *B. luzonica* was expressed as percentage based on peak area produced in the chromatogram.

2.4. Identification of chemical constituents

Bioactive compounds extracted from different extracts of *B. luzonica* were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

3. Results

3.1. Percentage yield

From three batches of approximately 1 kg of air dried powdered leaves, mean percentage yields of 3.64% (SD = 0.33) of *n*-hexane extract, 1.62% (SD = 0.05) of ethyl acetate extract and 6.34% (SD = 0.32) of methanol extract were obtained. Methanol extract gave the highest percentage yield. Most of the constituents were polar in nature.

3.2. Physical properties

The different crude extracts from *B. luzonica* leaves possessed unique physical characteristics. The *n*-hexane extract was yellow to yellow orange in color with agreeable odor; the pH was 5.79 in 10% aqueous solution and specific gravity was 1.02. The ethyl acetate extract was brown to dark brown in color with ammoniacal odor; the pH was 5.22 in 10% aqueous solution and specific gravity was 1.03. The methanol extract was green to dark green in color with sweetened agreeable odor; the pH was 4.07 in 10% aqueous solution having specific gravity of 2.4.

3.3. Bioactive compounds present in the extracts

The bioactive compounds present in methanol, *n*-hexane and ethyl acetate extracts obtained from *B. luzonica* leaves are shown in Tables 1–3. Their identification and characterization were based on their elution order in a HP-5MS column. The

Table 1
Biologically active chemical compounds of methanol extract from *B. luzonica* leaves.

Name of compounds (molecular formula)	Retention time (min)	%
Benzene, 1,2,3-trimethyl (C_9H_{12})	9.26	2.506
Phytol ($C_{20}H_{40}O$)	51.72	6.739
Tritriacontane ($C_{33}H_{68}$)	55.42	16.670
γ-Sitosterol ($C_{29}H_{50}O$)	55.81	14.754
β-Sitosterol ($C_{29}H_{50}O$)	56.08	3.065
Androst-5-en-17-ol, 4-4-dimethyl ($C_{21}H_{34}O$)	56.11	4.185
Hentriacontane ($C_{31}H_{64}$)	56.43	1.862
Lup-20(29)-en-3-one ($C_{30}H_{48}O$)	57.17	8.091
Lupeol ($C_{30}H_{50}O$)	57.70	21.973

Table 2
Biologically active chemical compounds of *n*-hexane extract from *B. luzonica* leaves.

Name of compounds (molecular formula)	Retention time (min)	%
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- ($C_{19}H_{32}O_2$)	51.56	4.421
Heneicosane ($C_{21}H_{44}$)	52.68	3.291
Tricosane ($C_{23}H_{48}$)	53.70	6.315
Tetracosane ($C_{24}H_{50}$)	54.62	8.626
Triacontane ($C_{30}H_{62}$)	55.46	7.341
Hexacosane ($C_{26}H_{54}$)	56.26	5.534
Octacosane ($C_{28}H_{58}$)	57.75	1.660
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- or squalene ($C_{30}H_{50}$)	58.06	29.028

Table 3
Biologically active chemical compounds of ethyl acetate extract from *B. luzonica* leaves.

Name of compounds (molecular formula)	Retention time (min)	%
1,2,3-Propanetriol, monoacetate ($C_5H_{10}O_4$)	20.08	21.211
1,2,3-Propanetriol, diacetate ($C_7H_{12}O_5$)	24.60	4.351
Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$)	50.11	2.386
Phytol ($C_{20}H_{40}O$)	51.70	20.288
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- ($C_{19}H_{32}O_2$)	51.56	2.465

(*continued on next page*)

Table 3 (*continued*)

Name of compounds (molecular formula)	Retention time (min)	%
9,12,15-Octatrienoic acid, ethyl ester, (Z,Z,Z)- ($C_{20}H_{34}O_2$)	52.30	2.753
3,7,11,15-Tetramethyl-2-hexadecen-1-ol ($C_{20}H_{40}O$)	52.90	6.765
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(all-E)- or squalene ($C_{30}H_{50}$)	58.00	6.853

elution time, molecular formula and the amount of these bioactive compounds were also presented. Based on abundance, the top three major compounds present in the methanolic extract were lupeol (21.973%), tritriacontane (16.670%) and γ-sitosterol (14.754%). The *n*-hexane crude extract contained squalene (29.028%) followed by tetracosane (8.626%) and triacontane (7.341%). The ethyl acetate crude extract had phytol (20.288%), 1,2,3-propanetriol, monoacetate (21.211%) and squalene (6.8%) as the top three major compounds. The GC chromatograms of the three extracts presented in Figures 1–3 show the retention

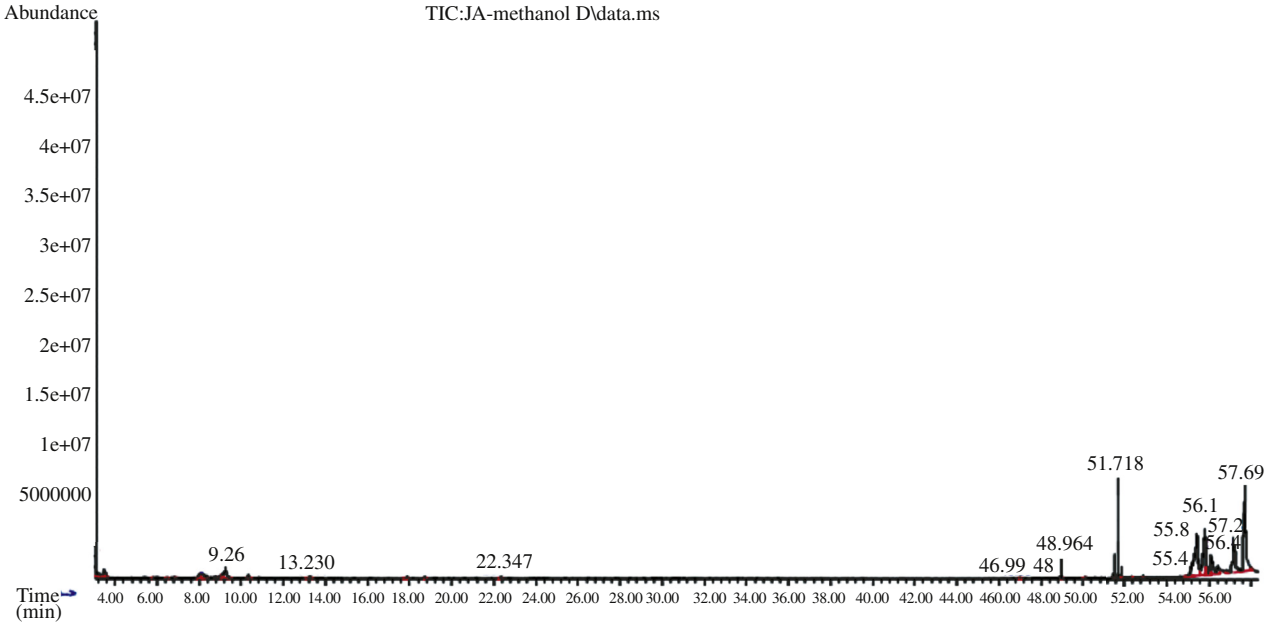

Figure 1. A typical chromatogram of the bioactive compounds present in methanol crude extract.

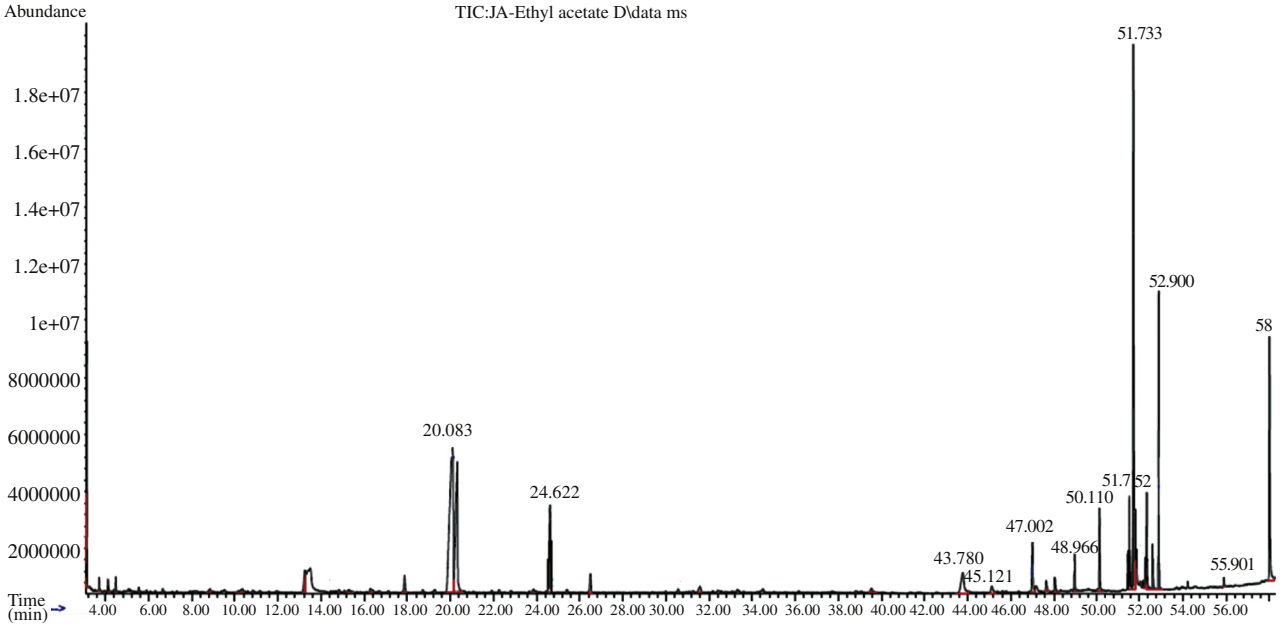

Figure 2. A typical chromatogram of the bioactive compounds present in ethyl acetate crude extract.

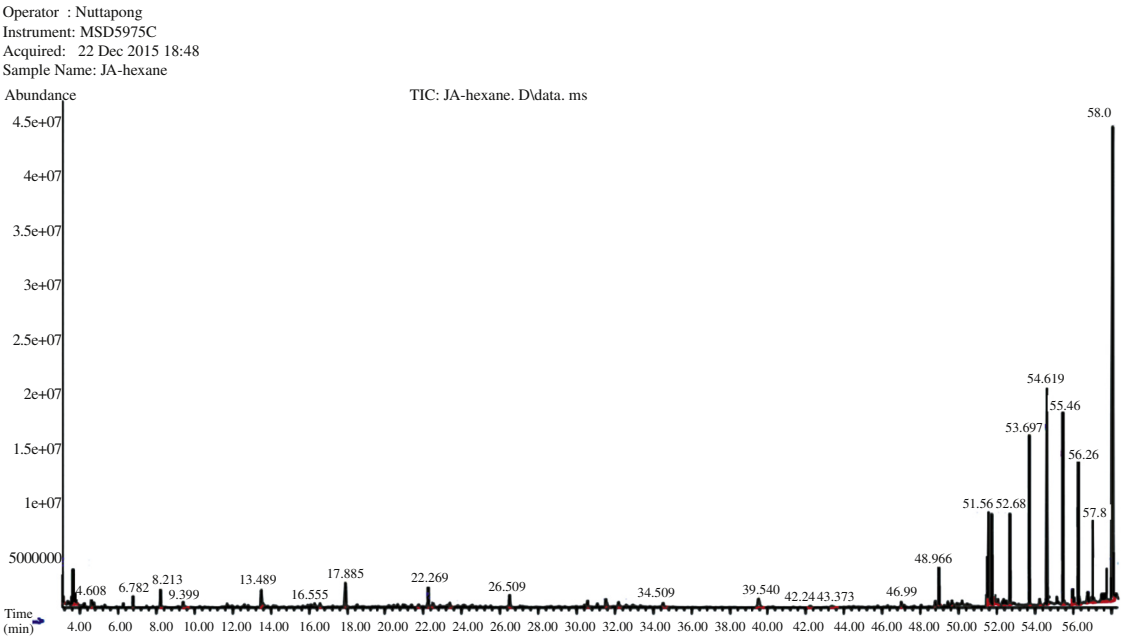

Figure 3. A typical chromatogram of the bioactive compounds present in *n*-hexane crude extract.

time in the column and the detected peaks which correspond to the bioactive compounds present in the extract.

4. Discussion

Different crude extracts were obtained from the leaves of *B. luzonica* through selective sequential extraction with solvents of increasing polarity, namely, *n*-hexane, ethyl acetate and methanol. GC–MS analysis of the *n*-hexane, ethyl acetate and methanol extracts revealed the presence of various bioactive compounds. Phytol is present both in methanol (6.739%) and ethyl acetate extracts (20.288%) but in different quantities. In addition, squalene and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- are both present in *n*-hexane and ethyl acetate extracts. The three crude extracts did not reveal a common major compound in them. Based on studies, some of the constituents revealed by GC–MS are biologically active compounds. They were proven to possess pharmacologic activities which may contribute to the healing potential of the plant. Phytol was proven to exhibit antioxidant and antinociceptive effects [11,12]. Phytol, precursor of synthetic vitamin E and vitamin K, was found to be cytotoxic against breast cancer cell lines (MCF7) [13,14]. Propanetriol monoacetate was confirmed to be present in other plants exhibiting antimicrobial, anti-inflammatory, diuretic and anticancer effects [15]. Propanetriol monoacetate was proven to be a precursor of tricetin (antifungal), but may also serve as a prodrug and vehicle for anticancer agents [16].

In addition, phytosterols such as α-sitosterol and β-sitosterol were proven to prevent cancer through targeting different mechanisms leading to cancer. They were able to prevent angiogenesis by increasing antioxidant enzymes and inhibiting reactive oxygen species production and oxidative stress. These sterols even blocked inflammatory cytokines and induced apoptosis [17–19].

Other studies revealed that lupeol, squalene, tetracosane and triacontane possess various pharmacological properties. Lupeol exhibited marked anti-inflammatory and anticancer properties [20]. Several investigations revealed that lupeol blocks tumorigenesis by affecting molecular growth pathways which are involved in cell proliferation and cell death [21,22]. *In vivo* and *in vitro* tests revealed potent anti-mutagenic property of lupeol [23–25]. Squalene has antioxidant, chemopreventive, antitumor and hypocholesterolemic activities [26,27]. Tetracosane showed cytotoxicity against AGS, MDA-MB-231, HT29 and NIH 3T3 cells [28]. Triacontane possesses antibacterial, antidiabetic and antitumor activities [29,30].

These biological activities of compounds present in *B. luzonica* leaf extract support the medicinal application of the plant. The study revealed major bioactive compounds present in all of the extracts. Identification of these compounds in the plant serves as the basis in determining the possible health benefits of the plant leading to further biologic and pharmacologic studies.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This study was supported by Faculty of Pharmacy and Office of Grants, Endowments and Partnerships in Higher Education; University of Santo Tomas, Manila, Philippines; Nanocast Lab, Department of Chemistry, Mahidol University, Bangkok, Thailand; Department of Science and Technology, Philippines; and Commission on Higher Education, Philippines.

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