International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 12, 2016

Original Article

PHYTOCHEMICAL CONSTITUENTS OF THE LEAVES OF *LEUCAENA LEUCOCEPHALA* FROM MALAYSIA

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Received: 08 Mar 2016 Revised and Accepted: 15 0ct 2016

ABSTRACT

Objective: This study was conducted to identify the phytochemical constituents of *Leucaena leucocephala* leaf extracts using gas chromatography-mass spectrometry (GC-MS).

Methods: Hexane, petroleum ether, chloroform, ethyl acetate and methanol leaves extract of *L. leucocephala* were analyzed using GC-MS, while the mass spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology (NIST) library.

Results: GC-MS analysis of *L. leucocephala* leaves revealed the presence of 30 compounds and the major chemical constituents were Squalene (41.02%), Phytol (33.80%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (30.86%) and 3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl (25.64%). Some of these compounds have been reported to possess various biological activities such as antioxidant, antimicrobial, hepatoprotective, antiparasitic, insecticide, nematicide, pesticide, anti coronary, antiarthritic, antiandrogenic, hypocholesterolemic, cancer preventive, anti-cancer, analgesic, anesthetic, allergenic and etc.

Conclusion: The findings of this study indicating that *L. leucocephala* leaves possess various potent bioactive compounds and is recommended as a plant of phytopharmaceutical importance.

Keywords: *Leucaena leucocephala*, Medicine, Phytochemical, Chromatography-mass spectrometry (GC-MS), Phytopharmaceutical, antioxidant.

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DOI: http://dx.doi.org/10.22159/ijpps.2016v8i12.11582

INTRODUCTION

Leucaena leucocephala trees or Petai belalang as it is locally known in Malaysia [1] is a tropical multipurpose tree species. Central America and Southeast Asia's genus, Leucaena (English name), formerly contained approximately 50 species of both trees and shrubs that belonged to the Mimosoideae's subfamily of the Fabaceae family. *L. leucocephala* was known as miracle tree because of its worldwide success as a long-lived and highly nutritious forage tree, used as firewood, timber, human food, green manure, shade and erosion control and it is estimated to cover 2-5 million ha worldwide [2, 3]. Almost every part of the *L. leucocephala* species is consumed as human food since the era of the Mayans [4]. In Indonesia, Thailand, and Central America, people eat the young leaves, flowers, and young pods in soups [5, 6]. In addition, it is one of the medicinal plants used to control stomach ache, like contraception and abortifacient. Bioactivity studies on this plant revealed its anthelmintic, antibacterial, anti-proliferative and antidiabetic activities [7].

Various phytochemical compounds have been identified from *L. leucocephala* using phytochemistry approaches to date. The phytochemical screening of leaf extract of *L. leucocephala* revealed the presence of various secondary metabolites as phylobatanins, alkaloid, cardiac glycosides, tannins, flavonoids, saponins and Glycosides [3]. The chemical constituents of the whole plants extracts of *L. leucocephala* from China were ficaprenol-11 (polyprenol), squalene, lupeol,-sitostenone, trans-coumaric acid, cis-coumaric acid, pheophytin-a, pheophorbide a methyl ester, methyl-132-hydroxy-(132-S)-pheophorbide-b and aristophyll-C [8], while the principal chemical constituents of the leaves extracts of the same plant from Mexico were 2(H)-benzofuranone-5,6,7,7a-tetrahydro-4,4,7a-trimethyl, pentadecanoic acid-14-methyl-methyl ester, and 6,10,14-trimethyl-2-pentadecanone a ketone [9].

To the best of our knowledge, since no information is available on the phytochemical screening of *L. leucocephala* leaves from Malaysia. Therefore, the present study was undertaken to investigate the extraction and phytochemical investigations of *L. leucocephala* leaves from Malaysia.

MATERIALS AND METHODS

Plant material

L. leucocephala leaves were collected from Stutong located between 1° 31'8"N Latitude and 110° 22'41"E longitude in Kuching, Sarawak, Malaysia. The plant materials were taxonomically identified and confirmed by Dr. Mohamed Zayed, Forestry and Wood Technology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt. Leaves were washed and dried at 30 °C in an oven until constant weights and ground to a powder with an electric blender.

Sample extraction

25 gm of the leaf powder of *L. leucocephala* for each solvent were weighted, transferred to a flask, treated with hexane, petroleum ether, chloroform, ethyl acetate and methanol until the powder was fully immersed and incubated overnight. The extracts were then filtered through Whatman filter paper No.1 along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted 95% ethanol. The filtrates were then air dried and subjected to gas chromatography-mass spectrometry analysis.

Gas chromatograph-mass spectroscopy (GC-MS)

GC-MS (Shimadzu QP 5000) was performed by using non-polar DB-5 cross-linked column (30 m long x 0.25 mm ID x 0.25 µm film thickness composed of 5% phenyl methyl polysiloxane). The initial temperature was programmed at 50 °C and held for two minutes, and then it was increased to 300 °C with the rate of 6.5 °C/min. The final temperature was held for ten minutes. The temperature of the injector and detector

were set up to 280 °C and 300 °C, respectively. Helium gas was used as a carrier gas. 1 µl of the fractions was diluted in 100 µl hexane and then injected into the GC-MS [24-25]. Interpretation of mass-spectrum was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular mass and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

This is the first study for the phytochemicals screening of *L. leucocephala leaves from* Malaysia. The components present in the hexane, petroleum ether, chloroform, ethyl acetate and methanol extracts of *L. leucocephala leaves* were identified by GC-MS analyzed (fig. 1, 2, 3, 4 and 5). 30 phytocomponents from the *L. leucocephala leaves* have been identified from all the solvent extracts after comparison of the mass spectra with NIST library (table 1) and the major chemical constituents were Squalene (41.02%), Phytol (33.80%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (30.86%) and 3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl (25.64%). In the present investigation, the compounds analyzed in the hexane extracts of *L. leucocephala leaves* were five important compounds and these include tetratetracontane, oxalic acid, allyl hexadecyl ester, squalene, octacosane and hexatriacontane (fig. 1). Meanwhile, six important compounds identified in the petroleum ether extract of *L. leucocephala leaves* as 5-octadecene, 1-octadecyne, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, pentadecanoic acid, 14-methyl-, methyl ester, 9,12-octadecadienoic acid, methyl ester and squalene (fig. 2). Four compounds were detected in the chloroform extract. The compounds were 3,7,11,15-tetramethyl-2-hexadecen-1-ol, hexadecanoic acid, 15-methyl-, methyl ester, 9,12,15-octadecatrienoic acid, methyl ester and 3,7,11-tridecatrienenitrile, 4,8,12-trimethyl (fig. 3). For ethyl acetate extract, nine active component detected were 2-dodecene, 7-hexadecene, 5-octadecene, 1-octadecyne, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 5-eicosene, 9,12,15-octadecatrienoic acid, methyl ester, 1-docosene and squalene (fig. 4). Six photo components were also successfully identified in the methanol extract of *L. leucocephala* leaves as 3,7,11,15-tetramethyl-2-hexadecen-1-ol, heptacosanoic acid, methyl ester, n-hexadecanoic acid, 9,12,15-octadecatrienoic acid, methyl ester, phytol and squalene (fig. 5).

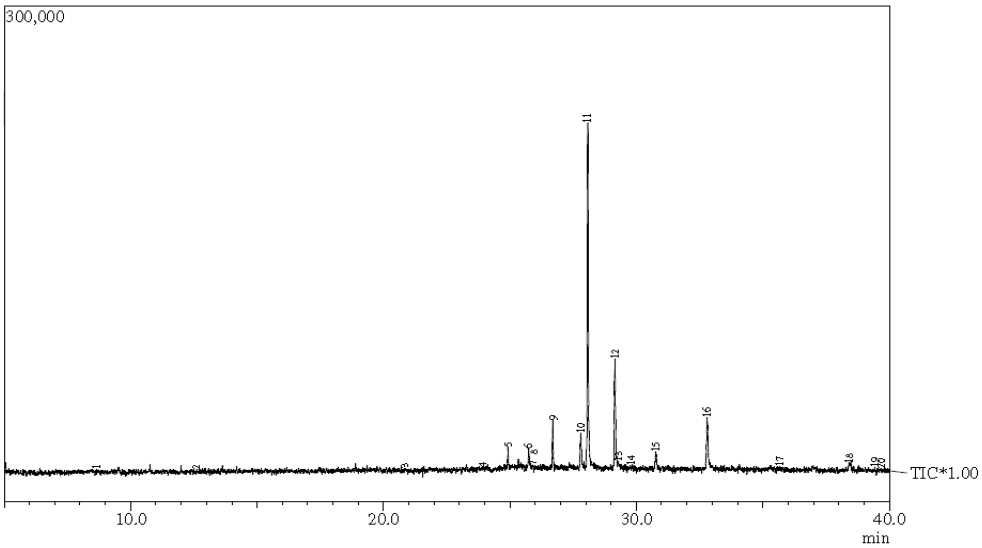

Fig. 1: Gas chromatogram of the hexane extract of the leaves of *L. leucocephala*. Tetratetracontane (9), (10) Oxalic acid, allyl hexadecyl ester, (11) Squalene, (12) Octacosane, (16) Hexatriacontane

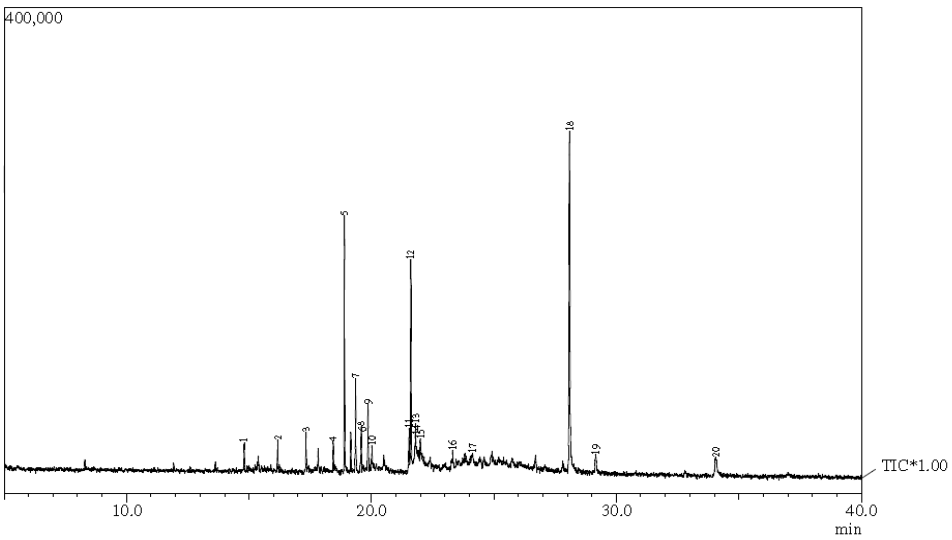

Fig. 2: Gas chromatogram of the petroleum ether extract of the leaves of *L. leucocephala*. 5-Octadecene (2), (3) 2-Bromotetradecane, (5) 1-Octadecyne, (7) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, (8) Eicosane, (9) Pentadecanoic acid, 14-methyl-, methyl ester, (12) 9,12-Octadecadienoic acid, methyl ester, (18) Squalene

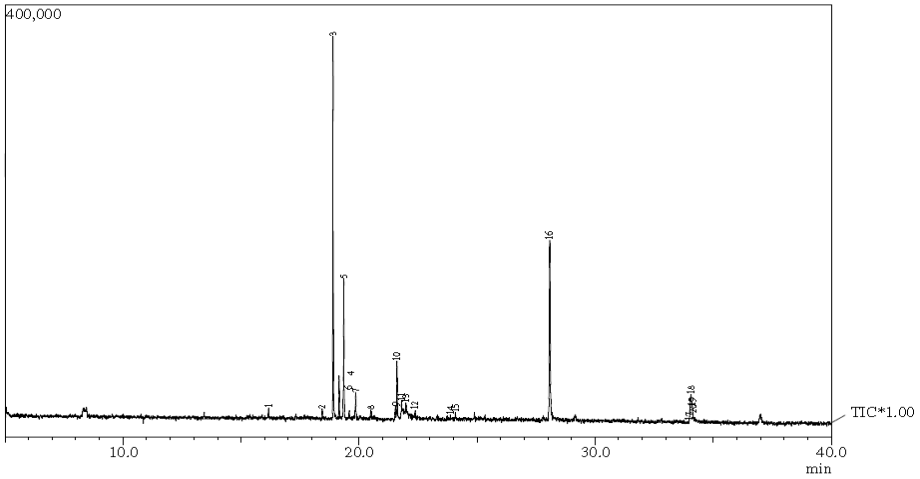

Fig. 3: Gas chromatogram of the Chloroform extract of the leaves of *L. leucocephala*. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (5), (7) Hexadecanoic acid, 15-methyl-, methyl ester, (10) 9,12,15-Octadecatrienoic acid, methyl ester, (16) 3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl

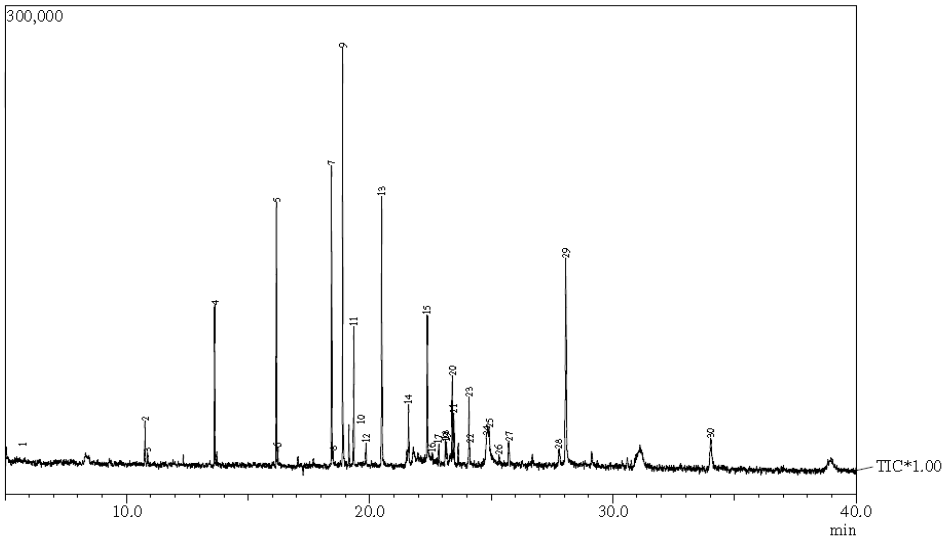

Fig. 4: Gas chromatogram of the ethyl acetate extract of the leaves of *L. leucocephala*. 2-Dodecene (2), (4) 7-Hexadecene, (7) 5-Octadecene, (9) 1-Octadecyne, (11) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, (13) 5-Eicosene, (14) 9,12,15-Octadecatrienoic acid, methyl ester, (23) 1-Docosene, (29) Squalene

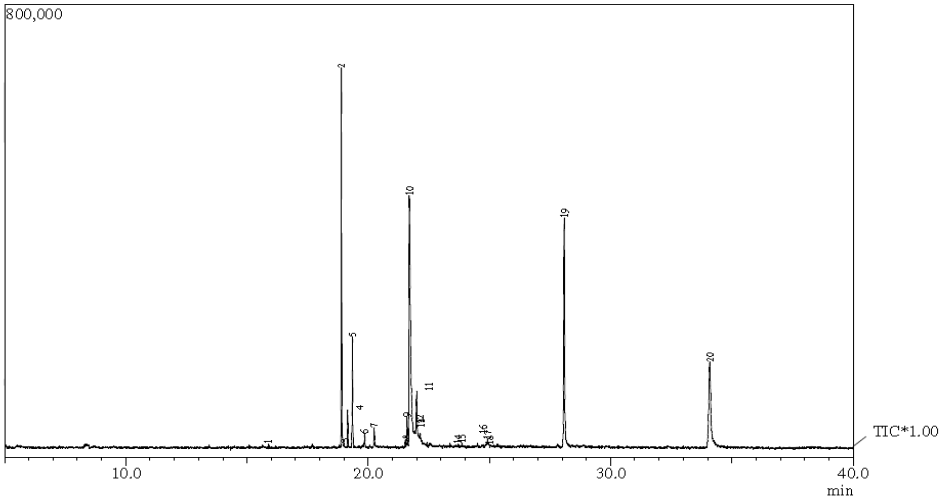

Fig. 5: Gas chromatogram of the methanol extract of the leaves of *L. leucocephala*. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (2), (6) Heptacosanoic acid, methyl ester, (7) n-Hexadecanoic acid, (9) 9,12,15-Octadecatrienoic acid, methyl ester, (10) Phytol, (19) Squalene

Table 1: Phytochemicals screening of solvent extracts of *L. leucocephala* leaves by GC-MS

No.	R. Time	Name of the compound	Molecular formula	Molecular weight	Peak area %
Hexane extract					
1	26.69	Tetratetracontane	$C_{44}H_{90}$	618	5.03
2	27.80	Oxalic acid, allyl hexadecyl ester	$C_{21}H_{38}O_4$	354	6.05
3	28.08	Squalene	$C_{30}H_{50}$	410	41.02
4	29.15	Octacosane	$C_{28}H_{58}$	394	16.00
5	32.80	Hexatriacontane	$C_{36}H_{74}$	506	9.50
Petroleum ether extract					
1	16.16	5-Octadecene	$C_{18}H_{36}$	252	1.83
2	18.90	1-Octadecyne	$C_{18}H_{34}$	250	12.10
3	19.35	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	5.93
4	19.86	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	3.77
5	21.53	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	2.38
6	28.08	Squalene	$C_{30}H_{50}$	410	30.47
Chloroform extract					
1	18.89	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	30.86
2	19.85	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284	2.08
3	21.60	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	5.88
4	28.08	3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl	$C_{16}H_{25}N$	231	25.64
Ethyl acetate extract					
1	10.75	2-Dodecene	$C_{12}H_{24}$	168	1.66
2	13.62	7-Hexadecene	$C_{16}H_{32}$	224	5.23
3	18.43	5-Octadecene	$C_{18}H_{36}$	252	9.59
4	18.89	1-Octadecyne	$C_{18}H_{34}$	250	13.67
5	19.35	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	5.27
6	20.49	5-Eicosene	$C_{20}H_{40}$	280	9.26
7	21.59	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	2.23
8	24.08	1-Docosene	$C_{22}H_{44}$	308	2.80
9	28.07	Squalene	$C_{30}H_{50}$	410	12.28
Methanol extract					
1	18.90	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	16.05
2	19.85	Heptacosanoic acid, methyl ester	$C_{28}H_{56}O_2$	424	0.57
3	20.24	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.95
4	21.60	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	1.55
5	21.70	Phytol	$C_{20}H_{40}O$	296	33.80
6	28.08	Squalene	$C_{30}H_{50}$	410	17.62

In accordance with the previous findings, most of the identified compounds from this study have also been reported elsewhere in other studies on the same species and other species such as squalene was identified in the hexane, petroleum ether, ethyl acetate and methanol extracts of *L. leucocephala* leaves was also reported in the whole plants extracts of the same plant from China [8]. Almost all the present phytochemical compounds have been detected in other species such as 9,12,15-Octadecatrienoic acid, methyl ester, phytol, squalene and 9,12-Octadecadienoic acid, methyl ester in *Cleome chelidonii* [10], Pentadecanoic acid, 14-methyl-, methyl ester in *indigofera suffruticosa* [11] and oxalic acid, allyl hexadecyl ester in *Aloe vera* [12]. Phytol and squalene were identified as the major compound of methanol extract of *L. leucocephala* leaves were also reported as the major compound of *vernonia amygdalina* leaf extracts [13]. Phytol, squalene and n-hexadecanoic acid have been detected in the methanol extract of *Cassia Italica* leaf [14]. Pentadecanoic acid,14-methyl-,methyl ester and n-Hexadecanoic acid were present in the ethanolic extract of *Indigofera suffruticosa* leaves [11]. n-hexadecanoic acid, Phytol, Squalene, and 3,7,11,15–tetramethyl-2-hexadecen-1-ol have been reported in the leaf of ethanol extract of *Hugonia mystax* [15]. Nine compounds were identified in the leaves of *Abrus precatorius* and n-hexadecanoic acid was one of these compounds [16]. Squalene, 9,12-octadecadienoic acid (Z,Z), n-hexadecanoic acid, ethyl ester, Phytol and hexadecanoic acid were detected in a leaf of *Pleiospermium alatum* [17]. The GC-MS analysis has identified 30 compounds from all the solvent extracts from the *L. leucocephala* leaves and some of them have been reported to possess many biological properties such as antimicrobial, anticancer, cancer preventive, diuretic, anti-inflammatory, antioxidant; antitumor, antihistaminic, nematicide, pesticide, antiandrogenic, hypo-cholesterolemic and hepatoprotective as summarized in table 2.

Table 2: Summary of phytochemical compounds identified from the *L. leucocephala* leaf extracts and their therapeutic activity

No	Compound	Secondary metabolite	Therapeutic activity
1	Phytol	Diterpene	Antimicrobial, anticancer, cancer preventive, diuretic, anti-inflammatory
2	Squalene	Triterpene	Antibacterial, antioxidant, antitumor; cancer-Preventive, chemopreventive; immunostimulant, lipoxygenase-inhibitor, perfumery, pesticide, sunscreen
3	n-Hexadecanoic acid	Palmitic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic, flavor, hemolytic, 5-alpha reductase inhibitor
4	Pentadecanoic acid, 14-methyl-, methyl ester	Palmitic acid methyl ester	Antioxidant.
5	Hexadecanoic acid, 15-methyl-, methyl ester	Fatty acid ester	Antioxidant, nematicide, pesticide, flavor, antiandrogenic
6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial
7	9,12,15-Octadecatrienoic acid, methyl ester	Linolenic acid ester	Antiinflammatory, insectifuge hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary
8	9,12-Octadecadienoic acid, methyl ester	Linolenic acid ester	Antiinflammatory, nematicide, insectifuge, hypocholesterolemic, cancer preventive, hepatoprotective, antihistaminic, antiacne, antiarthritic, antieczemic
9	Oxalic acid, allyl hexadecyl ester	Dicarboxylic acid	Acaricide, antiseptic, CNS-paralytic, fatal, hemostatic, irritant, pesticide, renotoxic, varroacide

Modified from Dr. Duke's: Phytochemical and Ethnobotanical Databases [18]

Among the identified phytochemicals, squalene is a triterpene that is an intermediate in the cholesterol biosynthesis pathway [19], and it is used in cosmetics as a natural moisturizer [14, 17]. Squalene is reported to be an antioxidant activity [17, 20, 21], anticancer activity [17, 22] and chemopreventive activity against colon carcinogenesis [17, 23, 24].

n-Hexadecanoic acid (Palmitic acid) is an intermediate in the biosynthesis of sexual pheromones of some insects [25, 26] and it is used as an insecticide and anti-microbial agents [26, 27]. It has the property of antioxidant activity [28] and it is used to decrease the hydrophobicity of virginiamycin in some drugs against Mycobacterium avium [26, 29, 30]. Other antioxidants present were pentadecanoic acid, 14-methyl-, methyl ester and hexadecanoic acid, 15-methyl-, methyl ester.

Phytol is one among the thirty compounds in L. leucocephala leaves which were also found to be effective at different stages of arthritis. It is used as preventive and therapeutic to against arthritis as well as it is a promising novel class of pharmaceuticals for the treatment of rheumatic arthritis and possibly other chronic inflammatory diseases [15, 17, 31]. In addition, it was observed to have antibacterial activities against Staphylococcus aureus by causing damage to cell membranes, as a result, there is a leakage of potassium ions from bacterial cells [14, 32]. It is used along with simple sugar or corn syrup as a hardener in candies and it is a key acyclic diterpene alcohol that is a precursor for vitamins E and K [14].

CONCLUSION

The present phytochemical study of *L. leucocephala* leaf extracts was studied for the first time using five different solvent extracts with increasing polarities. In fact, this is the first available information about the phytoconstituents of *L. leucocephala* from Malaysia. *L. leucocephala* leaf extracts have revealed the presence of many secondary metabolites. The findings of this study confirmed that the *L. leucocephala* leaf extracts could be used as antioxidant, anticancer, cancer-preventive, diuretic, antiandrogenic, antimicrobial, antitumor, antiacne, antiarthritic, antieczemic, anti-inflammatory, hypocholesterolemic, insecticide, nematicide and pesticide. The present study, which reveals the presence of components in *L. leucocephala* leaf extracts suggest that the contribution of these compounds on the pharmacological activity should be evaluated.

ACKNOWLEDGMENT

The authors are thankful to University Malaysia Sarawak for providing necessary facilities to carry out this work.

CONFLICT OF INTERESTS

Declared none

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How to cite this article

- Mohamed Zaky Zayed, Benedict Samling. Phytochemical constituents of the leaves of *Leucaena leucocephala* from malaysia. Int J Pharm Pharm Sci 2016;8(12):174-179.