Comparative Phytochemical Constituents of *Leucaena leucocephala* (Lam.) Leaves, Fruits, Stem Barks, and Wood Branches Grown in Egypt using GC-MS Method Coupled with Multivariate Statistical Approaches

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This work, for the first time, identified the phytochemical constituents of leaves, fruits, stem barks, and wood branches extracted from the tree pruning wastes of *Leucaena leucocephala* (Lam.) de Wit. grown in Egypt, showing 49, 29, 34, and 27 phytocomponents, respectively, as assayed by gas chromatograph-mass spectroscopy (GC-MS) analysis. The major components of leaves were 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (17.7%), betulin (15.7%), lupeol (14.7%), androstan-17-one,3-ethyl-3-hydroxy-, (5à)- (12.3%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (11.6%), betamethasone (9.7%), and β-sitosterol (9.1%). The major phytocomponents of fruits were β-sitosterol (55.7%), 3beta-hydroxy-5-cholen-24-oic acid (48.7%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (42.9%), lupeol (29.3%), betulin (15.8%), stigmasterol (12.8%), and campesterol (7.6%). The major phytocomponents of stem barks were 1,2-benzenedicarboxylic acid, diisooctyl ester (65.7%), β-sitosterol (27.2%), betulin (22.1%), lupeol (21.1%), and 9,12-octadecadienoic acid (Z,Z)-, methyl ester (8.8%). Wood branches contained β-sitosterol (60.1%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (47.2%), lupeol (22.5%), campesterol (15.6%), and stigmasterol (14.1%). Most of the identified compounds have been reported to possess important biological activities, such as antimicrobial, anti-inflammatory, anticancer, anti-arthritic, antioxidant, and antidiabetic activities. The four constituents of *L. leucocephala* were statistically independent in these phytocomponents. The phytocomponents in five solvents were mixed in describing the four constituents. These constituents of *L. leucocephala* are potential bioresources for phytopharmaceutics.

Keywords: Leucaena leucocephala (Lam.); Phytochemical constituents; GC-MS; Principal component analysis

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INTRODUCTION

Leucaena leucocephala (Lam.) de Wit. (Leguminosae), formerly known as *L. glauca* and leuceana as a local name in Egypt (Orwa *et al.* 2009), is a miracle tree or shrub due to its worldwide success as a long-lived, highly nutritious forage tree (Brewbaker and Sorensson 1990), as well as its medicinal efficacy and multipurpose nature (Abdelhady and

Abdallah 2016).

It is native to southern Mexico and northern Central America, but it is now naturalized throughout all continents of the world (Holm *et al.*1979). It is a multipurpose tree or shrub used to produce firewood, timber, human food, green manure, and shade, as well as to control erosion (Brewbaker *et al.* 1972). It is one of the medicinal plants used in traditional medicine to control stomachache, and as a contraceptive and abortifacient agent (Zayed and Samling 2016).

Various secondary metabolites have been identified from *L. leucocephala* using phytochemistry approaches, such as alkaloid, cardiac glycosides, tannins, flavonoids, saponins, and glycosides (Awe *et al.* 2013). The chemical constituents of the leaves of *L. leucocephala* from Malaysia are tetratetracontane, oxalic acid, allyl hexadecyl ester, squalene, octacosane, hexatriacontane, 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, hexadecanoic acid, 15-methyl-, methyl ester, 9,12,15-octadecatrienoic acid, methyl ester, 3,7,11-tridecatrienenitrile, 4,8,12-trimethyl, 2-dodecene, 7-hexadecene, 5-eicosene, 1-docosene, heptacosanoic acid, methyl ester, n-hexadecanoic acid, and phytol (Zayed and Samling 2016). The chemical composition of the leaf 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 (Salem *et al.* 2011), whereas the principal chemical constituents of the whole plant 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 (Chen and Wang 2010).

Medicinally, *L. leucocephala* has various biological effects such as antimicrobial, anthelmintic, antibacterial, anti-proliferative, antidiabetic, anticancer, cancer preventive, diuretic, anti-inflammatory, antioxidant, antitumor, antihistaminic, nematicide, pesticide, antiandrogenic, hypocholesterolemic, and hepatoprotective activities (Meena Devi *et al.* 2013; Zayed and Samling 2016). Almost the whole plant of the *L. leucocephala* species has been consumed as human food and traditional medicine since the era of the Mayans (Brewbaker *et al.* 1972; Zayed and Samling 2016). In Indonesia, Thailand, and Central America, people eat the young leaves, flowers, and young pods as an ingredient for soups and salads.

In the Philippines, the young pods are cooked as a vegetable, and roasted seeds are used as a substitute for coffee or popped like popcorn (Rushkin 1984; Zayed and Samling 2016). Seeds are considered as non-conventional sources of protein together with other leguminous seeds (Rushkin 1984; Zayed and Samling 2016). The bark is eaten for internal pain. A decoction of the root and bark is taken as a contraceptive and depilatory in Latin America (Duke 1983).

Different pharmacological properties of *L. leucocephala* have been reported. However, the phytochemical constituents remain unknown. Therefore, this is the first phytochemical study using GC-MS analysis of these four constituents from *L. leucocephala* to investigate and compare these four constituents grown in Egypt, using gas chromatography-mass spectrometry (GC-MS).

EXPERIMENTAL

Materials

Plant material

Fresh leaves, fruits, stem barks, and wood branches from the tree pruning wastes of *Leucaena leucocephala* (Lam.) de Wit were collected from the nursery of Forestry and Wood Technology Department, Faculty of Agriculture, Alexandria University, Egypt in the middle of September 2016, and the laboratory work was completed at the end of October 2017. The plant materials were taxonomically identified and confirmed by Prof. Dr. Ahmed A. El-Settawy, Head of Forestry and Wood Technology Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt. The samples were dried in the shade in open-air conditions for 6 to 12 days prior to extraction and ground to a powder with an electric blender.

Sample extraction

A total of 100 g of air-dried powder of the leaves, fruits, stem barks, and wood branches of *L. leucocephala* were used for the extraction with each of the following solvents with increasing polarities: hexane, petroleum ether, chloroform, ethyl acetate, and methanol. Approximately 250 mL of each solvent was poured in a 500-mL conical flask that held the air-dried leaves, fruits, stem barks, and wood branches. The flask was closed with a cotton wool plug and wrapped with aluminum foil. The extraction was continued for 72 h under laboratory conditions. The residue was extracted two more times using the same method to ensure complete extraction. The residue was discarded, and the filtrates were combined. The extracts were filtered using Whatman No. 1 filter paper along with 2 g sodium sulfate that was wetted with 95% ethanol to remove any sediments and traces of water in the filtrate. The solvents were evaporated to dryness using a rotary evaporator. All steps were performed three times in order to increase the effectiveness of the extraction process. All extracts were kept dry in sealed white vials and stored at 4 °C until they were used for GC-MS analysis.

Gas chromatograph-mass spectroscopy (GC-MS)

GC-MS analysis of the chemical composition of hexane, petroleum ether, chloroform, ethyl acetate, and methanol extracts of *L. leucocephala* leaves, fruits, stem barks, and wood branches grown in Egypt were performed on a GC-MS device Thermo Scientific Trace GC1300-TSQ 8000 evo equipped with TG-5MS (Waltham, MA, USA); the carrier gas was He with a flow of 1 mL/min. The GC-MS system contained a 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 2 min, and then it was increased to 300 °C at the rate of 6.5 °C/min. The final temperature was held for 10 min. The temperature of the injector and detector were 280 °C and 300 °C, respectively. Next, 1 μL of the fractions was diluted in 100 μL hexane and then injected into the GC-MS (Trabalon *et al.* 2005; Alagammal *et al.* 2012). Spectral data were interpreted using the National Institute Standard and Technology (NIST) database (https://www.nist.gov/). The name, molecular mass, and structure of the components of the test materials were ascertained.

Methods

Multivariate analysis

Principal component analysis (PCA) based on correlation matrix was performed to elucidate the correlated phytochemical composition in leaves, fruits, stem barks, and wood branches of *L. leucocephala.* The results were further analyzed by multivariate statistical approach employing a hierarchical cluster analysis (HCA, Pvclust function in R) associated with proximity score matrix represented as proximity heat-map.

RESULTS AND DISCUSSION

The components present in the hexane, petroleum ether, chloroform, ethyl acetate, and methanol extracts of the four constituents in *L. leucocephala* were identified by GC-MS (Supplementary Figs. 1 to 4). The active principles with their retention time (RT), molecular formula, molecular weight (MW), peak area in percentage, standard index (SI), and reverse standard index (RSI) are presented and the identified compounds are enlisted in Tables 1 through 4.

Phytochemical Constituents of Leaves of *L. leucocephala*

The phytochemical constituents of *L. leucocephala* leaves from Egypt identified from five solvent extracts after comparison of the mass spectra with NIST library (Table 1) were 49 phytocomponents, and the major components were 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (17.7%), betulin (15.7%), lupeol (14.7%), androstan-17-one,3-ethyl-3-hydroxy-, (5à)- (12.3%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (11.6%), betamethasone (9.7%), and β-sitosterol (9.1). These findings were in accordance with results in other studies on the same species and other species such as 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, which was identified as the major compound of petroleum ether extract of *L. leucocephala* leaves, and was also reported as the major compound of *Andrographis paniculata* (Krishnamoorthy and Kalaiselvan 2016) and *Gracilaria dura* (Cyriac and Eswaran 2015) leaf extracts. Squalene, phytol, 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-, n-hexadecanoic acid, and tetratetracontane have been reported in the leaf extract of the same species from Malaysia (Zayed and Samling 2016). Betulin, lupeol, androstan-17-one,3-ethyl-3-hydroxy-, (5à)- and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- were detected as the major compounds of *L. leucocephala* leaves, which were also reported as the major compounds of *Orthosiphon stamineus* (Hossain and Ismail 2013), *Broussonetia luzonica* (Casuga *et al*. 2016), *Andrographis paniculata* (Kalaivani *et al*. 2012), and *L. leucocephala* (Zayed and Samling 2016) leaf extracts, respectively.

Phytochemical Study of Fruits of *L. leucocephala*

Approximately 29 phytochemical compounds were identified in the fruits of *L. leucocephala* using five different solvents (Supplementary Fig. 2) (Table 2). GC-MS analysis of fruit extracts of *L. leucocephala* revealed that the major compounds were β-sitosterol (55.7%), 3beta-hydroxy-5-cholen-24-oic acid (48.7%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (42.9%), lupeol (29.3%), betulin (15.8%), stigmasterol (12.8%), and campesterol (7.6%).

Table 1. Phytochemical Screening of Solvent Extracts of *L. leucocephala* Leaves by GC-MS

Solvent	No.	Retention Time	Compound	Molecular Formula	Molecular Weight	Peak Area (%)	Standard Index(SI)	Reverse Standard Index (RSI)
Hexane	1	18.3	Benzene, (1-butylhexyl)-	$C_{16}H_{26}$	218	1.71	775	802
	2	21.66	Benzene, (1-pentylhexyl)-	$C_{17}H_{28}$	232	9.53	640	712
	3	23.92	Heneicosane, 11-phenyl-	$C_{27}H_{48}$	372	2.49	549	608
	4	36.19	Squalene	$C_{30}H_{50}$	410	1.56	738	842
	5	41.08	β-sitosterol	$C_{29}H_{50}O$	414	2.02	627	695
	6	41.94	Lupeol	$C_{30}H_{50}O$	426	3.08	667	751
	7	42.81	Astaxanthin	$C_{40}H_{52}O_4$	596	1.15	545	549
Petroleum ether	1	33.06	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	17.67	366	372
	2	36.19	2,2,4-Trimethyl-3-(3,8,12,16 -tetramethyl-heptadeca-3,7,11, 15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428	7.5	291	322
	3	36.91	Tetratetracontane	$C_{44}H_{90}$	618	1.95	443	494
	4	38.92	Cyclohexane,1,3,5-trimethyl-2-octadecyl-	$C_{27}H_{54}$	378	2.44	370	373
	5	40.52	Astaxanthin	$C_{40}H_{52}O_4$	596	2.37	497	506
	6	41.09	β-sitosterol	$C_{29}H_{50}O$	414	9.08	612	680
	7	41.21	Oleanolic acid	$C_{30}H_{48}O_3$	456	4.43	385	405
	8	41.7	Androstan-17-one,3-ethyl-3-hydroxy-, (5à)-	$C_{21}H_{34}O_2$	318	12.34	376	381
	9	41.95	Betulin	$C_{30}H_{50}O_2$	442	15.68	580	589
	10	43.58	Rhodoxanthin	$C_{40}H_{50}O_2$	562	5.6	370	374
Chloroform	1	2.22	Ethane, 1,1-diethoxy	$C_6H_{14}O_2$	118	3.02	371	375
	2	29.48	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	1.37	422	448
	3	32.04	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$	624	1.71	464	490
	4	33.06	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	6.81	543	655
	5	34.97	Stearic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{78}O_3$	594	2.88	368	373
	6	36.2	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428	3.96	358	363
	7	37.71	Oleanolic acid	$C_{30}H_{48}O_3$	456	0.42	329	353

	8	39.31	Vitamin E	$C_{29}H_{50}O_2$	430	1.09	375	485
	9	39.39	2-Butenoic acid, 2-methyl-, 2-(acetyloxy)	$C_{27}H_{38}O_8$	490	3.05	389	917
	10	41.1	β-sitosterol	$C_{29}H_{50}O$	414	4.97	549	666
	11	41.21	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	$C_{27}H_{42}O_4$	430	1.67	550	624
	12	41.71	Astaxanthin	$C_{40}H_{52}O_4$	596	3.55	426	437
	13	41.96	Lupeol	$C_{30}H_{50}O$	426	5.68	618	710
	14	42.18	Betamethasone	$C_{27}H_{42}O_5$	446	9.73	360	413
Ethyl acetate	1	2.24	Ethan 1,1-diethoxy-	$C_6H_{14}O_2$	118	3.65	389	427
	2	33.05	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	3.07	405	444
	3	40.31	Oleic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{76}O_3$	592	1.56	464	490
	4	41.09	psi., psi.,- Carotene, 1,1'2,2'-tetrahydro-1,1'-dimethoxy-	$C_{20}H_{24}O_6$	360	5.68	370	403
Methanol	1	20.17	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	3.85	522	713
	2	24.76	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.20	483	557
	3	25.28	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.28	470	542
	4	26.36	Phthalic acid, decyl methyl ester	$C_{19}H_{28}O_4$	320	7.32	460	513
	5	27.22	7,10-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	2.49	485	512
	6	27.32	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	11.63	421	483
	7	27.48	Phytol	$C_{20}H_{40}O$	296	1.00	641	726
	8	33.05	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	8.48	555	658
	9	39.31	Vitamin E	$C_{29}H_{50}O_2$	430	5.29	683	739
	10	41.09	β-sitosterol	$C_{29}H_{50}O$	414	5.36	604	695
	11	41.94	Lupeol	$C_{30}H_{50}O$	426	14.67	644	709
	12	42.82	Betulin	$C_{30}H_{50}O2$	442	3.14	448	479

Table 2. Phytochemical Screening of Solvent Extracts of *L. leucocephala* Fruits by GC-MS

Solvent	No.	Retention Time	Compound	Molecular Formula	Molecular Weight	Peak Area (%)	Standard Index (SI)	Reverse Standard Index (RSI)
Hexane	1	20.05	Benzene,(1-butylheptyl)-	$C_{16}H_{26}$	218	2.51	823	868
	2	20.22	Benzene, (1-propyloctyl)-	$C_{17}H_{28}$	232	2.12	775	802
	3	21.93	Benzene,(1-propylnonyl)-	$C_{18}H_{30}$	246	3.31	640	711
	4	33.05	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	2.56	520	580
	5	40.21	Campesterol	$C_{28}H_{48}O$	400	4.41	711	737
	6	40.52	Stigmasterol	$C_{29}H_{48}O$	412	6.35	778	787
	7	40.81	Tetratetracontane	$C_{44}H_{90}$	618	2.08	673	750
	8	41.09	β-sitosterol	$C_{29}H_{50}O$	414	27.34	733	754
	9	41.95	Lupeol	$C_{30}H_{50}O$	426	9.32	657	716
Petroleum ether	1	35.13	Tetratetracontane	$C_{44}H_{90}$	618	6.83	699	712
	2	40.21	Campesterol	$C_{28}H_{48}O$	400	7.51	737	758
	3	40.51	Stigmasterol	$C_{29}H_{48}O$	412	12.57	766	772
	4	41.09	3Beta-hydroxy-5-cholen-24-oic acid	$C_{24}H_{38}O_3$	374	48.70	571	580
	5	41.95	Lupeol	$C_{30}H_{50}O$	426	13.09	693	738
Chloroform	1	33.05	Scytalone	$C_{10}H_{10}O4$	194	6.61	752	999
	2	40.21	Campesterol	$C_{28}H_{48}O$	400	7.55	655	688
	3	40.52	Stigmasterol	$C_{29}H_{48}O$	412	12.83	696	715
	4	41.09	β-sitosterol	$C_{29}H_{50}O$	414	55.66	732	766
	5	41.95	Betulin	$C_{30}H_{50}O_2$	442	15.76	668	682
Ethyl acetate	1	33.06	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	42.86	510	560
	2	35.14	Nonacosane	$C_{29}H_{60}$	408	4.92	567	656
	3	40.21	Campesterol	$C_{28}H_{48}O$	400	3.90	634	668
	4	40.53	Stigmasterol	$C_{29}H_{48}O$	412	7.54	660	686
	5	41.09	β-sitosterol	$C_{29}H_{50}O$	414	31.37	736	777
	6	41.95	Lupeol	$C_{30}H_{50}O$	426	7.90	660	737
Methanol	1	40.21	Campesterol	$C_{28}H_{48}O$	400	6.60	667	699
	2	40.52	Stigmasterol	$C_{29}H_{48}O$	412	10.85	711	723
	3	41.09	β-sitosterol	$C_{29}H_{50}O$	414	53.22	752	783
	4	41.95	Lupeol	$C_{30}H_{50}O$	426	29.33	710	761

Table 3. Phytochemical Screening of Solvent Extracts of *L. leucocephala* Stem Bark by GC-MS

Solvent	NO.	Retention Time	Compound	Molecular Formula	Molecular Weight	Peak Area (%)	Standard Index (SI)	Reverse Standard Index (RSI)
Hexane	1	18.29	Benzene, (1-butylhexyl)-	$C_{16}H_{26}$	218	1.22	666	685
	2	20.05	Benzene, (1-butylheptyl)-	$C_{17}H_{28}$	232	6.65	670	694
	3	21.65	Benzene, (1-pentylheptyl)-	$C_{18}H_{30}$	246	8.84	848	853
	4	23.25	Benzene, (1-pentyloctyl)-	$C_{19}H_{32}$	260	6.68	744	756
	5	32.55	Phenol, 2,4-bis(1-methyl-1-phenylethyl)-	$C_{24}H_{26}O$	330	0.55	481	822
	6	33.05	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	0.96	566	661
	7	40.20	Campesterol	$C_{28}H_{48}O$	400	0.90	576	622
	8	40.51	Stigmasterol	$C_{29}H_{48}O$	412	1.06	653	672
	9	41.08	β-sitosterol	$C_{29}H_{50}O$	414	5.13	752	801
	10	41.94	Lupeol	$C_{30}H_{50}O$	426	4.29	670	724
Petroleum ether	1	33.05	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	11.74	530	612
	2	40.20	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	4.78	492	515
	3	40.51	Ergosta-5, 22-dien-3-ol, acetate, (3á,22E)- (Brassicasterol acetate)	$C_{30}H_{48}O_2$	440	5.43	622	688
	4	41.08	β-sitosterol	$C_{29}H_{50}O$	414	27.16	744	789
	5	41.93	Lupeol	$C_{30}H_{50}O$	426	21.08	633	702
Chloroform	1	33.05	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	59.13	520	589
	2	40.52	Stigmasterol	$C_{29}H_{48}O$	412	4.16	564	582
	3	41.09	β-sitosterol	$C_{29}H_{50}O$	414	19.13	750	787
	4	41.94	Lupeol	$C_{30}H_{50}O$	426	15.24	680	752
Ethyl acetate	1	33.05	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	65.73	535	574
	2	40.52	Stigmasterol	$C_{29}H_{48}O$	412	4.10	652	671
	3	41.09	β-sitosterol	$C_{29}H_{50}O$	414	18.62	738	784
	4	41.95	Lupeol	$C_{30}H_{50}O$	426	11.55	631	694
Methanol	1	24.76	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	5.16	480	547
	2	25.30	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.13	475	552
	3	26.36	Phthalic acid, methyl nonyl ester	$C_{18}H_{26}O_4$	306	3.73	440	523
	4	27.22	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	8.81	488	521
	5	27.31	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	6.74	435	493
	6	33.05	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	4.79	545	668
	7	39.31	Vitamin E	$C_{29}H_{50}O_2$	430	1.56	620	672
	8	40.21	Campesterol	$C_{28}H_{48}O$	400	4.76	745	770
	9	40.52	Stigmasterol	$C_{29}H_{48}O$	412	5.90	738	745
	10	41.09	β-sitosterol	$C_{29}H_{50}O$	414	22.56	750	782
	11	41.95	Betulin	$C_{30}H_{50}O_2$	442	22.06	626	642

Table 4. Phytochemical Screening of Solvent Extracts of *L. leucocephala* Wood Branches by GC-MS

Solvent	No.	Retention Time	Compound	Molecular Formula	Molecular Weight	Peak Area (%)	Standard Index (SI)	Reverse Standard Index (RSI)
Hexane	1	22.90	Benzene,(1-methylundecyl)-	$C_{18}H_{30}$	246	3.21	838	845
	2	23.25	Benzene,(1-pentyloctyl)-	$C_{19}H_{32}$	260	4.15	748	759
	3	40.21	Campesterol	$C_{28}H_{48}O$	400	10.36	737	761
	4	40.52	Stigmasterol	$C_{29}H_{48}O$	412	10.23	738	747
	5	41.09	β-sitosterol	$C_{29}H_{50}O$	414	46.92	753	775
	6	41.95	Lupeol	$C_{30}H_{50}O$	426	7.27	650	722
Petroleum ether	1	40.21	Campesterol	$C_{28}H_{48}O$	400	15.64	726	726
	2	40.51	Stigmasterol	$C_{29}H_{48}O$	412	14.09	761	771
	3	41.10	β-sitosterol	$C_{29}H_{50}O$	414	60.09	741	755
	4	41.95	Lupeol	$C_{30}H_{50}O$	426	10.18	708	758
Chloroform	1	33.06	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	7.38	570	677
	2	40.21	Campesterol	$C_{28}H_{48}O$	400	13.79	704	739
	3	40.52	Stigmasterol	$C_{29}H_{48}O$	412	12.36	691	704
	4	41.09	β-sitosterol	$C_{29}H_{50}O$	414	53.50	738	772
	5	41.95	Lupeol	$C_{30}H_{50}O$	426	11.09	614	696
Ethyl acetate	1	33.06	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	47.18	547	667
	2	40.21	Campesterol	$C_{28}H_{48}O$	400	6.73	677	710
	3	40.53	Stigmasterol	$C_{29}H_{48}O$	412	5.99	696	709
	4	41.09	β-sitosterol	$C_{29}H_{50}O$	414	33.71	733	763
	5	41.95	Lupeol	$C_{30}H_{50}O$	426	6.38	603	664
Methanol	1	24.76	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	4.94	473	544
	2	27.22	12,15-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	4.58	498	511
	3	27.31	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	352	5.32	465	498
	4	40.21	Campesterol	$C_{28}H_{48}O$	400	8.56	531	579
	5	40.51	Stigmasterol	$C_{29}H_{48}O$	412	7.45	561	579
	6	41.08	β-sitosterol	$C_{29}H_{50}O$	414	40.12	717	783
	7	41.95	Lupeol	$C_{30}H_{50}O$	426	20.53	634	699

There was no previous report on the phytochemical screening of hexane, petroleum ether, chloroform, ethyl acetate, and methanol extracts of *L. leucocephala* fruit by GC-MS. The major compounds were also reported elsewhere in different species, such as β-sitosterol in *Triphaladi rasayana* (Muthiah *et al.* 2017), 3beta-hydroxy-5-cholen-24-oic acid in *Moringa oleifera* (Agboke and Attama 2016), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester in *Andrographis paniculatas* (Hossain and Ismail 2013), lupeol in *Albizia adianthifolia* (Abubakar and Majinda 2016), betulin in *Orthosiphon stamineus* (Cyriac and Eswaran 2015), stigmasterol in *Albizia adianthifolia* (Abubakar and Majinda 2016), and campesterol in *Moringa oleifera* (Agboke and Attama 2016). These major compounds had important biological activities for medical application. β-sitosterol is known to reduce cholesterol and control benign prostrate hypertrophy as well as inflammation (Saleem 2009). Lupeol has anti-inflammatory and anti-cancer activities (Geetha and Varalakshmi 2001). Betulin has anticancer and apoptosis activities (Patočka 2003). Stigmasterol has thyroid inhibitory, antiperoxidative, and hypoglycemic effects (Panda *et al.* 2009). Campesterol is known to have cholesterol-lowering and anticarcinogenic effects (Choi *et al.* 2007).

Phytochemical Composition of Stem Barks of *L. leucocephala*

In the GC-MS analysis, 34 bioactive phytochemical compounds were identified in the five different solvents: hexane, petroleum ether, chloroform, ethyl acetate, and methanol extracts in the bark of *L. leucocephala* (Supplementary Fig. 3). The identification of phytochemical compounds was based on the peak area, molecular weight, molecular formula, standard index (SI), and reverse standard index (RSI). The identified high peak area (1,2-benzenedicarboxylic acid, diisooctyl ester) ($C_{24}H_{38}O_4$) with RT 33.05 had a peak area of 65.7%, β-sitosterol ($C_{29}H_{50}O$) with RT 41.1 had a peak area of 27.2%, betulin ($C_{30}H_{50}O_2$) with RT 42.0 had a peak area of 22.1%, lupeol ($C_{30}H_{50}O$) with RT 41.9 had a peak area of 21.1%, and 9,12-octadecadienoic acid (Z,Z)-, methyl ester ($C_{19}H_{34}O_2$) with RT 27.22 had a peak area of 8.8%. The results are presented in Table 3.

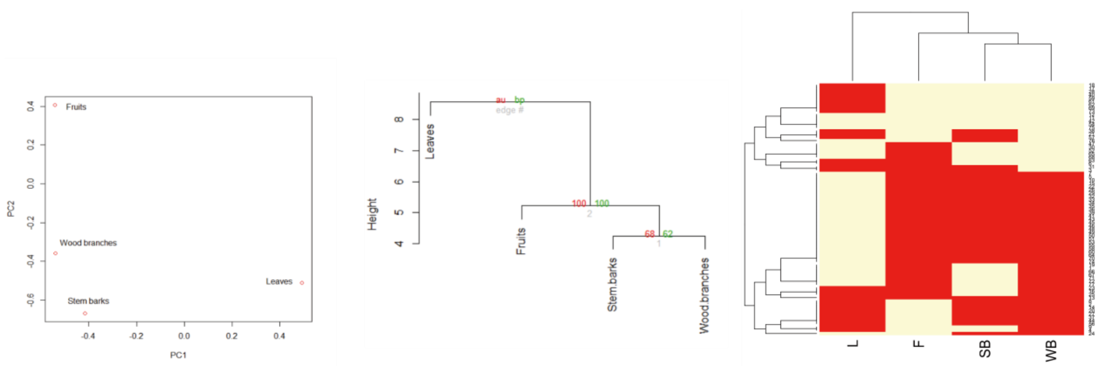
Similar compounds were found in stem bark extracts: 1,2-benzenedicarboxylic acid, diisooctyl ester, lupeol, and 9,12-octadecadienoic acid (Z,Z)-, methyl ester were observed in the bark of *Pterocarpus marsupium* (Maruthupandian and Mohan 2011). 1,2-Benzenedicarboxylic acid, diisooctyl ester was detected as one of the major compounds in the bark of *Pleiospermium alatum* (Parthipan *et al.*2015). 1,2-Benzenedicarboxylic acid, diisooctyl ester (48.8%) was found to be a major component followed by 9,12-octadecadienoic acid (Z,Z) in *Hugonia mystax* L. (Rajeswari *et al.* 2012). 1,2-Benzenedicarboxylic acid, diisooctyl ester and stegmasterol were detected in stem bark of *Kirganelia reticulate* (Reddy *et al.* 2017). Lupeol and 9,12-octadecadienoic acid (Z,Z)-, methyl ester were found in the bark of *Zanthoxylum rhetsa* (Santhanam *et al.* 2016). Lupeol and betulin were observed in the bark of *Alnus glutinosa* L. (Felföldi-Gáva *et al.* 2012). β-sitosterol, stigmasterol, and lupeol were found in the aerial parts of *Kirganelia reticulate* (Mohan *et al.*2013). β-sitosterol, stigmasterol, and 9,12-octadecadienoic acid (Z,Z)- were identified in the bark of *Dolichandrone atrovirens* (Deepa and Murugesh 2013).

Phytochemical Composition of Wood Branches of *L. leucocephala*

GC-MS analysis of hexane, petroleum ether, chloroform, ethyl acetate, and methanol extracts of *L. leucocephala* wood branches were presented in Supplementary Fig. 4 and Table 4. There were 27 compounds revealed in the hexane, petroleum ether, chloroform, ethyl acetate and, methanol extracts of *L. leucocephala* wood branches with the presence of β-sitosterol (60.1%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (47.2%), lupeol (22.5%), campesterol (15.6%), and stigmasterol (14.1%) (Table 4). This was the first study for the chemical composition of wood branches of *L. leucocephala* from Egypt. The major compounds of the hexane, petroleum ether, chloroform, ethyl acetate, and methanol extracts of wood branches of *L. leucocephala* were β-sitosterol, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, lupeol, campesterol, and stigmasterol. These major compounds were also found in species other than *L. leucocephala* such as *Dolichandrone atrovirens* (Deepa and Murugesh 2013), *Pleiospermium alatum* (Parthipan *et al.* 2015), *Dolichandrone atrovirens* (Deepa and Murugesh 2013), *Robinia pseudoacacia* (Hosseinihashemi and Kanani 2012), and *Kirganelia reticulate* (Mohan *et al.* 2013), respectively. In *Robinia pseudoacacia* heartwood extracts also were found to be 13.8% stigmasterol, 6.1% hexadecanoic acid, and 3.5% campesterol (Hosseinihashemi and Kanani 2012). Stigmasterol was one of the major compounds of *Albizia adianthifolia* heartwood extracts (Abubakar and Majinda 2016). Stigmasterol and campesterol were isolated from wood extracts of *Zanthoxylum quinduense* (Patiño Ladino and Cuca Suárez 2010).

Multivariate Statistical Analysis of the Phytochemical Constituents of Leaves, Fruits, Stem Barks, and Wood Branches of *L. leucocephala*

Multivariate statistical analysis revealed interconnected correlation patterns among the phytochemical components of the four constituents of *L. leucocephala* (Fig. 1).



(a) (b) (c)

Fig. 1. Multivariate statistical analysis of the phytochemical constituents of leaves, fruits, stem barks and wood branches**.** (a) Principal component analysis (PCA) of four traits (leaves, fruits, stem barks, and wood branches) in *L. leucocephala*. A PCA was based on the correlation matrix of phytochemical composition. The first and second principal components accounted for 53.1% and 25.8% of variation, respectively; (b) A consensus tree of the relationships among leaves, fruits, stem barks, and wood branches in *L. leucocephala*. Pvclust package in R was used to cluster these traits according to the Euclidean distance matrix. The numbers at the forks were the percentages of approximately unbiased (AU; in red) p-values and bootstrap probabilities (BP, in green) estimated from 1000 bootstrapping samples; (c) A heatmap showing cluster groups in both phytochemical composition and different traits of *L. leucocephala* (leaves, fruits, stem barks, and wood branches). Symbols “L” stands for leaves, “F” stands for fruits, “SB” stands for stem barks, and “WB” stands for wood branches. The numbers on the right side represent the codes for 77 phytochemical components within five solvents that showed a mixed pattern.

Partial convergence of phytochemical composition resulted due to synonymous primary and secondary metabolic pathways, which gave rise to identification of common phytochemicals or their derivatives in multiple parts of the plant. For instance, several compounds were identified in multiple parts of *L. leucocephala*, such as representing common or overlapping metabolic pathways. Spatial arrangement of the phytochemical composition in the four traits of *L. leucocephala* in the double-positive quadrant signified the presence of interrelated phytochemical composition (Fig. 1a). The loading plot of principal components 1 and 2 accounted for 53.1% and 25.8% of variance, respectively. Moreover, clustering of phytochemical composition exhibited a mixed pattern in describing the variation of the four traits of *L. leucocephala.* Furthermore, the presence of fairly distinct phytochemical composition in the leaves and other parts of *L. leucocephala* was highlighted by their isolated spatial location in the component plot as well as the correlation matrix (Table 5). It was interesting that the clustering analysis using a hierarchical method (Fig. 1b) showed a concordant pattern with PCA analysis. Phytochemical composition of the traits of *L. leucocephala* were grouped into various clusters according to their proximities. The proximity heat map (Fig. 1c) corroborated the correlation matrix demonstrating similar phytochemical composition in the four traits of *L. leucocephala*.

Table 5. Correlation Coefficient Matrix of Phytochemical Composition of *L. leucocephala* in Leaves, Fruits, Stem Barks, and Wood Branches

	Leaves	Fruits	Stem Barks	Wood Branches
Leaves	1.0000			
Fruits	-0.5882 (1.86×10^{-8})	1.0000		
Stem barks	-0.1691 (0.1414)	0.1934 (0.0919)	1.0000	
Wood branches	-0.3059 (0.0919)	0.4399 (6.25×10^{-5})	0.5185 (1.35×10^{-6})	1.0000

Note: p-values for statistical tests are in parentheses

Discussion

From the GC-MS analysis of all solvent extracts of *L. leucocephala* leaves, fruits, stem bark, and wood branches, 49, 29, 34, and 27 phytocomponents were identified from leaves, fruits, stem bark, and wood branch extracts, respectively. Some of the identified compounds contribute to various biological effects such as anti-microbial, anti-cancer, antimutagenic, antipeptic, antiseptic, antispasmodic, antiadrenogenic, and hypocholesterolemic activities, as summarized in Table 6 (Duke 2007).

Plasticizer compounds such as 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester and 1,2-benzenedicarboxylic acid, diisooctyl ester have been found as one of the major compounds in the four trait extracts of *L. leucocephala*. β-sitosterol, lupeol, and betulin were also identified as the major compounds of these four trait extracts of *L. leucocephala*. Stigmasterol and campesterol were present in fruits, stem barks, and wood branch extracts of *L. leucocephala*.

Phytol and squalene have been shown in leaf extracts of *L. leucocephala*. Vitamin E was found in the leaves and stem bark extracts of *L. leucocephala*. Hexadecanoic acid, methyl ester, n-hexadecanoic acid, and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z) were also found in the leaves, stem bark, and wood branch extracts of *L. leucocephala*.

Table 6. Summary of Chemical Compounds Identified from the Extracts of *L. leucocephala* Leaves, Fruits, Stem Barks, and Wood Branches and their General Biological Activities (Modified from Dr. Duke's: Phytochemical and Ethnobotanical Databases 2007)

Compound	Material	Secondary Metabolite	Biological Activities
Squalene	Leaves	Triterpene	Antibacterial, antioxidant, antitumor, cancer-preventive, chemopreventive, immunostimulant, lipoxygenase-inhibitor, perfumery, pesticide, sunscreen
β-sitosterol	Leaves, fruits, stem barks, wood branches	Steroids	Antimicrobial, anticancer, anti-inflammatory, anti-asthma, diuretic, antiarthritic
Lupeol	Leaves, fruits, stem barks, wood branches	Pentacyclic triterpene	Anti-inflammatory, antiarthritic, antimicrobial, antitumor, antiprotozoal, chemopreventive, antibacterial, antioxidant, cancer preventive, immunostimulant, lipoxygenase inhibitor, pesticide
Astaxanthin	Leaves	Tetraterpen	Antioxidant, anti-inflammatory
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	Leaves, stem barks, wood branches	Plasticizer compound	Antifouling, antimicrobial, antifungal, anti-retroviral, anti-tumor, anti-diabetic, anti-cancer, antioxtioxidant, anti-scabies, anti-inflammatory, potent antimicrobial agent
Tetratetracontane	Leaves, fruits	Alkane	Antioxidant, anti-inflammatory, antibacterial, antiulcerogenic
Cyclohexane,1,3,5-trimethyl-2-octadecyl-	Leaves	Alcohol	Anticancer
Oleanolic acid	Leaves	Pentacyclic triterpene	Antioxidant, antimicrobial, anti-inflammatory, antibacterial
Androstan-17-one,3-ethyl-3-hydroxy-, (5à)-	Leaves	Steroid	Neuroactive, analgesic, anesthetic
Betulin	Leaves, fruits, stem barks	Pentacyclic triterpene	Anticancer, anti-HIV, anti-bacterial, antimalarial, anthelmintic, antifeedant, antimicrobial
Ethane, 1,1-diethoxy	Leaves,	Ether	Flavoring agent
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	Leaves	Fatty acid	Antioxidant, hypocholesterolemic, antiandrogenic, hemolytic
Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	Leaves	Fatty acid	Hypocholesterolemic, antiarthritic, nematicide, 5-alpha reductase inhibitor, antiacne, hepatoprotective
Stearic acid, 3-(octadecyloxy)propyl ester	Leaves	Fatty acid methyl ester	5-alpha-reductase-inhibitor, cosmetic, flavor, hypocholesterolemic, lubricant, perfumery, propecic, suppository
Vitamin E	Leaves, stem barks	Vitamin	Antiageing, analgesic, antidiabatic, antiinflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary
Betamethasone	Leaves	Steroid	Anti-inflammatory

Oleic acid, 3-(octadecyloxy)propyl ester	Leaves	Fatty acid	Cancer preventive, flavor, hypocholesterolemic, 5-alpha reductase inhibitor, antiandrogenic, perfumery, insectifuge, anti-inflammatory, anemiagenic, dermatitigenic, choleretic
O-Methyl-d-glucose	Leaves	Glycosides	Preservative
Hexadecanoic acid, methyl ester	Leaves, stem barks, wood branches	Palmitic acid methyl ester (fatty acid methyl ester)	Antioxidant, nematicide, pesticide, flavor, antiandrogenic
n-Hexadecanoic acid	Leaves, stem barks	Palmitic acid (fatty acid)	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavor, hemolytic, 5-alpha reductase inhibitor
7,10-Octadecadienoic acid, methyl ester	Leaves	Unsaturated fatty acid methyl ester	Anti-inflammatory, nematicide, insectifuge, hypocholesterolemic, cancer preventive, hepatoprotective, antihistaminic, antiacne, antiarthritic, antieczemic

The founding compounds from the GC-MS analysis of all solvent extracts of the four constituents of *L. leucocephala* had important biological activities for medical applications, such as plasticizer compounds that were reported to have antifouling and antimicrobial activities (Sermakkani and Thangapandian 2012). β-sitosterol has the properties of anticancer, antimicrobial, antiasthma, diuretic, antiarthritic, and anti-inflammatory (Mohan *et al.* 2013). Lupeol is one of the triterpenoids that is used as anti-inflammatory activity (Saleem 2009) and anti-cancer (Geetha and Varalakshmi 2001). Betulin has been reported to have antimalarial, anticancer, anti-HIV, anthelmintic, anti-bacterial, antimicrobial, and antifeedant activities (Moghaddam *et al.* 2012). Stigmasterol has several pharmacological prospects such as antiosteoarthritic, antihypercholestrolemic, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory, and CNS effects (Marquis *et al.* 1977; Jeon *et al.* 2005). Campesterol and stigmasterol are the most commonly occurring phytosterols in the human diet; each contribute about 30% and 3% of diet contents, respectively (Weihrauch and Gardner 1978; Harcombe and Baker 2014). Campesterol has been reported to have cholesterol-lowering and anticarcinogenic effects (Kelly 1999). The diterpene phytol had anticancer, diuretic, antimicrobial, cancer preventive, and anti-inflammatory activities (Sermakkani and Thangapandian 2012).

CONCLUSIONS

1. In the present study, the leaves, fruits, stem barks, and wood branches supplied from the tree pruning wastes of *L. leucocephala* contained many important phytochemical components.
2. The GC-MS analysis of hexane, petroleum ether, chloroform, ethyl acetate, and methanol extracts of these four constituents of *L. leucocephala* showed the presence of 49, 29, 34, and 27 phytocomponents, respectively. Plasticizer compounds, β-sitosterol, lupeol, and betulin were identified as the major compounds of four constituents extracts of *L. leucocephala.* Stigmasterol and campesterol were present in fruits, stem barks, and wood branch extracts. Phytol and squalene have been shown in leaf extracts. Vitamin E was found in the leaves and stem bark extracts. Hexadecanoic acid, methyl este, n-hexadecanoic acid, and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z) were also found in these four constituents extracts of *L. leucocephala*.
3. Among the identified compounds, 24, 10, 14, and 8 compounds were from leaves, fruits, stem barks and wood branches extracts of *L. leucocephala*, respectively. These compounds have been reported to possess various biological activities such as antifungal, antimicrobial, antitumor, antidiabetic, anticancer, antioxidant, antiscabies, anti-inflammatory, thyroid inhibitory, antiperoxidative, antimicrobic, hypoglycemic, anticancer, diuretic, antiarthritic, antiasthma, antitetany, and antianemia, *etc*.
4. The presence of various bioactive compounds justified the use of the leaves, fruits, stem barks, and wood branches of *L. leucocephala* for various ailments by traditional practitioners. Therefore, the authors recommend using the leaves, fruits, stem barks and, wood branches of *L. leucocephala* as bioresources of phytopharmaceutical importance. However, further studies need to be undertaken to fully ascertain its bioactivity.

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