Pharmacogn J. 2018; 10(1):110-119
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Original Article

GC-MS Analysis of Phytochemical Compounds in *Syzygium polyanthum* Leaves Extracted using Ultrasound-Assisted Method

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History
- Submission Date: 02-10-2017;
- Review completed: 31-10-17;
- Accepted Date: 20-11-2017

DOI : 10.5530/pj.2018.1.20

Article Available online
http://www.phcogj.com/v10/i1

ABSTRACT

Context: *Syzygium polyanthum* has been traditionally formulated by the folklore for the treatment of diseases including diarrhea, rheumatism, diabetes mellitus, hypercholesterolemia, hypertension, gastritis and hyperuricemia. Normally, its phytochemicals are always extracted using solvent, maceration and steam distillation methods, but the use of ultrasound-assisted extraction (UAE) method is still not well documented. **Aims:** This study aims to extract the phytochemical compounds present in *S. polyanthum* leaves using UAE and to identify them by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. **Methodology:** The leaves were consecutively soaked with n-hexane, ethyl acetate and methanol in a bath sonicator to derive n-hexane (HSP), ethyl acetate (EASP), and methanol (MSP) extracts of *S. polyanthum* leaves and then the extracts were subjected to GC-MS analysis. Mass-spectral databases of peaks were compared with database from Wiley, NIST and FNSCC libraries for compound identification. **Results:** GC-MS analyses of HSP, EASP and MSP showed the presence of 21, 27, and 31 peaks, respectively. The major compound for HSP (31.912%), EASP (27.042%), and MSP (22.386%) were unknown compounds which were detected at retention time between 61.980 and 62.29 min, thus requires further characterization. Squalene and phytol were among the other major compounds present in all three extracts. Several identified compounds in the extracts such as squalene, phytol, hentriacontane, palmitic acid, α-pinene, nerolidol, linalool, α-tocopherol and β-tocopherol were known bioactive compounds. **Conclusion:** GC-MS analyses of n-hexane (HSP), ethyl acetate (EASP), and methanol (MSP) of *S. polyanthum* leaves extracts have revealed the presence of some known bioactive compounds with therapeutic importance.

Key words: *Syzygium polyanthum*, GC-MS, Bioactive compound, Ultra-sound-assisted, Sonication

INTRODUCTION

Syzygium polyanthum (Wight) Walp. or in Malay known as 'salam' or 'serai kayu' is an ethnomedicinal plant commonly used among Malay folks as fresh salad or as flavour enhancer in cuisines.[1] Other than that, the leaves are traditionally consumed for treating hypertension, diabetes mellitus, diarrhea, rheumatism, hypercholesterolemia, gastritis and hyperuricemia.[2] Few studies have discussed on the phytochemical constituents of *S. polyanthum* leaves. Amalina *et al*[3] reported the presence of α-pinene (30.88%) as the major compound, followed by octanal (18.30%) and α-caryophyllene (6.22%) while Hamad *et al* [4] reported another major compound which is cis-4-decanal (43.49%) in essential oil of *S. polyanthum* leaves. The later study also stated that the essential oil were mainly composed of aldehyde, hydrocarbons and some bioactive compounds such as β-caryophyllene, α-humulene, caryophyllene oxide, α-copaene, α-selinene and α-zingiberene.[4] Other than studies on the leaves essential oil, there were few studies which focused on the leaves crude extracts. Preliminary phytochemical screening analysis of crude methanolic *S. polyanthum* leaves extract showed the presence of tannins, flavonoids, glycosides, alkaloids, carbohydrates, steroids, triterpenoids and flavonoids.[5,6] Phenolic compounds such as caffeic acid and gallic acid were previously identified in crude macerated methanolic extract of *S. polyanthum* leaves by high performance liquid chromatography and liquid chromatography mass spectrometry analyses.[7] Analysis on the crude macerated methanolic extract of *S. polyanthum* leaves has detected squalene as the major compound.[5,8] Another analysis on the macerated n-hexane extract similarly found squalene as the major compound (30.45%) followed by n-hentriacontane (6.57%).[8]

Most phytochemicals previously studied were extracted using solvent maceration and steam distillation methods, however, the use of ultrasound-assisted extraction (UAE) method is not well-documented. The present study has utilized UAE method, a different extraction method from the other previous studies. Basically, UAE method

Cite this article: Rahim ENAA, Ismail A, Omar MN, Rahmat UN, Ahmad WANW. GC-MS Analysis of Phytochemical Compounds in *Syzygium polyanthum* Leaves Extracted using Ultrasound-Assisted Method. Pharmacog J. 2018;10(1):110-9.

enhances solvent penetration through plant cell with the aid of sound wave.[9] Its mechanistic action includes fragmentation, erosion, and destruction-detexturation of plant structures, thus, this will enhance the diffusion process by increasing the solute transfer rate.[10,11] This method is usually employed for its higher extraction efficiency and its shorter extraction time as compared to maceration and soaking with the less amount of solvent application.[9,10] Recently, UAE method was applied in many researches to enhance extractability of phenolic, thermo-labile and unstable compounds.[12,13] A recent study by Ramli *et al* [13] has utilized this method for extraction of *S. polyanthum* leaves, and has proven that the extract possess significant renal protective effect in Spontaneously Hypertensive Rats. Thus, this study aims to extract the phytochemical compounds in *S. polyanthum* leaves using ultrasound-assisted method and to analyse the phytochemical compounds using GC-MS analysis.

MATERIAL AND METHODS

Materials and Reagents

Ethyl acetate, n-hexane and methanol were purchased from Merck (Germany). *S. polyanthum* leaves were collected from the District of Ketereh, Kelantan, Malaysia in May 2016. The leaves (1.3 kg) were dried for a week at room temperature in the laboratory. The herbal specimen was authenticated and deposited into Forest Research Institute Malaysia herbarium (Specimen voucher number: PID-171011-10). The dried leaves were then ground into fine powder and kept in temperature of 1-5°C for further analysis.

Preparation of Extract

Preparation of extract was carried out based on ultrasound-assisted extraction (UAE) method previously reported by Ramli *et al.*[13] Briefly, 250 g of powdered *S. polyanthum* leaves were soaked in n-hexane (100 mL) and then sonicated at 24°C for 30 min using a sonicator (Sonicor SC221, USA). After filtration with Whatmann filter paper No. 1, the hexane layer was evaporated using a rotary evaporator (Buchi R-200, Switzerland) to afford the hexane extract (HSP). Similarly, the experiment was repeated by using the residue from the previous extraction, soaked and sonicated in ethyl acetate (100 mL) and finally repeated three times in methanol (3 × 100 mL), respectively. After filtration and evaporation, the ethyl acetate and methanol extracts afforded the extract of ethyl extract (EASP) and methanol (MSP), respectively. The crude extracts were stored at -20°C prior to further analyses.

Instrumentation

The GC-MS instrument (Shimadzu GCMS-QP2010 Ultra, Australia) with chromatographic system of GC-2010 was utilized in this analysis. All the three extracts were analysed on capillary column (BPX5-5% phenyl (equivalent)/95% methyl polysilphenylene/siloxane phase, 30 m × 0.25 µm x 0.25 µm, Shimadzu).

GC condition

The injector temperature was set at 250°C, column temperature program was set at 50°C (0 min) with an increasing rate of 3°C/min to 300°C (10 min). The carrier gas used was pure helium gas (99.999%) with its flow rate of 0.8mL/min. The split ratio used was 1:10.

MS condition

The ion source and ionization voltage used was electron ionization (EI) and 70eV respectively. The ion source temperature was set as 200°C and the detection voltage at 0.87kV. The interface temperature was 250°C with its solvent cut-off time of 2.0 min. The start time was set at 2.5 min and the end time was set at 93 min.

Identification of components

Compounds were identified based on molecular structure, molecular mass, and calculated fragments. Interpretation of data was based on mass spectral matching with standard compounds in Wiley 229, NIST11 (National Institute of Standards and Technology) and FFNSC1.3 (Flavour and Fragrance Natural and Synthetic Compounds) libraries. The retention time was matched, and the relative amounts of individual components were shown as the percentage peak areas relative to the total peak area. Only selected peaks with similarity index of 70% and above with Wiley, NIST or FFNSC libraries were chosen and identified.

RESULTS

Percentage yield

The percentage yield for HSP, EASP and MSP were tabulated in Table 1. Mean percentage yields were obtained for two batches of each extract. Among the three extracts, methanol extract gave the highest percentage yield while hexane extract gave the lowest yield.

GC-MS analysis for n-hexane extract of S. polyanthum leaves

A total of 21 peaks were identified in GC-MS chromatogram of HSP (Figure 1). Upon comparing of their mass-spectral databases with Wiley, NIST and FFNSC libraries, these phytochemical compounds were identified and characterized as listed in Table 2. There were five major compounds identified from the HSP chromatogram. The most abundant compound in HSP was an unknown compound with retention time of 62.093 min (31.912%), followed by squalene (8.776%), phytol (8.409%), α-pinene (4.921%) and lastly α-tocopherol (4.900%). The rest of compounds were present by the amount of less than 4%.

GC-MS analysis for ethyl acetate extract of S. polyanthum leaves

There were 27 peaks identified in GC-MS chromatogram of EASP (Figure 2). Upon comparing of their mass-spectral databases with the Wiley, NIST and FFNSC libraries, these phytochemical compounds were identified and characterized in Table 3. EASP extract was mainly composed of an

Table 1: Percentage yield of n-hexane, ethyl acetate and methanol extracts of *S. polyanthum* leaves.

Batch No.	Fresh weight (kg)	Yields After Drying		Final Yield					
		Dry Weight (kg)	% (Wet basis)	HSP		EASP		MSP	
				Weight (g)	% (Dry basis)	Weight (g)	% (Dry basis)	Weight (g)	% (Dry basis)
1	0.7	0.30	42.86	3.39	1.13	6.65	2.22	16.49	5.50
2	0.6	0.30	50.00	6.89	2.30	15.03	5.01	21.81	7.27
Total					1.72 ± 0.83		3.62 ± 1.97		6.39 ± 1.25

Note: Final yield was expressed as mean percentage ± standard deviation. HSP: n-Hexane extract of *S. polyanthum* leaves, EASP: Ethyl acetate extract of *S. polyanthum* leaves and MSP: Methanol extract of *S. polyanthum* leaves.

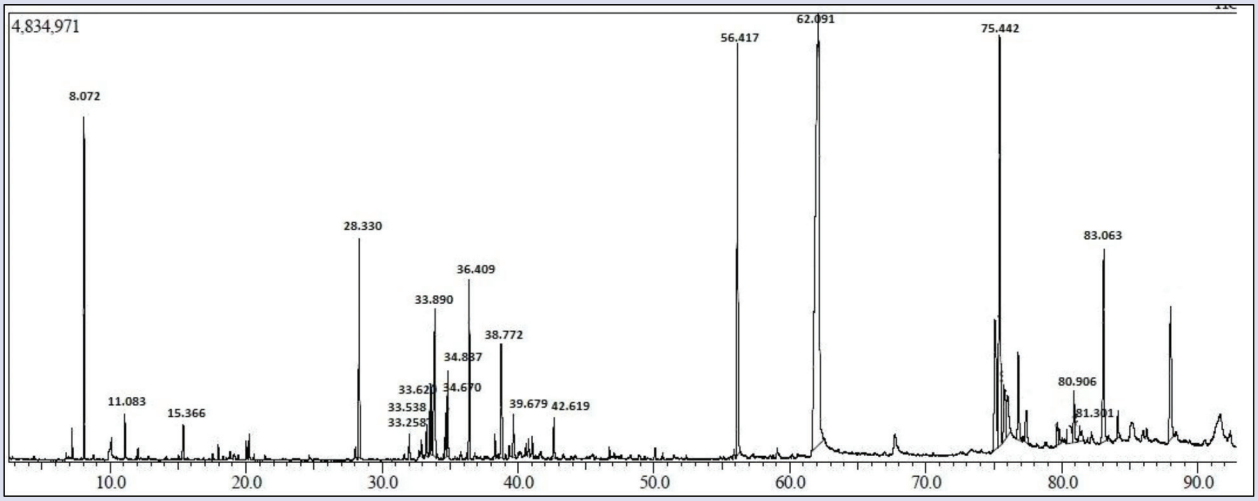

Figure 1: GC-MS chromatogram of n-hexane extract of *S. polyanthum* leaves.

Table 2: Phytochemical compounds in n-hexane extract of *S. polyanthum* leaves.

Name	Peak %	Chemical Classes	Molecular Formula	Retention Time (min)
α-Pipene	4.921	Monoterpene	$C_{10}H_{16}$	8.072
Octanal	0.573	Aldehyde	$C_8H_{16}O$	11.083
Linalool	0.448	Oxygenated monoterpene	$C_{10}H_{18}O$	15.366
α-Cubebene	3.633	Sesquiterpene	$C_{15}H_{24}$	28.330
2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	0.558	-	$C_{15}H_{24}$	33.258
Azulene	1.255	Sesquiterpene derivatives	$C_{15}H_{24}$	33.538
Valencene	1.097	Sesquiterpene	$C_{15}H_{24}$	33.620
β-Panasinsene	3.990	Volatile compounds	$C_{15}H_{24}$	33.892
δ-Cadinene	0.737	Bicyclic sesquiterpene	$C_{15}H_{24}$	34.670
α-Panasinsen	1.515	Sesquiterpene,	$C_{15}H_{24}$	34.848
Nerolidol	2.845	Sesquiterpene	$C_{15}H_{26}O$	36.417
Humulene epoxide II	2.060	Peroxide	$C_{15}H_{24}O$	38.772
Caryophyllene oxide	0.846	Oxygenated terpenoid/ sesquiterpene	$C_{15}H_{24}O$	39.679
Farnesol	0.715	Acyclic alcoholic sisquiterpene	$C_{15}H_{26}O$	42.619
Phytol	8.409	Diterpene alcohol	$C_{20}H_{40}O$	56.147
RT:62.093	31.912	-	-	62.091
Squalene	8.776	Triterpene	$C_{30}H_{50}$	75.442
β-Tocopherol	0.934	Tocopherol (methylated phenols)	$C_{28}H_{48}O_2$	80.906
γ-Tocopherol	0.934	Tocopherol (methylated phenols)	$C_{28}H_{48}O_2$	81.301
α-Tocopherol	0.340	Tocopherol (methylated phenols)	$C_{29}H_{50}O_2$	83.063
β-Sitosterol	0.676	Steroidal	$C_{29}H_{50}O$	87.992
Total = 77.174 %				

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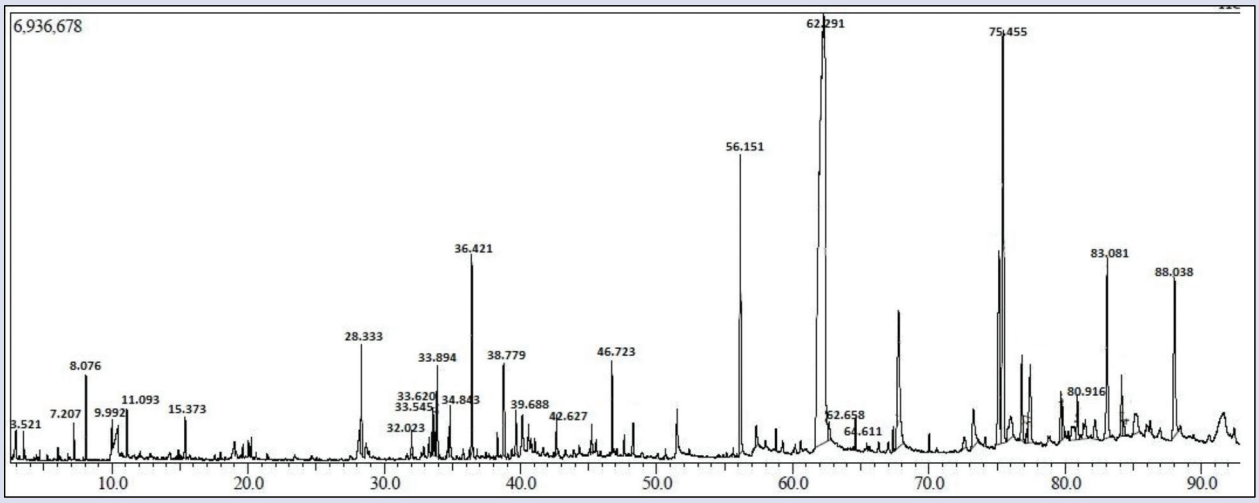

Figure 2: GC-MS chromatogram of ethyl acetate extract of *S. polyanthum*. Leaves.

Table 3: Phytochemical compounds in ethyl acetate extract of *S. polyanthum* leaves.

Name	Peak %	Chemical Classes	Molecular Formula	Retention Time (min)
Propylene glycol	0.144	Diol	$C_3H_8O_2$	3.521
n-Heptanal	0.317	Aldehyde	$C_7H_{14}O$	7.207
α-Pinene	1.068	Monoterpene	$C_{10}H_{16}$	8.076
Heptane	0.389	Alkane	C_7H_{16}	9.992
Octanal	0.563	Aldehyde	$C_8H_{16}O$	11.093
β-linalool	0.468	Terpene alcohol	$C_{10}H_{18}O$	15.373
α-Cubebene	1.690	Sesquiterpene	$C_{15}H_{24}$	28.333
α-Humulene	0.504	Monocyclic sesquiterpene	$C_{15}H_{24}$	32.023
β-Selinene	0.735	Sesquiterpene	$C_{15}H_{24}$	33.545
Valencene	0.548	Sesquirterpene	$C_{15}H_{24}$	33.620
1H-Cyclopropa[a]naphthalene	2.226	Acyclic alkene	$C_{15}H_{24}$	33.894
α-Panasinsen	0.887	Sesquirterpene	$C_{15}H_{24}$	34.843
Nerolidol	3.085	Sesquiterpene alcohol	$C_{15}H_{26}O$	36.423
Humulene epoxide II	1.557	Sesquiterpene	$C_{15}H_{24}O$	38.782
Caryophyllene oxide	0.824	Oxygenated terpenoid	$C_{15}H_{24}O$	39.688
Farnesol	0.645	Acyclic alcoholic sisquiterpene	$C_{15}H_{26}O$	42.628
Neophytadiene	1.347	Terpenoid	$C_{20}H_{38}$	46.723
Phytol	5.715	Diterpene alcohol	$C_{20}H_{40}O$	56.151
9,12,15-Octadecatrien-1-ol	1.037	Unsaturated alcoholic compound (Lignan)	$C_{18}H_{32}O$	57.341
Hentriacontane	0.303	Long chain alkane	$C_{31}H_{64}$	58.758
RT:62.290	27.042	-	-	62.291
2-Cyclohexen-3-ol-1-one, 2-[1-iminoethyl]-	0.107	-	-	62.658
n-Pentacosane	0.472	Aliphatic hydrocarbon alkane	$C_{25}H_{52}$	64.611
Squalene	8.345	Teriterpene hydrocarbon	$C_{30}H_{62}$	75.455
β-Tocopherol	0.693	Tocopherol (methylated phenols)	$C_{28}H_{48}O_2$	80.916
α-Tocopherol	4.660	Tocopherol (methylated phenols)	$C_{29}H_{50}O_2$	83.081
β-Sitosterol	4.959	Steroidal	$C_{29}H_{50}O$	88.038
	Total = 70.330 %			

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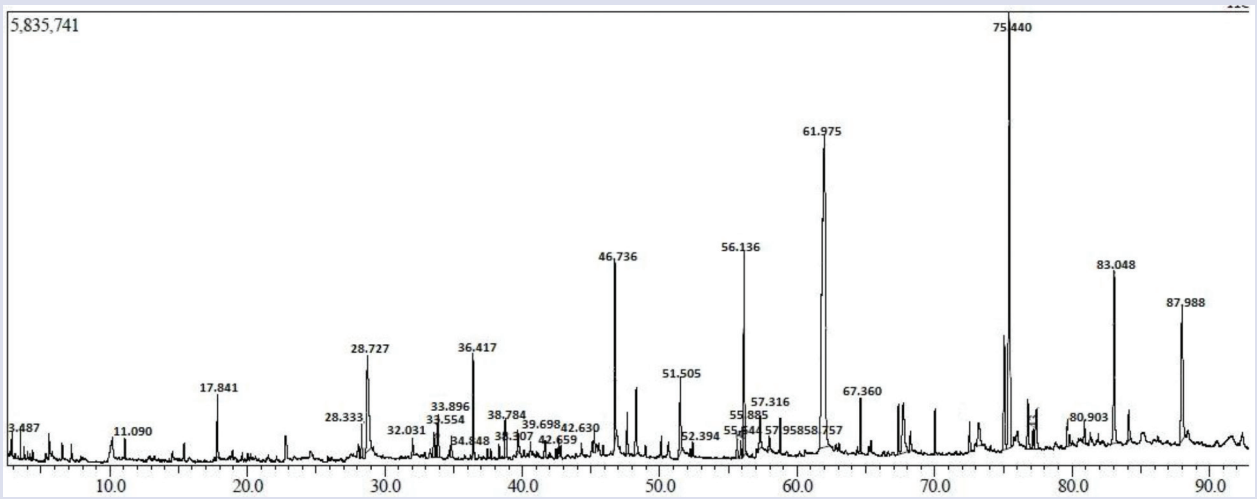

Figure 3: GC-MS chromatogram of methanol extract of *Syzygium polyanthum* leaves.

unknown compound found at retention time of 62.290 min (27.042%), followed by squalene (8.345%), another unknown compound at retention time of 67.797 min (5.747%), phytol (5.715%) and lastly β-sitosterol (4.959%). Other than these five main compounds, the rest of compounds were found in a relatively low amount of less than 4%.

GC-MS analysis for methanol extract of S. polyanthum leaves

GC-MS chromatogram of MSP showed presence of 31 peaks (Figure 3). These peaks which were identified and characterized by comparing their mass-spectral databases with the Wiley, NIST and FFNSC libraries was tabulated in Table 4. Similarly, the most abundant compound was an unknown compound with retention time of 61.98 min (22.386%); these was followed by squalene (10.913%), β-sitosterol (5.560%), pyrogallol (5.247%) and phytol (4.952%). The rest of the compounds were also not abundant with the amount of less than 4%.

DISCUSSION

Organic crude extracts from *S. polyanthum* leaves (hexane-HSP, ethyl acetate-EASP and methanol-MSP) were sequentially extracted using ultrasound-assisted extraction (UAE) method. Gas Chromatography–Mass Spectrometry (GC-MS) analyses on HSP, EASP and MSP showed that each crude extract was composed of different phytochemical compositions. Overall, they were basically composed of hydrocarbons, aldehydes, terpenoids, phenolics, fatty acids, monoterpenes, diterpenes, triterpenes and sesquiterpenes. Sesquiterpenes were found as highest in composition in which they have contributed to the total amount of 41.63%, 31.32 % and 22.58 % in HSP, EASP and MSP, respectively. There were 21, 27, and 31 compounds identified in the GC-MS chromatograms for HSP, EASP, and MSP, respectively. Nine compounds (nerolidol, caryophyllene oxide, farnesol, phytol, squalene, β-tocopherol, γ-tocopherol, α-tocopherol and β-sitosterol) were present in all three extracts. Certain compounds such as α-pinene and linalool co-exist in both HSP and EASP while α-humulene, β-selinene, 9,12,15-Octadecatrien-1-ol, hentriacontane, pentacosane, and octanal co-exist in both EASP and MSP.

The major compound in HSP, EASP and MSP was an unknown compound, each detected at retention time of 62.093, 62.2290 and 61.980 min, respectively. This may have suggested that this unknown compound might be the same compound, however, this will require further analysis for characterization. Terpenes, such as squalene (an isoprenoid compound) and phytol (a cyclic diterpene alcohol) were observed as among the next major components in all extracts. This finding was in agreement with previous studies that also found squalene as their major compound in macerated methanolic [5] and hexane [8] extracts of *S. polyanthum* leaves. Besides squalene, phytol was also identified as the most abundant compound in the macerated ethanolic extract of *S. polyanthum* leaves.[14] These findings have greatly supported our finding in which squalene and phytol are the main volatile compounds in *S. polyanthum* leaves. On contrary, n-hexatriacontane and n-triacontane which were reported by Hamad *et al* [8] in macerated hexane extract were not found in this study. Other than that, the unknown major compound which were detected in all three extracts in this study was not detected in these previous studies. Even so, these few differences in phytochemical composition might be contributed by the dissimilarities in the employed extraction method. Our study utilized sequential UAE method while the other previous studies utilized direct maceration method. In terms of geographic location, the plant materials used in this study was obtained from *S. polyanthum* leaves grown in Malaysia, while the other previous studies mainly harvested the leaves from *S. polyanthum* plant grown in Indonesia. Other than these major compounds, the rest of compounds only showed relative amount of less than 4%.

There were 20 bioactive compounds detected in HSP, EASP and MSP as summarized in Table 5. Few known antidiabetic compounds such as linalool [15] and β-sitosterol [16] were identified in the present study. As such, *S. polyanthum* leaves extracts were previously shown to exhibit anti-diabetic properties on alloxan-induced [17] and on streptozotocin-induced [5] diabetic rats. Antibacterial compounds identified in this present study include α-pinene which was active against *Staphylococcus aureus;*[18] linalool which was active against *Escherichia coli;*[19] and palmitic acid (n-hexadecanoic acid) which was active against *Salmonella typhii*[20] With relation to that, previous study also found that *S. polyanthum* leaves extracts possessed antibacterial activity against *Staphylococcus aureus,*[21] *Bacillus cereus* [6] and *Bacillus subtilis*.[22] Asides from antibacterial compounds, there were also some antifungal compounds found in the present study. This include nerolidol [23] and caryophyllene oxide [24] which were active against *Trichophyton mentagrophytes*; and farnesol which was active against *Candida albicans*.[25] Accordingly, *S. polyanthum* leaves extract has exhibited some antifungal activity, but against *Alternaria alternate* and *Colletotrichum capsicii*.[26]

Other than that, this study also found few cytotoxic compounds such as linalool and octanal;[19] α-humulene;[27] and pyrogallol (1,2,3-benzenetriol).[28]

Table 4: Phytochemical compounds in methanol extract of *S. polyanthum* leaves.

Name	Peak %	Chemical Classes	Molecular Formula	Retention Time (min)
Propylene glycol	0.272	Diol	$C_3H_8O_2$	3.487
Octanal	0.354	Aldehyde	$C_8H_{16}O$	11.090
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1.423	Flavanoid fraction	$C_6H_8O_4$	17.841
α-Copaene	0.636	Sesquiterpene	$C_{15}H_{24}$	28.333
Pyrogallol	5.247	Phenol	$C_6H_6O_3$	28.727
α-Humulene	0.576	Sesquiterpene	$C_{15}H_{24}$	32.031
β-Panasinsene	0.923	Sesquiterpene	$C_{15}H_{24}$	33.554
Selina-4,11-diene	1.519	-	$C_{15}H_{24}$	33.896
α-Panasinsen	0.677	Sesquiterpene,	$C_{15}H_{24}$	34.848
Nerolidol	2.267	Sesquiterpene	$C_{15}H_{26}O$	36.417
β-Selinene	0.321	Sesquiterpene	$C_{15}H_{24}$	38.307
Humulene epoxide II	0.910	Epoxide	$C_{15}H_{24}O$	38.784
Caryophyllene oxide	0.943	Oxygenated terpenoid	$C_{15}H_{24}$	39.698
Pentadecane, 2,6,10,14-tetramethyl-	0.386	Saturated terpenoid alkane	$C_{19}H_{40}$	41.659
Farnesol	0.421	Acyclic Sesquiterpene	$C_{15}H_{26}O$	42.630
Neophytadiene	0.581	Terpenoid	$C_{20}H_{38}$	46.735
Methyl palmitate	0.425	Palmitic acid ester	$C_{17}H_{34}O_2$	50.092
Palmitic acid	2.378	Fatty acid	$C_{16}H_{32}O_2$	51.507
Eicosane	0.294	Aliphatics alkane	$C_{20}H_{42}$	52.398
Methyl oleate	0.343	Unsaturated fatty acid Methyl ester	$C_{19}H_{36}O_2$	55.887
Phytol	4.952	Diterpene alcohol	$C_{20}H_{40}O$	56.137
9,12,15-Octadecatrien-1-ol	1.783	Unsaturated alcoholic compound	$C_{18}H_{32}O$	57.317
Stearic acid	0.604	Stearic acid Saturated fatty acid	$C_{18}H_{36}O_2$	57.963
Hentriacontane	0.674	Alkane	$C_{31}H_{64}$	55.647
RT:61.980	22.386	-	-	61.975
Pentacosane	1.282	Aliphatic hydrocarbon	$C_{31}H_{64}$	58.758
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)	0.525	Fatty acid	$C_{19}H_{38}O_4$	68.232
Squalene	10.913	Triterpene	$C_{30}H_{50}$	75.440
β-Tocopherol	0.548	Tocopherol compound (methylated phenols)	$C_{28}H_{48}O_2$	80.903
α-Tocopherol	4.933	Tocopherol (methylated phenols)	$C_{29}H_{50}O_2$	83.048
β-Sitosterol	5.560	Steroidal	$C_{29}H_{50}O$	87.988
	Total = 75.056 %			

: No data described for the section

Table 5: Bioactive compounds in n-hexane, ethyl acetate and methanol extracts of *S. polyanthum* leaves.

Bioactive compounds	HSP	EASP	MSP	Biological Activity
α-pinene	+ (4.921%)	+ (1.068 %)	-	Anti-inflammatory[43] Antibacterial[18, 44] Hypotensive[45]
Linalool	+ (0.448%)	+ (0.468%)	-	Antibacterial[46] Hepatoprotective[47] Anti-inflammatory[48] Antidiabetic[15] Cytotoxicity[19]
Azulene	+ (1.255%)	-	-	Anti-inflammatory[49]

Continued....

Table 5: Cont'd.

Nerolidol	+ (2.845%)	+ (3.085 %)	+ (2.267 %)	Antinociceptive[50] Anti-inflammatory[50] Antifungal[23] Antiulcer[51]
Caryophyllene oxide	+ (0.846%)	+ (0.824 %)	+ (0.943 %)	Analgesic[52] Anti-inflammatory[52] Antifungal[24]
Farnesol	+ (0.715%)	+ (0.645%)	+ (0.421%)	Anti-tumor[31] Antifungal[25] Antinociceptive[53] Anti-inflammatory[54] Antioxidant[55] Antimicrobial[56]
Phytol	+ (8.409%)	+ (5.715%)	+ (4.952 %)	Antimicrobial[57] Anti-inflammatory[34] Anti-cancer[58] Antinociceptive[59] Antioxidant[60]
Squalene	+ (8.776 %)	+ (8.345%)	+ (10.913 %)	Antioxidant[32] Antitumor[32] Chemopreventive effect[61]
β-Tocopherol	+ (0.934%)	+ (0.693 %)	+ (0.5485 %)	Antioxidant[41]
γ-Tocopherol	+ (0.340%)	-	-	Antineoplastic[62] Anti-carcinogenesis[63] Anti-inflammatory[64] Antioxidant[41]
α-Tocopherol	+ (4.900%)	+ (4.660 %)	+ (4.933%)	Antioxidant[41, 42]
β-Sitosterol	+ (0.676%)	+ (4.959%)	+ (5.560 %)	Antidiabetic[16]
α-Humulene	-	+ (0.504 %)	+ (0.576%)	Antifungal[65] Cytotoxicity[27] Anti-inflammatory[66]
Neophytadiene	-	+ (1.347 %)	-	Anti-inflammatory[67]
Hentriacontane	-	+ (0.303 %)	+ (0.361 %)	Anti-inflammatory[68]
Octanal	-	+ (0.563%)	+ (0.354%)	Cytotoxicity[19]
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	-	-	+ (1.423 %)	Antiproliferative[35] Antioxidant[39, 40]
Pyrogallol	-	-	+ (5.247 %)	Antioxidant[28] Cytotoxicity[28] Antiproliferative[28]
Methyl palmitate	-	-	+ (0.425%)	Phagocytosis inhibitor[69]
Palmitic acid	-	-	+ (2.378 %)	Anti-inflammatory[70] Antibacterial[20]

S. polyanthum leaves extract has also exhibited cytotoxic effect on HB4C5 human hybridoma and mouse colon 26 adenocarcinoma cells;[29] and on 3T3-L1 cell lines proliferation.[30] Some anti-tumour compounds such as farnesol[31] and squalene[32] were also found in this study. Previously, *S. polyanthum* leaves extract were reported to exhibit some anti-tumour promoting activity, but against Raji cells.[33] Moreover, an anticancer compound, phytol[34] and the anti-proliferative and pro-apototic compound, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one[35] were also found in this study.

S.polyanthum leaves extract was also known for their anti-inflammatory property.[36] Nerolidol, α-humulene, phytol, farnesol, azulene, caryophyllene oxide, neophytadiene and α-pipene were among the known terpenoids with anti-inflammatory properties found to be present in this study. A hypotensive compound α-pinene was found in HSP extract in this study. In agreement with that, *S. polyanthum* leaves extract was previously reported to reduce blood pressure of normotensive and hypertensive rats.[1] *S. polyanthum* leaves extract also possessed antioxidant properties.[14,37,38] Accordingly, this study has found some known phenolic compounds with anti-oxidant property such as 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one,[39,40] β-tocopherol,[41] pyrogallol (1,2,3,-benzenetriol)[28] and α-tocopherol.[41,42]

CONCLUSION

GC-MS analyses of n-hexane (HSP), ethyl acetate (EASP), and methanol (MSP) of *S. polyanthum* leaves extracted using ultrasound-assisted method have revealed the presence of few major compounds such as phytol, squalene and an unknown compound that requires further characterization. Some of the identified compounds in *S. polyanthum* leaves extract in this study are known bioactive compounds with therapeutic importance. Even though the association between these structure-activity relationships was not directly being proven yet, this study may serve as a basis for further investigation.

ACKNOWLEDGEMENT

We are thankful to International Islamic University Malaysia who have provided financial support for this research project through Research Initiative Grant Scheme (RIGS 15-039-0039).

CONFLICT OF INTEREST

There is no conflict of interest.

ABBREVIATION USED

EASP: Ethyl acetate extract of *Syzygium polyanthum* leaves; **GC-MS:** Gas chromatography-mass spectrophotometry; **HSP:** n-Hexane extract of *Syzygium polyanthum* leaves; **MSP:** Methanol extract of *Syzygium polyanthum* leaves; **RT:** Retention time; **UAE:** Ultrasound-assisted extraction.

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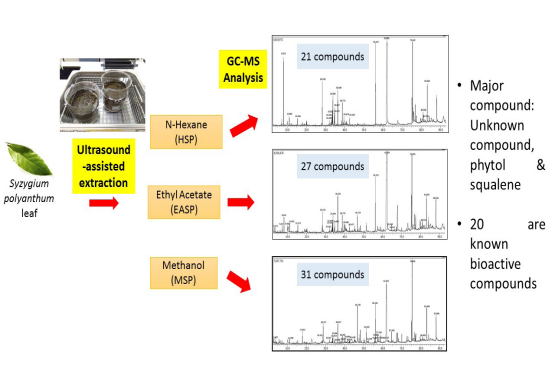
GRAPHICAL ABSTRACT



SUMMARY

- Phytol and squalene were among the major compounds in *Syzygium polyanthum* leaves which were extracted by using ultrasound-assisted extraction (UAE) method.
- Few known bioactive compounds were found in *S. polyanthum* leaves extracts. This has shown that this plant has vast pharma-therapeutic importance.

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Cite this article: Rahim ENAA, Ismail A, Omar MN, Rahmat UN, Ahmad WANW. GC-MS Analysis of Phytochemical Compounds in *Syzygium polyanthum* Leaves Extracted using Ultrasound-Assisted Method. Pharmacog J. 2018;10(1):110-9.