Review Article

Optimisation of Extraction Method and Phytochemical Compounds of Green *Christia vespertilionis* Leaves using GC-MS

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ABSTRACT

Christia vespertilionis (L. f.) Bakh. f. has been widely known in treating various contagious diseases. This plant is popular among researchers and locals to have anti-inflammatory, and anti-cancer properties. There are two types of *C. vespertilionis* which is green and red type. The green *C. vespertilionis* was extensively studied by many researchers and known by the public as a cure to the cancer. This study was carried out to identify major phytochemicals and optimise extraction method of green *C. vespertilionis* leaves in different extraction techniques (maceration and Soxhlet extraction) and solvents (methanol and ethanol) through GC-MS analysis. The green *C. vespertilionis* leaves extract was tested using Gas Chromatography Mass Spectrophotometer (GC-MS). The components were identified by comparing National Institute of Standards and Technology (NIST). Based on four samples which are green *C. vespertilionis* leaves using maceration of methanol (GMM), maceration of ethanol (GME), Soxhlet of methanol (GSM) and Soxhlet of ethanol (GSE), seventy phytochemical compounds were identified. Thirteen major phytochemical compounds (> 4 % of peak area) are acetic acid, butyl ester; 1-Butanol, 3-methyl-, acetate; 1,3-Diisobutyrin, trimethylsilyl; .alpha.-d-Mannofuranoside, methyl; 1-Tetradecene; 1-Hexadecene; 1-Octadecene; Hexanoic acid, 3-oxo-, ethyl ester; 4-O-Methylmannose; n-Hexadecanoic acid; Phytol; 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- and Squalene. Only ten out of thirteen compounds were reported to have biological activities. Among those samples, GMM and GSM were the most effective using correlation coefficient analysis between peak area (%) versus real time (min) with significant difference at $P < 0.001$.

Keywords: *Christia vespertilionis*, GC-MS, phytochemical compounds, maceration, Soxhlet extraction.

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INTRODUCTION

Since previous centuries, plant is one of the natural resources that can be used to treat diseases due to unique chemical diversity and various biological activity which has pharmacological properties. Since then, human developed knowledge about plant medicine and undergo interesting and important developments to be use for human consumption until synthetic drug invent at nineteenth century [1]. It is noted that plant medicine has been overshadowed by modern drug development. However, natural product has many secondary metabolites contain biological activity that can develop drug-like functioning to cure certain diseases.

Christia vespertilionis (L. f.) Bakh. F. (Family: Fabaceae) known as butterfly wing is widely used by local people to cure several diseases due to positive biological activities such as having anti-cancer, anti-inflammatory, anti-proliferative and anti-plasmodial properties [2,3,4]. Aside from that, this plant has been used to treat tuberculosis, scabies, snake bites, bronchitis, inflammation and pure blood circulation [5,6]. This plant origins from South-eastern China, India, Thailand, Cambodia, Laos, Vietnam, Indonesia and Malaysia. Long time ago, in Cambodia, the plant was used as medicine to cure fever and taken before meals [7]. Today, *C. vespertilionis* leaves are widely consumed by cancer patients and have gained a huge popularity among locals in Malaysia. Leaf part has commonly been used for many biomedical purposes as it is containing various biological activity [8,9].

As a medicinal plant that being popular in treating cancer, *C. vespertilionis* potentially to have various bioactive secondary compounds that widely used in food and pharmaceutical area [10]. Secondary compounds act as important role to physiological effects such as UV protection, pathogen defence mechanism, pollination and dissemination, symbiosis and allelopathic interactions [11,12]. Basically, biological secondary metabolites have been used for treating human diseases as it contains pharmacological effects since many centuries. Previous studies have revealed the presence of bioactive secondary compounds in *C. vespertilionis* such as phenols, alkaloids, triterpene, fatty acids and long chain alcohols [3]. The aerial part also is reported to have corynoxidine and palmitine, contains good anti-proliferative activity in MTC cells and good anti-plasmodial activity of aqueous-methanol extract [2,3]. In addition, two newly identified compounds which christene

and christanoate were recently found from n-Hexane extract [13].

To this day, people have high interest towards herbal remedies as substitute to the synthetic drugs. The origin of plant itself is natural and safe to use. However, more research needs to be examined to study the phytochemicals and toxicity towards human consumption. As many studies of *C. vespertilionis* has been revealed, there is no study available on phytochemical compounds of green *C. vespertilionis* in different solvents and extraction techniques using GC-MS. Therefore, this research aims to analyse the phytochemical compounds of green *C. vespertilionis* leaves and optimise the method of extraction in different solvent (methanol and ethanol) and techniques (maceration and Soxhlet extraction) using Gas Chromatography Mass Spectrophotometer (GC-MS).

MATERIALS AND METHODS

Reagents

Methanol (CH_3OH) was purchased from Merck (Germany) whilst absolute ethanol (C_2H_5OH) from VWR Chemicals (American). Both solvents used in this study were analytical grade.

Plant material

Green *C. vespertilionis* was collected from Floranika Nursery Sungai Buloh, Selangor (Malaysia) located at latitude and longitude of 3° 13' 6.7764'' N, 101° 34' 18.1704'' E. The voucher specimen was authenticated by Dr. Yong Kien Thai from Plant Taxonomy, Rimba Ilmu, University Malaya. The voucher specimen of green *C. vespertilionis* (KLU 50026) was deposited at the herbarium of University Malaya. Green *C. vespertilionis* leaves were air-dried at room temperature (26.9 °C) and humidity (63 %) for 7 to 8 days and the moisture loss content was above 70 – 80 %. The leaves were grinded with blender to obtain coarse powder and kept in the closed jar until further used.

Sample extraction

Maceration

1 g of dried leaves powder of green *C. vespertilionis* was immersed into a 100 ml of methanol (Merck, Germany) and absolute ethanol (VWR Chemicals, American) in 250 ml conical flask, separately. The samples were placed in a water bath with temperature of 40 °C for 48 hours. The extracts were filtered and evaporated with rotary evaporator with 45 °C of water bath temperature until concentrated and approximately 1 ml was left. The extracts were filtered again to remove any solid particles and kept in closed tightly microcentrifuge tube at 4 °C for further used.

Soxhlet extraction

1 g of dried leaves powder of green *C. vespertilionis* was extracted with 200 ml of methanol (Merck, Germany) and absolute ethanol (VWR Chemicals, American) using Soxhlet apparatus, separately for 8 hours until the reflux becomes clear which is approximately 10 cycles of reflux at temperature below boiling point based on respective solvent. The extracts were filtered and evaporated with rotary evaporator with 45 °C of water bath temperature until concentrated and approximately 1 ml was left. The extracts were filtered again to remove any solid particles and kept in closed tightly microcentrifuge tube at 4 °C for further used.

Gas chromatography-mass spectrometry (GC-MS) analysis

The extracts were diluted to 0.5 mg/ml or 500 ppm with respective solvent into 1.5 ml septa vial (HmbG Chemicals, Germany). The GC-MS analysis was done at the IPPP Central Laboratory Facilities, University Malaya, Kuala Lumpur, Malaysia. Model of GCMS-QP2010 Ultra (Shimadzu, Tokyo, Japan) was used for GC-MS analysis. 0.5 µl of sample was autoinjector into system. The system was equipped with capillary column of RTX5MS with 30.0 m × 0.25 mm (length × diameter) and 0.25 µm of thickness. The injection temperature was set to 200 °C and possessing a splitless injection mode. The initial temperature was 50 °C (3 min) with increasing rate of 10 °C/min to 300 °C (10 min). The carrier gas was Helium gas (99.999 %) with 47.8 cm/sec of linear velocity. Electron ionization (EI) mode at 70 eV with spectral range of 35 - 500 m/z was performed for mass spectra results. The ion source temperature was adjusted at 150 °C and the interface temperature was 230 °C with solvent cut-off time of 3 minutes. The start time was set at 3.0 minutes and the final time was set at 33.0 minutes. The total flow programmed was 21.6 ml/min with column flow of 1.69 ml/min. The compounds were identified based on the interpretation of mass spectrum with standard reference spectral using National Institute of Standards and Technology (NIST) databases.

Statistical analysis

The correlation between real time and peak area of the sample was determine using Pearson's correlation coefficient, the difference is considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

GC-MS chromatogram and phytochemical compounds

GC-MS is a technique to separate and identify different substances or chemical compounds in the sample. The GC-MS analysis showed seventy (70) phytochemical compounds from four (4) samples that consists of minor compounds and major compounds (> 4 % of peak area) [14]. The conditions of samples were shown in Table 1.

Table 1: Conditions of each sample

Plant	Method of extraction	Solvent	Sample code	Temperature (°C)	Time (hours)
Green C. *vespertilionis* leaves	Maceration	Methanol	GMM	40	48
		Ethanol	GME		
	Soxhlet	Methanol	GSM	< 64.7	8
		Ethanol	GSE	< 78.37	

GC-MS analysis for GMM resulted in total of twenty-five (25) peaks comparing their mass-spectral databases with NIST library (Figure 1a and Table 2). Major compounds were identified and listed in Table 3. Four (4) major compounds were detected are as follow which is acetic acid, butyl ester (49.578 %); 1-Butanol, 3-methyl-, acetate (24.321 %); 1,3-Diisobutyrin, trimethylsilyl (5.620 %) and .alpha.-d-Mannofuranoside, methyl (10.359 %).

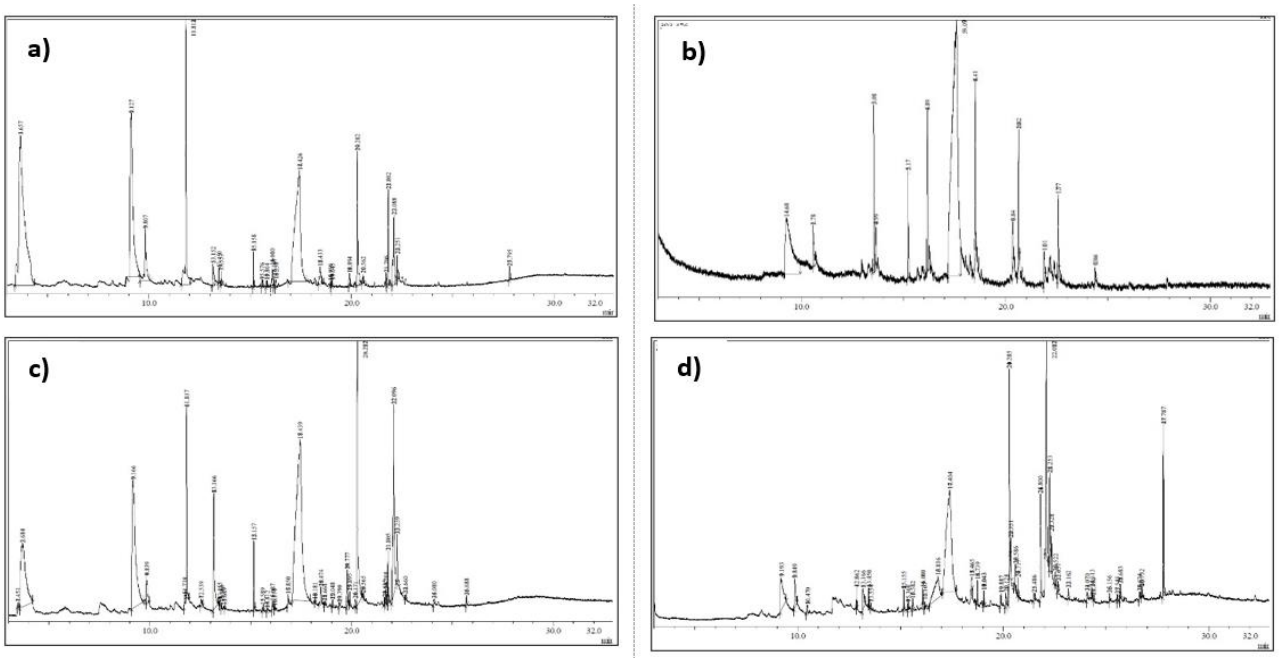


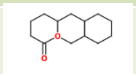
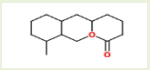
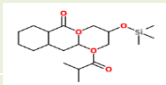
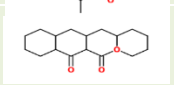
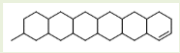
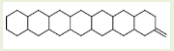
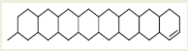
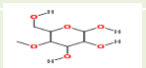
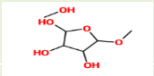
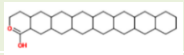
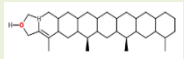
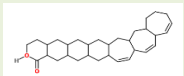
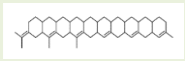
Figure 1: GC-MS chromatogram of a) green *C. vespertilionis* leaves methanolic extract in maceration (GMM); b) green *C. vespertilionis* leaves ethanolic extract in maceration (GME); c) green *C. vespertilionis* leaves methanolic extract in Soxhlet extraction (GSM); d) green *C. vespertilionis* leaves ethanolic extract in Soxhlet extraction (GSE).

Table 2: Phytochemical compounds found in green *C. vespertilionis* leaves extract based on real time and peak area.

	Phytochemical Compounds	RT (min)	Peak area (%)			
			Maceration		Soxhlet	
			Methanol (GMM)	Ethanol (GME)	Methanol (GSM)	Ethanol (GSE)
1	Acetic acid, butyl ester	3.683	49.578	-	32.372	-
2	1-Butanol, 3-methyl-, acetate	9.179	24.321	14.657	20.796	0.812
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.815	2.977	-	2.148	3.477
4	1-Dodecene	10.459	-	-	-	0.199
5	1-Undecene	10.566	-	1.448	-	-
6	1,2,3-Propanetriol, 1-acetate	11.733	-	-	2.740	-
7	1,3-Diisobutyrin, trimethylsilyl	11.817	5.620	-	4.602	-
8	2-Methoxy-4-vinylphenol	12.539	-	-	0.429	-
9	1,2-Dibutyroxy-1-ethoxyethane	12.864	-	-	-	0.985
10	Heptanoic acid, propyl ester	13.140	0.573	-	-	1.112
11	Hexanoic acid, 3-oxo-, ethyl ester	13.160	-	-	5.296	-
12	1-Dodecanol	13.167	-	-	3.421	-
13	1-Tridecene	13.446	0.146	-	-	-
14	1-Tetradecene	13.449	-	5.766	-	0.323
15	Tetradecane	13.555	0.238	-	0.114	0.343
16	Tridecane		-	1.891	-	-

17	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	13.690	-	-	0.032	-
18	Phenol, 3,5-bis(1,1-dimethylethyl)-	15.150	0.745	3.934	2.038	1.195
19	1-Nonene, 4,6,8-trimethyl-	15.574	0.109	-	-	-
20	1-Nonanol, 4,8-dimethyl-	15.581	-	-	0.059	-
21	Dihydroactinidiolide	15.582	-	-	0.023	0.338
22	1-Octanol, 2-butyl-	15.801	0.049	-	-	-
23	1-Hexadecene	16.072	-	5.388	0.411	-
24	1-Octadecene	16.077	-	4.314	-	-
25	1-Heptadecene	16.079	-	-	-	0.256
26	Hexadecane	16.170	-	-	-	0.295
27	Pentadecane, 7-methyl-	16.174	-	-	0.068	-
28	Ethylene brassylate	16.850	-	-	0.161	-
29	4-O-Methylmannose	17.404	-	-	12.813	39.054
30	.alpha.-d-Mannofuranoside, methyl	17.421	10.359	53.648	-	-
31	Cyclohexane, 2-butyl-1,1,3-trimethyl-	18.173	-	-	0.077	-
32	10-Heneicosene (c,t)	18.437	0.178	-	-	-
33	3,9-Epoxytricyclo[4.2.1.1(2,4)]decan-10-one, 9-methyl-	18.463	-	-	-	0.749
34	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	18.470	-	-	0.234	-
35	Tridecane, 3-methylene-	18.595	0.134	-	-	-
36	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	18.659	-	-	0.202	-
37	Octasiloxane	18.739	-	-	-	1.702
38	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	18.958	0.030	-	-	-
39	2-Pentadecanone, 6,10,14-trimethyl-	19.039	0.069	-	0.079	0.353
40	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	19.392	-	-	0.200	-
41	2-((2-Methoxyethoxy)carbonyl)benzoic acid	19.779	-	-	1.255	-
42	Farnesyl acetone	19.887	-	-	-	0.393
43	Hexadecanoic acid, methyl ester	19.895	0.186	-	0.105	-
44	Isophytol	20.124	-	-	-	0.935
45	Methyl di-tert- butylhydroxyhydrocinnamate	20.284	-	-	0.078	-
46	n-Hexadecanoic acid	20.291	1.662	1.312	3.972	4.679
47	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	20.354	-	-	-	3.301
48	3-Eicosene, (E)-	20.562	0.090	-	-	-
49	Octadecyl trifluoroacetate	20.565	-	-	0.090	-
50	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	20.718	-	-	-	0.761
51	1-Docosene	20.816	0.170	3.485	-	-
52	trans-Geranylgeraniol	21.485	-	-	-	0.172
53	9,12-Octadecadienoic acid (Z,Z)-	21.707	-	-	0.094	-
54	Phytol	21.798	1.468	1.290	1.098	4.715
55	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	22.090	0.106	-	0.183	-
56	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	22.092	0.558	-	-	7.243
57	cis,cis,cis-7,10,13-Hexadecatrienal	22.098	-	-	2.854	-
58	Octadecanoic acid	22.255	0.160	-	0.331	1.427
59	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	22.330	-	-	-	0.869
60	1-Tetracosanol	22.498	-	0.880	-	-
61	Octadecanoic acid, ethyl ester	22.523	-	-	-	0.434
62	4-Undecene, 3-methyl-	22.651	-	-	-	0.332
63	2,4-Pentadien-1-ol, 3-pentyl-, (2Z)-	22.659	-	-	0.060	-
64	4,8,12,16-Tetramethylheptadecan-4-olide	24.069	-	-	0.105	0.354
65	Oxirane	24.313	-	-	-	0.200
66	Heptasiloxane, hexadecamethyl-	24.377	-	-	-	0.416
67	2-methylhexacosane	25.154	-	-	-	0.355
68	Diisooctyl phthalate	25.679	-	-	0.276	0.897
69	Eicosane	26.730	0.149	-	-	-
70	Squalene	27.788	0.324	-	-	8.814
	Total identified compound		**25**	**12**	**35**	**33**

Table 3: Major phytochemical compounds of green *C. vespertilionis* leaves extract

	Name of compound/ chemical classes	RT (min)	Peak area (%)				Molecular formula	Molecular weight	Molecular structure	Biological activity
			Maceration		Soxhlet					
			Methanol (GMM)	Ethanol (GME)	Methanol (GSM)	Ethanol (GSE)				
1	Acetic acid, butyl ester (Carboxylic acid ester)	3.683	+ 49.578	-	+ 32.372	-	$C_{16}H_{12}O_2$	116		Antifungal [40] and antitumor [40].
2	1-Butanol, 3-methyl-, acetate (Carboxylic acid ester)	9.179	+ 24.321	+ 14.657	+ 20.796		$C_7H_{14}O_2$	130		Antimicrobial [30].
3	1,3-Diisobutyrin, trimethylsilyl (aliphatic ester)	11.817	+ 5.620	-	+ 4.602	-	$C_{14}H_{28}O_5Si$	304		No activity reported.
4	Hexanoic acid, 3-oxo-, ethyl ester (fatty acid ester)	13.160	-	-	+ 5.296	-	$C_8H_{14}O_3$	158		No activity reported.
5	1-Tetradecene (acyclic olefins)	13.449	-	+ 5.766	-		$C_{14}H_{28}$	196		Antimicrobial [31].
6	1-Hexadecene (unsaturated hydrocarbons)	16.072	-	+ 5.388		-	$C_{16}H_{32}$	224		Antibacterial [33,34,35], antifungal [33,34,35] and antioxidant [33,39].
7	1-Octadecene (Alkene hydrocarbon)	16.077	-	+ 4.314	-	-	$C_{18}H_{36}$	252		Anti-bacterial [33,36], antioxidant [33,36] and anti-cancer [33,42].
8	4-O-Methylmannose (aliphatic ether alcohol)	17.404	-	-	+ 12.813	+ 39.054	$C_7H_{14}O_6$	194		Antibacterial [24,37] and antifungal [41].
9	.alpha.-d-Mannofuranoside, methyl (methyl mannoside)	17.421	+ 10.359	+ 53.648	-	-	$C_7H_{14}O_6$	194		No activity reported.
10	n-Hexadecanoic acid (fatty acid)	20.291				+ 4.679	$C_{16}H_{32}O_2$	256		Anti-inflammatory[21], antibacterial[38] and antioxidant[32].
11	Phytol (diterpene alcohol)	21.798				+ 4.715	$C_{20}H_{40}O$	296		Antimicrobial [22], anti-inflammatory[22], anti-cancer [32], antioxidant[32] and antiarthritic[32].
12	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (fatty acids)	22.092		-	-	+ 7.243	$C_{18}H_{30}O_2$	278		Anti-inflammatory [23,24], cancer preventive [23,24] and antiarthritic [23,24].
13	Squalene (triterpene)	27.788		-	-	+ 8.814	$C_{30}H_{50}$	410		Antioxidant [27,29], antitumor [27,29] and anti-inflammatory [25,26].
Total major compound			**4**	**5**	**5**	**5**				

GC-MS analysis for GME was resulted in total of twelve (12) peaks (Figure 1b and Table 2). Five (5) major compounds (Table 3) were found which is 1-Butanol, 3-methyl-, acetate (14.657 %); 1-Tetradecene (5.766 %); 1-Hexadecene (5.388 %); .alpha.-d-Mannofuranoside, methyl (53.648 %) and 1-Octadecene (4.314 %).

GC-MS analysis for GSM was resulted in total of thirty-five (35) peaks with major of five (5) compounds (Figure 1c and Table 2). The major compounds (Table 3) were acetic acid, butyl ester (32.372 %); 1-Butanol, 3-methyl-, acetate (20.796 %); 1,3-Diisobutyrin, trimethylsilyl (4.602 %); Hexanoic acid, 3-oxo-, ethyl ester (5.296 %); 4-O-Methylmannose (12.813 %).

Lastly, GC-MS analysis for GSE was resulted in total of thirty-three (33) peaks with major of five (5) compounds (Figure 1d and Table 2). The major compounds (Table 3) were listed as 4-O-Methylmannose (39.054 %); n-Hexadecanoic acid (4.679 %); Phytol (4.715 %); 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (7.243%) and Squalene (8.814 %).

Using correlation between peak area and real time of the sample in GC-MS. Peak area was high in significant difference produced by maceration and only in methanol (GMM) with $P < 0.001$ (Figure 2). When using ethanol (GME), there is no significant difference which is at $P = 0.741$. In Soxhlet, the significant difference was high in methanol (GSM) with $P < 0.001$ and no significant difference in (GSE) which $P = 0.909$. The smaller P value the more significant it is. A total of seventy (70) peak area were analysed for each sample. The results indicated peak area increase when using either maceration and Soxhlet extraction particularly in methanol.

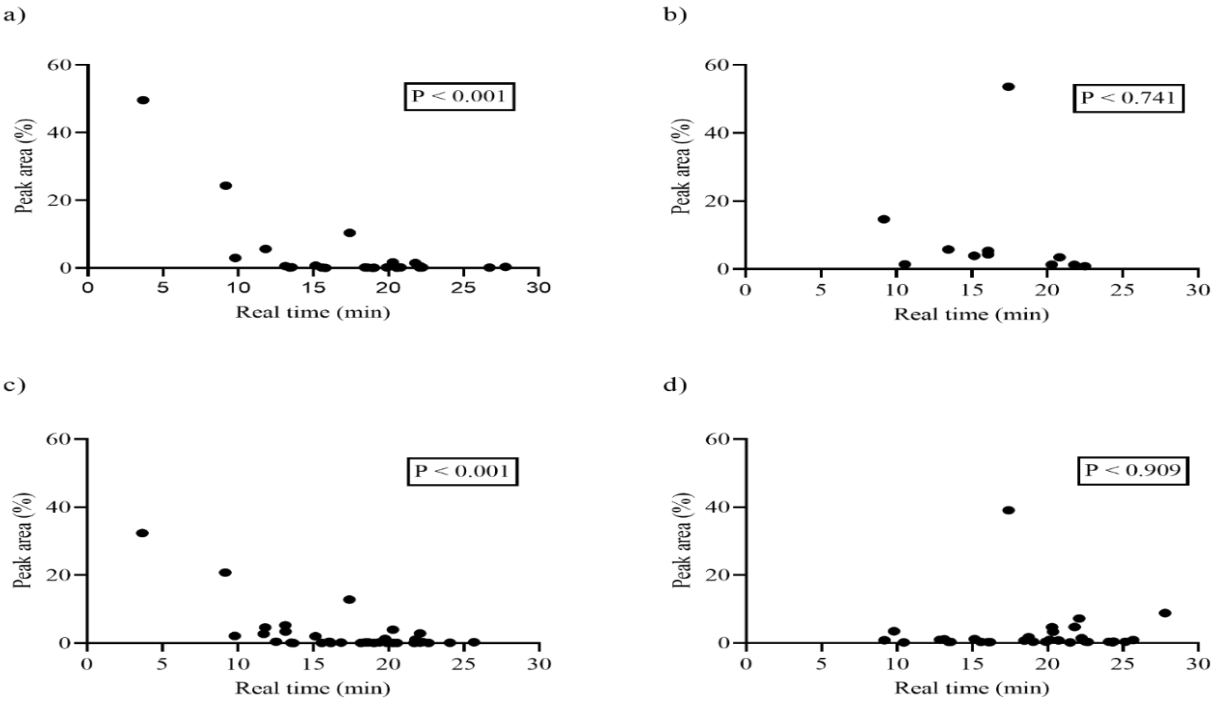


Figure 2: Correlation between peak area (%) of a) GMM b) GME c) GSM and d) GSE versus real time (min).

Highly polar solvent and water-soluble group such as methanol has high effectiveness to extract compounds compared to ethanol in general [15,16]. This is due to ability of methanol to allocate more polar compounds that enable the solubilisation of secondary metabolites than ethanol [17].

Peak area also increases when using both maceration and Soxhlet extraction. The compound produces by Soxhlet extraction commonly produces more significant difference. While maceration is a simple technique that does not involve complicated instruments yet gives a great efficiency. Previous study shows that Soxhlet also works well on aerial part of *Nepeta leucophylla* and *Potentilla atrosanguinea* when extracting the plant [17,10]. Meanwhile, maceration shows high percentage yield in methanol when extracting leaves of *Nephelium lappaceum*. L. (Sapindaceae) [18]. Overall, green *C. vespertilionis* leaves exhibits high peak area using both techniques and particularly in methanol.

Major phytochemical compounds analysis

The GC-MS analysis of four (4) samples (GMM, GME, GSM and GSE) of green *C. vespertilionis* leaves extract showed seventy (70) phytochemical compounds include major and minor compounds that are presented in Table 2. The peak area of each sample listed in Figure 1a, 1b, 1c and 1d. Table 3 showed thirteen (13) major compounds produce higher than 4 % of peak area from four (4) samples.

Overall, major compounds consist of carboxylic acid ester, aliphatic ester, fatty acids, acyclic olefins, methyl mannoside, diterpenes, triterpenes and hydrocarbons. Also stated by previous study, *C. vespertilionis* consists of

alkaloids, alkanes, triterpenes, unsaturated fats, phenols and long chain alcohols which have been categorised as main constituent [3,19,20]. Thirteen (13) major compounds are acetic acid, butyl ester (49.578 %); 1-Butanol, 3-methyl-, acetate (24.321 %); 1,3-Diisobutyrin, trimethylsilyl (5.620 %); Hexanoic acid, 3-oxo-, ethyl ester (5.296 %); 1-Tetradecene (5.766 %); 1-Hexadecene (5.388 %); 1-Octadecene (4.314 %); 4-O-Methylmannose (39.054 %); .alpha.-d-Mannofuranoside, methyl (53.648 %); n-Hexadecanoic acid (4.679 %); Phytol (4.715 %); 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (7.243 %) and Squalene (8.814 %).

n-Hexadecanoic acid [21], phytol [22], 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- [23,24] and squalene [25,26] possess anti-inflammatory activity. Squalene also proven to have chemopreventive activity in order to against colon carcinogenesis, having antioxidant and antitumor and also act as anti-inflammatory agent [25,27,28,29]. A study has been proven that squalene able to reduce damage on skin using Amaranthus cruentus oil extract [26]. The antimicrobial agents were included 1-Butanol, 3-methyl-, acetate [30], 1-Tetradecene [31] and phytol [22].

Several compounds had anticancer and antiarthritic which consists of 9,12,15-Octadecatrienoic acid, (Z,Z,Z) [23,24], phytol [32] and 1-Octadecene [33,42]. Four (4) compounds had antibacterial activity which is, 1-Hexadecene [33,34,35], 1-Octadecene [33,36], 4-O-Methylmannose [24,37] and n-Hexadecanoic acid [38]. Most of the compounds have anti-oxidant included squalene [27,29], phytol [32], n-Hexadecanoic acid [32], 1-Octadecene [33,36] and 1-Hexadecene [33,39]. Acetic acid, butyl ester was the most abundant compounds that beneficial as antifungal and antitumor [40]. Meanwhile, 1-Tetradecene was found as one of the compounds that having antimicrobial activity against bacteria and fungi from the genus Streptomyces [31]. Three compounds which are Acetic acid, butyl ester [40], 1-Hexadecene [33,34,35] and 4-O-Methylmannose [41] were discovered to having anti-fungal properties. Meanwhile, no biological activity has been reported for 1,3-Diisobutyrin, trimethylsilyl; Hexanoic acid, 3-oxo-, ethyl ester and .alpha.-d-Mannofuranoside, methyl. Therefore, only ten (10) out of thirteen (13) major compounds were found to be reported for having biological activities.

CONCLUSION

Green *C. vespertilionis* leaves extract possessed significant biological activity of anti-inflammatory, antitumor, anticancer, antiarthritic, antibacterial, antimicrobial, antioxidant and antifungal properties. The compounds were greatly exhibited from maceration and Soxhlet technique and particularly with methanol as a solvent. In the end, we can say that the study was achieved success in order to optimise the method of extraction as well as to identify phytochemical compounds include major compounds and its biological activities of green *C. vespertilionis* leaves. Hence, further study needs to be done in order to isolate phytochemical compounds to investigate its biological activities.

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