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Original Research Article

Chemical Composition and Cytotoxic Activities of Petroleum Ether Fruit Extract of Fruits of *Brucea javanica* (Simarubaceae)

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Abstract

Purpose: *To investigate the chemical composition and antitumor activity of the petroleum ether extract of the dried ripe fruits of Brucea javanica.*
Methods: *The composition of petroleum ether extract was analyzed by gas chromatography/mass spectrometric (GC/MS) and their antitumor activities were determined by MTT assay.*
Results: *GC/MS spectrometry results indicate that the petroleum ether extract was a mixture of esters, fatty acids, sterides, pregnanones, terpenes, alkaloids, alkenes, alcohols, ketones, aldehydes and other compounds. The results also revealed the significant antitumor activity of the extract with IC_{50} of 9.14, 12.45, 15.15, 16.13, 22.26, and 27.97 µg/mL against A549, CNE, MCF-7, NCI-H460, HepG2, and KB-3-1 cell lines, respectively.*
Conclusion: *The study establishes the chemical composition and cytotoxic activity of the petroleum ether extract of the plant fruits. There is need for further investigations to isolate more potent compounds and structurally modify the known compounds to retain activity and lower toxicity and thus lead to the possible development of Brucea javanica oil.*

Keywords: *Brucae javanica, Mass spectra, Cytotoxic activity, Anti-tumour.*

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INTRODUCTION

Natural products have long been an abundant source of therapeutic agents [1]. Recently, much attention has been paid to the *Brucea* genus and its chemical constituents because of its many-sided activities. Many chemical constituents have been isolated from *Brucea* genus, including quassinoids, alkaloids, triterpenoids, and flavonoids [2-5]. Its core components are quassinoids, which possess various biological activities including anti-tumor [4], anti-malarial and anti-babesial [6,7], anti-viral [8], anti-bacterial [9,10] and hyperglycemic [11] activities.

Brucea. javanica (L.) Merr., a Chinese herbal medicine called 'Yadanzi', is distributed in south of China (mainly in Guangxi and Guangdong Provinces) and shows significant antitumor and other activities mostly due to quassinoids, triterpenoids and alkaloids [12]. Recent research has focused on the constituents of the ethyl

acetate and n-butanol extracts of *B. javanica*. *Brucea javanica* oil (BJO) is a petroleum ether extract is mainly composed of fatty acids and fatty acid derivatives, which were extracted with petroleum ether from the fruits of *B. javanica* [13]. It has been used as anti-tumor agent to therapy hepatic, esophageal, rectal, pulmonic, renal, and prostatic carcinomas clinically [14]. However, the petroleum ether-soluble compositions of *B. javanica* seldom got attention, which is not conducive to the use of BJO on therapy. As a continuation of our search for naturally occurring bioactive substances from herb medicine in China, we investigated the constituents of the fruits from *B. javanica*.

Our objectives in the present work were, first, to determine the petroleum ether chemical compositions of *B. javanica* by GC/MS analysis and, second, to evaluate the antitumor efficacy of the crude extract and its petroleum ether fractions.

EXPERIMENTAL

Plant material

The air-dried fruits of *B. javanica* were purchased from Qingping Local Medicine Market at Guangzhou, China, in January 2008, identified by Prof. Yun-fei DENG of SCBG (South China Botanical Garden, Chinese Academy of Sciences) and a voucher specimen (no. MZH0173) has been deposited in the herbarium of SCBG.

Extraction and separation

The air-dried fruits (10 kg) were ground and extracted three times by maceration with 95 % ethanol at room temperature. After filtration, the extract was concentrated in vacuum at 40 ℃ to yield 2 L viscous liquid. The crude ethanol extract was suspended in warm distilled water (100 mL) to afford an aqueous ethanol solution (95%), then partitioned exhaustively with petroleum ether (3 × 2500 mL), followed by ethyl acetate (3 × 2500 mL), and n-butanol (3 × 2500 mL), to aff ord petroleum ester- (400 mL), ethyl acetate- (800 mL), n-butanol- (500 mL) and H2O-soluble viscous extracts respectively.

The petroleum ether-soluble extract was subjected to silica gel (80 – 100A, 400 g) column chromatography, and eluted with gradient mixtures of $CHCl_3$ - MeOH (from 1:0 to 0:1) to afford 300 fractions (250 mL each). Eight major subfractions (Fr1 - 8) were obtained by pooling fractions with similar TLC patterns. Each fraction (1 mg) was dissolved in and diluted to 1 mL distilled dichloromethane, which was identified by GC/MS analysis separately.

GC/MS conditions

GC/MS analysis was performed on Agilent AOC-20S Gas Chromatograph Mass Spectrometer. A petroleum ether phenomenex HP-5 fused silica column (30 m × 0.25 mm × 0.25 μm) was used with helium at a linear velocity of 36.8 cm/sec (65.2 psi) as a carrier gas. The GC oven temperature was programmed from 80 ℃, increasing at 2 ℃/min, up to 150 ℃ with a 2 min hold at 150 ℃, then programmed from 150 to 310 ℃ successively at 40 ℃/min with a 2 and 20 min hold at 250 ℃ and 310 ℃, respectively. The manual injection volume was 0.1 μL and split ratio was adjusted to 1:20. The Electronic ionization was at 70 eV and transfer line was heated at 220 ℃. A mass range of 10 – 400 amu was scanned. This GC/MS conditions were optimized from the methods used in previous studies [15].

The identification of volatile compounds was based on the comparison of their retention times and mass spectra with those obtained from the mass spectral reference library of National Insititute of Standards and Technology as well as those found in literature.

MTT cytotoxicity assay

Human lung cancer cell line (A549), human breast carcinoma cell line (MCF-7), human hepatoma cell line (HepG2), human lung cancer cell line (NCI-H460), human nasopharyngeal carcinoma cell line (CNE) and huaman epidermoid carcinoma cell line (KB-3-1) were obtained from Research Group of Pharmaceutical Sciences, Tropical Medicine Institute, Guangzhou University of Chinese Medicine, Guangzhou, China.

MTT assay, as previously described by *Mosmann et al [*16], was performed to assess the cytotoxicity of the plant extracts and petroleum ether fractions. Briefly, cells, grown in RPMI-1640 medium plus 10 % heat-inactivated foetal bovine serum, were plated in 96 well microtiter plates and incubated for 24 h at 37 °C, 5 % CO_2. When cells reached > 80 % confluence. The cells were treated with 100 μL petroleum ether fractions dissolved in dimethyl sulfoxide (DMSO) at serial concentrations of 50, 25, 12.5, 6.5, 3.125 and 1.56 μg/mL, while background wells were treated with only 100 μL culture medium.

After 72 h of incubation at 37 °C, 5 % CO_2, 10 µL MTT reagents (5 mg/mL) were mixed in each well and incubated at 37 ºC for a further 4 h. Then the medium was removed and 150 µL DMSO was acceded to each well after the plate was shaken thoroughly for 10 min. The absorbance was measured on a CENios microplate reader (TECAN, Austria) at a wavelength of 570 nm. MTT solution only with DMSO was used as blank and Doxorubicin as positive control. The half maximal inhibitory concentration (IC_{50}) values were calculated using SPSS software, version 16.0, by comparison with the reduction in absorbance in the control assay.

RESULTS

Chemical compositions of the petroleum ether fractions of *B. javanica*

The identified constituents of the petroleum ether fractions of *B. javanica* and their retention indices (RI) values, percentage composition are presented in Table 1. A total of 151 components were identified from these fractions. They were found to be a mixture of esters, fatty acids, alkenes, alcohols, ketones, aldehydes, terpenes, pregnanones, steroids, alkaloids and other compounds.

Table 1(a): Chemical constituents of petroleum ether fractions (Fr1-8) of *B. javanica*

Compound	RI	Fr / content (%)	Compound	RI	Fr / content (%)
Esters					
2-Ethyl-n-butyric acid ethyl ester	920	Fr6 / 2.21	Ethyl hexadecanoate	1978	Fr1 / 8.62
Butanedioic acid, monomethyl ester	1042	Fr7 / 0.77	Methyl 2-hydroxyhexadecanoate	2041	Fr3 / 5.36
Pentanedioic acid, monomethyl ester	1141	Fr6 / 0.33	n-Octadecanoic acid methyl ester	2077	Fr1 / 7.48
trans-2-Hexenyl Butyrate	1191	Fr2 / 0.26	(*Z*)-9-octadecenoic acid methyl ester	2085	Fr1,2,5,7 / 30.15, 1.97, 1.23, 1.37
3-Hydroxy-2-methylglutaric acid dimethyl ester	1249	Fr5 / 0.63	Methyl *cis,cis*-9,12-octadecadienoate	2093	Fr1,2,3 / 4.67, 1.72, 0.53
Methyl 8-hydroxyoctanoate	1326	Fr7 / 0.30	Methyl *cis*-9,10-epoxyoctadecanoate	2129	Fr4 / 13.28
Monomethyl pimelate	1340	Fr6 / 0.40	Ethyl n-octadecanoate	2177	Fr1 / 2.47
Dimethyl octanedioate	1350	Fr3,6 / 0.62, 0.36	(*Z*)-9-Octadecenoic acid ethyl ester	2185	Fr1 / 15.56
9-oxo-Nonanoic acid methyl ester	1371	Fr3,4,7 / 1.45, 0.90, 0.21	Ethyl *cis,cis*-9,12-octadecadienoate	2193	Fr1 / 3.11
4-hydroxy-Benzeneacetic acid methyl ester	1380	Fr4 / 0.95	2-[(2-nonylcyclopropyl)methyl]-Cyclopropanebutyric acid, methyl ester	2203	Fr4 / 3.84
Ethyl decanoate	1381	Fr1 / 0.02	Methyl 7-hydroxystearate	2239	Fr4 / 1.00
9-hydroxynonanoate	1425	Fr6 / 0.62	Eicosanoic acid methyl ester	2276	Fr1 / 0.97
Dimethyl nonanedioate	1449	Fr3,5,6 / 4.4, 9.46, 2.06	Methyl-(11*E*)-icosenoate	2284	Fr1 / 0.61
4-hydroxy-Benzeneacetic acid ethyl ester	1480	Fr4 / 0.36	Eicosanoic acid ethyl ester	2375	Fr1 / 0.31
Dodecanoic acid methyl ester	1481	Fr1 / 0.06	Methyl 9,10-dihydroxyoctadecanoate	2402	Fr3,4,7 / 2.78, 7.88,47.59
Dodecanoic acid ethenyl ester	1570	Fr6 / 1.28	Docosanoic acid methyl ester	2475	Fr1 / 0.58
Methyl myristate	1680	Fr1 / 0.35	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2498	Fr8 / 8.87
Ethyl myristate	1779	Fr1 / 0.13	8,11,14-Docosatrienoic acid, methyl ester	2499	Fr2,4 / 1.24, 5.56
Methyl n-pentadecanoate	1779	Fr1 / 0.29	Tetracosanoic acid, methyl ester	2674	Fr1 / 0.42
2-Phenylethyl cyclohexanecarboxylate	1820	Fr6 / 4.65	Ethyl iso-allocholate	3094	Fr3 / 1.74
n-Hexadecanoic acid methyl ester	1878	Fr1 / 22.89	1-O-Hexadecanoyl-3-O-(9*Z*-octadecenoyl) glycerol	4204	Fr5 / 20.82
(*Z*)-7-Hexadecenoic acid methyl ester	1886	Fr1,4,7 / 0.11, 1.59, 6.79	2,3-Bis[(9*E*)-9-octadecenoyloxy]	6149	Fr4,5,6,7,8/3.38, 15.62, 12.44,
Methyl 15-methylhexadecanoate	1914	Fr1 / 0.76	propyl(9*E*)-9-octadecenoate		4.02, 70.29

Table 1(b): Chemical constituents of petroleum ether fractions (Fr1-8) of *B. javanica* (contd)

Compound	RI	Fr / content (%)	Compound	RI	Fr / content (%)
Lactones					
Pantoyl lactone	1148	Fr6,7 / 0.72, 0.45	γ-Stearolactone	2178	Fr3 / 3.18
dihydroactinidolide	1426	Fr3,4 / 0.80, 1.19	4,8,12,16-Tetramethylheptadecan-4-olide	2258	Fr1 / 6.48
Acids					
Hexanoic acid	974	Fr3-7 / 4.26	3-Methoxy-4-Hydroxybenzoic acid	1560	Fr7 / 1.68
Heptanoic acid	1073	Fr4-6 / 1.09	Azelaic Acid	1629	Fr5 / 1.67
Benzenecarboxylic acid	1150	Fr3-5 / 4.57	Pentadecanoic acid	1869	Fr2,3,5,6 / 5.54, 6.40, 1.32, 2.48
Octanoic Acid	1173	Fr3 / 1.93	(*Z*)-9-Octadecenoic acid	2175	Fr2,3,5,6,7 / 29.63, 26.40, 5.96, 5.79, 3.33
Nonanoic acid	1272	Fr3 / 0.92	(*Z,Z*)-9,12-Octadecadienoic acid	2183	Fr2,3 / 27.74, 4.11
Cinnamic acid	1357	Fr3 / 0.89	*cis*-9,10-Epoxyoctadecanoic acid	2219	Fr4 / 4.36
8-Methoxy-8-oxooctanoic acid	1440	Fr6 / 4.15	Erythro-9,10-dihydroxyoctadecanoic acid	2491	Fr7 / 13.08
2-Oxoadipic acid	1466	Fr6 / 0.26			
Alkenes					
1,4-Cyclohexadiene	998	Fr1 / 0.01	1,3,12-Nonadecatriene	1916	Fr3 / 3.06
Cyclohexene	1018	Fr1 / 0.01	(17*E*)-17-Pentatriacontene	3508	Fr3 / 1.48
(7*E*)-7-Tetradecene	1421	Fr3 / 1.63			
Alcohols					
2-Methyl-1-penten-3-ol	747	Fr5 / 0.16	2,6-Dimethyl-1,7-octadiene-3,6-diol	1227	Fr7 / 1.08
Glycerin	967	Fr8 / 1.01	Cnidiol c	1255	Fr4,5,6 / 2.04, 0.32, 0.37
Isooctanol	995	Fr3 / 0.73	(2*E*)-2,6-Dimethyl-2,7-octadiene-1,6-diol	1325	Fr8 / 0.12
3,7-dimethyl-3-Octanol	1043	Fr4,5 / 0.31, 0.47	2-Cyclohexyl-hex-5-en-2-ol	1359	Fr2 / 0.47
3,7-dimethyl-1,6-Octadien-3-ol	1082	Fr2,7 / 0.14, 0.47	2-Butyloctanol	1393	Fr3 / 1.86
cis-*p*-Menth-2-en-1-ol	1109	Fr7 / 0.28	Hexadecane-1,2-diol	2017	Fr2,7 / 0.84, 0.94
Phenylethyl Alcohol	1136	Fr4,5,6,7 / 1.8, 0.98, 0.32, 0.53	2,6,10,15,19,23-Hexamethyl-tetracosa-2,10,14,18,22-pentaene-6,7-diol	3183	Fr5 / 1.5
(3*E*)-2,6-Dimethyl-3,7-octadiene-2,6-diol	1197	Fr5 / 1.25			
Ketones					
Acetophenone	1029	Fr1-7 / 1.73	9-Hydroxy-5-megastigmen-4-one	1647	Fr6 / 0.94
(3*E*)-3-Nonen-2-one	1060	Fr3 / 0.56	2-hydroxy-3-(1-propenyl)-1,4-Naphthalenedione	1917	Fr4 / 4.29
n-Nonaldehyde	1104	Fr4,5 / 0.15, 0.19	2-Hydroxycyclopentadecanone	2158	Fr4 / 0.94
Paroxypropiones	1349	Fr4 / 0.31			
Aldehydes					
(2*Z*)-2-Heptenal	913	Fr4-7 / 1.53	Vanillin	1392	Fr5 / 3.61
n-Nonaldehyde	1104	Fr6 / 0.11	(2*E*)-2-Tridecenal	1510	Fr4-8 / 4.32
(*E*)-2-Nonenal	1112	Fr7 / 0.95	2-Amylnonen-2-al	1586	Fr7 / 1.71
p-Hydroxybenzaldehyde	1203	Fr5 / 0.77	Tridecanedial	1690	Fr4 / 1.90
(*E,E*)-2,4-Decadienal	1220	Fr3,8 / 2.60, 0.14	Pentadecanal	1701	Fr1 / 0.03
Terpenes					
Copaene	1221	Fr1 / 0.03	α-Caryophyllene	1579	Fr1 / 0.04
Thujopsene	1416	Fr5 / 1.41	Widdrol	1651	Fr2 / 1.94
δ-Cadinene	1469	Fr1 / 0.08	Platambin	1813	Fr6 / 12.99
Caryophyllene	1494	Fr1 / 0.20	Simiarenol	2827	Fr2 / 1.94
Spathulenol	1536	Fr2 / 1.72	Lupeol acetate	2987	Fr3 / 4.96
Cedrol	1543	Fr2 / 3.37	Deacetylpapyriferic acid	3865	Fr4 / 1.34
Longiverbenone	1574	Fr5 / 0.88			
Pregnanones					
Pregn-4-ene-3,20-dione	2247	Fr5 / 1.71	3-hydroxy-Pregn-5-en-20-one	2264	Fr5 / 15.52
Steroids					
Lanosterol	2282	Fr8 / 18.31	Stigmasterol	2739	Fr4,5,6 / 8.97, 1.43, 5.60
(3β)-Cholesta-4,6-dien-3-ol	2579	Fr5 / 1.11	7-oxo-Cholesterol	2768	Fr7 / 4.12
(3β)-Ergost-5-en-3-ol	2632	Fr4 / 4.98	3β-Acetoxystigmasta-4,6,22-triene	2861	Fr5 / 1.38
(3β,5α,7β,8α,22E)-3',7-dihydro-Cycloprop[7,8]ergost-22-en-3-ol	2664	Fr7 / 6.43	5α-Stigmastane-3,6-dione	2875	Fr4 / 8.02
22-Stigmasten-3-one	2712	Fr4 / 5.95	(3β)-Lanosta-8,24-dien-3-ol	2882	Fr7 / 3.11
Stigmast-4-en-3-one	2714	Fr3 / 4.39	9,19-cyclo-9β-lanostane-3β,25-diol	2923	Fr5 / 2.93
(22*E*)-Stigmasta-4,6,22-trien-3-ol	2721	Fr6 / 3.49	3-Hydroxyergost-5-en-12-yl acetate	2959	Fr6 / 1.41

Table 1(d): Chemical constituents of petroleum ether fractions (Fr1 - 8) of *B. javanica* (contd)

Compound	RI	Fr / content (%)	Compound	RI	Fr / content (%)
Steroids					
(22*E*)-Stigmasta-4,22-dien-3-one	2722	Fr3 / 2.10	(3β,23*E*)-3-acetate-9,19-Cyclolanost-23-ene-3,25-diol	3071	Fr6 / 3.80
β-Sitosterol	2731	Fr4,5,6 / 13.05, 4.23, 2.39	22,23-Dibromostigmasterol acetate	3335	Fr5 / 1.40
Alkaloids or nitrogen-containing compounds					
Aniline	992	Fr5 / 0.11	N-(3-cyclopentylpropionyl)-1-Alanine methyl ester	1760	Fr6 / 3.66
1-(1H-pyrrol-2-yl)-Ethanone	1035	Fr5 / 0.48	4-Hydroxy-7-[2,3-dihydroxypropyl] pyrrolo[2,3-d]pyrimidine	1954	Fr5 / 2.01
Methyl nicotinate	1054	Fr5 / 0.28	N-(3-cyclopentylpropionyl)-l-Leucine methyl ester	1995	Fr7 / 1.52
3-Phenylpyridine	1361	Fr5 / 1.72	(Z)-9-Octadecenamide	2228	Fr5,8 / 1.17, 0.76
3-Oxo-4-phenylbutyronitrile	1473	Fr4 / 0.50	Benzoyl-3-hydroxy-2-(3-nitrophenyl)-4-imidazolidinone	2913	Fr5 / 6.36
2-Amino-1-(3-hydroxy-4-methoxyphenyl)ethanone	1682	Fr4 / 0.74			
Others compounds					
Carbon hexachloride	995	Fr1-7 / 0.78	(+)-Ambraketal	1774	Fr3 / 1.19
4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol	1457	Fr6 / 4.32	13-hydroxykaur-16-en-18-oate	2206	Fr6 / 1.58
1,2,3,5-tetraisopropyl-Cyclohexane	1503	Fr2 / 20.93	2-(12-Pentadecynyloxy)tetrahydro-2H-pyran	2254	Fr6 / 1.13

Note: *Fr / content (%) = the percentage content of every compound in the related fraction, for an example, Fr2 / 20.93, that means the percentage content of 1,2,3,5-tetraisopropyl-Cyclohexane in Fraction 2 is 20.93, and so on.*

Fr1 was characterized by its high proportion of esters (99.56 %), of which the most abundant compounds were determined to be (*Z*)-9-octadecenoic acid methyl ester (30.15 %), n-hexadecanoic acid methyl ester (22.89 %). Fatty acids (62.91 %), others compounds (21.05 %), terpenes (8.97 %), and esters (5.19 %) predominate in Fr2, and consisted mainly of (*Z*)-9-octadecenoic acid (29.63 %), (*Z, Z*)-9, 12-octadecadienoic acid (27.74 %), and 1, 2, 3, 5-tetraisopropyl-cyclohexane (20.93 %). Thirty-one components, including five repeated compounds, were found in Fr3, and consisted of fatty acids (47.89 %), esters (16.88 %), lactones (10.46 %), sterides (6.49 %), alkenes (6.17 %), terpenes (4.96 %), aldehydes (2.6 %) and alcohols (2.59 %). The major components of Fr3 were (*Z*)-9-octadecenoic acid (26.4 %), 4, 8, 12, 16-tetramethylheptadecan-4-olide (6.48 %), methyl 2-hydroxyhexadecanoate (5.36 %), lupeol acetate (4.96 %). Fr4 was marked by sterides (40.97 %), esters (38.74 %), ketones (5.92 %), fatty acids (4.86 %), alcohols (4.15 %), and aldehydes (2.6 %). The most affluent constituents were methyl cis-9, 10-epoxyoctadecanoate (13.28 %), *β*-sitosterol (13.06 %), stigmasterol (8.97 %), 5α.-stigmastane-3,6-dione (8.02 %), methyl 9,10-dihydroxyoctadecanoate (7.88 %), and 22-stigmasten-3-one (5.95 %). The analysis of the Fr5 gave forty compounds, which were primarily 1-O-hexadecanoyl-3-O-(9Z-octadecenoyl) glycerol (20.82 %), vanillin (3.6 %) and thujopsene (1.41 %). Thirty-five compounds were found in Fr6 and had the main constituents as esters (24.35 %), pregnanones (17.23 %), sterides (16.69 %), terpenes (12.99 %), fatty acids (12.94 %), others (7.20 %), alkaloids (3.66 %) and aldehydes (2.39 %). The chief compounds were 3-hydroxy-pregn-5-en-20-one (15.52 %), platambin (12.99 %), 2, 3-bis [(9*E*)-9-octadecenoyloxy] propyl (9*E*)-9-octadecenoate (12.44 %), (*Z*)-9-octadecenoic acid (5.79 %), and stigmasterol (5.60 %). Fr7 was discernible by esters (61.05 %), fatty acids (18.31 %), sterides (13.66 %), alcohols (3.30 %), and aldehydes (2.87 %). Except for the repeated constituents, the richest components were erythro-9,10-dihydroxyoctadecanoic acid (13.08%), (*Z*)-7-hexadecenoic acid methyl ester（6.79 % and (3β,5α,7β,8α,22*E*)-3',7-dihydro-cycloprop[7,8]ergost-22-en-3-ol （6.43 %）. Fr8 mainly contained 2, 3-bis [(9*E*)-9-octadecenoyloxy] propyl (9*E*) -9-octadecenoate (70.29 %), lanosterol (18.31 %), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (8.87 %).

From our results, chemical compositions of the petroleum ether extract of B. javanica included a high amount of esters, fatty acids and sterides. This is the first full report on the liposoluble constituents of B. javanica by GC/MS analysis.

Cytotoxicity assay

Six human tumor cell lines A549, MCF-7, HepG2, NCI-H460, CNE and KB-3-1 were used to investigate the in vitro antitumor effects of the different extracts and fractions of B. javanica. The IC_{50} values of extracts and Fr1 - 8 on the viability of cancer cells after 72 h of incubation are presented in Table 2.

Table 2: Cytotoxic activities of various fractions of *B. javanica* on six cell lines

Fraction	IC_{50} μg/mL					
	A549	***MCF-7***	***HEPG2***	***NCI-460***	***CNE***	***KB-3-1***
Fr1	71.29	＞100	67.80	-	83.89	＞100
Fr2	13.26	16.83	22.73	-	6.78	84.57
Fr3	36.25	78.89	31.08	-	42.69	25.19
Fr4	27.98	64.29	32.28	-	＞100	＞100
Fr5	6.04	18.26	18.20	33.77	9.24	30.05
Fr6	8.34	33.87	18.23	17.97	7.90	24.14
Fr7	7.21	21.93	16.17	24.68	11.59	30.87
Fr8	9.75	13.57	15.50	25.26	18.09	23.82
BjEE	8.79	15.12	33.31	19.67	12.81	21.72
BjP	9.14	15.15	22.26	16.13	12.45	27.97
BjE	0.02	3.28	4.14	5.79	0.48	9.66
BjB	17.47	30.92	27.16	25.75	24.29	24.08
Doxorubicin	0.16	2.37	0.52	0.31	0.37	0.17

Note: *BjEE = Ethanol Extract of Brucea javanic; BjP = Petroleum ether Extract of Brucea javanica; BjE = Ethyl acetate Extract of Brucea javanica; BjB = n-butyl alcohol Extract of Brucea javanica; "-" means no inhibition to tumor cell.*

As shown in *Table 2*, the ethanol extract (BjEE) of *B. javanica* exhibited moderate cytotoxicity against all the tested cell lines with IC_{50} values ranging from 8.79 to 33.31 μg/mL. Moreover, there were almost the same general tendencies of antitumor activity as to BJEE, when treated on the tested cell lines with the further petroleum ether (BjP), ethyl acetate (BjE) and n-butyl alcohol (BjB) extracts, respectively. Among them, BJE exhibited the highest cytotoxicity with IC_{50} values ranging from 0.02 to 9.66 μg/mL against the tested tumor cell lines, followed by BJP (8.79 to 33.31 μg/mL) and BJB (17.47 to 30.92 μg/mL). In addition, the BjEE, BjP, BjE, BjB showed the most significant cytotoxicity against A549 cell lines with IC_{50} value of 8.79 μg/mL, 9.12 μg/mL, 0.02 μg/mL, 17.47 μg/mL respectively.

Among the petroleum ether fractions of petroleum ether extract of *B. javanica*, Fr1-8 exhibited significant or moderate cytotoxicity against all the tested cell lines, except that Fr1-4 presented no inhibition to NCI-H460 cell lines. Fr1 showed inconspicuous antitumor activity on the tested cell lines with the IC_{50} values all more than 50 μg/mL, whereas, Fr2 displayed moderate cytotoxic activity on CNE, A549, MCF-7, HepG2 and KB-3-1 cell lines with IC_{50} values of 6.78, 13.26, 16.83, 22.73 and 84.57 μg/mL, separately. Fr3 & 4 exposed a modest suppression in the proliferation of A549, MCF-7 and HepG2 cell lines with IC_{50} values ranging from 27.98 to 78.89 μg/mL, Fr3 showed cytotoxic effect against KB-3-1 and CNE cell lines with the IC_{50} values of 25.19 and 42.69 μg/mL, while Fr4 had no cytotoxic activity against these two cell lines with IC_{50} values > 100 μg/mL. Fr5 & 7 displayed the same general tendencies of antitumor activity with highest IC_{50} values on A549 (6.04 and 7.21 μg/mL, respectively), followed by CNE (9.24 and 11.59 μg/mL, respectively), HepG2 (18.20 and 16.17 μg/mL), MCF-7(18.26 μg/mL and 21.93 μg/mL), NCI-H460 (33.77 μg/mL and 24.68 μg/mL) and KB-3-1(30.05 μg/mL and 30.87 μg/mL). Fr6 proved the antiproliferative rate against the tested cell lines with IC_{50} ranging from 8.34 to 33.87 μg/mL. Fr8 exhibited a potent cytotoxicity against all the tested cell lines with IC_{50} values extending from 9.75 to 25.26 μg/mL, of which were the highest values on MCF-7 (13.57 μg/mL).

DISCUSSION

It is well known that medicinal plants contain excellent antitumor compounds and they are ancient weapons in the defense against malignant neoplasms [1]. Antitumor agents destroy or inhibit the growth of tumors and over 50% of the currently used anti-cancer agents are derived from natural sources [4]. According to Geran et al., a crude extract having an IC_{50} value ≤ 20 μg/mL is considered active [17]. Under the concentration of 20 μg/mL, the ethanol, petroleum ether, ethyl acetate and n-butyl alcohol extracts of *B. javanica* all exhibited a potent selected cytotoxicity against the tested cell lines, A549, CNE, MCF-7 and NCI-H460 in

particular. Different classes of organic compounds, like quassinoids, alkaloids and triterpenoids, have been isolated and identified in the present research, and these compounds may be responsible for the cytotoxicity actions [12]. As a result of that, the ethyl acetate extract tend to be more active than the ethanol and petroleum ether extracts.

Brucea javanica oil extracted with petroleum ether from the fuits of *B. javanica*, is a complex mixture of fatty acids, which were reported to be cytotoxic active constituents [13]. The cytotoxic activity of petroleum ether fractions can be explained partly, by the high concentration of fatty acids and minor components such as sterides, pregnanones [18] and alkaloids. The large proportion of fatty acids might have contributed to the activities of the petroleum ether fractions against the tumor cell lines, because hexadecanoic acid and octadecenoic acid are known to possess cytotoxic activity [19]. However, Fr1 extract exhibited IC_{50} values almost more than 100 µg/mL on the tested cell lines, indicating that the esterification of fatty acids decrease the cytotoxicity. The decrease of esters and increase of pregnanones, sterids and alkaloids in Fr6, induced the improvement of cytotoxic capacity comparing to Fr5.

Despite the cytotoxic activities of petroleum ether fractions are lower than that of the positive control, the present results revealed their antitumor potential and further support the applications on clinical.

CONCLUSION

The present work has determined the chemical compositions of petroleum ether extract of *B. javanica* fruits by GC/MS analysis, and evaluated its cytotoxic activity. The results showed that the crude extracts and the petroleum ether fractions were significant or moderate active against the tested cell lines. Further studies should be undertaken next step to ascertain the phytochemical components and their bioactivities. This will be helpful to establish the foundation for clinical application of *Brucea javanica* oil.

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REFERENCES

1. *Lee K-H. Discovery and Development of Natural Product-Derived Chemotherapeutic Agents Based on a Medicinal Chemistry Approach. J Nat Prod 2010; 73(3): 500-516.*
2. *Pan L, Chin YW, Chai HB, Ninh TN, Soejarto DD, Kinghorn AD. Bioactivity-guided isolation of cytotoxic constituents of Brucea javanica collected in Vietnam. Bioorg Med Chem 2009; 17(6): 2219-2224.*
3. *Chen H, Bai J, Fang ZF, Yu SS, Ma SG, Xu S, Li Y, Qu J, Ren JH, Li L, et al. Indole Alkaloids and Quassinoids from the Stems of Brucea mollis. J Nat Prod 2011; 74(11): 2438-2445.*
4. *Fiaschetti G, Grotzer MA, Shalaby T, Castelletti D, Arcaro A. Quassinoids: From Traditional Drugs to New Cancer Therapeutics. Curr Med Chem 2011; 18(3): 316-328.*
5. *Yu YN, Li X. Studies on the chemical constituents of Brucea javanica (L.) Merr. Yao Xue Xue Bao 1990; 25(5): 382-386.*
6. *Nakao R, Mizukami C, Subeki YK, Bawm S, Yamasaki M, Maede Y, Matsuura H, Nabeta K, Nonaka N, Oku Y, et al. Evaluation of Efficacy of Bruceine A, a Natural Quassinoid Compound Extracted from a Medicinal Plant, Brucea javanica, for Canine Babesiosis. J Vet Med Sci 2009; 71(1): 33-41.*
7. *Anderson MM, O'Neill MJ, Phillipson JD, Warhurst DC. In vitro cytotoxicity of a series of quassinoids from Brucea javanica fruits against KB cells. Planta Med 1991; 57(1): 62-64.*
8. *Chen QJ, Ouyang MA, Tan QW, Zhang ZK, Wu ZJ, Lin QY. Constituents from the seeds of Brucea javanica with inhibitory activity of Tobacco mosaic virus. J Asian Nat Prod Res 2009; 11(6): 539-547.*
9. *Rahman S, Fukamiya N, Okano M, Tagahara K, Lee KH. Anti-tuberculosis activity of quassinoids. Chem Pharm Bull (Tokyo) 1997; 45(9): 1527-1529.*
10. *Sawangjaroen N, Sawangjaroen K. The effects of extracts from anti-diarrheic Thai medicinal plants on the in vitro growth of the intestinal protozoa parasite: Blastocystis hominis. J Ethnopharmacol 2005; 98(1-2): 67-72.*
11. *NoorShahida A, Wong TW, Choo CY. Hypoglycemic effect of quassinoids from Brucea javanica (L.) Merr (Simaroubaceae) seeds. J Ethnopharmacol 2009; 124(3): 586-591.*
12. *Liu JH, Jin HZ, Zhang WD, Yan SK, Shen YH. Chemical Constituents of Plants from the Genus Brucea. Chem Biodivers 2009; 6(1): 57-70.*
13. *Cui Y, Wu Z, Liu X, Ni R, Zhu X, Ma L, Liu J. Preparation, safety, pharmacokinetics, and pharmacodynamics of liposomes containing Brucea javanica oil. AAPS PharmSciTech 2010; 11(2): 878-884.*
14. *Yu YL, Lu Y, Tang X, Cui FD. Formulation, preparation and evaluation of an intravenous emulsion containing Brucea Javanica Oil and Coix Seed Oil for anti-tumor application. Bio Pharm Bull 2008; 31(4): 673-680.*
15. *Wu HW, Liu YQ, Yan ZJ, Wei SL, Ye JQ. A Gas Chromatography-Mass Spectrometry Analysis of the Essential Oils from Brucea javanica Extracted with Different Methods. Fine Chemicals 2011; 28(7): 668.*
16. *Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65(1-2): 55-63.*

17. Geran R. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother Rep 1972; 3: 51-61.
18. Chen YY, Pan QD, Li DP, Liu JL, Wen YX, Huang YL, Lu FL. New Pregnane Glycosides from Brucea javanica and Their Antifeedant Activity. Chem Biodivers 2011; 8(3): 460-466.
19. Tronstad KJ, Bruserud O, Berge K, Berge RK. Antiproliferative effects of a non-beta-oxidizable fatty acid, tetradecylthioacetic acid, in native human acute myelogenous leukemia blast cultures. Leukemia 2002; 16(11): 2292-2301.