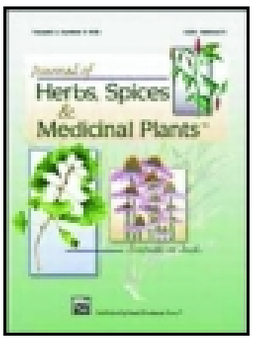

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Phytochemical Screening and Cytotoxic Effects of Crude Extracts of *Pereskia Bleo* Leaves

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ABSTRACT

The phytocompounds in crude solvent extracts of *Pereskia bleo* leaves were identified and their cytotoxic effects on cancer cell lines were determined. Crude extracts were obtained *via* maceration and subjected to GCMS analysis. Then, each extract was incubated with HeLa, MDA-MB-231, SW480, and NIH/3T3 cell lines for 72 h. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay was done to determine IC_{50} values of each extract. Terpenoids, sterols, alkaloids, fatty acids and phenolic compounds were identified from the crude extracts of *P. bleo* leaves. Other compounds identified were γ-sitosterol, β-tocopherol, and γ-tocopherol. The ethyl acetate extract had potent cytotoxic effect against HeLa and MDA-MB-231 cancer cells as noted by the lowest IC_{50} values

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KEYWORDS
GCMS; phytocompounds; antiproliferative; anti-cancer; maceration

Introduction

Cancer, a malignant tumor caused by uncontrolled proliferation of abnormal cells in the body is the second cause of death in the world.[1] Chemotherapy is costly and causes detrimental side effects to the patients due to low selectivity of target cells.[2] Due to the development of drug resistance, chemotherapy has become a less effective treatment option and hence there is a need to find a safer and sustainable treatment for cancer. Today, plants have gained the attention of researchers as a good source of anti-cancer agents[3] as they are easily available, less costly and have insignificant adverse side effects. *Pereskia bleo* (Cactaceae) locally known as "Jarum tujuh bilah" or "Cak Sing Cam" in Malaysia[4], has been studied for health promoting properties. The leaves of *P. bleo* are effective in traditional cancer treatment when consumed raw or as tea,[5–7] and also used for treating hypertension, diabetes mellitus, rheumatism, inflammation, gastric pain, and ulcer.[5–7]

Phytochemical studies on the leaves of *P. bleo* commonly use fractions instead of crude extracts. In traditional medicine, crude extract is preferred

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instead of isolated single compounds because the synergies of all compounds that are present in the plant offers greater effectiveness.[8] A crucial step in the isolation of bioactive compounds from plants is the extraction process. Soxhlet extraction, common practice for plant leaves extraction to demonstrate their cytotoxic effect,[4,9,10] requires heating at high temperature leading to the loss of thermolabile compounds.[11] In contrast, maceration is a simple procedure that involves the soaking of plant materials in the solvent for a minimum of 3 d with frequent shaking at room temperature.[12] The low extraction temperature in maceration can preserve the phytocompounds from degradation.[13]

Gas chromatography mass spectrometry (GCMS) offers rapid analysis with high sensitivity and selectivity, better resolution, high throughput, and broad coverage.[14,15] Previous studies involving GCMS on crude methanol extract of *P. bleo* leaves revealed the presence of β-sitosterol and stigmasterol though high amount of sugar and fatty acids were found in the aqueous extract.[16] In this study, the maceration extraction technique was adopted to explore the therapeutic potential of *P. bleo* leaves crude extracts. The present research was carried out to identify the phytochemicals present in the crude extracts of *P. bleo* leaves by using hexane, ethyl acetate, methanol, and aqueous *via* GCMS technique and test the extracts on HeLa, MDA-MB-231, and SW480 cancer cell lines.

Materials and Methods

Preparation of Plant Extracts

The leaves of *P. bleo* were collected from Kota Bharu, Kelantan, verified and a voucher specimen (Voucher No: 11575) was deposited at the herbarium in the School of Biology, Universiti Sains Malaysia (USM), Penang. *P. bleo* leaves were cleaned, oven-dried (50°C) and powdered. The powder (10 g) was soaked in 500 mL of hexane, ethyl acetate and methanol successively for ~30 d. Then, the extracts were filtered (Whitmann paper no. 1) and concentrated by using rotary evaporator. Another 10 g of the leaf powder was boiled in 450 mL water (50°C) until it was reduced to one-third of its initial volume and filtered. Subsequently, the aqueous extract was frozen (−20°C) overnight and dried using freeze dryer. All the extracts were stored at −20°C until use.

Cancer Cell Lines

MDA-MB-231 (breast cancer), HeLa (cervical cancer), SW480 (colon cancer), and NIH 3T3 (normal mouse fibroblast) cell lines used in this study were obtained from ATCC. All the cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) supplemented with 10% of fetal

bovine serum (FBS; Gibco) and 1% of penicillin-streptomycin (Gibco) under atmospheric humidity (5% CO_2 at 37°C).

Gas Chromatography Mass Spectrometry (GCMS) Analysis

The GCMS analysis was carried out by using Hewlett Packard 6890 Gas Chromatograph with 5973N Mass Selective Detector. The column was a fused silica capillary, HP-5 column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Agilent Technologies, USA). The carrier gas was helium with a flow rate of 1.0 mL min^{-1} with the oven temperature programmed from 50°C (5 min) to 300°C (10 min) at a rate of 25°C min^{-1}. Both injection and interface temperatures were set at 280°C. One μL sample was injected in split-less mode and analyzed in MS full scan mode (m/z 40–650). The electron ionization was fixed at 70 eV. Acquisition of data was performed using Chemsation software. Identification of phytochemical constituents was accomplished based on mass spectral matching with National Institute of Standards and Technology (NIST02) and Wiley 275 libraries (≥ 80% match).

Cytotoxicity Assay

In vitro cytotoxicity activity of *P. bleo* leaves crude extracts were determined by colorimetric assay of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), which is based on the ability of viable cells to convert soluble MTT (yellow) to insoluble formazan product (dark purple) through mitochondrial enzymatic activity. A total of 100 μL cell suspension (5×10^4 cells) was seeded in 96 well microplates for 24 h. The medium was removed from each well and 200 μL of complete medium was added. The cells were then treated with 2 μL of *P. bleo* leaves extracts that had been dissolved in dimethyl sulfoxide (DMSO) at various concentrations (3–990 μg mL^{-1}) and also with tamoxifen (positive control). After 72 h of incubation period, the medium was pipetted out and gently replaced with 20 μL of MTT solution (5 mg mL^{-1}). After 4 h, 200 μL of DMSO was added to dissolve the formazan crystal product. Absorbance was recorded at 570 nm using enzyme-linked immunosorbent assay (ELISA) plate reader. Results were obtained from three independent experiments with triplicate for each experiment. IC_{50} value (concentration that inhibit 50% of cell proliferation) was determined as:

$$\text{Cell viability } (\%) = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control}} \times 100\%$$

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) and analyzed by repeated measure one-way ANOVA analysis ($p < 0.05$) using GraphPad PRISM (ver. 7).

Results

GCMS Analysis of P. bleo Leaves Extracts

Twenty-four compounds were identified from the hexane extract of *P. bleo* leaves consisting of terpenoids, sterols, phenolic compounds, fatty acids, and others (Tables 1–4). Sterol was identified at the greatest amount representing 23.25% of total hexane extract with the main compound γ-sitosterol. Phenolic compounds consisted of β-tocopherol and γ-tocopherol representing 9.32% from the total amount of the extract (Table 1).

Ethyl acetate extract of *P. bleo* leaves showed the presence of terpenoids, phenols, sterols and fatty acids. Terpenoids were the greatest with 24.75% from the total ethyl acetate extract of *P. bleo* leaves consisted majority of phytol. New compounds such as loliolide and neophytadiene in addition to γ-sitosterol were also identified (Table 2).

Phytochemicals identified from the methanol and aqueous extracts of *P. bleo* leaves included terpenoids, sterols, phenols, alkaloids, and fatty acids. Fatty acids were the highest in both methanol (9.8%) and aqueous (5.51%) extracts of *P. bleo* leaves (Tables 3 & 4).

Cytotoxicity Activity Assay

The leaf extracts of *P. bleo* were subjected to cytotoxicity assay on selected cancer and normal cell lines *via* MTT assay. Tamoxifen was used as control positive for this study. The ethyl acetate extract of *P. bleo* leaves exhibited the strongest cytotoxic effect on HeLa cells at IC_{50} value of 17.51 ± 8.6 μg mL^{-1} (Table 5). The number of HeLa cells were reduced after 72 h incubation with ethyl acetate extract. In addition, it was also active toward MDA-MB-231 cells at 19.39 ± 1.26 μg mL^{-1}. Meanwhile, in SW480 cells, it exhibited moderate cytotoxic effect with an IC_{50} value of 31.80 ± 16.1 μg mL^{-1}.

Discussion

Phytochemical studies on the leaves of *P. bleo* extracted with solvents of different polarity detected terpenoids, sterols, alkaloids, fatty acids, sugars, and phenols. Extraction methods are important in the discovery of phytochemicals from plants because different extraction techniques will isolate different compounds and heating will eliminate heat-sensitive compounds.[11]

Table 1. Phytochemical Compounds Identified in the Hexane Extract of *Pereskia bleo* Leaves

Compound name	Retention time	% of total	Molecular weight (g mol^{-1})	Molecular formula	Compound nature
2(4H)-benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	10.195	0.22	180	$C_{11}H_{16}O_2$	Others
Cyclopentaneacetic acid,3-oxo-2-pentyl-, methyl ester	10.657	0.13	226	$C_{13}H_{22}O_3$	Fatty acid
(-)-Loliolide	11.280	0.42	196	$C_{11}H_{16}O_3$	Terpenoids
3-Eicosyne	11.610	0.37	279	$C_{20}H_{38}$	Others
Hexadecanoic acid, methyl ester	11.855	1.90	270	$C_{17}H_{34}O_2$	Fatty acid
9,12-Octadecadienoic acid, methyl ester	12.513	2.55	294	$C_{19}H_{34}O_2$	Fatty acid
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	12.541	2.69	292	$C_{19}H_{32}O_2$	Fatty acid
Phytol	12.583	5.22	297	$C_{20}H_{40}O$	Terpenoids
Octadecanoic acid, methyl ester	12.625	0.67	294	$C_{19}H_{34}O_2$	Fatty acid
4,8,12,16-tetramethylheptadecan-4-olide	13.444	0.48	325	$C_{21}H_{40}O_2$	Alkene hydrocarbon
5,9,13-Pentadecatrien-2-one,6,10,14-trimethyl-	13.535	0.19	262	$C_{18}H_{30}O$	Others
Tetracosanoic acid, methyl ester	14.578	0.46	383	$C_{25}H_{50}O_2$	Others
Squalene	14.879	1.05	411	$C_{30}H_{50}$	Terpenoids
2H-1-Benzopyran-6-ol,3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-	15.244	2.15	403	$C_{27}H_{46}O_2$	Phenol
β-t ocopherol	15.538	1.04	417	$C_{28}H_{48}O_2$	Phenol
γ-tocopherol	15.594	6.13	417	$C_{28}H_{48}O_2$	Phenol
1-Heptacosanol	15.706	2.72	397	$C_{27}H_{56}O$	Fatty acid
Vitamin E	15.902	13.31	431	$C_{29}H_{50}O_2$	Vitamin E
Campesterol	16.364	4.39	401	$C_{28}H_{48}O$	Sterols
Stigmasterol	16.511	1.13	413	$C_{29}H_{48}O$	Sterols
n-Tetracosanol-1	16.560	1.20	355	$C_{24}H_{50}O$	Fatty acid
γ-sitosterol	16.819	17.53	415	$C_{29}H_{50}O$	Sterols
Stigmast-7-en-3-ol, (3β,5α)-	17.106	0.20	415	$C_{29}H_{50}O$	Sterols
Neophytadiene	18.121	5.84	279	$C_{20}H_{38}$	Terpenoids

Table 2. Phytochemical Compounds Identified in the Ethyl Acetate Extract of *Pereskia bleo* Leaves

Name of compound	Retention time	% of total	Molecular weight (g mol^{-1})	Molecular formula	Compound nature
4-vinyl-2-methoxy-phenol	8.977	0.10	150	$C_9H_{10}O_2$	Phenol
1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	9.467	0.08	206	$C_{13}H_{18}O_2$	Others
(-)Loliolide	11.287	2.73	196	$C_{11}H_{16}O_3$	Terpenoids
Neophytadiene	11.504	5.12	279	$C_{20}H_{38}$	Terpenoids
Hexadecanoic acid, ethyl ester	12.121	0.16	284	$C_{18}H_{36}O_2$	Fatty acid
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	12.541	5.90	292	$C_{19}H_{32}O_2$	Fatty acid
Phytol	12.590	16.09	297	$C_{20}H_{40}O$	Terpenoids
9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	12.786	1.18	306	$C_{20}H_{34}O_2$	Fatty acid
9-Octadecenamide, (Z)-	12.849	0.55	281	$C_{18}H_{35}NO$	Fatty acid
3,4-Dimethyl-3-cyclohexene-1-carbaldehyde	13.332	1.74	138	$C_9H_{14}O$	Others
4,8,12,16-Tetramethylheptadecan-4-olide	13.444	0.51	323	$C_{21}H_{40}O_2$	Alkene hydrocarbon
Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	13.941	2.54	331	$C_{19}H_{38}O_4$	Fatty acid
Nonanoic acid,9-(3-hexenylidenecyclopropylidene)-2-hydroxy-1-(hydroxymethyl)	14.522	6.04	353	$C_{21}H_{36}O_4$	Fatty acid
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	14.795	1.99	278	$C_{18}H_{30}O_2$	Fatty acid
2H-1-Benzopyran-6-ol,3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-	15.243	1.82	403	$C_{27}H_{46}O_2$	Phenol
γ-t ocopherol	15.594	2.42	417	$C_{28}H_{48}O_2$	Phenol
1-Heptacosanol	15.706	2.39	397	$C_{27}H_{56}O$	Fatty acid
Stigmastan-3,5-diene	15.818	1.31	397	$C_{29}H_{48}$	Sterols
Vitamin E	15.895	3.49	431	$C_{29}H_{50}O_2$	Vitamin E
Campesterol	16.364	3.23	401	$C_{28}H_{48}O$	Sterols
Stigmasterol	16.511	0.82	413	$C_{29}H_{48}O$	Sterols
Octacosyl acetate	16.56	1.13	453	$C_{30}H_{60}O_2$	Fatty acid
γ-sitosterol	16.812	9.63	415	$C_{29}H_{50}O$	Sterols

Table 3. Phytochemical Compounds Identified in the Methanol Extract of *Pereskia bleo* Leaves

Compounds name	Retention time	% of total	Molecular weight (g mol^{-1})	Molecular formula	Compound nature
Butyrolactone	4.096	0.18	86	$C_4H_6O_2$	Others
Pyridine,2,4,6-trimethyl-	6.085	0.06	121	$C_8H_{11}N$	Others
2-Pyrrolidinone	7.268	0.69	85	C_4H_7NO	Others
Methyl salicylate	8.186	5.66	152	$C_8H_8O_3$	Others
4-vinyl-phenol	8.403	2.18	120	C_8H_8O	Phenol
1H-Pyrrole-2,5-dione,3-ethyl-4-methyl-	8.473	1.16	139	$C_7H_9NO_2$	Others
Indole	8.865	0.18	117	C_8H_7N	Alkaloids
2-Methoxy-4-vinylphenol	8.977	2.07	150	$C_9H_{10}O_2$	Phenol
Cyclopropane, octyl-	9.292	0.25	154	$C_{11}H_{22}$	Others
1-Hexadecanol	9.831	0.56	242	$C_{16}H_{34}O$	Fatty acid
2-Naphtalenamine	10.251	0.54	143	$C_{10}H_9N$	Aromatic amine
3-Methyl-4-phenylpyrrole	10.405	0.45	157	$C_{11}H_{11}N$	Others
4-(1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	11.189	0.92	180	$C_{10}H_{12}O_3$	Others
(-)-Loliolide	11.308	2.39	196	$C_{11}H_{16}O_3$	Terpenoids
Neophytadiene	11.512	1.22	279	$C_{20}H_{38}$	Terpenoids
2-Pentadecanone,6,10,14-trimethyl-	11.533	0.64	268	$C_{18}H_{36}O$	Others
Hexadecanoic acid, methyl ester	11.855	1.70	270	$C_{17}H_{34}O_2$	Fatty acid
Cyclotetradecane	12.415	0.87	196	$C_{14}H_{28}$	Others
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	12.541	2.13	292	$C_{19}H_{32}O_2$	Fatty acid
Phytol	12.583	1.75	297	$C_{20}H_{40}O$	Terpenoids
Methyl 16-methyl-heptadecanoate	12.625	0.34	299	$C_{19}H_{38}O_2$	Others
Cyclohexyl-15-crown-5	12.695	0.24	302	$C_{16}H_{30}O_5$	Others
Bis(2-ethylhexyl) maleate	12.723	0.27	341	$C_{20}H_{36}O_4$	Others
Decyltetraglycol	12.786	0.84	335	$C_{18}H_{38}O_5$	Others
2-Butanedioic acid (E)-, bis(2-ethylhexyl) ester	12.996	0.98	341	$C_{20}H_{36}O_4$	Others
4,8,12,16-Tetramethylheptadecan-4-olide	13.437	0.25	325	$C_{21}H_{40}O_2$	Terpenoids
Hexagol	13.724	0.91	282	$C_{12}H_{26}O_7$	Others
Hexadecanoic acid, 2,3-dihydroxypropyl ester	13.941	3.52	331	$C_{19}H_{38}O_4$	Fatty acid
13-tetradecenal	14.501	1.44	210	$C_{14}H_{26}O$	Others
Hexaethylene glycol monododecyl ether	15.062	0.89	451	$C_{24}H_{50}O_7$	Others
1-Octacosanol	15.699	0.38	411	$C_{28}H_{58}O$	Fatty acid
Vitamin E	15.895	0.23	431	$C_{29}H_{50}O_2$	Vitamin E
Campesterol	16.357	0.13	401	$C_{28}H_{48}O$	Sterols
Stigmasterol	16.504	0.10	413	$C_{29}H_{48}O$	Sterols
n-tetracosanol-1	16.553	0.28	355	$C_{24}H_{50}O$	Fatty acid
β-sitosterol	16.798	0.93	415	$C_{29}H_{50}O$	Sterols

Table 4. Phytochemical Compounds Identified in the Aqueous Extract of *Pereskia bleo* Leaves

Name of compound	Retention time	% of total	Molecular weight (g mol^{-1})	Molecular formula	Compound nature
Pyrazine, trimethyl-	6.218	0.34	122	$C_7H_{10}N_2$	Others
Thiazolidine,2-isobutyl-	8.067	1.90	145	$C_7H_{15}NS$	Others
Benzofuran,2,3-dihydro-	8.389	0.82	120	C_8H_8O	Others
2-Methoxy-4-vinylphenol	8.977	1.96	150	$C_9H_{10}O_2$	Phenol
4-methyl-2,5-dimethoxybenzaldehyde	10.314	0.76	198	$C_{10}H_{12}O_3$	Others
1,2,3,4-Tetrahydro-cyclopenta(b)indole	10.405	1.80	157	$C_{11}H_{11}N$	Alkaloids
Methyl dihydrojasmonate	10.65	2.30	226	$C_{13}H_{22}O_3$	Fatty acid
Octanal, 2-(phenylmethylene)-	11.162	1.28	216	$C_{15}H_{20}O$	Others
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	11.932	0.84	210	$C_{11}H_{18}N_2O_2$	Alkaloids
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	12.534	0.38	292	$C_{19}H_{32}O_2$	Fatty acid
Phytol	12.583	1.31	296	$C_{20}H_{40}O$	Terpenoids
γ-tocopherol	15.587	2.38	416	$C_{28}H_{48}O_2$	Phenol
n-Tetracosanol-1	15.699	1.56	354	$C_{24}H_{50}O$	Fatty acid
Vitamin E	15.888	0.52	430	$C_{29}H_{50}O_2$	Vitamin E
1-Heptacosanol	16.553	0.91	396	$C_{27}H_{56}O$	Fatty acid
γ-sitosterol	16.791	2.98	414	$C_{29}H_{50}O$	Sterols

Table 5. IC_{50} Values of Cytotoxic Activity from *Pereskia bleo* Leaf Crude Solvent Extracts Against Cancer (HeLa, MDA-MB-231, and SW480) and NIH/3T3 Normal Cell Lines

Extract solvents	IC_{50} values (μg mL^{-1})			
	HeLa	MDA-MB-231	SW480	NIH/3T3
Hexane	278.01 ± 12.8	95.75 ± 27.9	154.0 ± 2.0	275.0 ± 16.0
Ethyl acetate	17.51 ± 8.6	19.39 ± 1.26	31.80 ± 16.1	182.0 ± 23.0
Methanol	683.47 ± 15.7	213.23 ± 27.7	> 990	631.0 ± 22.0
Aqueous	100.40 ± 2.3	224.31 ± 25.6	128.2 ± 7.5	359.5 ± 27.5
Tamoxifen	2.71 ± 0.88	2.24 ± 0.95	2.66 ± 0.22	3.78 ± 1.46

GCMS investigation of crude hexane extract of *P. bleo* leaves showed the presence of terpenoids, sterols and phenolic compounds. Sterols were the highest in the hexane extract comprising of γ-sitosterol (17.53%) as the main compound followed by phenolic compounds. Earlier literatures recorded phenolic compounds and β-sitosterol from the hexane fraction.[5,17] γ-sitosterol has been previously reported to influence cholesterol synthesis in liver and intestinal cell lines.[18] It also acts as a cytotoxic sensitizing agent.[19] In addition, γ-sitosterol from *Strobilanthes crispus* leaves extract was cytotoxic against colon and liver cancer cell lines.[20] Additionally, β-tocopherol and γ-tocopherol identified in this extract, are well known for their antioxidant properties.[21] Recent studies also have reviewed the benefits of these compounds such as anticancer, anti-inflammatory, and cancer preventive effects.[22,23]

Phytol was the major compound isolated from the ethyl acetate extract of *P. bleo* leaves. The results showed that the ethyl acetate extract exerted the most potent cytotoxic effect against HeLa cells followed by MDA-MB-231 cells.

According to the National Cancer Institute (NCI), a plant crude extracts should have an IC_{50} of less than 20 μg mL^{-1} for potent cytotoxic effect.[24] Similar findings were reported by previous studies where this extract was cytotoxic against human nasopharynx cancer (KB) cell lines.[6,25] Phytol is believed to have anticancer properties,[26] triggering apoptosis in liver and lung cancer cells activated *via* caspase 3 and 9 pathway.[27,28] Besides phytol, other prominent compounds such as loliolide, neophytadiene and γ- sitosterol were found in this study. Loliolide has been reported for its antioxidant[29] and antiproliferative effects.[30] Meanwhile neophytadiene was widely known for its antioxidant properties.[31] The cytotoxic effect of this extract toward different cancer cell lines maybe due to the synergistic effect of all the compounds.

The methanol extracts also had terpenoids, sterols, alkaloids, phenols, and fatty acids and did not exert cytotoxic effects in the tested cell lines, which is contrary to previous reports of cytotoxicity against breast cancer (T47-D) cell lines.[10] This may be because the phytochemicals are selectively sensitive toward different cell lines.

Sim et al.[17] reported the presence of phenolic compound in the aqueous extract of *P. bleo* leaves which only measured total phenolic content while the present study has elucidated single compounds from the phenolic group namely 2-methoxy-4-vinylphenol and γ-tocopherol. Sharif et al.[16] reported that aqueous extract of *P. bleo* leaves contained fatty acids and high content of myo-inositol and sugars (galactose and phenanthrene). On the contrary, GCMS analysis in this study revealed the presence of alkaloids, terpenoids, fatty acids and phenolic compounds. Phenol was the major compound identified in the aqueous extract of *P. bleo* leaves. These differences might be due to the different temperature used during extraction. Sharif et al.[16] carried out extraction at 30°C while the temperature used in this study was 50°C. Extraction at a higher temperature releases higher phenolic compound compared to lower temperature.[32]

The present study showed that the ethyl acetate extract of *P. bleo* leaves had the highest cytotoxic effect toward HeLa and MDA-MB-231 cell lines. Potent biological effects of this plant extract are associated with the phytochemical compounds present in the plant. Further analysis is required to investigate the mechanism of cancer cell death induced by this plant.

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Conflict of interest statement

None to be declared by all authors.

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