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Original Research Article

Psiadia punctulata (DC.) Vatke induces cell apoptosis in highly metastatic MDA-MB-231 cells

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Abstract

Purpose: *This study assessed the in vitro cell migration inhibitory and cell apoptotic effects of P. punctulata stem (PPS (and leaf hexane) PPL (extracts on breast cancer cell lines (MDA-MB-231 and MCF-7 cells).*

Methods: *Cytotoxicity was quantified using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LD) release test after 48 h exposure of MDA-MB-231 cells to 0 – 200 µg/mL of PPS and PPL hexane extract. Cell apoptosis was determined using Muse™ cell cytometry, while the phytoconstituents of PPS and PPL hexane extracts were identified by gas chromatography–mass spectrometry.*

Results: *The half-maximal inhibitory concentration (IC_{50}) for PPS and PPL hexane extracts against MDA-MB-231 cells was 44.33 and 52.16 µg/mL, respectively. T, whereas the IC_{50} of PPS and PPL hexane extracts was 102.22 and 59.53 µg/mL against MCF-7 cells, sequentially. Treatment with 100 and 200 µg/mL of PPS and PPL hexane extract increased late apoptosis in MDA-MB-231 cells to 16.005 ± 1.155 and 52.58 ± 3.02 %, respectively, for PPS hexane extract and 77.34 ± 0 % and 95.21 ± 1.61 %, respectively, for PPL hexane extract, when compared to control cells (3.81% ± 0.79%). PPL hexane extract decreased cell migration and filled ~15.5 % of the wound gap on MDA-MB-231 cells after 24 h, while PPS hexane extract decreased cell migration by ~35 and ~42.5 % at 24 and 48 h, respectively. PPS and PPL hexane extracts contained several phytocompounds. Stem and leaf extracts of P. punctulata showed significant ($p < 0.05$) cell apoptotic and migration inhibition activities.*

Conclusion: *The extracts P. punctulata exhibit potent cytotoxic activity against the tested breast cancer cells. Further research is required to assess the acute and subacute toxicity of the extracts.*

Keywords: *Psiadia punctulata, Apoptosis, Cell migration, Caspase-3/7*

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INTRODUCTION

Breast cancer (BC) is the prevalent tumor malignancy in women globally. Despite the different treatments available for BC, it causes the highest number of cancer-related deaths among women. In 2018, an estimated 627,000 women died due to BC [1].

Worldwide, around 80% of people use plant-derived products to treat several diseases [2], including cancer, due to safety, low cost, availability and multitargeting ability. In addition, 60% of currently approved anticancer drugs (vincristine, vinblastine, etoposide, paclitaxel, camptothecin, topotecan, aplidine, dolastatin, dactinomycin, bleomycin and doxorubicin) are derived from natural sources, such as microorganisms and plants [3].

Psiadia punctulata (DC) Vatke (family Asteraceae) is a shrub found in Africa and Asia [4], including Saudi Arabia. The plant is traditionally used to treat abdominal pain, cold, rheumatoid arthritis, fever, malaria, skin infection and scabies, and is also used as an analgesic and expectorant [4,5]. The plant has antimicrobial, anti-trypanosomal, anti-leishmanial, anti-plasmodial and cytotoxic activities [4,6]. Phytochemical analysis of *P. punctulata* has showed the presence of several biologically active compounds, such as flavonoids, coumarins, phenylpropanoids, diterpenes and terpenoids [4,6].

This study assessed the antiproliferative ability of stem and leaf hexane extracts of *P. punctulata* (PPS and PPL hexane extracts) grown wild in Saudi Arabia against two BC cell lines (MDA-MB-231 and MCF-7). We assessed the in vitro cell migration inhibitory and cell apoptotic effects of PPS and PPL Hex on MCF-7 and MDA-MB-231 cells.

EXPERIMENTAL

Plant collection and identification

P. punctulata was collected from Al suda (the Asir region; 18°19'09.3"N, 42°20'27.9"E) in south of Saudi Arabia between October and November 2019. A herbarium voucher specimen (no. KSU/BRC-050) was deposited for reference purposes at the Bioproducts Research Chair, Riyadh, Saudi Arabia

Phytochemical extraction

The *P. punctulata* leaves (PPL) and stems (PPS) were detached from the plants, washed several times with distilled water, and air-dried at 25°C. Subsequently, 50 g of dry leaves and stems were separately ground and extracted with 500 mL of solvent (hexane, chloroform, ethyl acetate and methanol) using Soxhlet apparatus for 24 h. Thereafter, the solvent was rotary evaporated (Heidolph, Germany) and transferred to a pre-weighed flask. The same process was repeated sequentially with three other solvents: chloroform ($CHCL_3$), ethyl acetate (EtoAC) and methanol (MeOH).

Cell culture

MDA-MB-231 and MCF-7 cells were seeded in Dulbecco's modified Eagle's medium (DMEM). All media were complemented with foetal bovine serum (10 %) (Invitrogen, USA) and 1 % antibiotics (Gibco, USA) at 37 °C in a CO_2 incubator.

Cell viability assay

Cytotoxicity was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay. MDA-MB-231 and MCF-7 cells were separately grown in 24-well plates at 5×10^5 cells/well and then incubated for 48 h with different dilutions of *P. punctulata* stem and leaf extracts in triplicate (concentration range = 10 – 200 µg/mL). Next, the cells were incubated for 2 h with 10 % MTT at 37 °C. Finally, the absorbance of the converted dye was recorded using a multi-well plate reader (Thermo Fisher Scientific, Multiskan, China) with a 540 nm filter, and the data collected were used to calculate the medial lethal concentration (LC_{50}) using OriginPro version 8.5 (OriginLab Corporation, Northampton, USA).

Lactate dehydrogenase release assay

The cytotoxic effect of PPS and PPL hexane extracts on the cell membrane integrity was analysed by detecting lactate dehydrogenase (LDH) release to the extracellular medium. Briefly, MCF-7 and MDA-MB-231 cells were seeded and treated (LC_{50}), as described earlier. LDH cytotoxicity assay was performed according to the kit manufacturer's instructions. Absorbance at 490 nm was measured using a multiwell plate reader (Thermo Fisher Scientific). Finally, cytotoxicity was expressed absorbance (A) values. The LDH release assay was performed in triplicate.

Cell morphology

MDA-MB-231 and MCF-7 cells were incubated in LC_{50} of PPS and PPL hexane extracts for 48 h and their morphology was observed.

Wound-healing assay

MDA-MB-231 cells were grown in a 24-well plate until they reached 90% confluence and then wounded using a pipette tip (P-10) to create a physical gap. Thereafter, they were treated with

a noncytotoxic concentration (one-fourth of the half-maximal inhibitory concentration [IC_{50}]) of PPS and PPL hexane extracts for 24 and 48 h. Images of cells migrating into denuded areas were taken using a Leica microscope (Leica Camera, Germany) on each day of treatment (0, 24 and 48 h). Finally, the images were analysed using Image J software (National Institutes of Health, Bethesda, MA, USA). The wound-healing assay was performed in triplicate.

Cell apoptosis

MDA-MB-231 cells were treated for 48 h with 100 and 200 µg/mL of PPS and PPL hexane extracts and 0.01% dimethyl sulfoxide (DMSO) as a control. Next, the cells were stained with Muse™ annexin V and dead cell reagent to assess the percentage of apoptotic cells, as previously described [7]. Lastly, cell analysis was performed using a Muse™ flow cytometry analyser (Millipore, Burlington, MA, USA).

Caspase-3/7 activity assay

MDA-MB-231 cells were treated with PPS and PPL hexane extracts and then further incubated with CellEvent™ caspase-3/7 green detection reagent (5µM). Following the treatment, the cells were photographed using a fluorescence microscope.

Gas chromatography–mass spectrometry (GC-MS)

PPS and PPL hexane extracts were analysed for phytocompounds using **GC-MS** (PerkinElmer, MA, USA) and identified, as previously described [7].

Statistical analysis

The data were analysed using OriginPro 8.5, and are presented as mean ± standard deviation (SD). Statistical comparisons between the control, and extract-treated groups were by Student's two-tailed t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Extract yield

The plant powders prepared from *P. punctulata* leaves and stems were extracted using four different solvents (hexane, $CHCl_3$, EtoAC and MeOH), and their yields are shown in Table 1. MeOH produced the highest yield, while EtoAC provided the lowest yield of PPS and PPL hexane extracts. All extracts were sticky and medium to dark green in colour.

Effect of extracts on cell viability

In cytotoxicity assay, the highest cytotoxic activity was reported for PPS hexane extract (IC_{50} = 44.33 µg/mL) and PPL hexane extract (IC_{50} = 52.16 µg/ml), followed by *P. punctulata* leaf chloroform extract (IC_{50} = 68.11 µg/mL) against MDA-MB-231 cells. The IC_{50} of PPS and PPL hexane extracts was 102.22 and 59.53 µg/mL, respectively against MCF-7 cells. LDH release assay showed a significant ($p \leq 0.05$) release of LDH after 48 h treatment at IC_{50} of PPS and PPL hexane extracts. Therefore, PPS and PPL hexane extracts were selected for subsequent assays (Figure 1 and Figure 2).

Cell morphology

Compared to control cells, most of the treated MDA-MB-231 and MCF-7 cells changed from spindle shaped to round shaped. In addition, they detached, shrunk, blebbed and formed apoptotic bodies (Figure 3). These morphological alterations are characteristics of apoptosis.

Cell migration

Control cells closed ~36.5 % of the wound after 24 h and ~55 % after 48 h. Compared to control cells, PPL hexane extract treatment (one-fourth of the IC_{50}) decreased cell migration time-dependently and filled ~15.5 and ~24 % of the wound gap in MDA-MB-231 cells after 24 and 48 h, respectively (Figure 4), while PPS hexane extract treatment (one-fourth of the IC_{50}) decreased cell migration by ~35 and ~42.5 % at 24 and 48 h, respectively (Figure 5).

Cell apoptosis

PPL hexane extract was cytotoxic to MDA-MB-231 cells (Figure 6 a and b), and treatment of MDA-MB-231 cells with PPL hexane extract increased the late apoptotic cells percentage to 77.34 ± 0 % (100 µg/mL) and 95.21 ± 1.61 % (200 µg/mL) compared to control (3.81 ± 0.79 %), indicating that PPL hexane extract inhibits MDA-MB-231 cell viability through the apoptotic pathway. Similarly, PPS hexane extract treatment (100 and 200 µg/mL) increased the percentage of late apoptotic cells to 16.001 ± 1.15 and 52.58 ± 3.02%, respectively, compared to control cells (3.81 ± 0.79 %) (Figure 6 c and d), indicating that the apoptotic effect of PPS hexane extract increases depending on the concentration.

Table 1: Yield of *Psiadia punctulata* leaf and stem extracts in solvents with different polarities ($CHCl_3$, chloroform; EtOAc, ethyl acetate; MeOH, methanol)

Plant	Plant part (g)	Extract yield, g (%)			
		n-Hexane	$CHCL_3$	EtOAc	MeOH
Psiadia punctulata	Leaves (41.34)	1.88 (4.54)	2.44 (5.90)	0.75 (1.81)	6.5 (15.72)
	Stem (48)	1.3 (2.70)	1.31 (2.72)	0.23 (0.47)	7.3 (15.20)

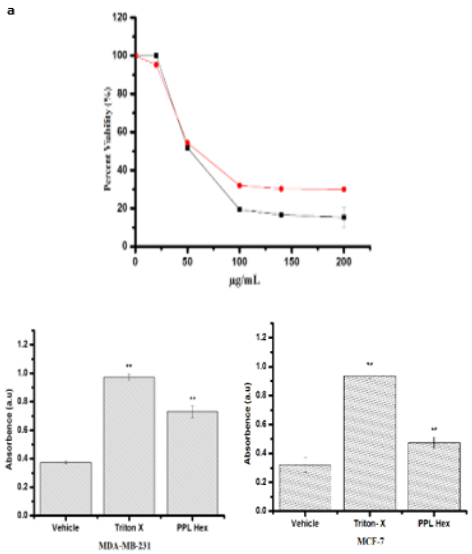

Figure 1: Cytotoxic effect of PPL Hex and (b) (PPS Hex) on MDA-MB-231 and MCF-7 cells using MTT and LDH release assays. (Top) Dose–response curves of the effects of five (a) PPL Hex and (b) PPS Hex concentrations after 48 h treatment

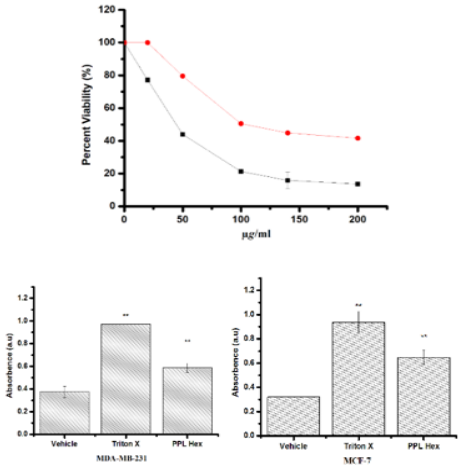

Figure 2: (Bottom) LDH release assay showing significant cytotoxicity of (a) PPL Hex and (b) PPS Hex on cells. Data represent the mean ± SD ($p < 0.05$) from triplicate experiments. PPL, *Psiadia punctulata* leaf; PPS, *Psiadia punctulata* stem; Hex, hexane extract; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; LDH, lactate dehydrogenase; SD, standard deviation. ■ MDA-MB-231 ○ MCF-7

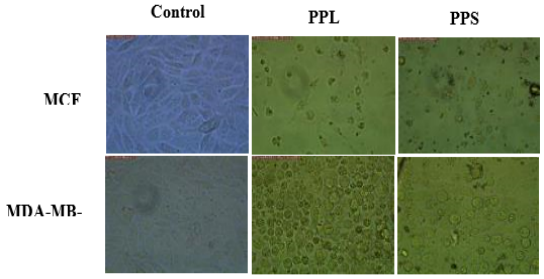

Figure 3: Morphological alterations of MCF-7 and MDA-MB-231 cells after 48 h treatment with PPL Hex and PPS Hex (magnification 200x). PPL, *Psiadia punctulata* leaf; PPS, *Psiadia punctulata* stem; Hex, hexane extract

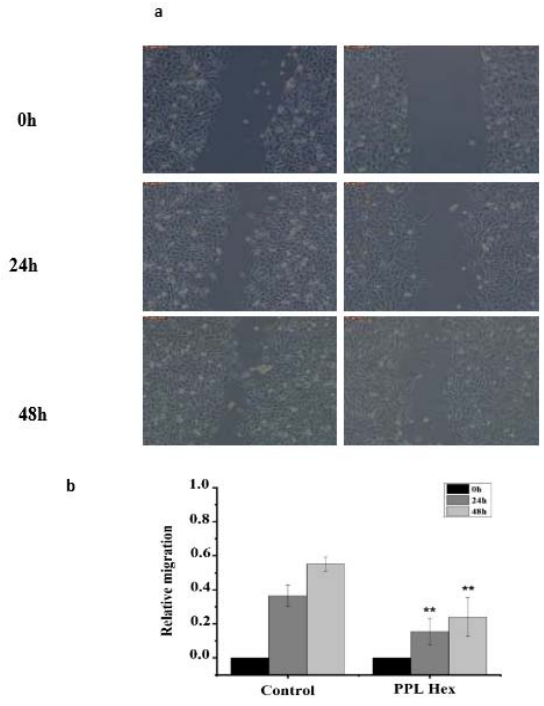

Figure 4: (a) *In vitro* scratch assay of MDA-MB-231 cells post treatment with PPL Hex extract. Cells were incubated with one-fourth of the IC_{50} and pictures were taken at 0, 24 and 48 h. **(b)** Results are presented as mean ± standard deviation from triplicate experiments; $^{**}p < 0.05$ vs. control cells (Student's two-tailed *t*-test). PPL, *Psiadia punctulata* leaf; Hex, hexane extract; LC_{50}, half-maximal inhibitory concentration

Caspase-3/7 fluorescence results

Apoptosis is frequently associated with caspases activation. In this experiment, the effect of PPL and PPS hexane extracts on caspase-3/7 activation using fluorescent microscopy was investigated. MDA-MB-231 cells treated with IC_{50} of PPL and PPS hexane extracts caused an increase in caspase-3/7 expression in the treated cells, as shown by a bright green fluorescence when compared to the control (low fluorescence intensity) (Figure 7).

Phytochemical content of *Psiadia punctulata*

GC-MS profile of PPL hexane extract indicated the presence of 37 phytoconstituents when their mass spectra were compared with the NIST library (Table 2). The most abundant compounds were 2-methyl-heptane (5.55 %), 3-methyl-heptane (5.56 %), 2-β-pinene (11.77 %), 2, 6, 10, 14, 18, 22-tetracosahexaene (7.78%) and nonacosane (8.60 %). GC-MS result of PPS hexane extract indicated the presence of 40 phytoconstituents (Table 3). The most abundant compounds were 1, 2, 4-trimethyl-benzene, tetratetracontane and pentacosane.

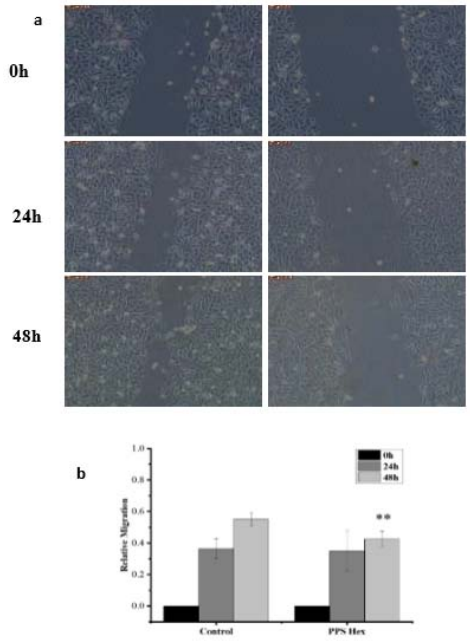

Figure 5: (a) *In vitro* scratch results of MDA-MB-231 cells post treatment with PPS Hex extract. Cells were incubated with one-fourth of the LC_{50}, and pictures were taken at 0, 24 and 48 h. **(b)** Results are presented using the mean ± standard deviation from triplicate experiments; $^{**}p < 0.05$ vs. control cells (Student's two-tailed *t*-test). PPS, *Psiadia punctulata* stem; Hex, hexane extract; LC_{50}, half-maximal inhibitory concentration

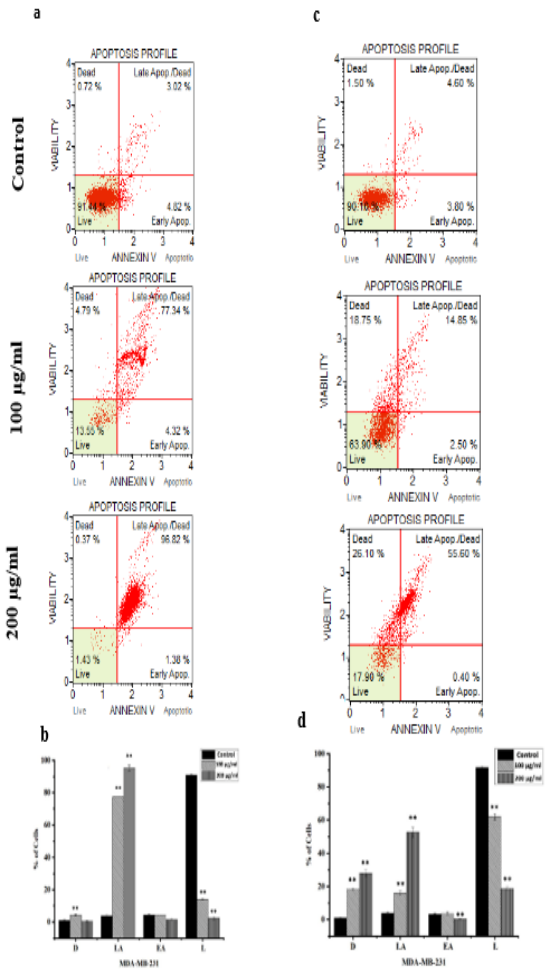

Figure 6: (a) Apoptotic potential of two different concentrations of PPL Hex extract (100 and 200 µg/mL) was assessed using Muse™ cell analyser. (b) Bar charts revealing the total apoptotic percentage of cells post treatment with PPL Hex extract. PPL, *Psiadia punctulata* leaf; Hex, hexane extract**. (c)** Apoptotic potential of two different concentrations of PPS Hex extract (100 and 200 µg/mL) was assessed using Muse™ cell analyzer. **(d)** Bar charts revealing the total apoptotic percentage of cells post treatment with PPS Hex extract PPS, *Psiadia punctulata* stem, Hex and hexane extract

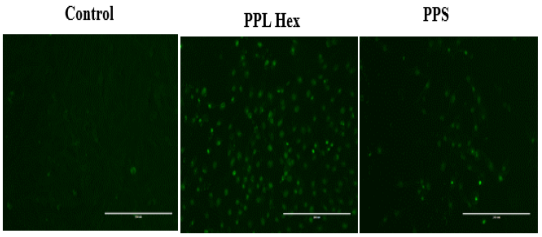

Figure 7: Caspase-3/7 activation in MDA-MB-231 cells. Caspase-3/7 was activated posttreatment with PPL and PPS Hex. PPL, *Psiadia punctulata* leaf; PPS, *Psiadia punctulata* stem; Hex, hexane extract

DISCUSSION

Plants are a "treasure" of bioactive compounds that can be used to treat numerous human diseases. In the past few decades, many plant-based drugs have been commercialised [8]. This study provides details of the apoptotic potential of the leaf and stem extracts of *P. punctulata*. Bader *et al* [9] reported that treatment with a *P. punctulata* root hexane extract has a cytotoxic effect on HeLa and Jurkat cells with an IC_{50} of 71 ± 3.1 and 73 ± 2.5 µg/mL, respectively [9]. Studies have also reported the cytotoxic effect of a methanol extract of *P. punctulata* aerial parts on 5637 and MCF-7 cells with an IC_{50} of 41.9 ± 0.2 and 93 ± 4.8 µg/mL, respectively [10]. However, there are no detailed reports on apoptosis and cell migration activities of *P. punctulata*. In this study, we performed both MTT and LDH release assays in order to confirm the cytotoxicity of PPS and PPL hexane extracts. Both PPS and PPL hexane extracts showed concentration-dependent cytotoxicity by decreasing the viability of the cancer cells tested. LDH release is an indication of irreversible death attributed to cell membrane damage [11], which is caused by the plant's cytotoxic nature and confirms its anticancer potential [12].

Antiproliferative investigation showed that both MCF-7 and MDA-MB-231 cells were successfully inhibited by the extracts of *Psiadia punctulata*. *P. punctulata* extracts induced morphological changes on both cell lines tested at 24 h of treatment. Among the signs of apoptosis process is the presence of cell shrinkage, nuclear fragmentation, blebbing and chromatin condensation [13],

Table 2: Molecular weight (MW), retention time (RT), and area percentage found in *Psiadia punctulata* leaf hexane extract (PPL Hex)

No.	Name of compound	Chemical formula	Molecular weight (g/mol)	Retention time	Area (%)
1	3,3-dimethyl- hexane	C_8H_{18}	114.23	3.75	0.990
2	2,4-dimethyl-hexane	C_8H_{18}	114.23	3.61	3.110
3	2,3-dimethyl- hexane	C_8H_{18}	114.23	4.04	2.280
4	2-methyl-heptane	C_8H_{18}	114.23	4.12	**5.550**
5	3-methyl-heptane	C_8H_{18}	114.23	4.25	**5.560**
6	Octane	C_8H_{18}	114.23	4.69	0.850
7	1,4-dimethyl-benzene	C_8H_{10}	106.17	6.03	1.680
8	1-ethyl-3-methyl-benzene	C_9H_{11}	119.18	7.65	1.160
9	2-beta-pinene	$C_{10}H_{16}$	136.23	7.92	**11.770**
10	Beta-myrcene	$C_{10}H_{16}$	136.23	8.08	4.400
11	1,2,4-trimethyl-benzene	C_9H_{12}	120.19	8.25	3.360
12	DL-limonene	$C_{10}H_{16}$	136.23	8.80	0.360
13	Alpha-copaene	$C_{15}H_{24}$	204.35	13.98	0.780
14	Alpha-gurjunene	$C_{15}H_{24}$	204.35	14.41	0.300
15	Trans-caryophyllene	$C_{15}H_{24}$	204.35	14.62	1.820
16	Alpha-humulene	$C_{15}H_{24}$	204.35	15.08	1.680
17	3,7-guaiadiene	$C_{15}H_{24}$	204.35	15.32	0.910
18	Germacrene-d	$C_{15}H_{24}$	204.35	15.40	4.450
19	Beta-selinene	$C_{15}H_{24}$	204.35	15.51	0.490
20	3,7-guaiadiene	$C_{15}H_{24}$	204.35	15.57	2.980
21	(-)-caryophyllene oxide	$C_{15}H_{24}O$	220.35	16.69	3.390
22	1h-cycloprop[e]azulen-4-ol	$C_{15}H_{26}O$	222.37	17.19	1.820
23	Megastigma-5,7-diene	$C_{19}H_{30}O_8$	386.44	18.04	0.340
24	3,5,7-nonatrien-2-one	$C_9H_{12}O$	136.19	18.35	0.670
25	Beta-ionone	$C_{13}H_{20}O$	192.3	18.54	1.000
26	Aristolen	$C_{15}H_{24}$	204.35	18.89	0.270
27	Phytol acetate	$C_{22}H_{42}O_2$	338.6	19.36	1.150
28	Falcarinol	$C_{17}H_{24}O$	244.37	22.38	0.710
39	Phytol	$C_{20}H_{40}O$	128.17	23.15	0.870
31	3-cyclohexene-1-ethanol	$C_8H_{14}O$	126.2	26.56	1.000
31	Kauren-19-yl-acetate	$C_{22}H_{34}O_2$	330.5	27.51	2.380
32	(-)-obtusane	$C_{15}H_{23}$	203.35	27.70	0.690
33	1-methylene-2b-hydroxymethyl	$C_{15}H_{26}O$	222.37	27.84	5.670
34	Methyl alpha-ketopalmitate	$C_{17}H_{32}O_3$	284	28.45	1.440
35	2,6,10,14,18,22-tetracosahexae	$C_{24}H_{38}$	326.6	29.33	**7.780**
36	Nonacosane	$C_{29}H_{60}$	408.6	29.92	**8.600**
37	Kauran-18-al	$C_{22}H_{34}O_3$	346.5	30.25	0.350

Table 3: Molecular weight (MW), retention time (RT), and area found in *Psiadia punctulata* stem hexane extract (PPS Hex)

No.	Name of compound	Chemical formula	Molecular weight (g/mol)	Retention time	Area (%)
1	2,4-dimethyl-hexane	C_8H_{18}	114.23	3.60	1.820
2	3,3-dimethyl- hexane	C_8H_{18}	114.23	3.73	0.590
3	2,3-dimethyl- hexane	C_8H_{18}	114.23	4.01	1.500
4	2-methyl-heptane	C_8H_{18}	114.23	4.10	3.330
5	3,4-dimethyl-hexane	C_8H_{18}	114.23	4.17	0.510
6	3-methyl-heptane	C_8H_{18}	114.23	4.22	4.580
7	Octane	C_8H_{18}	114.23	4.66	0.970
8	Ethyl-benzene	C_8H_{10}	106.17	5.83	0.600
9	1,4-dimethyl-benzene	C_8H_{10}	106.17	6.01	2.580
10	Propyl-benzene	C_9H_{12}	120.2	7.47	1.000
11	1-ethyl-2-methyl benzene	C_9H_{12}	120.2	7.61	2.780
12	1-ethyl-4-methylbenzene	C_9H_{11}	119.18	7.66	1.150
13	(-)-Beta-pinene	$C_{10}H_{16}$	136.23	7.88	1.400
14	Beta-myrcene	$C_{10}H_{16}$	136.23	8.04	0.230
15	1,2,4-trimethyl- benzene	C_9H_{12}	120.19	8.22	**6.580**
16	2-ethyl-1,4-dimethyl-benzene	$C_{10}H_{14}$	134.22	9.25	0.300
17	Alpha-copaene	$C_{15}H_{24}$	204.35	13.97	0.220
18	(-)-Beta-elemene	$C_{15}H_{24}$	204.35	14.13	0.220
19	Trans-caryophyllene	$C_{15}H_{24}$	204.35	14.59	0.790
20	Alpha-humulene	$C_{15}H_{24}$	204.35	15.05	0.570
21	Germacrene-d	$C_{15}H_{24}$	204.35	15.37	1.420
22	Eudesma-4(14),11-diene	$C_{15}H_{24}$	204.35	15.48	0.160
23	Alpha-muurolene	$C_{15}H_{24}$	204.35	15.54	0.440
24	Aromadendrene 2	$C_{15}H_{24}$	204.35	16.34	0.130
25	1,6-Germacradien-5-ol	$C_{15}H_{26}$	222.366	16.58	0.220
26	(-)-Caryophyllene oxide	$C_{15}H_{24}O$	220.35	16.67	0.580
27	Gamma-himachalene	$C_{15}H_{24}$	204.35	17.17	0.340
28	(-)-Caryophyllene oxide	$C_{15}H_{24}O$	220.35	17.50	0.120
29	Juniper camphor	$C_{15}H_{26}O$	222.37	17.66	0.380
30	(-)-Caryophyllene oxide	$C_{15}H_{24}O$	220.35	17.87	0.160
31	(+-)-Pentalenene	C_8H_6	102.13	18.86	0.120
32	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	22.22	2.330
33	Falcarinol	$C_{17}H_{24}O$	244.37	22.37	0.810
34	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.47	24.61	0.650
35	Cholesta-8,24-dien-3-ol	$C_{30}H_{50}O$	426.7	27.52	0.620
36	Octadecanal	$C_{18}H_{36}O$	268.5	28.06	0.640
37	Tetratetracontane	$C_{44}H_{90}$	619.2	29.98	**5.650**
38	Caryophyllan-2,6-beta-oxides	$C_{15}H_{26}O$	222.37	30.22	0.840
39	(-)-Alpha-costol	$C_{15}H_{24}O$	220.35	31.01	0.590
40	Pentacosane	$C_{25}H_{52}$	352.7	31.60	**45.450**

which can be observed using light and fluorescent microscopy. Cell death can take place via necrosis and apoptosis. Most anticancer drugs show their cytotoxicity by inducing apoptosis in malignant cells [14]. Plant extracts induce apoptosis in different cancer cell lines [15]. Changes to cellular morphological and nuclear features are the first signs of cell death.

One other important indicator of apoptosis is the translocation of phosphatidylserine (PS), which is detected by annexin V [16]. In the present investigation, apoptosis increased concentration-dependently posttreatment with PPS and PPL Hex are extracts. In addition, apoptosis was confirmed by fluorescent microscopy using the caspase-3/7 kit. Caspases are important mediators of apoptosis [17]. Treatment with PPL and PPS hexane extracts resulted in bright nuclei (Figure 4), an indicator of apoptotic cells.

Although huge progress has been made in breast cancer detection and therapy, the five-year survival rate and prognosis are unsatisfactory [18]. The high mortality rate of breast cancer is due to metastasis, which is a complex process involving several steps: matrix degradation, migration, adhesion and invasion [19]. Inhibition of cancer cell migration is considered a potential approach to treating cancer [20]. Although *P. punctulata* showed cytotoxicity in different cancer cell lines, there are no reports on the effects of *P. punctulata* on cancer cell migration. This study showed that PPL and PPS hexane extracts at noncytotoxic concentrations (one-fourth of the IC_{50}) significantly inhibited MDA-MB-231 cell

migration, which have a strong capacity for migration and invasion. GC-MS analysis of the obtained extracts can be an interesting tool to identify the amount and nature of some active compounds in plants extracts used in drugs.

In this study, GC-MS analysis showed the presence of 37 phytoconstituents in PPL hexane extract (Table 2) and 40 phytoconstituents in PPS hexane extract (Table 3), some of which individually or combined possess anticancer activity. The other major phytoconstituents of PPL hexane extract do not have anticancer activity. However, minor phytoconstituents of PPL hexane extract, such as phytol, induce apoptosis in A549 (lung cancer) cells [21]. D-limonene inhibits the growth of A549 and H1299 cells and inhibits the tumour growth in mice. Expression of apoptosis genes increases in cancer cells after D-limonene treatment [21] which also induces apoptosis against gastric and prostate cancers, both in vivo and in vitro [22].

Hexadecanoic acid shows anticancer activity against HCT-116 cells (colorectal cancer) [23]. However, a few phytoconstituents of plants extracts do not show anticancer activity as individual compounds; however, when they are combined with other compounds, they exude anticancer activity. For example, β-selinene, aromadendrene and α-terpinolene together show cytotoxicity against MCF-7 cells [24].

CONCLUSION

The stem and leaf extracts of *P. punctulata* exert cell migration inhibition and apoptotic activities on MDA-MB-231 cells. Combinations of the phytoconstituents appear to target multiple pathways, and hence, the extract combination is a promising approach to overcome multidrug resistance of cancer cells.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Abutaha conceived and designed the study, Abutaha, Almalki, AL-Mekhlafi1, Al-Keridis, El Hadi, and Wadaan collected and analysed the data, Abutaha wrote the manuscript and all authors read and approved the manuscript.

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