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Original Article

Investigation of Secondary Metabolites and Cytotoxicity of *Jacquemontia pentantha* (Jacq.)

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ABSTRACT

Introduction: The aim of this study is to isolate and identify sterols and terpenes from the chloroform/methanol extract (3:1) of aerial parts of *Jacquemontia pentantha* (Jacq.) and evaluation of cytotoxic activity of crude extract and phytol for the first time from this plant. **Methods**: Different chromatographic techniques for the aerial parts of *Jacquemontia pentantha* extract were used resulting in isolation of eight compounds. Their structures were elucidated by spectroscopic methods including ^{1}HNMR, ^{13}CNMR, EI/MS spectrometry and by comparing their data with those reported in the literature. The cytotoxicity was evaluated using MTT assay. The mode of action of the extract was predicted by using Enzyme-linked Immunosorbent Assay Kit for Tubulin beta (TUBb). **Results**: Eight compounds for the first time from this plant were identified as Palmitic acid (1), Phytol (major) (2), Stigmast-4-en-3-one (3), mixture of α-amyrin (4) and β–amyrin (5), 1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl (all-E) (6) and mixture of α– amyrin acetate (7) and β-amyrin acetate (8). The extract showed potent cytotoxic activity on MCF-7 breast carcinoma cell line as well as HCT-116 colon carcinoma cell line at different concentrations (100-6.25 ug/ml) with IC_{50} (21.8 ± 0.9) and (40.9 ± 1.3) respectively. Phytol showed potent cytotoxic activity on MCF-7 cell line at different concentrations (100-12.5 ug/ml) with IC_{50} (60 ± 2.4), while it had no cytotoxic effect on HCT-116 cell line. The extract showed significant TUBb polymerization inhibition activity. **Conclusion**: The extract of aerial parts of *Jacquemontia pentantha* (Jacq.) and also phytol compound has cytotoxic activity due to the presence of phytochemicals such as sterols and terpenes.

Key words: Cytotoxic activity, enzyme-linked immunosorbent assay, Jacquemontia pentantha, MTT assay, sterols, terpenes.

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INTRODUCTION

Jacquemontia pentantha (Jacq.) belongs to family Convolvulaceae, which includes 55 genera and 1650 species, distributed in both tropical and temperate regions of the world.[1] *Jacquemontia pentantha* is an attractive ornamental plant, spreading in tropical and subtropical countries including the West African Region.[2] Chemically, many species of this family include phytoconstituents as; alkaloids,[3] flavonoids, as well as terpenoids and coumarins.[4] Bioactivities, reported in these species were antioxidant,[5] antimicrobial,[6] anti-inflammatory,[7] antidiabetic,[8] and anticancer activity.[9] Convolvulaceae importance as a source of food and drugs has been reported.[1] Worldwide cancer is a life- threatening disease affecting human populations globally. There is a constant need for discovering new candidate to treat and prevent cancer. Scientific research interest is directed towards plant kingdom due to their wide diversity of naturally-occurring secondary metabolites with less toxic side effects compared to current chemotherapeutic drugs used in pharmaceutical market. Continuing our work on this species,[2,10] this study aims to characterize the chemical constituents (sterols and terpenes) of *Jacquemontia pentantha* aerial parts, cultivated in Egypt, for the first time from this plant, to support the possibility of its uses as a natural resource in therapeutics. Also to demonstrate the cytotoxic activity of the extract and phytol, which hasn't been investigated before for this plant.

MATERIALS AND METHODS

Collection of plant material

The aerial parts of *Jacquemontia pentantha* (Jacq.) (Family Convolvulaceae) were collected from El–Orman garden, Giza, Egypt, in July 2017. The plant was identified by Mm. Tressa Labib, Taxonomist, El-Orman garden, Giza, Egypt. The plant samples were air–dried, powdered and kept for phytochemical and bioactivity studies.

Instrumentation

NMR spectra were recorded on a Jeol Ex-600 MHz NMR spectrometer, using TMS as internal standard. UV spectrometer (Schimadzu UV-240) and mass spectra EI-MS (Varian MAT at 70ev) were used. Column chromatography (CC) was carried out on silica gel F254 (Merck) in glass blades. TLC was performed with silica gel 60 GF254 plates (Merck, Darmstadt, Germany),

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then the plates were visualized using UV light and by spraying with vanillin in H_2SO_4.

Isolation and extraction of the compounds

The air-dried powdered aerial parts *of Jacquemontia pentantha* (1 kg) was extracted with chloroform-methanol (3:1) till exhaustion. The extract was evaporated under reduced pressure to yield 100 g crude extract. A portion of this extract (70 g) was subjected to silica gel (60-120 mesh, Merck) column chromatography, eluted with petroleum ether, followed by methylene chloride step–gradient, starting with 100% petroleum ether. Then, the column was eluted with gradient of methylene chloride/methanol (100:0, 9:1, 7:3, 1:1 and 0:100). The elutes were monitored using TLC, and were viewed under UV light (254 and 365 nm) and by spraying with 1% vanillin/ 5% H_2SO_4/ EtOH reagent, followed by heating at 100°C. The fractions obtained were collected and combined on the basis of TLC profiles, to give five major fractions from which 8 compounds were obtained. **Fraction 1**: It was subjected to silica gel column, eluted by 20% methylene chloride in petroleum ether and was purified by TLC on silica gel "G" plate using petroleum ether: ethyl acetate (9:1) as developing system, giving compound **1** (3.7 mg)**. Fraction 2**: It was subjected to silica gel column, eluted by 30% methylene chloride in petroleum ether and was purified by TLC on silica gel "G" plate using petroleum ether: ethyl acetate (9 : 1) as developing system, giving major compound **2** (65.4 mg)**. Fraction 3:** It was subjected to silica gel column, eluted by 30% methylene chloride in petroleum ether and was purified by TLC on silica gel "G" plate using petroleum ether: ethyl acetate (9 : 1) as developing system, giving compound **3** (5.3 mg)**. Fraction 4**: It was subjected to silica gel column, eluted by 30% methylene chloride in petroleum ether and was purified by TLC on silica gel "G" plate using petroleum ether : ethyl acetate (8 : 2) as developing system, giving mixture of compound **4,5** (13.6 mg), then it was eluted by 40% methylene chloride in petroleum ether and was purified by TLC on silica gel "G" plate using petroleum ether : ethyl acetate (8 : 2) as developing system, giving compound **6** (5.8 mg)**. Fraction 5:** It was subjected to silica gel column, eluted by 50% methylene chloride in petroleum ether and was purified by TLC on silica gel "G" plate using petroleum ether: ethyl acetate (8:2) as developing system, giving mixture of compound **7** and compound **8** (10.3 mg). The isolated compounds were identified on the basis of UV, Mass spectrum, ^{1}HNMR and ^{13}CNMR data and compared with literature.

Cell lines

Human breast carcinoma (MCF-7 cell line), colon carcinoma (HCT-116 cell line) were obtained from Karolinska Center, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden.

Cell culture

The procedure was done in a sterile area using a laminar air flow cabinet biosafety class II level. Culture was maintained in RPMI 1640 medium with 1% antibiotic-antimycotic mixture (10.000 U/ml potassium penicillin, 10.000 µg/ml streptomycin sulfate and 25 µg/ml amphotericin B), 1% L-glutamine, and supplemented with 10% heat inactivated fetal bovine serum. Culturing and subculturing were carried out according to.[11] Doxorubicin was used as a positive control. A negative control composed of DMSO was also used.

Cell viability assay

This was done according to,[12] as described by Mosmann T.[13] Following culturing for 10 days, the cells were seeded at concentration of 10×10^3 cells per well in case of MCF-7 and 20×103 cells/well in a fresh complete growth medium in case of HCT-116 cell lines, using 96-well microtiter plastic plates at 37 °C for 24 hours under 5% CO2, in a water jacketed carbon dioxide incubator. Fresh medium (without serum) was added, and cells were incubated either alone (negative control) or with samples, to give a final concentration of 100 µg/ml. After 48 hours incubation, the medium was aspirated and then 40 µl MTT salt (2.5 mg/ml) were added. To stop the reaction and dissolve the formed crystals, 200 µl 10% sodium dodecyl sulphate (SDS) was added. The absorbance was measured using a microplate multi-well reader at 595 nm and a reference wavelength of 690 nm.

Determination of IC_{50} values

IC_{50} values were calculated using probit analysis and utilizing the SPSS computer program (SPSS for windows, statistical analysis software package /version 9/ 1989 SPSS Inc., Chicago, USA).

All experimental data stated in this work were expressed as the average ± standard deviation (SD) for n = 3 and were analyzed using standard analysis of Student's t-test. The level of significance (p-value) is set at < 0.05.

Enzyme-linked immunosorbent assay kit for tubulin beta (TUBb)

MCF-7 cells were cultured using DMEM (supplemented with 10% FBS and with 1% antibiotic-antimycotic mixture (10.000 U/ml potassium penicillin, 10.000 µg/ml streptomycin sulfate and 25 µg/ml amphotericin B). Plate cells (cells density 1.2 – 1.8×10.000 cells/well) were kept in a volume of100 µl complete growth medium + 100 ul of the tested compound per well in a 96 well plate for 18-24 hours, before the enzyme assay for Tubulin. The microtiter plate provided in this kit had been pre-coated with an antibody specific to TUBb. Standards or samples were then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to TUBb. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and were incubated. After TMB substrate solution was added, only those wells that contain TUBb, biotin-conjugated antibody and enzyme-conjugated Avidin would exhibit a change in color. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The concentration of TUBb in the samples was then determined by comparing the O.D. of the samples to the standard curve.[14]

RESULTS

Eight compounds from the extract chloroform-methanol (3:1) of aerial parts of *Jacquemontia pentantha* were identified for the first time as: Palmitic acid (1), Phytol (major) (2), Stigmast-4-en-3-one (3), mixture of α-amyrin (4) and β–amyrin (5), 1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl (all-E) (6) and mixture of α– amyrin acetate (7) and β-amyrin acetate (8). The isolated compounds were identified on the basis of UV, Mass spectrum, ^{1}HNMR and ^{13}CNMR data and compared with literature. The following compounds were identified with their physical and spectroscopic data:

***Palmitic acid* (1):** Colorless powder, [M]+ *m/z* 256. Molecular formula $C_{16}H_{32}O_2$. UV: λ max 220 nm. ^{1}H NMR [$CDCl_3$: 600 MHz] δ [ppm]: 0.87 [t, *J* = 6.5 Hz. H_3:16], 1.22-1.28 (24H, $2H_4\rightarrow2H_{15}$), 1.62 (2H, *J* = 7.5 Hz, 2H:3), 2.21 [t, *J* = 6.7 Hz. 2H:2]. ^{13}CNMR [$CDCl_3$, 150 MHz] δ [ppm]: 24.4, 28.7, 31.8 [C (1, 2, 3)] respectively, 28.6 [m, C:4 to C:13], 25.86, 34.2, 179.2 [C (14, 15, 16)] respectively. Compound was confirmed to be palmitic acid by comparing its spectral data with the literature.[15,16]

***Phytol* (2):** Yellow oil, [M]+ *m/z* 296, Molecular formula $C_{20}H_{40}O$. ^{1}HNMR [$CDCl_3$: 600 MHz]: δ 1.67 [s, OH], δ 4.13 [d, H:1], δ 5.38 [t, H:2], δ 1.96 [t, H:4], δ 1.42- δ 1.33 [m,H: 7, H:11], δ 1.31 – δ 1.02 [m, H:6, 8, 9,10,12,13], δ 1.64 [s, H:20]. ^{13}CNMR [$CDCl_3$, 150 MHz]:

δ59.47, 123.07, 140.11, 40.02, 25.1 [C (1, 2, 3, 4, 5)] respectively, 35.11, 31.12, 37.37, 24.88, 37.41 [C (6, 7, 8, 9, 10)] respectively, 33.12, 37.25, 25.21, 39.78, 27.88 [C (11, 12, 13, 14, 15)] respectively, 22.62, 22.68, 19.65, 19.73, 16.15 [C (16, 17, 18, 19, 20)] respectively. Comparison of the spectral data with literature confirmed compound as phytol.[17,18]

Stigmast-4-en-3-one **(3):** Colorless oily matter; [M+] *m/z* 412, Molecular formula $C_{29}H_{48}O$.

^{1}HNMR [$CDCl_3$: 600 MHz]: δ 5.71 [d, J = 2.2 Hz, H:4], δ 1.17 [s, 3H, 19:CH3], δ 0.91 [d, 3H, J=6.4 Hz, 29:CH3], δ 0.81~ δ 0.82 [9H, m, 3CH_3:21, 26, 27], δ 0.73 [3H, S, CH_3:18].^{13}CNMR [$CDCl_3$:150 MHz] spectrum: δ 34.7, 32.7, 198.4, 122.6, 170.4 [C (1, 2, 3, 4, 5)] respectively, 31.8, 31.1, 34.5, 52.6, 37.4 [C (6, 7, 8, 9, 10)] respectively, 22.1, 38.4, 41.2, 54.7, 25.1 [C (11, 12, 13, 14, 15)] respectively, 27.0, 54.7, 10.7, 18.1, 35.1 [C (16, 17, 18, 19, 20)] respectively, 16.1, 32.6, 23.0, 43.7, 26.1 [C (21, 22, 23, 24, 25)] respectively, 18.5, 17.4, 22.9, 10.7 [C (26, 27, 28, 29)] respectively. It was confirmed as stigmast-4-en-3-one by comparison the spectral data of the compound with the literature.[19,20]

α-Amyrin **(4):** Colorless amorphous solid. [M+] *m/z* 426, UV: 274, 257, 243 nm. Molecular formula $C_{30}H_{50}O$. ^{1}HNMR [$CDCl_3$: 600 MHz]: δ 5.10 [H:12, t, *J* = 3.5 Hz], 3.19 [H:3, dd, *J* = 5.0, 11.0 Hz], 1.01, 0.94, 0.93, 0.89, 0.72, 0.71 [CH_3 (27, 28, 23, 26, 24, 25), s], 0.85 [CH3: 29, d, *J* = 6.0 Hz], 0.74 [CH3: 30, d, *J* = 7.0 Hz]. ^{13}C NMR [150 MHz: $CDCl_3$]: δ 124.4 [HC=, C:12], 139.5 [C:13], 38.5, 28.6, 79.1, 38.7, 55.2 [C (1, 2, 3, 4, 5)] respectively, 18.3, 32.6, 40.0, 47.6, 36.8 [C (6, 7, 8, 9, 10)] respectively, 23.4, 42.1, 27.8, 26.5, 33.5, [C (11, 14, 15, 16, 17)] respectively, 59.2, 39.5, 39.5, 31.2, 41.5 [C (18, 19, 20, 21, 22)] respectively, 28.0, 15.6, 15.6, 16.9, 23.1 [C (23, 24, 25, 26, 27)] respectively, 28.7, 17.4, 21.3 [C (28, 29, 30)] respectively. The data for this compound was assigned by comparison with the corresponding in the published spectrum of α-Amyrin.[21-23]

β-Amyrin **(5):** Colorless amorphous solid. [M+] *m/z* 426, UV: λ max 245 nm. Molecular formula $C_{30}H_{50}O$. ^{1}H NMR: [$CDCl_3$, 600 MHz]: δ 5.15 [H:12, t, *J*=3.2 Hz], 3.21 [H:3, dd, J=11.0, 5.2 Hz], 0.77-1.11 [s, CH3], 1.84 [H:22, m], 1.88 [H:15, m]. ^{13}C NMR [150 MHz: $CDCl_3$]: δ 121.7 [HC=, C:12], 145.2 [C:13], 38.6, 27.2, 79.0, 38.5, 55.1 [C (1,2,3,4,5)] respectively, 18.4, 32.9, 39.8, 47.6, 36.9 [C (6,7,8,9,10)] respectively, 23.5, 41.7, 27.3, 26.2, 32.9 [C(11,14,15,16,17)] respectively, 47.2, 46.8, 31.1, 34.8, 37.1 [C(18,19,20,21,22)] respectively, 28.1, 15.4, 15.6, 16.8, 25.9 [C (23,24,25,26,27)] respectively, 28.1, 33.3, 23.7 [C(28,29,30)] respectively. The spectral data was compared with those previously reported.[21]

1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl-, (all-E) **(6):** Colorless oil. [M]+ *m/z* 426, molecular formula $C_{30}H_{50}O$. ^{1}HNMR [600 MHz, $CDCl_3$]: δ 5.42 [1H (3), d, J = 6.9 Hz], 5.12 [5H (7, 14, 11, 18, 22), t, *J* = 6.9 Hz], δ 5.01 [2H (1), d, *J* = 6.9 Hz], 1.91 - 2.05 [10H, m], 1.97 [10H (4, 5, 8, 9, 12, 13, 16, 17, 20, 21) m], 1.67 [3 H (24 - Me), s], 1.61 [18H (C (2 - Me, 6 - Me, 10 - Me, 15-Me, 19 - Me, 23-Me), s]. ^{13}CNMR [150 MHz:$CDCl_3$]: 116.10 [C:1], 148.2 [C:2], 131.2 [C:23], 25.70 [C:24], 134.5 [C - C (7, 11, 15, 19)] respectively, 124.4, 124.3, 124.3, 124.2, 124.2 [CH - C (6, 10, 14, 18, 22)] respectively, 39.74, 39.73, 39.71, 37.47, 37.01 [CH_2 - C (5, 9, 13, 17, 21)] respectively, 26.79, 26.77, 26.71, 26.68, 26.62 [CH_2 - C (4, 8, 12, 16, 20)] respectively, 17.67, 16.45, 16.03, 16.01, 15.95, 15.89 [CH_3 -C (25, 26, 27, 28, 29, 30)] respectively. Compound was confirmed by comparing its spectral data with squalene and its derivatives literature.[24,25] The spectroscopic data of this compound is reported here for the first time as it was identified previously only by GC/MS of plant *Sauropus bacciformis* Blume.[24-26]

a-amyrin acetate **(7):** White powder. [M]+ *m/z* 468. Molecular formula $C_{32}H_{52}O_2$. ^{1}HNMR [$CDCl_3$, 600 MHz] *δ* [ppm]: 5.1 [dt, J = 8 Hz and J = 4 Hz, H:12], 4.4 [m, H:3], 0.80 [s], 0.83 [s], 0.88 [s], 0.92 [s], 0.98 [s], 1.0 [s], 2.05 [s]. ^{13}CNMR [$CDCl_3$, 150 MHz] δ [ppm]: 124.3 [C:12], 140.1 [C:13], 37.80, 23.75, 80.59, 38.14, 55.50 [C (1,2,3,4,5)] respectively, 18.59, 32.69, 39.6, 48.47, 36.4, [C (6,7,8,9,10)] respectively, 22.07, 42.10, 26.68, 27.42, 33.7 [C (11,14,15,16,17)] respectively, 59.30, 39.5, 31.1, 41.5, 14.53 [C (18, 19, 21, 22, 23)] respectively, 28.19, 16.13, 16.14, 19.02, 18.25, 18.57 [C (24,25,26,27,28,30)] respectively, 172.02 [C:1'], 21.42 [CH3]. The spectral data was compared with those previously reported.[27,28]

β-amyrin acetate **(8):** White powder. [M]+ *m/z* 468. Molecular formula $C_{32}H_{52}O_2$. ^{1}HNMR [600 MHz, $CDCl_3$,] *δ* [ppm]: 5.13 [t, H:12], 4.4 [m, H:3], 1.13 [s, CH_3:27], 0.962 [s, CH3:26], 0.96 [s, CH_3:25], 0.87 [s, CH_3:29 and CH3:30], 0.86 [s, CH_3:24], 0.83 [s, CH_3:23], 0.82 [s, CH_3:28], 2.2 [H:2']. ^{13}CNMR [$CDCl_3$, 150 MHz] δ [ppm]: 121.65 [C:12], 145.21 [C:13], 38.10, 22.27, 80.28, 37.10, 55.20 [C (1,2,3,4,5)] respectively, 18.71, 32.50, 39.70, 47.50, 36.91 [C (6,7,8,9,10)] respectively, 23.50, 41.70, 26.20, 26.21, 32.5 [C (11,14,15,16,17)] respectively], 47.10, 46.79, 31.1, 34.6, 37.1 [C (18,19,20,21,22)] respectively, 16.78, 28.40, 15.51, 25.92, 23.40, 33.20 [C (23,24,25,27,28,29)] respectively, 171.08 (C:1'), 34.75 (C:2'). All these assignments were consistent with the data obtained from literature for beta-amyrin acetate.[27]

These compounds were isolated here from *Jacquemontia pentantha* for the first time. Spectral data of the isolated compounds were in accordance with previously reported spectral data. Phytol is reported here as a major compound.

Cell viability assay (MTT assay)

The results revealed that the crude extract showed potent cytotoxic activity on both breast and colon human cell lines at different range of concentration (100-6.25 ug/ml) with IC_{50} (21.8 ± 0.9) and (40.9 ± 1.3) respectively. The antiproliferative potentency represented by IC_{50} value, the IC_{50} value is reverse proportional with the activity (i.e.,: the less IC_{50} value the more potent antiproliferative activity). The crude extract showed more potency than the drug reference doxorubicin on breast tumor cell line as shown in (Figure 1).

Enzyme-linked immunosorbent assay kit for tubulin beta (TUBb)

To elucidate the possible mode of action of antiproliferative effect of crude extract of *Jacquemontia pentantha* on breast tumor cell line as shown in (Figure 1), Enzyme-linked Immunosorbent Assay Kit for Tubulin beta (TUBb) assay was done. The percentage inhibition of tubulin (TUBb) polymerization against breast cancer cell lines was

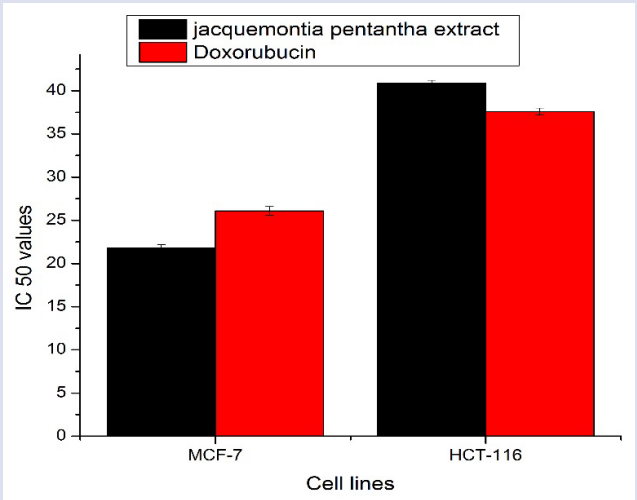

Figure 1: IC_{50} (the concentration required to kill 50% of the cell population) of *Jacquemontia pentantha* crude extract on cell lines of, breast carcinoma (MCF-7) and colon carcinoma (HCT-116) compared with reference drug Doxorubicin. Each result is a mean of 3 replicate samples.

measured using colchicine as a reference drug. The tested extract of *Jacquemontia pentantha* showed significant TUBb polymerization inhibition activity with statistically significant ($p<0.05$). Its activity was higher than colchicine as TUBb polymerization inhibitors (Figures 2 and 3, Table 1) in accordance with the *in vitro* anti-cancer activity of this extract against MCF-7 cell line. In conclusion: *Jacquemontia pentantha* extract has antiproliferative effect on two tumor cell line namely (MCF-7 and HCT-116). The anti- proliferative effect on breast cell line is through affecting microtubule-polymer mass resulting in cell death.

Cytotoxic effect of phytol

The cytotoxic activity of phytol was screened on both colon and breast cell line at concentration (100-12.5 ug/ml). The results revealed that the compound had no cytotoxic effect on colon HCT-116 cell line, on the other hand it possessed potent cytotoxic activity on MCF-7 breast cell line, with IC_{50} (60 ± 2.4) as seen in (Figure 4). According to the above results, it can be concluded, that *Jacquemontia pentantha* has potent cytotoxic effect on breast and colon tumor cell line.

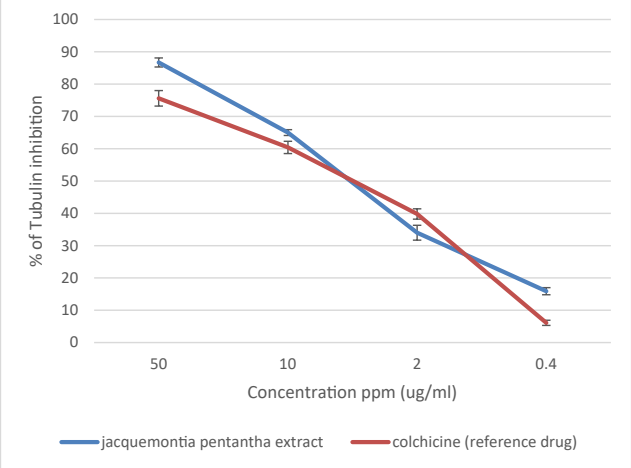

Figure 2: Tubulin polymerization inhibition of *Jacquemontia pentantha* crude extract on MCF-7 at different concentration compared with colchicine reference drug. Each result is a mean of 3 replicate samples.

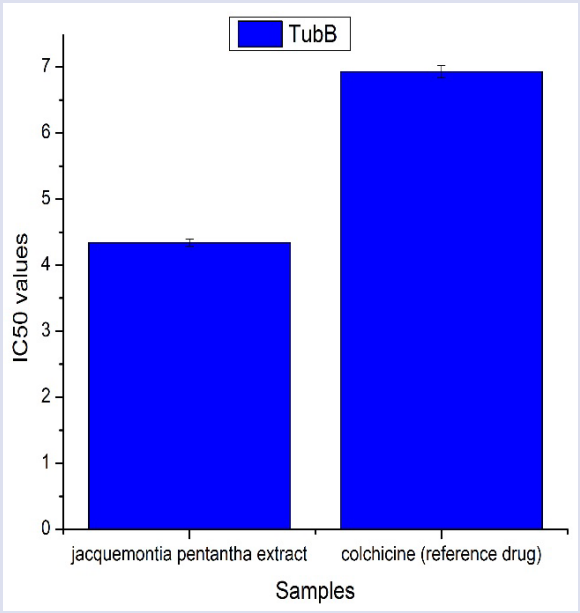

Figure 3: IC_{50} of *Jacquemontia pentantha* extract on TubB inhibition using (MCF-7). $p<0.05$. Each result is a mean of 3 replicate samples.

Table 1: Tubulin polymerization inhibition of crude extract on MCF-7. $p < 0.05$.

MCF-7			
IC50 values ug/ml	Tubulin% inhibition	conc. ug/ml	Cpd. code.
4.34	86.73123	50	Crude Extract
	65.02825	10	
	34.03551	2	
	15.89992	0.4	
6.93	75.64169	50	REF (colchicine)
	60.43066	10	
	39.78467	2	
	6.115418	0.4	

MCF-7: Human breast carcinoma; IC_{50}: The concentration required to kill 50% of the cell population; Conc: Concentration; Cpd: Compound; REF: Reference.

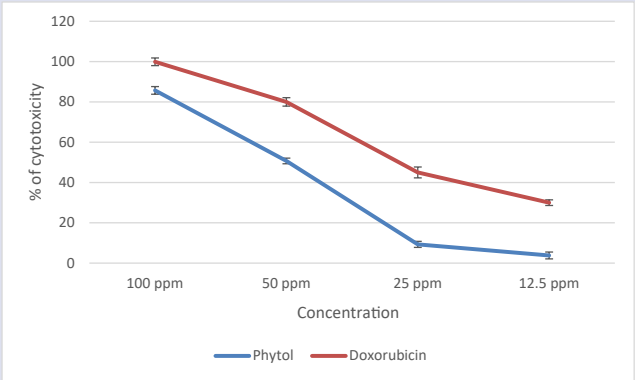

Figure 4: Percentage of cytotoxic effect of phytol on breast human tumor cell line MCF-7 at different concentration (100-12.5 ppm) compared with the reference drug Doxorubicin. Each result is a mean of 3 replicate samples.

DISCUSSION

The aerial parts of the plant were extracted with chloroform- methanol (3:1) till exhaustion. The extract was subjected to silica gel column chromatography. The fractions obtained were examined using TLC and the similar fractions were collected to give five major fractions, from which 8 compounds were isolated from this plant. By means of spectroscopic analysis, they were identified as, Palmitic acid (1), Phytol (2), Stigmast-4-en-3-one (3), α-amyrin (4), β-amyrin (5), 1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23- hexamethyl (all-E) (6), α-amyrin acetate (7), β-amyrin acetate (8). The isolated terpenes and sterols were identified on the basis of UV, Mass spectrum, ^{1}HNMR and ^{13}CNMR data and compared with literature.

Cell-based assays are often used for investigation proliferated profile of studied plant extract, which is a reliable indicator of cellular energy capacity of the cell through enzymatic reducing effect of 3-[4, 5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) to MTT-formazan governed by Mitochondria enzyme succinate dehydrogenase. So, in the present study we explore the proliferative effect of crude extract of *Jacquemontia pentantha* on breast and colon human tumor cell line namely MCF-7, HCT-116.

Microtubules are dynamic protein filaments represented major structural components of the cytoskeleton assembled from polymers of α- and β-tubulin subunits, which play a key role for cell signaling, cell

migration, and cell proliferation. The critical role of microtubules in cell division and other cellular functions makes them an attractive target for cancer chemotherapy. Microtubule-targeting agents such as the taxanes and the vinca alkaloids represent a successful class of anticancer drugs affect their dynamics by inhibiting cell proliferation.[29] *Jacquemontia pentantha* extract has antiproliferative effect on two tumor cell line namely (MCF-7 and HCT-116) is through affecting microtubule-polymer mass resulting in cell death. As cancer is a life-threating disease, phytol was found to exhibit a potent antitumor on MCF-7 breast cell line. Phytol can be used as a precursor for the manufacture of synthetic forms of vitamin E,[30] and vitamin K.[31]

CONCLUSION

These eight compounds (sterols and terpenes) were isolated here for the first time from the crude extract of *Jacquemontia pentantha*. The results revealed that the crude extract showed potent cytotoxic activity on both breast and colon human cell lines. Phytol is reported here as a major compound and was found it has a potential role in the cytotoxic effect of *Jacquemontia pentantha* on breast cell line, along with other compounds. Further investigation needs to be done to elucidate the compounds mode of action responsible for the antiproliferative effect for these compounds could be promising cytotoxic agents in treatment of human cancer.

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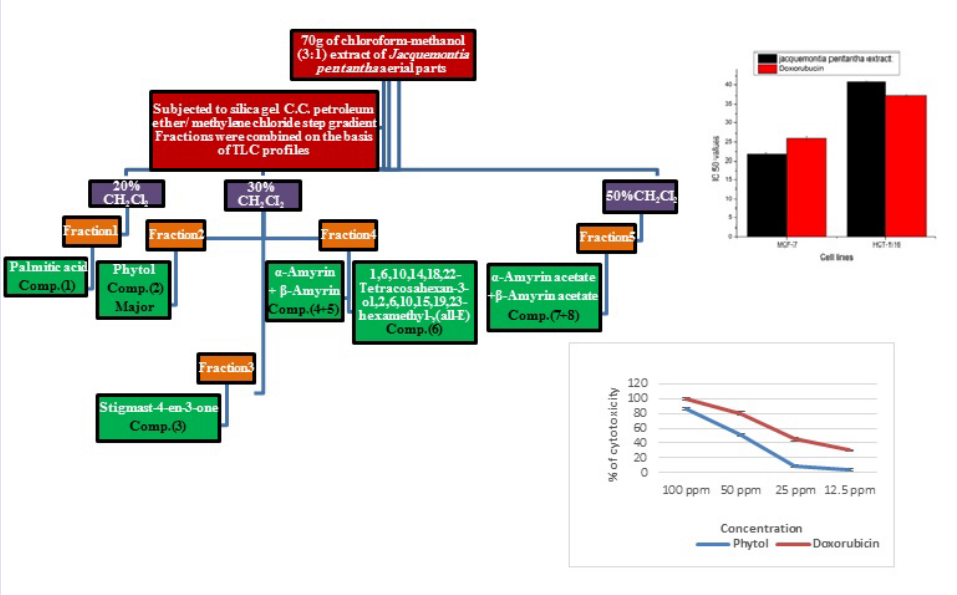
GRAPHICAL ABSTRACT



SUMMARY

The aim of this study is to isolate and identify sterols and terpenes from the chloroform/methanol extract (3:1) of aerial parts of *Jacquemontia pentantha* (Jacq.) and evaluation of cytotoxic activity of crude extract and phytol for the first time from this plant. Different chromatographic techniques were used resulting in isolation of eight compounds for the first time from this plant, were identified as: Palmitic acid, Phytol (major), Stigmast-4-en-3-one, mixture of α-amyrin and β–amyrin, 1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl (all-E) and mixture of α–amyrin acetate and β-amyrin acetate .Their structures were elucidated by spectroscopic methods including ^{1}HNMR, ^{13}CNMR, EI/MS spectrometry and by comparing their data with those reported in the literature. Cytotoxicity was evaluated using MTT assay. Mode of action of extract was predicted by using Enzyme-linked Immunosorbent Assay Kit for Tubulin beta (TUBb). The extract showed potent cytotoxic activity on MCF-7 breast carcinoma cell line as well as HCT-116 colon carcinoma cell line at different concentrations (100-6.25 ug/ml) with IC_{50} (21.8 ± 0.9) and (40.9 ± 1.3) respectively. It showed more potency than drug reference doxorubicin on breast tumor cell line. Phytol showed potent cytotoxic activity on MCF-7 cell line at different concentrations (100-12.5 ug/ml) with IC_{50} (60 ± 2.4), while it had no cytotoxic effect on HCT-116 cell line. Extract showed significant TUBb polymerization inhibition activity. It has antiproliferative effect on tumor cell line namely (MCF-7, HCT-116), through affecting microtubule-polymer mass resulting in cell death. Cytotoxic activity due to presence of phytochemicals as sterols and terpenes.

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