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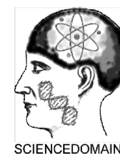

Antiproliferative Effect of *Portlandia* Extracts and Their Fractions on Breast Cancer Cells

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CC, MLV and MAB designed the study, performed the statistical analysis and wrote the protocol. Author HAP prepared all plant extracts. Authors MAB, BJ and JFO wrote the first draft of the manuscript. Authors CC, KC and TC managed the analyses of the study. Authors MAB, BJ and JFO managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: Rubiaceae is one of the largest families of plants and it includes the Jamaican genus *Portlandia.* This family has been used in several ethnomedicinal practices. Thus, in this study we investigated whether extracts of two species of *Portlandia* affect *In vitro* proliferation of breast cancer cells.
Study Design: Metastatic (MDA-MB-231 and HeLa) cells were incubated in the absence or in the presence of *Portlandia* extracts. A human non-metastatic (MCF-10A) and normal (ATCC PCS-600-010) epithelial cell lines were used as control.
Methodology: At the end of the study all cell lines were incubated with *Portlandia* extracts (-and its subfractions-) and then analyzed on proliferation, thymidine incorporation and mitogenic and survival signaling pathways on metastatic and non-metastatic cells.
Results: It was found that *Portlandia* methanol extract inhibited proliferation of MDA-MB-231 in a dose-dependent manner attenuating the phosphorylation of Erk1/2 and Akt1

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without affecting the proliferation of MCF-10A cells. [^{3}H] thymidine incorporation was also decreased by *Portlandia* methanol extract. This study suggests that *Portlandia* extracts selectively affected the proliferation of metastatic breast cancer cells through the modulation of Erk1/2 and Akt1 activities that play a critical role during cell survival and proliferation.

Conclusion: *Portlandia* leaf extracts contain active compounds, which strongly repress cancer cell proliferation in a dose-dependent manner and selectively down-regulate phosphorylation of both Erk1/2 and Akt1 activities.

Keywords: Portlandia; breast cancer cells; proliferation; signaling transduction pathways.

1. INTRODUCTION

Breast cancer includes a group of very heterogeneous diseases, which can be demonstrated at the molecular and histopathologic levels [1]. Breast cancer cell lines have been utilized extensively providing valuable insights into many aspects of the biology of this breast anomaly [2,3]. These cell lines provide the most critical tools to study tumor formation [4,5] signal transduction pathways [6,7], and to discover new therapeutic targets for breast carcinoma [8,9]. These signaling pathways involve, at least, phosphorylated Akt1 and Erk1/2 that are commonly used as readouts of proliferative signaling [4]. Metastatic breast cancer is the leading cause of breast cancer related deaths in the US [10]. MDA-MB-231 cell (a metastatic human epithelial cell line derived from mammary gland), is one of the most metastatic breast cancer cell lines available for cancer research. It is usually triple negative estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and without Her2/neu overexpression. Patients suffering from this type of cancer have poor prognosis as they do not respond to current therapeutic regimen [11]. Moreover, HeLa cells (a metastatic human epithelial cell line derived from cervical carcinoma) are one of the oldest and most widely used cell lines in research and they hey express several major cell signaling pathways, and MAPK, p38, JNK, Akt signaling. In contrast, MCF-10A cells belong to immortalized, non-transformed and non-metastatic epithelial cell line derived from mammary gland, and primary mammary epithelial cells, which proliferation depends on exogenous growth factors [11,12].

Phytochemicals are classes of compounds derived from plant extract that can be used to treat numerous diseases particularly cancers. One of the most successful FDA-approved phytochemical drugs used for the treatment of metastatic breast cancer is paclitaxel [13], an extract isolated from the bark of the Pacific yew tree [14]. Other anti cancer phytochemical drugs in different phases of clinical trial includes resveratrol [15], epigallocatechin gallate (EGCG) [16], gingerol [17] and myricetin [18]. While the modes of action of some of these plants derived drugs are still been studied, *in vitro* and *in vivo* evidences indicate that they modulate cell signaling processes that lead to growth and metastatic arrest of tumor cells including those of breast cancer [19,20].

Rubiaceae is one of the largest flowering families and it includes more than 7,000 species being used in several ethnomedicinal practices [21]. The family is characterized by the production of several classes of secondary metabolites with a great pharmacological potential, mainly alkaloids, terpenes, flavonoids, and coumarins with antibacterial properties [22]. Secondary metabolites of Rubiaceae plants have also been investigated scientifically for antiviral activities [21,22]. *Portlandia* (Rubiaceae) is a small genus (six species) endemic

of Jamaica characterized by large flowers and multiseeded capsules. In this study, we investigated the effects of *Portlandia* extracts on *In vitro* proliferation and the changes in signaling transduction pathways in HeLa cells, MDA-MB-231 cells, primary mammary epithelial and MCF-10A cells.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh leaves of *P. platantha* Hook. f. and *P. coccinea* Sw. (taxonomy follows the recent Catalogue of Seed Plants of the West Indies [23]) were collected in winter 2011 by Dr. J. Francisco-Ortega at the Fairchild Tropical Botanical Garden (FTG) in Miami, Florida, USA. The plant material was collected and stored at -20ºC and analyzed within the following days. Plants were taxonomically identified by Dr. B. Jestrow and Dr. Francisco-Ortega. Vouchers JFO-12-01 (FTG 149980) for *P. platantha* and JFO-12-02 (FTG 149981) for *P. coccinea* were deposited at the FTG herbarium.

2.2 Sample Preparation and Fractionation

Extracts were prepared from leaves of two species of *Portlandia* as previously described [24].

2.2.1 Preparation of the methanol extract

Leaves (2.5 g for both species, fresh weight) from one individual plant were cut into small pieces and extracted with methanol (100 mL) in a 250 mL round bottom flask connected to a reflux condenser heated at 70ºC for 45 minutes (min). The leaves were filtered, and plant material was washed twice with methanol (25 mL). The extract and washes were pooled. Evaporation of the methanol extract by rotorvapor/desiccation yielded a residue of 540 mg and 537 mg for *P. coccinea* and *P. platantha*, respectively.

2.2.2 Fractionation of the methanol extract

The individual plant methanol extract was partitioned among hexane, ethyl acetate and water. Briefly, leaves were extracted with methanol as indicated above. An aliquot (300 mg for *P. coccinea* and 302 mg for *P. platantha*) of the residue obtained by methanol extraction was partitioned between methanol and hexane. The hexane was removed from the methanolic phase and then it evaporated and concentrated by rotorvapor/desiccation as previously described [24]; the residue was partitioned between water and ethyl acetate. These fractions were then individually evaporated and concentrated by rotorvapor/desiccation as previously described [24]. The hexane, ethyl acetate, and water extracts yielded 24, 78, and 150 mg of residue for *P. coccinea*, representing 84% of the initial methanol extract. For *P. platantha,* the hexane, ethyl acetate, and water extracts yielded 25, 76, and 140 mg of residue representing 80% of the initial methanol extract. All extracts were dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 100 mg/ml plant extract.

2.3 Cell Culture

Cell lines including HeLa (ATCC-CCL-2), MCF-10A (ATCC-CRL-10317), MDA-MB-231 (ATCC-HTB-26) and primary mammary epithelial (ATCC-PCS-600-010) cells were purchased from ATCC (Manassas, VA, USA) and cultured for less than 2 months splitting them twice a week. HeLa and MDA-MB-231 cells were grown in Dulbecco's modified Eagle's medium (DMEM) from Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS), 50 U/ml penicillin, and 50 g/ml streptomycin in a humidified incubator with 5% CO_2 at 37ºC. MCF-10A cells were grown in DMEM supplemented with 10% FBS, 50 U/ml penicillin, 50 μg/ml streptomycin, 10 ng/ml human epidermal growth factor (hEGF), 100 ng/ml Cholera toxins, 5 μg/ml bovine insulin and 500 ng/ml hydrocortisone in a humidified incubator with 5% CO_2 at 37ºC. PCS-600-010 cells were grown in mammary epithelial cell basal medium supplemented with 10% FBS, 50 U/ml penicillin, 50 μg/ml streptomycin,100 ng/ml recombinant human transforming growth factor-alpha (rhTGF-α), 5 μg/ml Apo-transferrin, 1 μM epinephrine, 5 μg/ml recombinant human insulin (rhI) and 100 ng/ml hydrocortisone in a humidified incubator with 5% CO_2 at 37ºC. The cells were harvested after reaching 70-80% confluence and were plated for either subsequent passage or treatments.

2.4 Immunoblotting Analysis and Sodium Dodecyl Sulfate-polyacrylamide Gel Electrophoresis (SDS-PAGE)

Cells ($1x10^5$) were plated in 6-well plates in DMEM containing 10% FBS, starved for 12 h in the absence or in the presence of 300 μg/ml *P. coccinea* hexane fraction, and then stimulated in the absence or in the presence of 2 ng/ml EGF for 8 min at 37°C. After incubation, treated and non-treated cells were washed twice with ice-cold PBS, pelleted and lysed by freeze/thaw in extraction buffer (25 mM HEPES, pH 7.5, 1 mM EDTA, 150 mM NaCl, 10 mM β-glycerophosphate, 1 mM NaF, 1% Triton X-100, 10% glycerol containing protease inhibitors) for 30 min on ice. The cell lysates were centrifuged at 800 x g for 5 min at 4ºC, and supernatants were collected. Protein concentrations were determined by the Bradford method [25]. Cell extracts were separated by SDS-PAGE, transferred onto nitrocellulose membranes (BioRad Laboratories, Hercules, CA, USA), and incubated with antibodies specific to tyrosine-phosphorylated (p)-extracellular-signal-regulated kinases (Erk)1/2 and total (t)-Erk1/2, p-RAC-alpha serine/threonine-protein kinase (Akt)1, t-Akt1, p-p38 mitogen-activated protein kinases(p38), t-p38, p-Jun N-terminal kinases(Jnk) and t-Jnk for 2 h at room temperature. Subsequently, membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies. A SuperSignal West Pico chemiluminescent substrate kit (Pierce/ThermoFisher Scientific Inc., Rockford, IL, USA) was used to visualize protein bands. Band densities were determined using the Image J64 software.

2.5 Viability Assay

Cells ($1x10^5$) were plated in 6-well plates in DMEM containing 10% FBS, starved for 12 h, and then stimulated with 2 ng/ml EGF for 24 h in the absence or in the presence of different amounts of *Portlandia* methanol extracts (100 to 600 μg/ml). After incubation, the cells were removed from the 6-well plate by trypsinization. Cell suspensions were aspirated, centrifuged at 500 x g for 5 min and washed twice with sterile PBS (pH 7.4). The cell suspension was then treated with trypan blue dye (0.4% solution) and placed in heamocytometer. Live and dead cells were counted using a 63x magnification in phase-

contrast inverted microscope. The untreated cells (control) were also processed simultaneously under the identical condition. As a positive control, cells were treated with different amounts of wortmannin (25 to 100 μg/ml) and then processed as described above.

2.6 Proliferation Analysis

Cells (5 x10^5) were plated in 6-well plates in DMEM containing 10% FBS. Cells were starved for 12 h, and then stimulated with 2 ng/ml hEGF for 24 h in the absence or in the presence of different amounts (100 to 600 μg/ml) of methanolic extracts of *P. coccinea* and *P. plantantha* hexane, ethyl acetate and water fractions of the methanol extract of *P. coccinea.* Cells were exposed to 10 mg/ml MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] for the last 4 h. After incubation, the medium was removed and formazan crystals were dissolved in detergent reagent following the manufacturer's instructions (ATCC). Optical density for each condition was determined at 570 nm. MTT incorporation was expressed as percentage of the control in the absence or in the presence of 2 ng/ml EGF. DMSO (0.65 %) was used as control since these plant extracts were dissolved on it at 100 mg/ml.

2.7 Analysis of DNA Synthesis

After the specified treatment, cell growth was measured by thymidine incorporation into DNA. Serum starved-cells were incubated with 1 mCi/ml of methyl-[^{3}H] thymidine (GE Healthcare Bio-sciences, Pittsburgh, PA, USA) for 4 h in the absence or in the presence of EGF and washed three times with PBS. Cold 10% (w/v) trichloroacetic acid was then added, and cells were solubilized with 1 M NaOH. Tritium (^{3}H) was measured by scintillation counting (PerkinElmer, Waltham, MA, USA) [26,27].

2.8 Statistical Analysis

Mean ± SD from the three independent experiments are shown. Statistical analyses were performed using one-way ANOVA or paired two-tailed student's *t*-test. Differences were considered significant for $^*P$ values <0.001.

3. RESULTS

3.1 Effect of *Portlandia* Extracts on Cell Proliferation

To investigate the effect of the *Portlandia* extracts on cell viability, we incubated cells in the absence or in the presence of the *Portlandia* extracts as described in Material and Methods. For these experiments, we utilized MDA-MB-231 and MCF-10A cells, a metastatic and immortalized non-metastatic cell lines, respectively. We also used HeLa cells, a metastatic human epithelial cell line derived from cervical carcinoma. Addition of *Portlandia* extracts inhibited proliferation of HeLa and MDA-MB-231 cells in a dose-dependant manner (Fig. 1A-B). Specifically, the methanol extract prepared from *P. coccinea* (Fig. 1A) showed strong anti-viability activity against MDA-MB-231 cells with a more than 90% inhibition at 600 μg/ml. At a concentration of 100 μg/ml, the addition of *P. coccinea* methanol extract inhibited ~50% of the proliferation activity for both HeLa and MDA-MB-231 cells with an IC_{50} of 89±6 μg/ml and 132±6 μg/ml, respectively. *P. platantha* also showed anti-viability activity for HeLa cells (90 % inhibition at 600 μg/ml with an IC_{50} of 121±10 μg/ml), but not for MDA-MB-231 cells (Fig. 1B). As expected, proliferation of MCF-10A cells was not affected by any of the

prepared *Portlandia* extracts (Fig. 1A-B). The trypan blue dye exclusion assay indicated that cell viability was unaffected by these methanol extracts (non-treated cells: 100±12%, DMSO-treated cells: 96±7%, *P. platantha* methanol extract-treated cells: 93±10% and *P. coccinea* methanol extract-treated cells: 95±10%). Consistent with this observation, we found that both methanolic extracts from *P. coccinea* and *P. platantha* were unable to affect the viability of primary normal mammary epithelial cells (PCS-600-010) (compare viability of non-treated cells (92±8%) with cells treated with 600 μg/ml of methanol extract obtained from *P. coccinea* (96±9%) and *P. platantha* (91±8%), respectively. As a positive control, we used wortmannin, a potent inhibitor of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3-kinase) activity, which also inhibited proliferation of MDA-MB-231 and HeLa cells with an IC_{50} of 51±6 μg/ml, and 32±6 μg/ml, respectively (Fig. 1C). Addition of wortmannin, did not affect the viability of MCF-10A cells (Fig. 1C). These results indicated that *Portlandia* extracts selectively altered viability of both HeLa and MDA-MB-231 cells without affecting the viability of normal epithelial PCS-600-010 and non-metastatic epithelial MCF-10A cells.

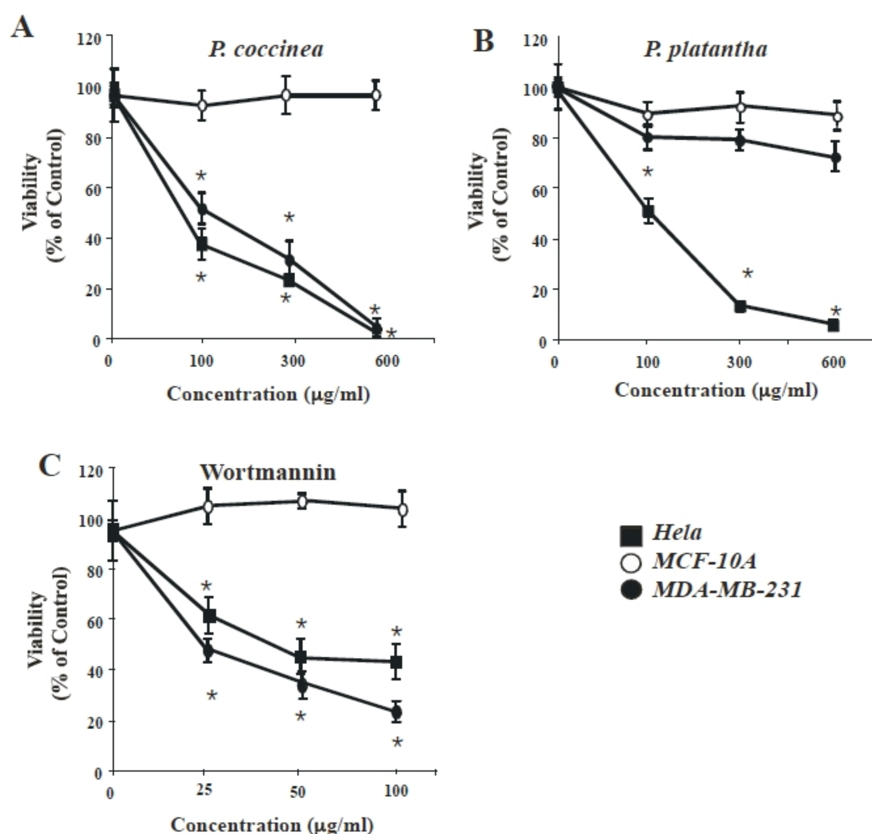

Fig. 1. A dose-dependent inhibitory effect of *Portlandia* methanol extracts on cell viability

*Viability of MCF-10A (✦), MDA-MB-231 (✧) and HeLa (■) cells were treated with either several concentrations (100 to 600 μg/ml) of two Portlandia species (A: P. coccinea, B: P. platantha) or wortmannin (25 to 100 μg/ml) (C), and quantified by spectrophotometrical analysis as described in Material and Methods. Data represent the mean ± SD of three independent experiments. Results were expressed as percentage of non-treated cells (control) *P<0.001 by student's t-test compared to non-treated cells*

3.2 Effect of Hexane, Ethyl Acetate and Water *Portlandia* Extracts on Cell Proliferation

Our data indicate that the methanol extract of *P. coccinea, but not the* methanol extract of *P. platantha,* showed a strong inhibition (>90% inhibition) of proliferation at 600 µg/ml with a significant inhibition (62±6 % inhibition) at 100 µg/ml. These results correspond to MDA-MB-231 cell line. Therefore, to gain information about the nature of the active compounds in *P. coccinea* leaves, the methanol extract, a mixture of high complexity, was further fractionated as described in the Materials and Methods. Three fractions (named as hexane, ethyl acetate and water fractions) were obtained. As expected, none of these fractions were active against MCF-10A cells (Fig. 2A). The hexane fraction exhibited the most potent inhibitory effect (89±5 % inhibition at 100 µg/ml) on cell proliferation as compared with the ethyl acetate fraction (Fig. 2B).

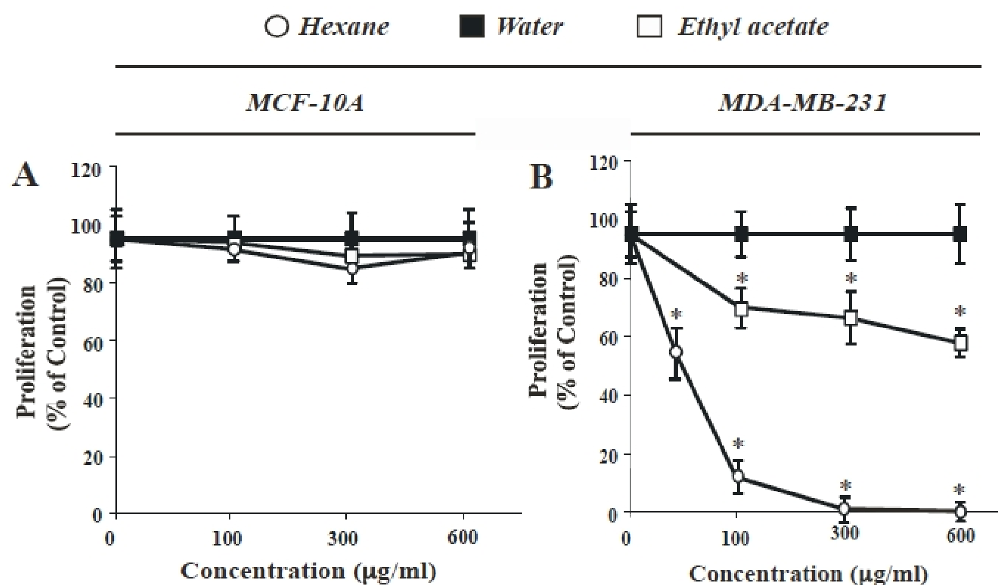

Fig. 2. Differential effect of fractions of the *P. coccinea* methanol extracts on cell proliferation

*MCF-10A (A) and MDA-MB-231 (B) cells were treated with several concentrations (100 to 600 µg/ml) of hexane (✦), ethyl acetate (✧) and water (■) fractions of the methanol extract of P. coccinea. Cell proliferation was quantified by spectrophotometrical analysis as described in Material and Methods. Results were expressed as percentage of non-treated cells (control). Data represent the mean±SD of three independent experiments. *P<0.001 by student's t-test compared to non-treated cells*

The ethyl acetate fraction also blocked cell proliferation (33±6 % inhibition at 100 µg/ml and 40±3 % inhibition at 600 µg/ml) but in lesser extent than the hexane fraction (Fig. 2B). The water fraction did not block proliferation (Fig. 2B). Consistent with this observation, we also found that the incorporation of [^{3}H] thymidine in MDA-MB-231 cells was drastically inhibited by the hexane fraction of *P. coccinea* (69±5 % inhibition), while the ethyl acetate fraction of *P. coccinea* only inhibited 15±5 % the incorporation of [^{3}H] thymidine in MDA-MB-231 cells (Fig. 3). The water fraction of *P. coccinea* was unable to block [^{3}H] thymidine incorporation (Fig. 3). In addition, we also found that the hexane fraction of *P. coccinea*, but neither ethyl

acetate nor water fractions, strongly blocked proliferation of HeLa cells (compare proliferation of non-treated cells [100±8%] with cells treated with 300 μg/ml of hexane [94±5% inhibition], ethyl acetate [33±5% inhibition], and water [4±3% inhibition] extracts of *P. coccinea*, respectively) (data not shown).

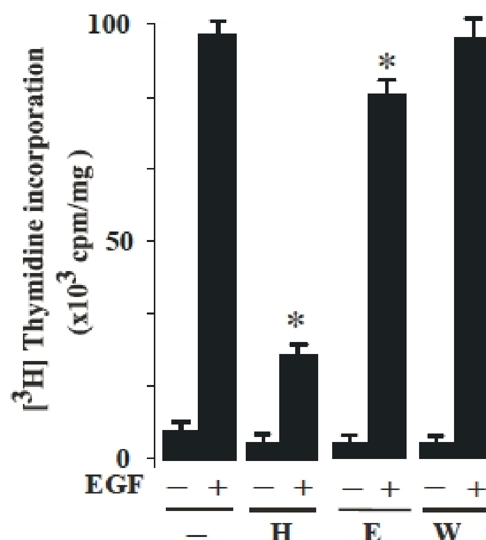

Fig. 3. Effect of the *P. coccinea* fractions on [^{3}H] thymidine incorporation

*MDA-MB-231 cells were incubated in the absence or in the presence of EGF, treated with 300 □g/ml of hexane (H), ethyl acetate (E), and water (W) fractions of P. coccinea, and the incorporation of [^{3}H]thymidine was quantified as described in Material and Methods. Results were expressed as percentage of non-treated cells (control). Data represent the mean ± SD of three independent experiments. *P<0.001 by student's t-test compared to non-treated cells*

3.3 Selective Inhibitory Effect of *Portlandia* Extracts on Signaling Markers

Our results indicate that the hexane fraction of *P. coccinea* strongly decreases the incorporation of [^{3}H] thymidine in MDA-MB-231 cell lines. Thus, it is possible that the hexane fraction of *P. coccinea* may selectively block pathways that regulate cell proliferation mediated by EGF [28]. To investigate whether the hexane fraction of *P. coccinea* affects the expressions of Erk1/2 and Akt1, cells were treated with EGF in the absence or presence of the hexane fraction of *P. coccinea,* and then harvested for Western blot analysis with anti-Erk1/2 and anti-Akt1 antibodies. In Fig. 4A and B, we observed that the addition of the hexane fraction of *P. coccinea* at 300 μg/mL attenuated the phosphorylation of Erk1/2 (Fig. 4A, 48±5% inhibition) and significantly decreased the phosphorylation of Akt1 (Fig. 4B, 32±1% inhibition). Furthermore, we also show that addition of the hexane fraction of *P. coccinea* at 300 μg/mL does not alter the phosphorylation of p-38 and p-Jnk (Figs. 4C and D). These data suggest that the hexane fraction of *P. coccinea* blocked the phosphorylation of Akt1 and Erk1/2 kinases, without affecting the activities of p-38 and p-Jnk.

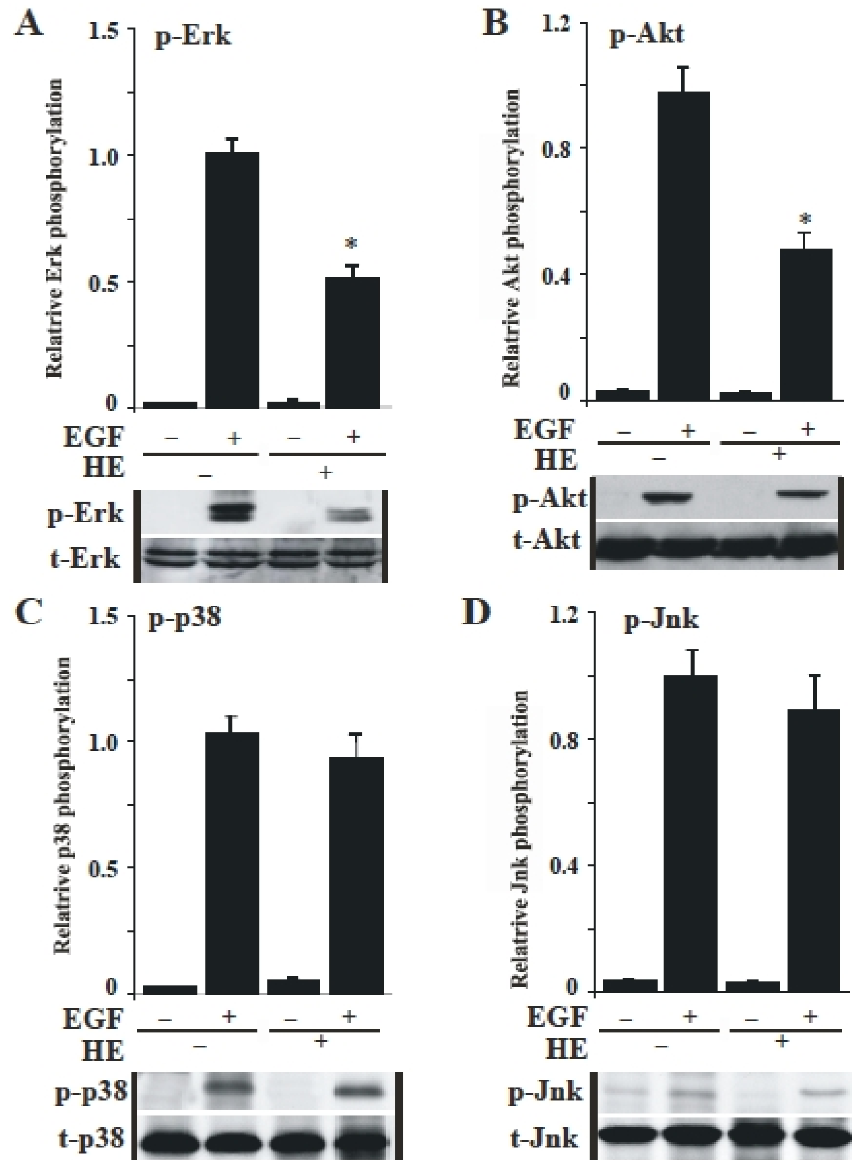

Fig. 4. Selective inhibitory effect of *P. coccinea* hexane extract on several signaling pathways

*MDA-MB-231 cells were incubated in the absence or in the presence of 300 μg/ml P. coccinea hexane extract (HE) for 24 h, and then stimulated with 2 ng/ml EGF for 8 min as described in Material and Methods. The proteins were subset to SDS-PAGE, blotted to a nitrocellulose, and antibodies specific to tyrosine-phosphorylated (p)-Erk1/2 and total (t)-Erk1/2 (A), p-Akt and t-Akt (B) p-p38 and t-p-38 (C), and p-Jnk and t-Jnk (D) were used to visualize these proteins by Western blot analysis. Relative levels of phoshorylated proteins were determined by densitometry as described in Material and Methods and they were expressed as a ratio of phosphorylated/total proteins. Data represent the mean ± SD of three independent experiments. *P<0.001 by student's t-test compared to EGF-stimulated cells in the absence of P. coccinea hexane extract (control: 1±0.05)*

4. DISCUSSION

In this study, we investigated the effect of methanol extract derived from the leaves of *Portlandia* species on the viability of MDA-MB-231 breast cancer cells and HeLa epithelial adenocarcinoma cells. Methanol extract obtained from *P. coccinea* showed the strongest inhibitory effect on the viability of both HeLa and MDA-MB-231 cells. At a concentration of 600 μg/ml, the methanol extract obtained from *P. coccinea* strongly inhibited the proliferation of both HeLa and MDA-MB-231 cells (>90% inhibition) (Fig. 1A, B). However, the methanol extract obtained from *P. platantha* blocked the proliferation of HeLa cells (>90% inhibition) without affecting the proliferation of MDA-MB-231 (19±5 % inhibition). In addition, methanol extract obtained from *P. platantha and P. coccinea* did not affect the viability of MCF-10A cells (Fig. 1A, B). Therefore, we chose *P. coccinea* for further fractionation and testing. Furthermore, we also observed that both methanol extracts obtained from *P. coccinea* and *P. platantha* were unable to affect the viability of primary normal mammary epithelial cells (ATCC PCS-600-010) (compare viability of non-treated cells (92±8 %) with cells treated with 600 μg/ml of methanol extract obtained from *P. coccinea* (96±9%) and *P. platantha* (91±8%). This indicated that the anti-proliferative properties of *P. coccinea* may be restricted to certain types of cancer cells, as is generally the case with different herbal extracts [19-22]. The anti-proliferative property of methanol extracts from the two species of *Portlandia* was compared with wortmannin [29] indicating the presence of highly active compound(s) in the extracts.

In order to investigate the nature of the active compounds in *P. coccinea*, components of the methanol extract were partitioned among hexane, ethyl acetate and water. The components of the hexane fraction exhibited the most potent inhibitory effect (Fig. 2). The ethyl acetate fraction also showed inhibitory activity. Our results clearly indicate that the addition of *P. coccinea* extract blocked proliferation of MDA-MB-231 cells, which is consistent with an inhibition of the phosphorylation of both Erk1/2 and Akt1. This inhibitory effect by *P. coccinea* extract seems to show some selectivity since affected the Erk and Akt pathways, but not the Jnk and p-38 pathways. The Erk and Akt pathways work coordinately or synergistically to promote cell proliferation, inhibit pro-apoptotic signals, cell survival and progression through the cell cycle [4,28]. Because the Erk and Akt pathways are pleiotropic and interconnected, it is difficult to determine the distinct contribution of each to the overall proliferative response to growth factors [28]. Both phosphorylated Akt1 and Erk1/2 are frequently used as readouts of proliferative or oncogenic signaling [4,28]. Its down-regulation is an essential part to block proliferation of cancer cells [28]. Because of our finding that hexane fraction of *P. coccinea* extract inhibits cell proliferation in of metastatics cell lines and their Erk and Akt signaling pathways, these results may have interesting therapeutic implications and applications. Thus, it is possible that compounds in this extract may work by altering the cycle of phosphorylation and/or dephosphorylation of proteins in signaling pathways driven by growth factor receptor. It is also possible that these *Portlandia* extracts may affect the synthesis of signaling proteins (for example; Erk and Akt proteins). However, this is unlikely since the addition of *Portlandia* extract did not affect their expression as observed in Fig. 4. Constitutive phosphorylation of Erk represents a common deregulated signaling route in metastatics cells [30,31]. One objective of our study was to determine whether *Portlandia* extracts exert inhibitory effects on this pathway in metastatics cell lines, particularly phosphorylated Erk. Moreover, survival signals, including Akt, may also be involved in determining the radiosensitivity of human cancer cells [31,32], and plant extracts treatment of these cells may enhance their chemosensitivity via inhibition of their survival signals [33]. In this study, we have demonstrated the inhibitory effect of *Portlandia* extracts on phosphorylated of Akt. Consistent with our findings is the recent observation that incubation

with compounds derived from tea, broccoli and other plant sources [34-36], blocked poliferation of several cancer cell lines without affecting normal cells. Our Western blot analyses showed that the proliferation blockage of MDA-MB-231 cells was associated with a significant inhibition of the phosphorylation of both Erk1/2 and Akt1 (Fig. 4). This result suggests that these enzymes may be the selective target genes in the reduction of proliferation by *P. coccinea* active principles. However, the regulation of other genes by *P. coccinea* components (methanol extracts) cannot be ruled out since the incorporation of thymidine was also affected (Fig. 3).

Previous reports on Rubiaceae taxa that produce essential oils indicated that they mainly contain terpenes [21,22,37]. Previous phytochemical analyses revealed that species of *Portlandia* also synthesize terpenes [24,37]. Our results indicated that the monoterpene alcohols linalool and borneol have been identified in these *Portlandia* extracts [24]. In addition, we have described the presence of volatile organic compounds (hexanal and (E)-2-hexenal) in both *P. coccinea and P. platantha* [24]. Interestingly, these terpenes have been implicated in the inhibition of proliferation multidrug resistant breast cancer cell and also in the production of tissue inhibitor of metalloproteinase 1 [33,38], which can help us to explain, at least in part, the inhibitory effect of *Portlandia* extracts.

5. CONCLUSION

Portlandia species leaf extracts contain active compounds, which strongly repress cancer cell proliferation in a dose-dependent manner. These results suggest that inhibition was caused by non-polar active components that strongly and selectively down-regulate phosphorylation of both Erk1/2 and Akt1 activities. Further studies are underway to determine the nature of the active component(s) of *Portlandia* leaf extracts, and also to understand how these beneficial changes would aid in the treatment of breast cancer.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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