# Screening of Anti-breast Cancer and Anti-oxidant Potentials of Selected Medicinal Plants from Sri Lanka

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*This work was carried out in collaboration among all authors. Author NSW performed the research, analyzed the data, and wrote the first draft of the manuscript. Author TJ analyzed the data and edited the manuscript. Author KSS supervised the research and edited the manuscript. Author KHT contributed to the design of the study and edited the manuscript. Author DNK supervised the work and edited the manuscript. Author SRS designed the work, and contributed to editing and supervising the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To identify anti- breast cancer potential of medicinal plants used in Sri Lanka.

**Study Design:** The anti-proliferative and cytotoxic potentials of solvent extracts from leaves and bark of seven medicinal plants from Sri Lanka were investigated against estrogen receptor (ER) positive (MCF-7 cell line), triple negative (MDA-MB-231 cell line) breast cancer subtypes and normal mammary epithelial cells (MCF-10A cell line) *in-vitro*. Additionally, the anti-oxidant activity, phenolic, and flavonoid contents of the extracts were determined.

**Methodology:** The anti-proliferative activity of the extracts was determined using Sulforhodamine B assay. The anti-oxidant activity was measured using 2, 2-di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) radical scavenging assay and Ferric ion reducing antioxidant power (FRAP) assay. The Flavonoid and polyphenol contents were assessed using $AlCl_3$ and Folin-Ciocalteu reagents, respectively.

**Results:** Extracts of *Erigeron sp*, *Gardenia crameri, Canarium zeylanicum*, *Elaeocarpus subvillosus* and *Angiopteris evecta* exerted high anti-proliferative potentials (half maximal inhibition concentration $IC_{50}$ <100 µg/ mL) with less cytotoxicity to normal mammary epithelial cells. Most selective, potent extracts against MCF-7 and MDA-MB-231 cancer cell lines, were dichloromethane extracts of *A. evecta* leaves ($IC_{50}$ = 43.74 µg/ mL) and *C. zeylanicum* bark ($IC_{50}$ = 44.75 µg/ mL) respectively. All methanol extracts, except those from *A. evecta* and *A. variabilis* exhibited potent anti-oxidant activity and high poly-phenolic content in Galic acid equivalents (>50 mg/g). Comparatively high flavonoid quercetin equivalents (> 100 mg/ g) content was observed in dichloromethane extracts of *Erigeron sp*. leaves and bark.

**Conclusion:** Five of the seven studied plants demonstrate potential for use in cancer treatment. The phytochemicals responsible for the anti-cancer activity of these plants may not include polyphenols and flavonoids. However, the extracts with high anti-oxidant potentials, primarily attributed to the polyphenolic compounds present. In the future, these extracts could be used to isolate potential anti-breast cancer and anti-oxidant compounds for drug development. Furthermore, the study adds scientific value for traditional remedies and decoctions.

## 1. INTRODUCTION

Breast cancer is a malignancy of the breast tissue mainly recorded in women. Globally, breast cancer is the leading cause of cancer-associated mortality and morbidity in women, with an estimated 2.3 million new cases (24.5% of all cancer cases in females) [1-2]. Based on hormone receptor expression on their cell surface, which can serve as therapeutic targets, breast cancer cells can be classified into three main molecular sub-types. These include luminal subtypes expressing estrogen receptors (ER) and varying degree of progesterone receptor (PR); HER2-enriched subtypes over-expressing human epidermal growth receptor 2 (HER2); and basal-like (also known as triple negative) subtypes that do not express any of these hormone receptors (ER, PR, and HER2) [3-4].

About 70% of breast cancer incidents are hormone receptor positive breast cancers (HRPBC), which generally have a better prognosis due to the presence of either estrogen (ER) or progesterone (PR) receptors through which HRPBC cells respond to hormone therapy drugs that lower hormone levels or block receptors from binding to the hormones. Although the growth rate of HRPBC is relatively low and indicate better outlook in short period of time, nearly one third of HRPBC patients who initially respond to hormone therapy drugs, later develop resistant to treatment. Moreover, recurrence of HRPBC occurs in some patients even after several years of post-treatment with currently available therapeutics [5]. Such incidences of HRPBC are associated with poor prognosis and remain an area of unmet clinical need.

Triple-negative breast cancer (TNBC) is the second most abundant type. TNBC cells do not express ER nor PR while the expression of HER2 is extremely low or absent. Hence TNBCs do not respond to hormone therapy drugs or drugs that block these receptors making it difficult to manage. Therefore, TNBCs give poorer prognosis [6-9]. Currently, surgery,

hormonal therapy, radiotherapy and chemotherapy are used separately or in combination for the treatment of breast cancer [9]. There is an urgent requirement to find new drug leads to treat breast cancer patients due to the continuous rise of resistance to standard therapies.

Nature is the leading source of compounds in medicinal chemistry due to the variety of novel compounds obtained from natural sources like micro-organisms, plants and animals for molecular modification and drug synthesis in medicine [10]. Among all cancer therapeutics, over 25% are derived from natural products. Vincristine, paclitaxel, etoposide, and irinotecan, are some of the diverse structured natural products used in cancer therapy [11].

Anti-oxidants are also used for the prevention of cancer. The anti-oxidant activity is mainly due to the presence of alkaloids, polyphenols and flavonoids in natural products. Flavonoids and polyphenolic compounds are suggestive for use in prevention of cancer and heart diseases [12-13]. As traditional medicines have been consumed by humans for many hundreds of years without any known contraindications, the plant-derived compounds from traditional medicinal plants are presumed to be safer than synthetic compounds [14-15].

Given that plants with claimed anticancer properties have been well researched, the current study aimed to screen the anti-breast cancer activity of commonly used Sri Lankan medicinal plant species (including three endemic species) for which anticancer activity has not been traditionally claimed or scientifically studied. However, plants used in the study belonged to Acanthaceae, Asteraceae, Burseraceae, Elaeocarpaceae, Marattiaceae, and Rubiaceae families where some plants in each family have scientific evidence supporting their anticancer activity [16-23]. The flavonoid and phenolic content of these plant species was also determined to identify their correlation with anti-oxidant and anti-cancer properties of extracts.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

Dulbecco's Modified Eagle Medium (DMEM - Cat. No. 30-2002, American Type Cell Culture (ATCC; VA, USA).), Leibovitz' 15 (L15-Cat. No. 30-2008, American Type Cell Culture (ATCC; VA, USA).), fetal bovine serum (FBS-Cat. No. 30-2020, American Type Cell Culture (ATCC; VA, USA).), Penicillin and Streptomycin (Cat. No. 30-2300, American Type Cell Culture (ATCC; VA, USA).), trypsin-EDTA (Cat. No. 30-2101, American Type Cell Culture (ATCC; VA, USA).) and cell lines [MCF-7 (ATCC HTB-22), MDA-MB-231(ATCC HTB-26) and MCF-10A (ATCC CRL-10317)] were purchased from American Type Cell Culture (ATCC; VA, USA). Mammary epithelial cell growth medium BulletKitTM (MEGM-CC-3150) was purchased from Lonza PLC (GA, USA), Sulforhodamine B (SRB) powder (Cat. No. 230162 Sigma Aldrich (St. Louis, MO, USA), 2, 2-di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) (Cat No. D9132 Sigma Aldrich (St. Louis, MO, USA), quercetin (Cat. No. Q0125 Sigma Aldrich (St. Louis, MO, USA), gallic acid (Cat. No. G7384 Sigma Aldrich (St. Louis, MO, USA), dimethyl sulfoxide (DMSO) (Cat No. D8414 Sigma Aldrich (St. Louis, MO, USA), trichloro acetic acid (TCA) (Cat No. T6399 Sigma Aldrich (St. Louis, MO, USA), acetic acid (Cat No. A 6283 Sigma Aldrich (St. Louis, MO, USA), aluminium chloride (Cat No. 563919 purchased from Sigma Aldrich (St. Louis, MO, USA).

### 2.2 Collection of Plant Material and Preparation of Extracts

Seven plants comprising four endemic plants and three native plants utilized in Sri Lankan traditional system of medicine for various disease conditions were selected for the present study (Table 1). Native medicinal plant *Erigeron sp* was collected from Pilimathalawa in the Kandy district of Sri Lanka while all other plants were collected from Pitigala in the Galle district of Sri Lanka. Plant materials were collected according to the guidelines of Department of Wild Life conservation and Department of Forest conservation of Sri Lanka. Plant materials were authenticated by botanists of the National Herbarium of the Department of National Botanic Garden, Peradeniya, Sri Lanka. The voucher specimens were deposited at the same herbarium.

Fresh plant materials were air-dried in a drying cabinet for 72 h and then ground in to powder. Dry powder of leaves or bark (5 g of each) from each plant were subjected to sequential solvent extraction using hexane, dichloromethane, ethyl acetate and methanol respectively. (The exudate at the buds of *G. crameri* was extracted directly to dichloromethane). Each extract was prepared by repeated ultra-sonication of 5 g of the plant

**Table 1. Plant species- used in the current study and their traditional medicinal uses in Sri Lanka (Endemic plants are given in bold letters)**

| | Plant species | Family | Specimen number | Medicinal uses in Sri Lanka |
|---|---|---|---|---|
| 1 | *Wendlandia bicuspidate* **Weight& Arn** | Rubiaceae | 13/NBG/PDN | Treatment for-<br>Dysentery<br>Fever<br>Diarrhoea<br>Ulcers<br>Rheumatism |
| 2 | *Canarium zeylanicum* **(Retz.) Blume** | Burseraceae | 7/NBG/PDN | Nutritious edible oil |
| 3 | *Elaeocarpus subvillosus* **Arn** | Elaeocarpaceae | 12/NBG/PDN | Treatment for-<br>Rheumatism |
| 4 | *Asystasia variabilis* (Nees) Trimen | Acanthaceae | 3/NBG/PDN | Treatment for-<br>Abscesses<br>Ulcers<br>Wounds |
| 5 | *Angiopteris evecta* (Forst.) Hoffm | Marattiaceae | 4/NBG/PDN | Treatment for-<br>Wounds |
| 6 | *Erigeron sp* | Asteraceae | 10/NBG/PDN | Treatment for-<br>Wounds |
| 7 | **Gardenia crameri** | Rubiaceae | 5/NBG/PDN | Wounds<br>Pneumonia<br>Asthma<br>Bronchitis<br>Cough<br>Bronchitis<br>Abdominal pain<br>Hiccups<br>Splenomegaly<br>Haemorrhoids |

*NBG/PDN: National Herbarium, National Botanic Gardens, Peradeniya*

material three times, each with 50 mL of fresh solvent for 1 h at 27ºC. For each solvent, three extracts thus obtained were combined and dried under reduced pressure to obtain the hexane, dichloromethane, ethyl acetate and the methanol extracts. Stock solutions (200 mg/mL in DMSO) of each extract were prepared and stored at -20ºC until future use.

## 2.3 Cell Culture and Exposure to Extracts

Two breast cancer cell lines MCF-7 (ER positive), and MDA-MB-231(triple negative) along with the normal mammary epithelial cells (MCF-10A) were cultured and maintained according to ATCC recommendations in DMEM, L15, and MEGM media respectively with 10% FBS and 0.1% streptomycin-penicillin antibiotic mixture. MCF-7 and MCF-10A cells were cultured in T25 cell culture treated flasks by incubating at 37 °C at a 95% air and 5% $CO_2$ atmosphere with 95% humidity whereas MDA-MB-231 cells were cultured and maintained in an air tight T25 cell culture treated flasks and incubated at 37 °C.

Breast cancer cells and normal mammary epithelial cells were trypsinized and separately seeded in 96 well plates with 5000 cells/well which were incubated for 24 h at 37ºC under 5% $CO_2$ atmosphere with 95% humidity. Cells were then observed under the phase-contrast microscope in order to confirm their characteristic shape. Different concentrations of each extract prepared with the respective culture medium were added to the wells in triplicates (25 - 200 µg/mL final concentration) and further incubated for 48 h under the same conditions.

## 2.4 Morphological Observations

After 48 h post incubation with different concentrations of each extract, morphological changes in cells were observed using phase-contrast microscope (OLYMPUS CKX 41). Changes were compared with control cells to identify any morphological changes caused due to the treatment.

## 2.5 Anti-proliferative Assay-Sulforhodamine B Assay

After 48 h post incubation with different concentrations of each extract, Sulforhodamine B (SRB) assay was performed according to the procedure described by Thusyanthan J [24]. The treated cells were washed 3 times with PBS by aspirating the wells, air dried and 40 µL of trichloro acetic acid (TCA) was added to each well with 160 µL of respective culture medium (without FBS) to fix the cells. After 1 h of incubation at 4ºC, the plates were washed with tap water and air-dried. The dried wells were stained with SRB dye and were incubated for 15 min in the dark. The excess dye was then washed with 1% acetic acid and the plate was air dried. Then tris- base (10 mM) was added to the dried wells, which were then shaken for 1 h at 300 rpm and the absorbance was measured at 540 nm using Synergy™ HT Multi-Mode Microplate Reader (BioTek, USA). Paclitaxel and culture medium with 0.1% DMSO were used as the positive and negative controls respectively. $IC_{50}$ values were calculated using nonlinear regression curve using Prism 6.0 (Graph Pad Software Corporation, Inc, San Diego, California, USA). For the extracts which exert potent anti-proliferative activity ($IC_{50}$ <100 µg/mL) on at least one breast cancer cell line, experiment was repeated with normal mammary epithelial cells (MCF-10A) to identify the toxicity on normal mammary epithelial cells. Selectivity index of the extracts was defined as $IC_{50}$ against normal cells / $IC_{50}$ against cancer cells.

## 2.6 2, 2-di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The 2, 2-di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) radical scavenging assay was performed according to the procedure described by Samarakoon et al. [25]. Briefly, a concentration series (7.8-1000 µg/mL) of each extract was prepared in triplicates by dissolving in DMSO in a 96 well plate. A mixture of 60 µL of DPPH solution (2 mg/ mL) and 90 µL of methanol was added to 50 µL of each solution in the concentration series of the extracts. The absorbance was measured at 512 nm after 10 min of incubation in the dark using Synergy™ HT Multi-Mode Microplate Reader. Trolox served as the positive control. The assay was carried out in triplicate. Percentage free radical scavenging ability was calculated according to the formula:

Percentage free radical scavenging ability = ((A control – A sample) / (A control)) × 100

Where, A= absorbance

## 2.7 Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out as described by Benzie and Devaki [26] with some

modifications. Working FRAP reagent was prepared by mixing acetate buffer (30 mM pH 3.6), 2, 4, 6- tripyridyl-s-triazine (TPTZ) (10 mM) and ferric chloride solution (FeCl3.6H2O) (20 mM) in a ratio of 10: 1: 1 (v/v) and the solution was heated to 37⁰C before use. Acetate buffer (pH 3.6, 30 µL) and 150 µL of FRAP reagent was added to a 96 well plate and the absorbance was measured at 600 nm. Then 20 µL from each diluted extract (7.8 -1000 µg/mL) was added to the wells and incubated for 8 min at room temperature. The absorbance was measured again at 600 nm using Synergy™ HT Multi-Mode Microplate Reader (BioTek, USA). The standard curve was plotted by repeating the assay for Trolox with a concentration series (1- 8 µg/ mL). The ferric reducing antioxidant power of the sample was expressed as Trolox equivalents TE in mg/ g of extract using the standard curve of Trolox.

## 2.8 Determination of the Total Flavonoid Content (TFC)

The total flavonoid content of the extracts was determined according to the method described by Samarakoon et al. [27]. Briefly, different concentrations (7.8 - 1000 µg/ mL) of each extract was prepared in methanol in a 96 well plate and the absorbance was measured at 415 nm using Synergy™ HT Multi-Mode Microplate Reader (BioTek, USA). Then, 100 µL of 2% aluminium chloride solution prepared with distilled water was added to each solution in the concentrations series and incubated at room temperature for 10 min. After the incubation, absorbance was measured again at 415 nm using Synergy™ HT Multi-Mode Microplate Reader (BioTek, USA). The standard curve was plotted for Quercetin and the total flavonoid content of the sample was expressed as Quercetin equivalents (in QE mg /g) of extracts using the standard curve of Quercetin.

## 2.9 Determination of the Total Polyphenol Content (TPC)

The total polyphenol content of the extracts was determined by the methods described by Samarakoon et al. [27] with certain modifications. Briefly, a dilution series of the extracts (7.8 - 1000 µg/ mL) was prepared in a 96 well plate by diluting the 200 mg/mL stock solutions in distilled water, and 20 µL of each diluted extract was mixed with 110 µL of Folin-Ciocalteu reagent. The absorbance was measured at 765 nm using the Synergy™ HT Multi-Mode Microplate Reader

(BioTek, USA). Then, 70 µL of 10% sodium carbonate solution was added to each reaction mixture and incubated at room temperature for 30 min. After incubation, the absorbance was measured again at 765 nm using Synergy™ HT Multi-Mode Microplate Reader (BioTek, USA). Gallic acid was used as the standard. The standard curve was plotted for Gallic acid and the total phenolic content of the sample was expressed as Gallic acid equivalents in GAE mg/ g of extract using the standard curve of Gallic acid.

## 2.10 Statistical Analysis

All experiments were conducted in three independent trials. The best fit curve with high $r^2$ was obtained with the mean and standard deviation data from the three trials in a non-linear regression. The statistical analysis was done using the Prism 6.0 (Graph Pad Software Corporation, Inc, San Diego, California, USA).

## 3. RESULTS AND DISCUSSION

### 3.1 Anti-proliferative Assay- Sulforhodamine B Assay

Plant extracts having $IC_{50}$ values less than 100 µg/ mL against MCF-7 and/or MDA-MB-231 breast cancer cells were considered as anti-proliferative [28]. Therefore, those extracts having $IC_{50}$ < 100 µg/mL were further evaluated by repeating in triplicate and testing on normal mammary epithelial cells (MCF-10A) to investigate the selectivity of the extracts towards breast cancer cells. Out of the fifty-seven extracts from seven medicinal plants tested, seventeen extracts exhibited anti-proliferative activity ($IC_{50}$ <100 µg/ mL) against one or both breast cancer cell lines (MCF-7 and MDA-MB-231) used in the study. When compared to the anti-proliferative activity against normal mammary epithelial cells, dichloromethane and ethyl acetate extracts of *C. zeylanicum* bark exhibited selective activity towards MDA-MB-231 cells (selectivity index > 10) while the anti-proliferative activity of dichloromethane and ethyl acetate extracts of *A. evecta* leaves and the ethyl acetate extract of *A. evecta* bark exhibited high selectivity (selectivity index > 10) towards MCF7 cell line. Bark ethyl acetate extract of *Erigeron sp* exhibited high selectivity (selectivity index ≥ 12) toward both cancer cell lines (Table 2). Although, different extracts of *G. crameri* (except hexane extracts of leaves and bark), bark ethyl acetate and methanol extracts of *E. subvillosus* and leaf

**Table 2. The IC$_{50}$ values of the plant extracts screened for anti-proliferative activity on breast cancer cell lines (MCF-7 and MDA-MB-231) and normal mammary epithelial cells (MCF-10 A) as assessed by SRB assay**

| Scientific name | Plant part | Extract | IC$_{50}$ (µg/ mL) value (and selectivity index*) | | |
| --- | --- | --- | --- | --- | --- |
| | | | MCF-7 cell line | MDA-MB-231 cell line | MCF-10A cell line |
| *W. bicuspidate* | Leaves | Hexane | 204.4 | 248 | - |
| | | Dichloromethane | 204 | 500 | - |
| | | Ethyl Acetate | 341.4 | 407.9 | - |
| | | Methanol | 104.5 | 500.5 | - |
| | Bark | Hexane | 208.4 | 779.9 | - |
| | | Dichloromethane | >1000 | 240.7 | - |
| | | Ethyl Acetate | 615.3 | 101.5 | - |
| | | Methanol | 227.4 | 150.3 | - |
| *C. zeylanicum* | Leaves | Hexane | 287.4 | >1000 | - |
| | | Dichloromethane | 339 | 933.8 | - |
| | | Ethyl Acetate | 589.3 | 185.4 | - |
| | | Methanol | 163.6 | >1000 | - |
| | Bark | Hexane | 88.04±9.31 (2.42) | 69.15± 6.22 (3.08) | 213.2 |
| | | Dichloromethane | 348.7 (>2.86) | 44.75± 3.75 (>22.30) | >1000 |
| | | Ethyl Acetate | 520.0 (1.48) | 67.80± 5.21 (11.32) | 767.6 |
| | | Methanol | >1000 | 800.96 | - |

*\* Selectivity index = IC50 for normal cell line (MCF-10A) / IC50 for cancer cell line. Only the extracts giving IC50 < 100 for cancer cell lines were tested against normal cell line*

| Scientific name | Plant part | Extract | IC$_{50}$ (µg/ mL) value (and selectivity index*) | | |
|---|---|---|---|---|---|
| | | | MCF-7 cell line | MDA-MB-231 cell line | MCF-10A cell line |
| *A. evecta* | Leaves | Hexane | 240.70 | 220.70 | - |
| | | Dichloromethane | 43.74±8.36 **(>22.9)** | 157.50 **(> 6.35)** | >1000 |
| | | Ethyl Acetate | 18.84± 6.12 **(16.8)** | 115.50 **(2.74)** | 317.00 |
| | | Methanol | 160.20 | 505.00 | - |
| | Bark | Hexane | 414.10 | 626.50 | - |
| | | Dichloromethane | 85.64±8.96 **(8.74)** | 119.30 **(6.27)** | 748.60 |
| | | Ethyl Acetate | 93.81± 10.32 **(10.66)** | 202.90 **(4.92)** | >1000 |
| | | Methanol | 441.20 | 192.60 | - |
| *A. variabilis* | Leaves | Hexane | 192.00 | 209.90 | - |
| | | Dichloromethane | 209.50 | >1000 | - |
| | | Ethyl Acetate | 199.00 | >1000 | - |
| | | Methanol | >1000 | 616.40 | - |
| | Bark | Hexane | 304.20 | >1000 | - |
| | | Dichloromethane | 302.10 | 296.20 | - |
| | | Ethyl Acetate | 230.50 | 242.20 | - |
| | | Methanol | 268.30 | 831.50 | - |

*\* Selectivity index = IC50 for normal cell line (MCF-10A) / IC50 for cancer cell line. Only the extracts giving IC50 <100 for cancer cell lines were tested against normal cell line*

| Scientific name | Plant part | Extract | IC $_{50}$ (µg/ mL) value (and selectivity index*) | | |
|---|---|---|---|---|---|
| | | | MCF-7 cell line | MDA-MB-231 cell line | MCF-10A cell line |
| *G. crameri* | Leaves | Hexane | 250.5 | 178.5 | - |
| | | Dichloromethane | 19.78±3.26 **(0.62)** | 21.60±2.64 **(0.56)** | 12.17 |
| | | Ethyl Acetate | 36.86± 7.25 **(2.05)** | 41.54± 3.18 **(1.81)** | 75.55 |
| | | Methanol | 772 | 206.9 | - |
| | Bark | Hexane | 232.8 | >1000 | - |
| | | Dichloromethane | 72.23± 8.99 **(1.04)** | 58.5± 5.61 **(1.28)** | 75.19 |
| | | Ethyl Acetate | 102.6 **(3.68)** | 55.51± 7.16 **(6.81)** | 378.00 |
| | | Methanol | 166.5 **(0.57)** | 94.00±10.32 **(1.02)** | 96.40 |
| | Resin | Dichloromethane | 51.79± 1.35 **(1.23)** | 44.02±2.95 **(1.45)** | 64.04 |
| *E. subvillosus* | Leaves | Hexane | 355.5 | 482.2 | - |
| | | Dichloromethane | 144.7 | 440.2 | - |
| | | Ethyl Acetate | 204.8 | 186.8 | - |
| | | Methanol | 806.6 | >1000 | - |
| | Bark | Hexane | 355.5 | 201.9 | - |
| | | Dichloromethane | >1000 | 630.1 | - |
| | | Ethyl Acetate | 212.9 **(1.44)** | 83.15± 6.35 **(3.70)** | 306.0 |
| | | Methanol | 212.2 **(0.57)** | 48.78± 5.86 **(2.50)** | 122.4 |

* Selectivity index = IC50 for normal cell line (MCF-10A) / IC50 for cancer cell line. Only the extracts giving IC50 <100 for cancer cell lines were tested against normal cell line

| Scientific name | Plant part | Extract | IC$_{50}$ (µg/ mL) value (and selectivity index*) | | |
|---|---|---|---|---|---|
| | | | MCF-7 cell line | MDA-MB-231 cell line | MCF-10A cell line |
| *Erigeron sp* | Leaves | Hexane | 169.7 | 127.4 | - |
| | | Dichloromethane | 230.3 | >1000 | - |
| | | Ethyl Acetate | 47.52± 1.23 **(1.51)** | 75.74± 6.92 **(0.95)** | 72.23 |
| | | Methanol | >1000 | >1000 | - |
| | Bark | Hexane | >1000 | 248.5 | - |
| | | Dichloromethane | 108.74 | 101.35 | - |
| | | Ethyl Acetate | 53.45±4.62 **(16.92)** | 65.1±6.67 **(13.89)** | 904.4 |
| | | Methanol | 403.62 | 363.1 | - |

* Selectivity index = IC50 for normal cell line (MCF-10A) / IC50 for cancer cell line. Only the extracts giving IC50 <100 for cancer cell lines were tested against normal cell line

ethyl acetate extract of *Erigeron sp* exerted high anti-proliferative activity against both cancer cell lines, their selective activity towards cancer cells is very low and hence these extracts are potentially toxic to normal mammary epithelial cells *in vitro*.

None of the extracts of *W. bicuspidate* and *A. variabilis* exhibited anti-proliferative activity ($IC_{50}$ > 100 µg/ mL) against MCF-7 or MDA-MB-231 cells. The dose response curves constructed for the anti-proliferative activities exerted by the most potent extracts (extracts with $IC_{50}$ < 50 µg/mL), that are extracts of *Erigeron sp*, *G. crameri*, *A. evecta*, *C. zeylanicum* and *E. subvillosus* on cancer cell lines provides further evidence for their potent anti-proliferative and cytotoxic activities (Figs. 1 and 2).

## 3.2 Morphological Observations

Cytosol shrinkages, vacuole formation at the cytosol and separation of the cytosol from the membrane linkages was clearly observed in a dose dependent manner up to 200 µg/mL in both cancer cell lines when observed under phase contrast microscope after exposure of cells to most potent extracts ($IC_{50}$ < 50 µg/mL) for 48 h. (Fig. 3 and 4). Such morphological changes were not visible in control cells.

Seven medicinal plants tested in the present study belonged to Acanthaceae, Asteraceae, Burseraceae Elaeocarpaceae, Marattiaceae and Rubiaceae families. Four out of seven plants (*G. crameri*, *W. bicuspidate*, *C. zeylanicum* and *E. subvillosus*) were endemic to Sri Lanka while three (*A. evecta*, *Erigeron sp*, *A. variabilis*) were native plants. Out of the fifty-six leaves and bark and one resinous extract of *G. crameri*, extracts of seven medicinal plants, seventeen extracts exhibited anti-proliferative activity ($IC_{50}$ <100 µg/ mL). Current study reports the anti-proliferative activity of these plant species for the first time. However, several other plants of each of these families are known to possess anti-proliferative and cytotoxic activities.

Leaves of *Strobilanthes crispus* (Acanthaceae family) has been reported to have cytotoxicity *in vitro* and used in Asian folk medicine to treat cancer [16]. *Conyza canadensis* is a species of Asteraceae family and has exhibited anticancer properties on human lung cancer cells *in vitro* [17]. A member of Burseraceae family, *Canarium schweinfurthii* have been used in traditional medicine by the people of tropical countries. Chemo preventive and *in vitro* cytotoxic abilities of *C. schweinfurthii* extracts have been scientifically proven against different cell lines [18-19].

*Elaeocarpus floribundus* belonging to Elaeocarpaceae family also have shown *in vitro* anticancer properties [20]. It has been reported that water boiled with small pieces of rhizome of *Angiopteris evecta* (belongs to Marattiaceae family) have been used to treat cancer by the Temuan tribe in Borga village of Malaysia [21]. *Gardenia jasminoides* and *Ixora coccinea* which belong to the family Rubiaceae found to have many biological activities including *in vitro* anticancer activity against different cell lines [22-23]. In the present study, dichloromethane and ethyl acetate extracts of *C. zeylanicum* bark exhibited over 10 times more anti-proliferative activity to triple negative breast cancer cells MDA-MB-231 cells while the anti-proliferative activity of dichloromethane and ethyl acetate extracts of *A. evecta* leaves and the ethyl acetate extract of *A. evecta* bark were over 10 times more selective toward MCF 7 cells when compared to the anti-proliferative activity of these extracts against normal mammary epithelial cells. This difference in activities opens up a window for specifically targeting these cancer cells. Moreover, bark ethyl acetate extract of *Erigeron sp* exhibited over 12 times more anti-proliferative activity toward both cancer cell lines (Table 2) compared to anti-proliferative activity against normal cells. In general, test substances having *in vitro* selectivity index ≥ 10 is considered as a potential substance for anti-cancer drug development [29]. Therefore, further *in vivo* efficacy and safety studies as well as active ingredient identification studies of these extracts will provide vital materials for anti-breast cancer drug development.

In a previous study, *G. crameri* and *W. bicuspidate* collected from a different location: Bulathsinghala (Western Province, Sri Lanka) have been tested and reported to possess no significant anti-proliferative activity after 24 h post incubation with MCF 7 or MDA-MB-231 breast cancer cells ($IC_{50}$ >100 µg/mL) [30]. In the present study, *G. crameri*, collected from Pitigala (Southern Province, Sri Lanka) exhibited potent anti-proliferative activity in leaves extracts, bark extracts and extract of exudate at the buds against both MCF-7 and MDA-MB-231 cells. *W. bicuspidate* did not exert any anti-proliferative activity which is consistent with the results of the said previous study.
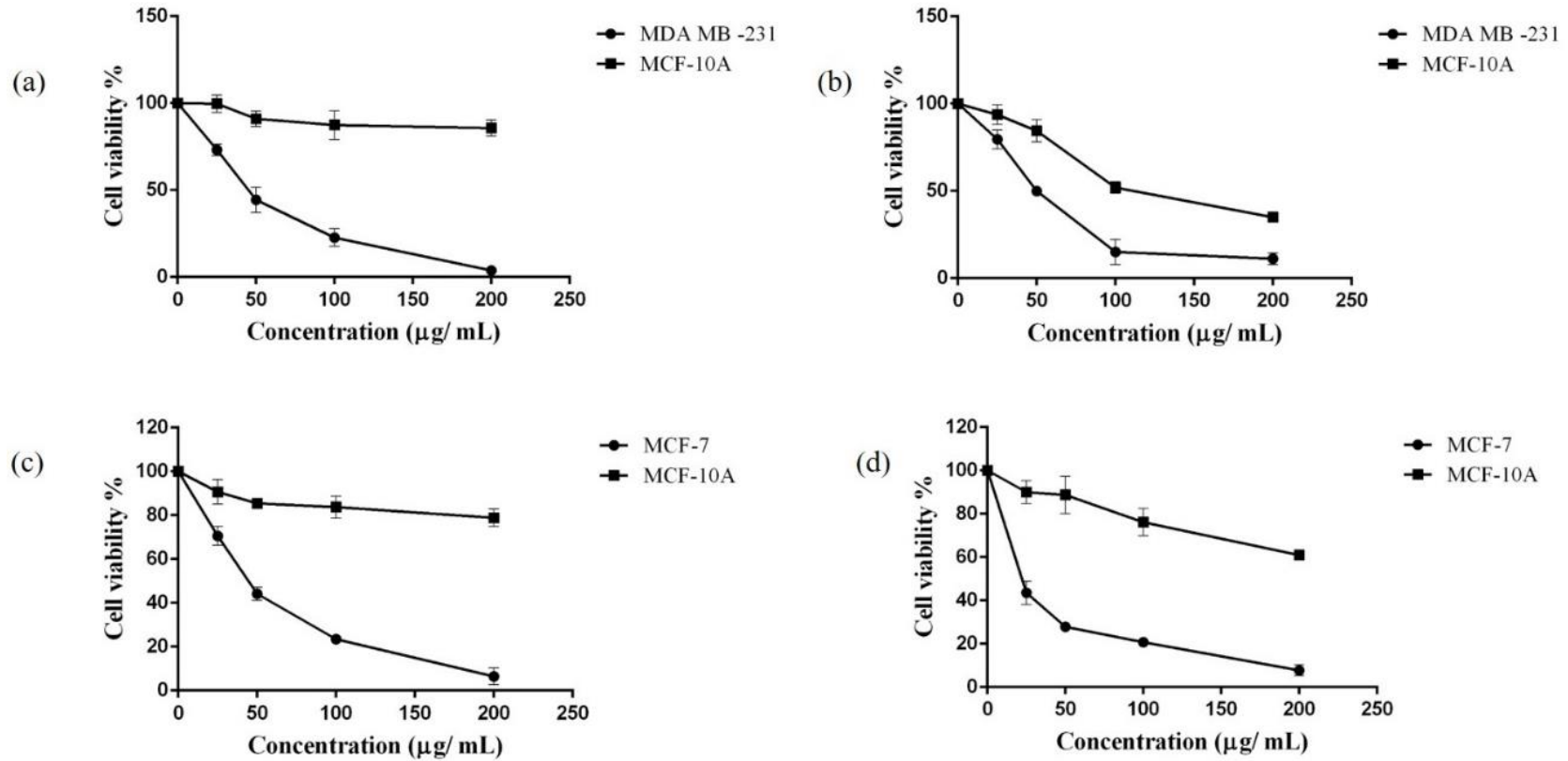
**Fig. 1. Dose response curves for the percentage cell survival in plant extract 48h post incubation treatment on MCF-7 and/or MDA-MB-231 cells with IC$_{50}$ < 50 µg/mL with compared to the normal mammary epithelial cells MCF-10A (a. *C. zeylanicum* bark dichloromethane extract, b. *E. subvillosus* bark methanol extract, c. *A. evecta* leaf dichloromethane extract   d. *A. evecta* leaf ethyl acetate extract)**
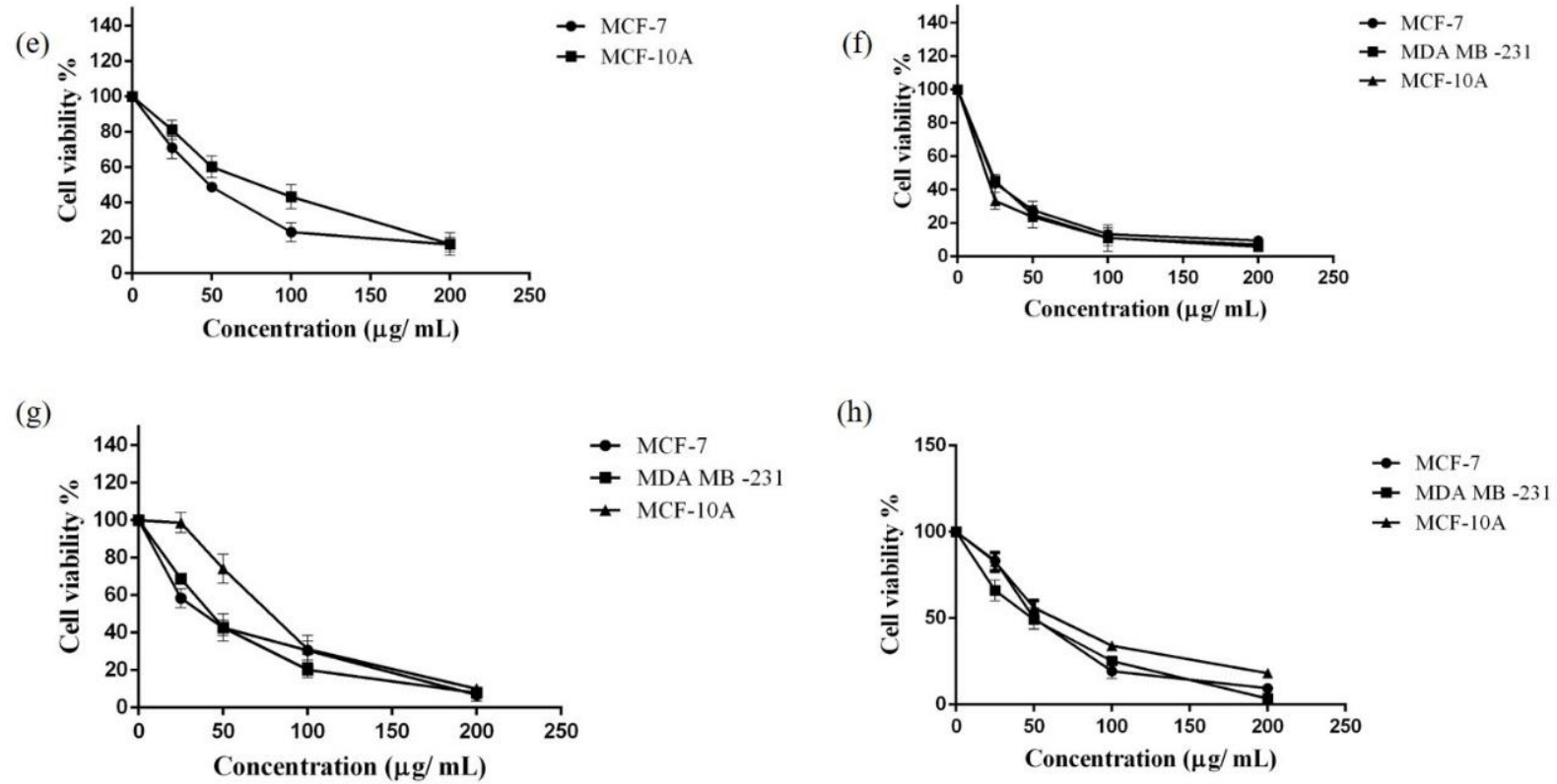
**Fig. 2. Dose response curves for the percentage cell survival in plant extract 48 h post incubation treatment on MCF-7 and/or MDA-MB-231 cells with IC$_{50}$ < 50 µg/mL with compared to the normal mammary epithelial cells MCF-10A (e. *Erigeron sp* leaf ethyl acetate extract f. *G. crameri* leaf dichloromethane extract g. *G. crameri* leaf ethyl acetate h. *G. crameri* exudate dichloromethane extract)**
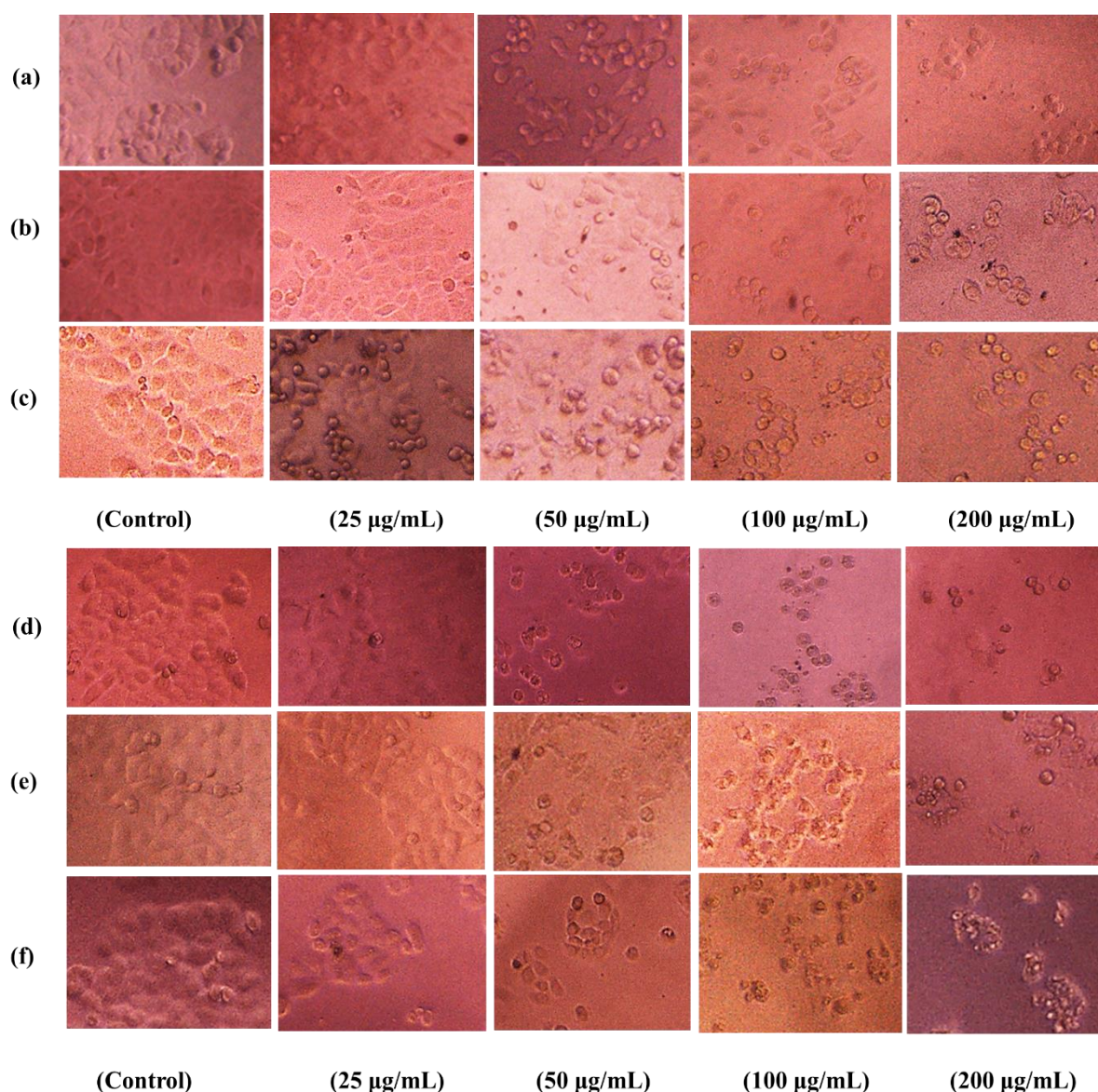
**Fig. 3. Morphological changes of MCF-7 cells treated with active plant extracts having an IC$_{50}$ < 50 μg/mL at 48 h of post incubation. (a) *Erigeron sp* leaf ethyl acetate extract, (b) *A. evecta* leaf dichloromethane extract, (c) *A. evecta* leaf ethyl acetate extract, (d) *G. crameri* leaf dichloromethane extract (e) *G. crameri* leaf ethyl acetate extract and (f) *G. crameri* exudate dichloromethane extract doses of 25, 50, 100 and 200 μg/mL were used (Magnification 200X)**

In general, plants traditionally used for their medicinal properties are thought to have fewer toxic effects. Plants used in the current study have long been used traditionally to treat many different disease conditions and some of those medicinal properties are scientifically proven. *Erigeron sp* has been reported to exert potent peripheral and centrally acting analgesic effects, antifungal and anti-inflammatory activities on acute inflammatory processes [31-33]. *A. evecta*, commonly known as giant fern has been reported to exhibit a wide spectrum of anti-

bacterial and anti-fungal activities [34]. Moreover, the methanol extract of *A. evecta* leaves has been reported to lower blood glucose levels and alleviate pain [35]. Wijerathna et al. [36] have reported anti-bacterial activity of *A. variabilis* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* confirming its medical importance.

Findings of the current study open a new window for selectively targeting breast cancer cells using plant extracts. However, due to inherent

limitations of *in vitro* cell culture-based studies such as inability to provide pharmacokinetic and organ toxicity information, further *in vivo* efficacy and safety studies are necessary for evaluating their potential as anti-cancer agents.

### 3.3 Antioxidant Activity, Total Phenolic and Flavonoid Contents of the Extracts

Methanol extract of leaves and bark of *W. bicuspidate*, *C. zeylanicum*, *G. crameri*, *E. subvillosus*, showed comparatively high anti-oxidant activity (EC$_{50}$ < 50 µg/mL in DPPH assay and TE> 50 mg /g in FRAP assay). Methanol

extract of the leaves and bark of *W. bicuspidate*, *C. zeylanicum*, *G. crameri*, *E. subvillosus* and methanol extract of bark of *Erigeron sp* had comparatively higher phenolic content (GAE> 100 mg /g). Ethyl acetate extract of leaves of *C. zeylanicum*, dichloromethane and ethyl acetate extracts of the leaves of *E. subvillosus*, dichloromethane and methanol extracts of leaves of *G. crameri*, dichloromethane and ethyl acetate extracts of leaves and dichloromethane extract of the bark of *Erigeron sp* showed comparatively higher flavonoid content (QE> 20 mg/g) (Table 3) Moreover, there were differences in the antioxidant activity and phenol and flavonoid contents of *G. crameri* extracts when compared
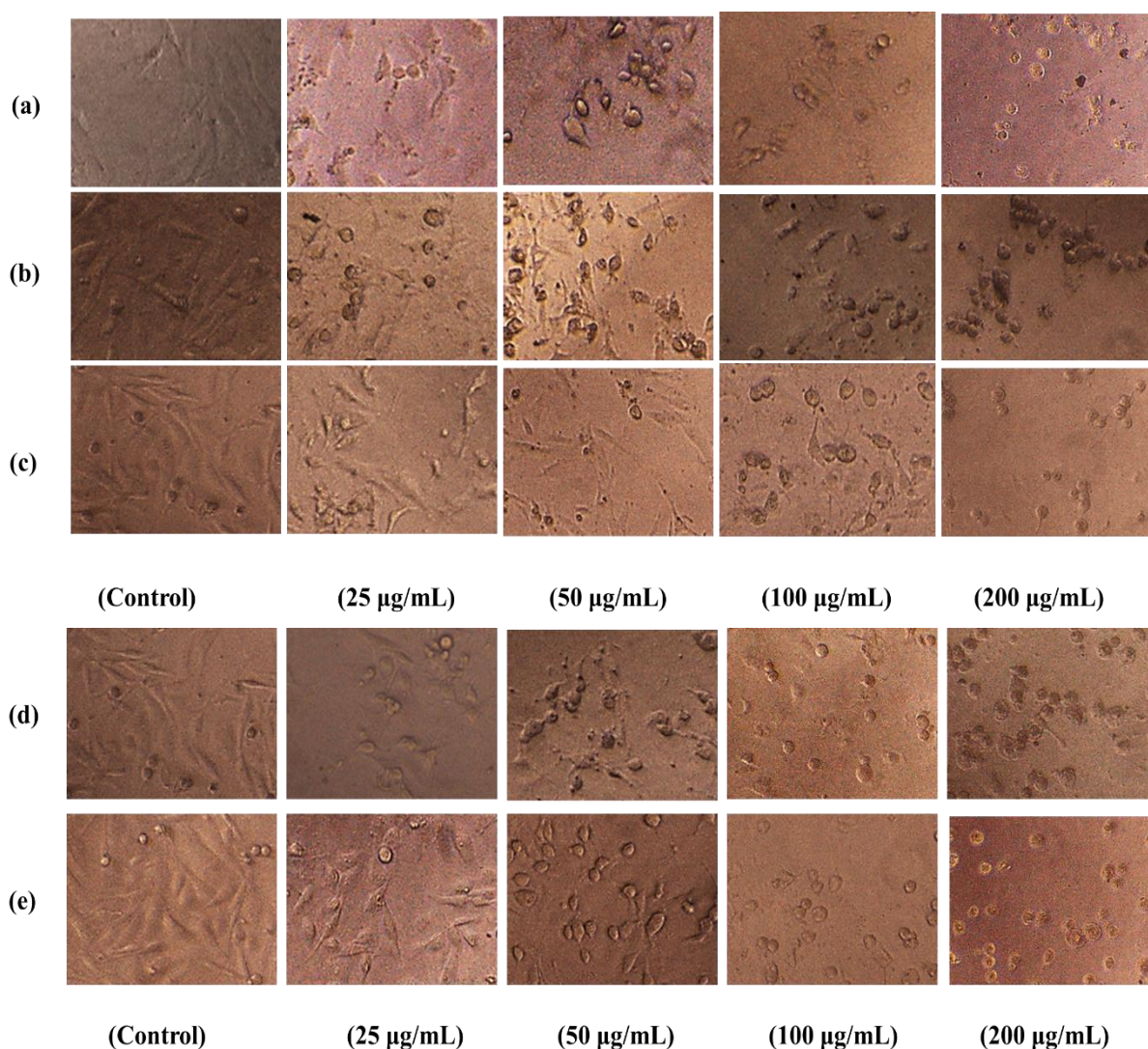


**Fig. 4. Morphological changes of MDA-MB-231 cells treated with active plant extracts having IC$_{50}$ < 50 µg/mL at 48 h of post incubation. (a) *C. zeylanicum* bark dichloromethane extract, (b) *E. subvillosus* bark methanol extract, (c) *G. crameri* leaf dichloromethane extract (d) *G. crameri* leaf ethyl acetate extract and (e) *G. crameri* exudate dichloromethane doses of 25, 50, 100 and 200 µg/mL were used (Magnification 200X)**

**Table 3. The results of anti-oxidant, total phenolic content and flavonoid content of plant leaf and bark extract**

| Scientific name | Plant part | Extract | Anti-oxidant activity | | Total poly phenolic content (GAE mg/ g) | Total flavonoid content (QE mg/ g) |
|---|---|---|---|---|---|---|
| | | | DPPH radical scavenging activity EC $_{50}$ (µg/mL) | FRAP (TE mg/ g) | | |
| *E. subvillosus* | Leaves | Hexane | 466.28±38.06 | 0.43±0.09 | 3.57±1.39 | 3.71±0.99 |
| | | Dichloromethane | >1000 | 0.31±0.01 | 6.70±1.35 | 61.08±12.30 |
| | | Ethyl Acetate | 211.4±22.78 | 2.92±0.12 | 8.25±4.06 | 23.47±0.94 |
| | | Methanol | 12.55±1.083 | 104.51±5.03 | 210.91±25.07 | 4.77±1.50 |
| | Bark | Hexane | >1000 | 0.44±0.25 | 0.53±0.38 | 5.30±2.42 |
| | | Dichloromethane | >1000 | 0.64±0.25 | 1.72±0.64 | 1.15±1.06 |
| | | Ethyl Acetate | 171.01±1.61 | 10.12±0.39 | 16.65±2.76 | 11.10±2.02 |
| | | Methanol | 6.08±0.20 | 78.95±4.65 | 282.17±34.20 | 10.56±2.90 |
| *A. evecta* | Leaves | Hexane | >1000 | 0.22±0.04 | 0.24±0.12 | 3.31±1.43 |
| | | Dichloromethane | 548.25±52.68 | 1.16±0.84 | 1.36±0.48 | 2.02±2.14 |
| | | Ethyl Acetate | >1000 | 0.92±0.25 | 8.18±0.88 | 12.87±2.37 |
| | | Methanol | 323.70±15.9 | 1.75±0.19 | 13.38±1.60 | 8.37±0.46 |
| | Bark | Hexane | >1000 | 0.66±0.44 | 0.06±0.01 | 0.96±0.06 |
| | | Dichloromethane | 880.72±66.60 | 2.24±0.10 | 3.51±0.55 | 1.02±0.85 |
| | | Ethyl Acetate | >1000 | 0.06±0.02 | 2.38±0.00 | 0.39±0.00 |
| | | Methanol | 666.71±21.08 | 0.25±0.04 | 6.59±0.03 | 3.06±1.74 |

| Scientific name | Plant part | Extract | Anti-oxidant activity | | Total poly phenolic content (GAE mg/ g) | Total flavonoid content (QE mg/ g) |
|---|---|---|---|---|---|---|
| | | | DPPH radical scavenging activity EC$_{50}$ (µg/mL) | FRAP (TE mg/ g) | | |
| *G. crameri* | Leaves | Hexane | >1000 | 1.99±0.57 | 2.93±0.87 | 6.11±2.20 |
| | | Dichloromethane | 582.75±10.67 | 3.56±0.16 | 12.33±0.59 | 30.36±7.23 |
| | | Ethyl Acetate | 426.42±37.76 | 0.49±0.27 | 20.21±2.90 | 8.96±2.92 |
| | | Methanol | 43.79±18.93 | 69.75±10.18 | 178.10±3.17 | 32.18±2.68 |
| | Bark | Hexane | >1000 | 0.38±0.38 | 0.46±0.11 | 12.78±7.33 |
| | | Dichloromethane | >1000 | 2.71±0.55 | 5.94±1.05 | 9.55±1.72 |
| | | Ethyl Acetate | 411.08±24.23 | 3.89±0.20 | 11.73±2.35 | 13.09±1.75 |
| | | Methanol | 31.87±4.62 | 130.03±6.08 | 192.73±13.22 | 15.39±2.93 |
| | Resin | Dichloromethane | 666.05±73.12 | 0.13±0.13 | 13.59±1.6 | 12.10±1.87 |
| *C. zeylanicum* | Leaves | Hexane | 340.4±11.41 | 0.87±0.03 | 6.35±0.78 | 5.83±0.41 |
| | | Dichloromethane | >1000 | 3.75±0.04 | 13.38±0.40 | 14.16±1.81 |
| | | Ethyl Acetate | 142.26±7.30 | 7.00±0.18 | 42.83±1.34 | 99.85±7.66 |
| | | Methanol | 0.01±0.00 | 88.16±1.76 | 327.27±48.11 | 11.49±0.51 |
| | Bark | Hexane | >1000 | 0.73±0.37 | 5.31±4.98 | 0.91±0.10 |
| | | Dichloromethane | 296.86±47.88 | 2.42±0.69 | 17.69±4.04 | 10.80±0.88 |
| | | Ethyl Acetate | 88.42±3.08 | 34.71±3.46 | 25.07±8.03 | 4.96±2.76 |
| | | Methanol | 24.63±2.10 | 257.77±15.18 | 394.25±18.43 | 9.25±0.74 |

| Scientific name | Plant part | Extract | Anti-oxidant activity | | Total poly phenolic content (GAE mg/ g) | Total flavonoid content (QE mg/ g) |
|---|---|---|---|---|---|---|
| | | | DPPH radical scavenging activity EC $_{50}$ (µg/mL) | FRAP (TE mg/ g) | | |
| *W. bicuspidate* | Leaves | Hexane | >1000 | 0.24±0.09 | 0.71±0.00 | 3.77±2.86 |
| | | Dichloromethane | >1000 | 0.66±0.04 | 0.11±0.00 | 18.59±3.12 |
| | | Ethyl Acetate | 452.68±42.22 | 2.02±0.73 | 13.38±1.70 | 4.96±0.93 |
| | | Methanol | 0.01±0.00 | 391.13±50.72 | 476.20±17.66 | 7.21±1.43 |
| | Bark | Hexane | 695.62±72.55 | 0.55±0.05 | 6.30±0.46 | 4.47±3.75 |
| | | Dichloromethane | >1000 | 0.21±0.04 | 9.79±4.99 | 1.78±0.58 |
| | | Ethyl Acetate | 222.54±15.13 | 3.65±0.72 | 4.32±0.43 | 3.02±0.31 |
| | | Methanol | 81.08±2.02 | 50.47±2.65 | 342.03±21.72 | 2.55±2.84 |
| *Erigeron sp* | Leaves | Hexane | 837.72±22.14 | 0.63±0.03 | 21.42±0.92 | 2.62±0.00 |
| | | Dichloromethane | 806.14±68.98 | 3.86±0.52 | 6.46±0.91 | 197.53±19.80 |
| | | Ethyl Acetate | 290.92±17.64 | 4.93±0.34 | 17.55±0.93 | 34.86±5.29 |
| | | Methanol | 150.15±12.00 | 9.95±0.85 | 78.68±5.83 | 15.67±47.93 |
| | Bark | Hexane | 802.46±13.31 | 0.34±0.02 | 10.96±2.68 | 1.53±0.01 |
| | | Dichloromethane | 260.98±20.59 | 2.06±0.16 | 3.25±1.06 | 100.65±7.65 |
| | | Ethyl Acetate | 140.35±2.62 | 5.24±0.34 | 20.89±2.95 | 22.32±2.93 |
| | | Methanol | 90.98±2.36 | 9.03±0.90 | 101.52±8.53 | 10.39±1.62 |

| Scientific name | Plant part | Extract | Anti-oxidant activity | | Total poly phenolic content (GAE mg/ g)) | Total flavonoid content (QE mg/ g) |
| --- | --- | --- | --- | --- | --- | --- |
| | | | DPPH radical scavenging activity $EC_{50}$ (µg/mL) | FRAP (TE mg/ g) | | |
| *A. variabilis* | Leaves | Hexane | >1000 | 0.13±0.02 | 0.98±0.90 | 4.89±1.90 |
| | | Dichloromethane | >1000 | 0.53±0.47 | 7.97±1.89 | 2.78±0.50 |
| | | Ethyl Acetate | >1000 | 0.27±0.03 | 1.45±0.40 | 12.72±2.54 |
| | | Methanol | 232.47±10.88 | 4.78±0.42 | 19.70±1.16 | 10.22±2.37 |
| | Bark | Hexane | >1000 | 0.23±0.02 | 0.85±0.54 | 4.52±2.13 |
| | | Dichloromethane | >1000 | 1.54±0.44 | 1.53±0.82 | 15.30±4.45 |
| | | Ethyl Acetate | >1000 | 0.30±0.09 | 1.09±0.52 | 7.28±4.23 |
| | | Methanol | 348.94±2.89 | 9.54±1.15 | 16.48±1.68 | 1.08±0.08 |

to the previous study done by Jayarathna and co-authors [30]. Observed difference of the anti-proliferative activities as well as phenol and flavonoid contents of *G. crameri* collected from different locations may be due to geographical or seasonal variations in their secondary metabolites.

Antioxidant and anti-cancer properties are thought to relate to each other as most antioxidants are found to be regulators of cancer development [37]. According results of the present study, no significant relationship was observed between the anti-proliferative properties and antioxidant properties as none of the potent anti-proliferative extracts showed considerable anti-oxidant properties. However, all extracts having antioxidant properties showed high phenolic content, which suggests that the antioxidant activity of the extracts might be due to the phenolic compounds present in the extract [38]. A high flavonoid content was observed in dichloromethane extracts and ethyl acetate extracts of leaves and bark (> 20 mg/g) of *Erigeron sp.* Flavonoids may be responsible for the observed anti-proliferative effect of these extracts on both breast cancer cell lines.

The present study preliminary focusses on screening the anti-breast cancer and antioxidant activities of medicinal plants grown in Sri Lanka. However, further evaluation on the individual compounds is necessary to identify potent anti-breast cancer active drug leads from these extracts. There are several limitations to the further analysis on these extracts. For instance, the geographic availability and conservation status of the plant and the plant part selected for the extraction may impact the feasibility of large-scale extraction required for compound-level-studies.

## 4. CONCLUSION

In the present study, dichloromethane and ethyl acetate extracts of *A. evecta* leaves and the ethyl acetate extract of *A. evecta* bark exhibited potent phenotype selective anti-proliferative activity against ER positive breast cancer molecular subtype (MCF-7 cells). Dichloromethane and ethyl acetate extracts of *C. zeylanicum* bark exhibited phenotype selective anti-proliferative activity against triple negative breast cancer molecular subtype (MDA-MB-231 cells). Bark ethyl acetate extract of *Erigeron sp* exhibited selective anti-proliferative activity toward both cancer cell lines when compared to anti-proliferative activity against normal cells. Anti-oxidant compounds of these extracts may not be responsible for anti-proliferative activity as none of the potent anti-proliferative extracts tested showed considerable anti-oxidant properties. Selective anti-proliferative activity of these extracts opens up a window for specifically targeting these cancer cells. However, further *in vivo* efficacy and safety studies are necessary for evaluating their potential as anticancer agents.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## DATA AVAILABILITY STATEMENT

Data set supporting this manuscript is included within the manuscript. Any further information should be requested from the corresponding author.

## CONSENT AND ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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