European Journal of Medicinal Plants
15(1): 1-15, 2016, Article no.EJMP.26591
ISSN: 2231-0894, NLM ID: 101583475

SCIENCEDOMAIN *international*
www.sciencedomain.org

Cytotoxic, Antioxidant and Apoptotic Effects of Twenty Sri Lankan Endemic Plants in Breast Cancer Cells

Panchima P. Jayarathna[1], Kamani H. Tennekoon[1*], Sameera R. Samarakoon[1], Ira Thabrew[1], Eric H. Karunanayake[1], E. Dilip de Silva[2] and Meran K. Ediriweera[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, No: 90, Cumaratunga Munidasa Mawatha, Colombo, Sri Lanka.
[2]Department of Chemistry, Faculty of Science, University of Colombo, Colombo, Sri Lanka.

Authors' contributions

This work was carried out in collaboration between all authors. Authors KHT, SRS, IT, EHK and EDS designed the study and supervised experimental work. Author PPJ collected plant material and carried out experiments. Author MKE carried out some biological assays. Authors PPJ and SRS carried out data analysis. Author PPJ wrote the first draft of the manuscript. Authors KHT, IT and SRS revised the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/26591
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Michel Mansur Machado, Federal University of Pampa, Brazil.
(2) Özlem Sultan Aslantürk, Adnan Menderes University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/14690

Original Research Article

Received 25th April 2016
Accepted 10th May 2016
Published 18th May 2016

ABSTRACT

Aims: Main aim of this study was to evaluate cytotoxic, antioxidant and apoptotic properties of Sri Lankan endemic plants in estrogen receptor positive (MCF-7) breast cancer and triple negative breast cancer (MDA-MB-231) cells.
Place and Duration of Study: At the Institute of Biochemistry, Molecular Biology and Biotechnology, between June 2013 and May 2015.
Methodology: We investigated leaf and bark extracts (hexane, chloroform, ethyl acetate, methanol) of twenty endemic plants for possible cytotoxicity against breast cancer (on estrogen receptor positive MCF-7 and triple negative MDA-MB-231 breast cancer) cell lines and normal

**Corresponding author: E-mail: kamani@ibmbb.cmb.ac.lk;*

mammary epithelial cells (MCF-10A). Cytotoxic extracts were also investigated for possible apoptotic effects on MDA-MB-231 cells by caspase 3/7 estimation. Total polyphenols, flavonoids and the free radical scavenging activity of the extracts were also measured.

Results: Of the 156 solvent extracts from 20 plants studied only 35 extracts from 14 plants were significantly cytotoxic [18 extracts to MCF-7 cells (IC_{50} 31.43 to 97.50 µg/mL), 7 extracts to MDA-MB-231 cells (IC_{50} 16.11 to 95.83 µg/mL) and 10 extracts (IC_{50} 0.51 to 99.35 µg/mL) to both cell lines] with only some of these being less cytotoxic to normal breast epithelial cells. Caspase 3/7 was activated by some of the cytotoxic extracts. Polyphenol and free radical scavenging activity were high in most of the methanol and ethyl acetate extracts. Flavonoids were present in all the extracts in varying quantities.

Conclusion: The cytotoxic and apoptotic potential demonstrated by some plants endemic to Sri Lanka, indicates strong leads for future drug discovery for combating breast cancer.

Keywords: Cytotoxicity; apoptotic effect; phytochemical constituents; antioxidant activity; breast cancer; Sri Lankan endemic plants.

1. INTRODUCTION

Cancer is a leading cause of death due to lack of early detection methods and poor prognosis when detected late especially in developing countries. Breast cancer is a leading cause of mortality among women worldwide [1]. Based on the molecular profiling different forms of breast cancer have been recognized. Breast cancer cells expressing estrogen receptor alpha (ER) in their nuclei, ER-positive breast cancer is the common form. They have a better prognosis as they respond to anti-estrogen therapy [2]. Approximately 15% of breast cancers do not express estrogen, progesterone and human epidermal growth factor (HER2/neu) receptors and are known as triple-negative [3]. These are more aggressive and survival of patients is poor due to lack of effective targeted therapies [4]. Oxidative stress, resulting from an imbalance between formation and neutralization of highly reactive free radicals is implicated in carcinogenesis [5]. Stabilization of free radicals that occur in healthy cells through electron pairing with macromolecules causes protein and DNA damage as well as lipid peroxidation leading to carcinogenesis [6].

Programmed cell death (apoptosis) is necessary for maintenance of homeostasis and elimination of damaged cells but this process is hindered in tumor cells [7]. The apoptotic cascade can be initiated via two major pathways, the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. A specific family of cysteine proteases, the caspases, is needed for both pathways [8]. The extrinsic and intrinsic pathways converge on the same execution pathway initiated by the cleavage of caspase-3. At the end of the execution pathway, apoptotic bodies formed are engulfed by adjacent parenchymal cells, neoplastic cells or macrophages [9].

In view of adverse effects of currently available treatments, many individuals are resorting to natural remedies for cancer treatment. Plants are known to contain a diverse range of secondary metabolites including anti cancer compounds and several antioxidants. Natural antioxidants such as flavonoids and polyphenols are explored for their anti-oxidative properties of hydrogen donating, radical scavenging and metal chelating activities for prevention and treatment of cancer. Therefore much attention has been directed towards the development of novel anticancer drugs from plant sources [10-14]. Paclitaxel which is a currently used drug for cancer treatment was also derived from a plant.

The World Conservation Monitoring Centre has designated Sri Lanka as a hotspot of biodiversity as the flora of Sri Lanka occurs in a wide range of environments. The indigenous flora of Sri Lanka comprises about 7,500 plant species and of the 3360 flowering plants, about 830 (25%) species are endemic to the island [15]. These provide an excellent source to discover novel anticancer agents. In the present study we evaluated 20 plants endemic to Sri Lanka for possible cytotoxic and apoptotic effects on breast cancer cell lines as well as the free radical scavenging activity, total polyphenol content and flavonoid content of their solvents extracts.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

DMEM, L-15, fetal bovine serum (30-2020), penicillin and streptomycin (30-2300) were

purchased from American type cell culture collection (ATCC) Manassas, VA, USA. Sulforhodamine B (SRB) powder (3520-42-1), DPPH (1, 1-Diphenyl-2-Picrylhydrazyl, D9132), quercetin (Q0125), gallic acid (G7384), dimethyl sulfoxide (DMSO, D8414), trichloro acetic acid (T 6399) and acetic acid (A 6283) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Plant Materials

From the plants identified as endemic, we selected twenty plants that can be collected easily. The plants identified were collected from Bulathsinhala area in the Kalutara district of the Western province, Sri Lanka. Although the traditional medicine recommends use of leaves, bark and roots, collections were limited to leaves and bark in order to comply with guidelines of Wild Life Department and Forest Department of Sri Lanka and also to minimize damage to plants. Approval from the Forest Department, Sri Lanka, was obtained prior to collection. Plants were identified by the Botanists either at the National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka or at the Bandaranayake Memorial Ayurvedic Research Institute, Maharagama, Sri Lanka. Voucher specimens were deposited in the publicly accessible herbaria in these two Institutes and the reference numbers are given in Table 1.

2.3 Preparation of Solvent Extracts of Plants

Collected leaves and bark were dried completely and ground into small pieces. The dried ground leaves and barks (5 g each) were subjected to sequential extraction using different solvents. Four solvents with increasing polarity namely hexane, chloroform, ethyl acetate and methanol were selected for sequential extraction in order to maximize the extraction of different compounds/secondary metabolites in the plants. Hexane being strongly non polar, such compounds will be extracted to hexane, compounds with increasing polarity to chloroform and to ethyl acetate followed by the highly polar compounds into methanol. The extracts were filtered individually and evaporated to dryness. Dried extracts were stored at 4℃ until used.

2.4 Biological Assays

2.4.1 Cell lines and maintenance of cell culture

The two human breast-cancer cell lines used in this study were estrogen receptor positive MCF-7 (ATCC HTB-22) and triple negative (oestrogen receptor, progesterone receptor and epidermal growth factor receptor HER2/neu negative) MDA-MB-231 (ATCC HTB-26) cells. MCF 10A (ATCC CRL-10317) was the normal breast epithelial cell line used. These cell lines were purchased from American type culture collection (ATCC).

All the cell lines were maintained according to ATCC recommendations. MCF-7 and MCF 10A were maintained in DMEM (ATCC Catalog No. 30-2002) and MDA-MB-231 cells were maintained in ATCC-formulated Leibovitz's L-15 Medium (ATCC Catalog No. 30-2008) at 37℃. All media were supplemented with 10% fetal bovine serum, 50 IU/mL penicillin and 50 µg/mL streptomycin. Except triple negative cells, the others were maintained in 5% CO_2 atmosphere.

2.4.2 Assessment of cytotoxicity of the extracts by sulforhodamine B (SRB) assay

MCF-7, MDA-MB-231 and normal breast epithelial (MCF 10A) cells were plated on 96 well plates (5000 cells/well) with 200 µL of suitable growth medium. Cells cultured in vitro were exposed to plant extracts (doses ranging from 25 to 400 µg/mL and in triplicates) for 24 h and cytotoxicity assessed by Sulforhodamine B assay. SRB assay was performed for MCF 10A cells for the extracts which were cytotoxic (IC_{50}<100 µg/mL) even to a single cancer cell line. The assay was performed as previously described [16,17]. Supernatant was removed and cells washed with PBS. The cells were fixed with Trichloroacetic acid (10%) and washed with tap water. SRB (dissolved in 1% TCA) was added to each well and incubated for 15 min. After removing the unbound dye using acetic acid, the protein bound dye was solubilized with Tris base (10 mM; pH 7.5). The absorbance was then measured at 540 nm using Synergy™ HT Multi-Mode Microplate Reader (BioTek, USA). The percentage viability of cells was calculated and half maximal inhibitory concentration (IC_{50}) determined.

Table 1. Cytotoxicity (IC_{50} values), polyphenol content, flavonoid content and free radical scavenging activity (Mean±SD) of four solvent extracts (hexane, chloroform, ethyl acetate, and methanol) of bark and leaves of twenty endemic plants from Sri Lanka

Plant / Family (Voucher number / place of deposition)	Part	Extract	Cytotoxicity/ IC_{50} value (µg/mL)		Polyphenol content mg gallic acid equivalents /g of extract	Flavonoid content mg quercetin equivalents /g of extract	DPPH radical scavenging activity EC_{50} values (µg/mL)
			MCF-7	MDA-MB-231			
Anacardiaceae							
Campnosperma zeylanica	Leaves	Hexane	32.76±0.4	36.37±0.3	1.60±0.03	0.40±0.009	224 .80±0.3
		Chloroform	38.69±0.1	76.58±0.4	0.78±0.02	0.27±0.002	256.70±0.4
		Ethyl Acetate	22.10±0.2	86.49±0.3	17.43±0.09	1.10± 0.01	16.89±0.1
(S1PDA0110 BG/PDN)		Methanol	31.43±0.4	800.02±0.1	27.79±0.04	0.55±0.003	10.64±0.3
	Bark	Hexane	1.93±0.3	0.51±0.3	2.69±0.02	0.17±0.001	290.80±0.3
		Chloroform	21.67±0.09	1.79±0.4	2.30±0.06	0.72±0.001	291.00 ±0.2
		Ethyl Acetate	34.30±0.2	126.70±0.2	28.85±0.02	0.33 ±0.002	21.45±0.1
		Methanol	270.00±0.2	> 1000	14.03±0.03	0.12±0.001	17.85±0.2
	Leaves	Hexane	514.25±0.09	425.90±0.2	2.82±0.02	0.64±0.002	146.70± 0.2
Semecarpus subpeltata		Chloroform	521.00±0.4	308.45±0.1	1.16±0.03	2.12±0.0 02	> 1000
		Ethyl Acetate	784.95±0.1	251.40±0.3	6.28±0.03	2.38 ±0.009	267.50±0.3
		Methanol	308.60±0.3	444.53±0.4	21.21±0.04	1.68±0.00 4	35.67±0.2
(1574B1707 BMARI)	Bark	Hexane	664.30±0.2	> 1000	3.07±0.05	1.05±0.001	> 1000
		Chloroform	> 1000	359.20±0.4	1.21±0.04	3.42±0.009	> 1000
		Ethyl Acetate	> 1000	258.45±0.3	68.68±0.2	1.68±0.002	175.30±0.1
		Methanol	> 1000	546.80±0.09	33.84±0.1	1.81±0.09	151.30±0.2
Apocynaceae							
Wrightia zeylanica	Leaves	Hexane	> 1000	> 1000	0.29±0.01	0.58±0.002	626.20±0.3
		Chloroform	> 1000	> 1000	1.02±0.01	2.78±0.001	> 1000
(S4PDA0110 BG/PDN)		Ethyl Acetate	> 1000	> 1000	0.22±0.02	0.51±0.002	974.90±0.4
		Methanol	> 1000	> 1000	2.08±0.01	1.32±0.002	584.00±0.2
	Bark	Hexane	751.00±0.3	587.00±0.2	0.01±0.002	0.39±0 .001	> 1000
		Chloroform	548.00±0.2	> 1000	0.69±0.02	0.63±0.001	> 1000
		Ethyl Acetate	> 1000	> 1000	0.59±0.01	0.50±0.002	> 1000
		Methanol	468.00±0.1	> 1000	2.17±0.03	0.41±0.003	553.50±0.2

Plant / Family (Voucher number / place of deposition)	Part	Extract	Cytotoxicity/ IC_{50} value (µg/mL)		Polyphenol content mg gallic acid equivalents /g of extract	Flavonoid content mg quercetin equivalents /g of extract	DPPH radical scavenging activity EC_{50} values (µg/mL)
			MCF-7	MDA-MB-231			
Celastraceae							
Bhesa ceylanica	Leaves	Hexane	> 1000	452.00±0.1	0.91±0.01	3.50±0.02	> 1000
		Chloroform	> 1000	367.30±0.09	0.68±0.02	5.10±0.1	> 1000
(1575B1707 BMARI)		Ethyl Acetate	578.90±0.09	414.90±0.4	0.20±0.01	3.19±0.07	> 1000
		Methanol	428.30±0.4	598.70±0.2	7.80±0.03	1.91±0.06	146.20±0.4
	Bark	Hexane	> 1000	286.50±0.3	0.11±0.01	2.00±0.06	> 1000
		Chloroform	280.10±0.3	232.50±0.2	1.61±0.02	2.61±0.02	> 1000
		Ethyl Acetate	53.55±0.2	90.93±0.2	4.40±0.02	2.71±0.02	375.00±0.1
		Methanol	162.00±0.2	181.20±0.2	57.90±0.1	0.60±0.005	1.04±0.01
Clusiaceae							
Calophyllum calaba	Leaves	Hexane	270.80±0.1	143.30±0.1	0.92±0.02	0.57±0.004	353.10±0.2
		Chloroform	45.73±0.09	127.80±0.2	1.91±0.01	1.65±0.003	279.90±0.4
(S6PDA0110 BG/PDN)		Ethyl Acetate	63.81±0.1	219.70±0.4	12.79±0.2	1.79±0.002	12.99±0.3
		Methanol	343.50±0.4	> 1000	20.42±0.3	2.34±0.005	10.95±0.1
	Bark	Hexane	691.10±0.09	198.50±0.2	2.74±0.09	1.06±0.006	> 1000
		Chloroform	166.60±0.2	311.60±0.2	3.90±0.2	3.77±0.009	383.20±0.1
		Ethyl Acetate	81.48±0.3	129.60±0.1	11.79±0.4	4.44± 0.1	237.70±0.1
		Methanol	793.60±0.3	395.4±0.4	60.95±0.09	3.22±0.3	90.25±0.2
Calophyllum moonii	Leaves	Hexane	222.50±0.1	82.22±0.3	0.80±0.01	0.12±0.002	1.66±0.02
(1576B1707 BMARI)		Chloroform	149.70±0.2	195.40±0.2	0.92±0.01	0.86±0.003	> 1000
		Ethyl Acetate	61.79±0.3	126.60±0.4	13.42±0.09	0.45±0.004	64.69±0.2
		Methanol	42.73±0.1	75.72±0.2	32.16±0.2	1.17±0.009	18.27±0.3
	Bark	Hexane	74.91± 0.4	177.40±0.1	7.32±0.1	1.00±0.002	> 1000
		Chloroform	159.40±0.2	343.70±0.2	6.21±0.09	3.38±0.002	144.80±0.3
		Ethyl Acetate	108.60±0.3	179.80±0.3	9.29±0.2	2.50±0.001	74.87±0.4
		Methanol	90.52±0.3	166.40±0.2	29.30±0.3	0.23±0.002	6.99±0.2
Calophyllum	Leaves	Hexane	> 1000	128.20±0.1	2.64±0.03	0.17±0.003	309.20±0.3
tomentosum Wight		Chloroform	451.78±0.3	165.60±0.1	2.90±0.02	2.38±0.002	287.80±0.1
		Ethyl Acetate	464.61±0.2	157.70±0.3	28.30±0.2	2.31±0.002	4.03±0.1
(1577B1707 BMARI)		Methanol	515.04±0.4	149.90±0.2	32.99±0.3	0.37±0.001	1.53±0.01
	Bark	Hexane	321.30±0.1	172.10±0.09	0.47±0.02	0.04±0.001	> 1000

Plant / Family (Voucher number / place of deposition)	Part	Extract	Cytotoxicity/ IC_{50} value (µg/mL)		Polyphenol content mg gallic acid equivalents /g of extract	Flavonoid content mg quercetin equivalents /g of extract	DPPH radical scavenging activity EC_{50} values (µg/mL)
			MCF-7	MDA-MB-231			
		Chloroform	53.96±0.2	184.40±0.3	2.33±0.01	1.08±0.002	> 1000
		Ethyl Acetate	288.20±0.2	334.10±0.4	17.35±0.3	0.67±0.004	10.55±0.1
		Methanol	225.80±0.3	179.30±0.2	37.98±0.4	0.31±0.001	1.75±0.04
Connaraceae							
Connarus championii	Leaves	Hexane	72.05±0.3	38.90±0.4	1.11±0.01	0.93±0.002	242 .60±0.3
		Chloroform	178.56±0.2	29.83±0.08	1.54±0.02	4.14±0.1	349.90±0.4
(S8PDA0110 BG/PDN)		Ethyl Acetate	152.81±0.2	138.60±0.2	47.89±0.3	4.71 ±0.3	8.67±0.2
		Methanol	298.82±0.3	> 1000	29.18±0.2	0.32±0.001	5.68±0.2
	Bark	Hexane	696.10±0.4	> 1000	0.82±0.01	0.02±0.001	474.10±0.2
		Chloroform	214.90±0.1	225.20±0.2	2.34±0.02	1.15±0.002	715.00±0.3
		Ethyl Acetate	> 1000	492.80±0.1	4.38±0.01	0.16±0.002	217.00±0.3
		Methanol	> 1000	> 1000	60.08±0.2	0.22±0.003	1.69±0.02
Dilleniaceae							
Schumacheria castaneifolia	Leaves	Hexane	159.23±0.2	404.50±0.3	0.02±0.0009	0.65±0.002	> 1000
		Chloroform	201.89±0.1	146.40±0.2	0.28±0.01	1.62±0.001	> 1000
		Ethyl Acetate	526.23±0.4	95.83±0.08	1.74±0.01	0.77±0.001	> 1000
(S30PDA0110 BG/PDN)		Methanol	547.75±0.3	191.80±0.4	5.99±0.02	0.67±0.002	87.35±0.3
	Bark	Hexane	316.30±0.09	105.10±0.2	0.27±0.01	0.15±0.003	> 1000
		Chloroform	390.90±0.1	33.55±0.2	0.30±0.02	0.61±0.002	> 1000
		Ethyl Acetate	176.20±0.3	16.11±0.1	1.69±0.01	0.91± 0.003	525.30±0.3
		Methanol	36.66±0.3	63.26±0.4	24.15±0.4	0.11±0.001	1 1.60 ±0.2
Dipterocarpaceae							
Doona macrophylla	Leaves	Hexane	589.12±0.2	379.90±0.1	0.23±0.01	0.14±0.009	> 1000
		Chloroform	430.36±0.09	286.40±0.3	1.14±0.01	2.42±0.08	> 1000
(1578B1707 BMARI)		Ethyl Acetate	182.56±0.3	420.40±0.4	30.92±0.3	0.86±0.05	94.65±0.2
		Methanol	199.42±0.1	127.30±0.3	14.70±0.2	2.55±0.1	1 6.24±0.3
	Bark	Hexane	316.30±0.4	309.90±0.4	0.28±0.01	0.0087± 0.0001	> 1000
		Chloroform	390.90±0.2	232.60±0.3	0.84±0.02	1.28±0.002	> 1000
		Ethyl Acetate	176.20±0.3	602.10±0.3	23.25±0.4	0.27±0.01	36.78±0.2
		Methanol	36.66±0.4	568.71±0.3	58.68±0.1	2.32±0.03	1 .12±0.03

Plant / Family (Voucher number / place of deposition)	Part	Extract	Cytotoxicity/ IC_{50} value (µg/mL)		Polyphenol content mg gallic acid equivalents /g of extract	Flavonoid content mg quercetin equivalents /g of extract	DPPH radical scavenging activity EC_{50} values (µg/mL)
			MCF-7	MDA-MB-231			
Vateria copallifera	Leaves	Hexane	561.70±0.2	628.60±0.2	0.53±0.01	1.51±0.01	> 1000
		Chloroform	143.90±0.3	461.90 ±0.3	2.26±0.02	7.55±0. 1	> 1000
(S29PDA0110		Ethyl Acetate	318.20±0.4	> 1000	38.24±0.3	1.47±0.02	96.64±0.2
BG/PDN)		Methanol	465.20±0.2	> 1000	25.62±0.4	1.45±0.02	98.76±0.1
	Bark	Hexane	379.60±0.2	449.10±0.4	0.06±0.01	0.52±0. 01	> 1000
		Chloroform	406.60±0.3	194.70±0.2	0.89±0.02	1.58±0.0 2	> 1000
		Ethyl Acetate	> 1000	458.30±0.1	41.16±0.3	1.51±0.01	110.60±0.3
		Methanol	> 1000	472.13±0.3	34.99±0.4	0.77±0.03	86.74±0.1
Euphorbiaceae							
Chaetocarpus	Leaves	Hexane	259.71±0.2	> 1000	1.19±0.02	1.36±0.01	238.10±0.2
coriaceus		Chloroform	458.90±0.3	> 1000	0.35±0.01	1.50±0.02	> 1000
		Ethyl Acetate	670.1±0.4	> 1000	0.64±0.02	0.53±0.009	844.00±0.4
(S15PDA0110		Methanol	621.85±0.2	576.00±0.09	28.20±0.2	1.20±0.00 7	12.88±0.3
BG/PDN)	Bark	Hexane	702.14±0.09	761.90±0.09	0.02±0.009	0.04±0.002	> 1000
		Chloroform	280.60±0.1	423.10±0.3	1.12±0.01	0.76±0.0 08	378.70±0.3
		Ethyl Acetate	120.60±0.2	271.00±0.4	6.60±0.2	0.17± 0.02	27.70±0.2
		Methanol	52.10±0.3	210.70±0.2	19.27±0.3	0.23±0.001	18.97±0.3
Lauraceae							
Actinodaphne	Leaves	Hexane	970.14±0.4	> 1000	0.79±0.01	0.87±0.02	> 1000
stenophylla		Chloroform	290.20±0.2	219.00±0.3	2.61±0.02	3.90±0.2	961.80±0.2
		Ethyl Acetate	141.10±0.3	115.30±0.2	26.94±0.03	5.6 9±0.4	47.13±0.4
(1579B1707 BMARI)		Methanol	856.66±0.2	351.30±0.3	18.56±0.2	2.60±0.2	2 7.17±0.2
	Bark	Hexane	384.10±0.3	233.40±0.2	0.30±0.01	0.071±0 .0002	> 1000
		Chloroform	901.20±0.2	592.80±0.3	0.32±0.01	0.25±0.0 02	> 1000
		Ethyl Acetate	863.20±0.1	670.80±0.2	0.50±0.01	0.34 ±0.001	> 1000
		Methanol	> 1000	441.30±0.3	0.97±0.01	0.03±0.0009	> 1000
Melastomaceae							
Lijndenia capitellata	Leaves	Hexane	> 1000	636.20±0.3	0.38±0.009	2.29±0.1	> 1000
		Chloroform	> 1000	447.7±0.4	0.05±0.004	2.73±0.2	> 1000
(S11PDA0110		Ethyl Acetate	97.50±0.3	315.00±0.1	41.10±0.4	2.09± 0.2	12.18±0.2
BG/PDN)		Methanol	175.10±0.3	886.80±0.2	28.02±0.2	1.53±0.09	10.55±0.3

Plant / Family (Voucher number / place of deposition)	Part	Extract	Cytotoxicity/ IC_{50} value (µg/mL)		Polyphenol content mg gallic acid equivalents /g of extract	Flavonoid content mg quercetin equivalents /g of extract	DPPH radical scavenging activity EC_{50} values (µg/mL)
			MCF-7	MDA-MB-231			
	Bark	Hexane	> 1000	804.4 ±0.4	0.08±0.002	0.63±0.008	> 1000
		Chloroform	> 1000	> 1000	0.07±0.003	0.72±0.005	> 1000
		Ethyl Acetate	122.90±0.09	104.70±0.3	9.65±0.09	0.70±0.01	180.40±0.4
		Methanol	139.70±0.3	276.98±0.2	36.04±0.3	0.60±0.03	11.42±0.2
Memecylon rostratum	Leaves	Hexane	> 1000	> 1000	5.03±0.03	2.48±0.2	100.20±0.3
		Chloroform	328.00±0.1	202.60±0.2	2.24±0.09	3.06±0.3	565.30±0.1
(1582B1707 BMARI)		Ethyl Acetate	90.33±0.1	> 1000	31.17±0.2	0.59±0.1	6.07±0.2
		Methanol	183.70±0.2	> 1000	35.54±0.3	1.54±0.2	4.19±0.3
	Bark	Hexane	47.14±0.3	99.35±0.2	12.37±0.4	1.64±0.1	74.57±0.2
		Chloroform	185.40±0.3	169.70±0.2	9.78±0.2	1.58±0.2	218.80±0.4
		Ethyl Acetate	75.89±0.4	151.30±0.1	42.52±0.4	4.10 ±0.2	1.2±0.02
		Methanol	233.30±0.1	294.40±0.3	34.00±0.2	1.56±0.09	8.93±0.1
Myrtaceae							
Cleistocalyx nervosum	Leaves	Hexane	989.00±0.2	> 1000	1.79±0.02	0.14±0.02	302.10±0.2
		Chloroform	393.26±0.1	285.70±0.2	2.04±0.09	0.18±0.01	354.85±0.1
(1580B1707 BMARI)		Ethyl Acetate	308.40±0.2	65.78±0.3	2.11±0.01	0.22± 0.03	458.90±0.3
		Methanol	> 1000	71.33±0.2	2.08±0.09	0.19±0.02	399.20±0.2
	Bark	Hexane	> 1000	700.20±0.3	1.90±0.01	0.15±0.009	315.80±0.3
		Chloroform	102.10±0.3	300.50±0.1	2.00±0.01	0.17±0.007	348.90 ±0.2
		Ethyl Acetate	55.86±0.4	204.90±0.2	2.21±0.02	0.20± 0.001	436.01±0.4
		Methanol	72.91±0.3	270.78±0.3	2.10±0.01	0.16±0.002	389.50±0.4
Nepenthaceae							
Nepenthes distillatorya	Leaves	Hexane	196.80±0.2	388.00±0.3	6.28±0.02	0.82±0.002	117.80 ±0.4
	and	Chloroform	148.00±0.3	389.10±0.2	0.45±0.03	0.53±0.001	3.56±0.3
(S22PDA0110	Pitcher	Ethyl Acetate	105.60±0.1	> 1000	5.71±0.02	1.02±0.09	64.47±0.4
BG/PDN)		Methanol	273.20±0.3	242.80±0.09	46.12±0.02	2.32±0.1	1.08±0.01
Ochnaceae							
Ochna jabotapita	Leaves	Hexane	210.80±0.2	649.40±0.4	0.04±0.002	0.15±0.01	> 1000
(S23PDA0110		Chloroform	80.1± 0.09	355.60±0.1	3.15±0.02	5.62±0.09	> 1000
BG/PDN)		Ethyl Acetate	110.30±0.3	204.70±0.3	10.27± 0.3	2.34±0.4	66.10±0.2
		Methanol	> 1000	> 1000	42.47±0.4	1.11±0.2	1.7±0.01

Plant / Family (Voucher number / place of deposition)	Part	Extract	Cytotoxicity/ IC_{50} value (µg/mL)		Polyphenol content mg gallic acid equivalents /g of extract	Flavonoid content mg quercetin equivalents /g of extract	DPPH radical scavenging activity EC_{50} values (µg/mL)
			MCF-7	MDA-MB-231			
	Bark	Hexane	> 1000	> 1000	0.10±0.002	0.22±0.009	> 1000
		Chloroform	> 1000	203.70±0.2	2.06±0.02	0.79±0.02	> 1000
		Ethyl Acetate	180.80±0.1	305.00±0.2	19.74±0.2	0.29±0.01	149.50±0.2
		Methanol	> 1000	847.23±0.1	61.36±0.2	0.27±0.009	2.66±0.04
Rubiaceae							
Gardenia crameri	Leaves	Hexane	196.50±0.3	180.80±0.2	0.52±0.02	1.04±0.2	645.10±0.2
		Chloroform	106.30±0.2	176.90±0.3	5.73±0.02	2.11±0.3	> 1000
(1581B1707 BMARI)		Ethyl Acetate	309.30±0.3	270.90±0.4	2.63±0.01	1.26±0.1	> 1000
		Methanol	> 1000	549.71±0.2	21.23±0.01	1.78±0.1	31.26±0.3
	Bark	Hexane	114.50±0.4	112.90±0.3	0.69±0.02	0.71±0.02	> 1000
		Chloroform	131.90±0.2	156.40±0.2	3.62±0.01	2.02±0.2	> 1000
		Ethyl Acetate	57.95±0.3	153.60±0.2	9.95±0.03	1.33±0.3	219.80±0.3
		Methanol	157.50±0.1	551.70±0.2	0.73±0.01	0.73±0.02	> 1000
Wendlandia bicuspidate	Leaves	Hexane	832.60±0.2	687.30±0.2	0.01±0.002	0.43±0.02	854.10±0.2
		Chloroform	226.50±0.2	828.00±0.4	0.38±0.01	2.38±0.4	> 1000
		Ethyl Acetate	301.10±0.3	> 1000	1.46±0.01	0.73±0.008	> 1000
(S28PDA0110 G/PDN)		Methanol	386.50±0.4	879.10±0.2	21.01±0.2	0.79±0.01	16.21±0.4
	Bark	Hexane	745.10±0.09	> 1000	0.01±0.002	0.34±0.005	> 1000
		Chloroform	442.00±0.2	> 1000	0.02±0.001	0.70±0.003	> 1000
		Ethyl Acetate	167.7±0.3	615.10±0.2	1.75±0.01	0.43±0.001	> 1000
		Methanol	509.40±0.2	207.45±0.4	11.71±0.2	0.44±0.002	19.15±0.1

BG/PDN: National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka; BMARI: Bandaranayake Memorial Ayurvedic Research Institute, Maharagama, Sri Lanka

2.4.3 Assessment of caspase 3/7 activation by ApoTox-Glo™ Triplex Assay

Plant extracts which had an IC_{50} value less than 100 µg/mL were selected for determination of caspase 3/7 activation in MDA-MB-231 cells. Caspase 3/7 activation was determined using ApoTox-Glo™ Triplex Assay (Promega, Madison, USA) according to the manufacturer's protocol. MCF- 7 cells do not express caspase 3; hence the test was not performed on MCF-7 cell line. MDA-MB-231 cells were plated on 96 well plates (5000 cells/well) with 100 µL of Leibovitz's L-15 medium at 37℃ in a humidified atmosphere and cultured overnight. Cells in triplicates were exposed to the plant extracts (doses used were determined based on the IC_{50} values) for 24 h. Activation of caspase 3/7 was determined by adding the substrate containing a tetra peptide sequence DEVD. When the enzymatic reaction occurs, the luminescence signal formed is directly proportional to the amount of caspase 3/7 produced in the cell. The luminescence was measured using Synergy™ HT Multi-Mode Microplate Reader.

2.4.4 Determination of total polyphenol content

Total polyphenol content of the extracts was determined by Folin- Ciocalteau method as described in Samarakoon et al. [18]. Serial dilutions of the plant extracts were prepared from the stock solution (2 mg/mL), using deionized water. Briefly, sodium carbonate solution (10%; 70 µL) was added to each plant extract (20 µL) with Folin-Ciocalteau reagent (0.2 N) and incubated for 30 minutes at room temperature. The absorbance was measured at 765 nm using Synergy™ HT Multi-Mode Microplate Reader. Gallic acid solution was used as the standard. A dose response linear regression was generated by using the gallic acid standards and polyphenol content in the extracts were expressed as gallic acid equivalents (mg of GAE/g of extract). Triplicate experiments were carried out for each extract.

2.4.5 Determination of total flavonoid content

The total flavonoid content of the plant extracts was determined by the modified Aluminium chloride colorimetric method [18]. Stock solutions (2 mg/mL) of the plant extract were diluted with methanol to obtain serial dilutions. Aluminium chloride (2%; 100 µL) was mixed with the plant extract (100 µL) and incubated for ten minutes at room temperature. The absorbance was measured at 415 nm. The calibration curve was constructed using quercetin and values expressed as quercetin equivalents (mg of QE/g of extract). Triplicate experiments were carried out for each extract.

2.4.6 Assessment of free radical scavenging property by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay

The DPPH assay was performed as described by Samarakoon et al. [18]. Plant extracts (50 µL) were mixed with 60 µL of DPPH in methanol (0.02 mg/mL), and the final volume was made up to 200 µL with methanol. The mixture was allowed to stand for 10 min in the dark at room temperature. The absorbance was read at 517 nm. Methanol was used as the negative control, and L-Ascorbic acid was used as the positive control. The percentage inhibition of DPPH free radicals by the extracts was calculated according to the following formula:

$$\% \text{ free radical scavenging activity} = [(A_{control} - A_{sample}) / A_{control}] \times 100\ \%$$

Where A is absorbance at 517 nm

The effective concentration of the plant extract required to scavenge DPPH radical by 50% (EC_{50}) was obtained using linear regression analysis. The analysis was done individually in triplicates.

2.5 Data Analysis

Data were analyzed using Prism 5.0 (Graph pad Prism) statistical software package. One way ANOVA with Dunnett's multiple comparison test was used to compare fold change in caspase 3/7 activity in cells treated with different doses of a particular extract with the control. The value of $P<0.05$ was considered significant.

3. RESULTS

3.1 Cytotoxicity against Breast Cancer Cell Lines

Of the 156 extracts from 20 plants studied only 35 extracts from 14 plants showed significant cytotoxicity (IC_{50}<100 µg/mL) against MCF-7 cells and/or to MDA-MB-231 cells in the SRB assay (Table 1). These were *Calophyllum calaba, Calophyllum tomentosum Wight, Connarus championii, Schumacheria*

castaneifolia, Doona macrophylla, Chaetocarpus coriaceus, Memecylon rostratum, Cleistocalyx nervosum, Campnosperma zeylanica, Ochna jabotapita, Lijndenia capitellata, Calophyllum moonii, Bhesa ceylanica, Gardenia crameri. Neither the leaf nor the bark extract of the remaining six plants were significantly cytotoxic to either cancer cell line. Those plants were namely *Wrightia zeylanica* (Apocynaceae), *Semecarpus subpeltata* (Anacardiaceae), *Actinodaphne stenophylla* (Lauraceae), *Nepenthes distillatorya* (Nepenthaceae), *Vateria copallifera* (Dipterocarpacea) and *Wendlandia bicuspidate* (Rubiaceae). Eighteen plant extracts were cytotoxic only to MCF-7 cells (IC_{50} values ranging between 31.43 – 97.50 µg/mL), seven extracts were cytotoxic only to MDA-MB-231 cells (IC_{50} values ranging between 16.11 – 95.83 µg/mL) and ten extracts were cytotoxic to both MCF-7 and MDA-MB-231 cells (IC_{50} values ranging between 0.51 – 99.35 µg/mL). Of these only *C. nervosum, O. jabotapita, C. calaba, D. macrophylla, C. coriaceus, C. tomentosum Wight, B. ceylanica, L. capitellata*, hexane, ethyl acetate and methanol extracts of leaves of *C. zeylanica* and *C. moonii,* ethyl acetate extract of leaves of *S. castaneifolia*, ethyl acetate extracts of the bark and leaves of *M. rostratum* and hexane extract of leaves of *C. championii* were less cytotoxic to normal breast epithelial cell line MCF-10A, than to cancer cells to which they were cytotoxic.

3.2 Total Polyphenol and Flavonoid Contents and Free Radical Scavenging Activity

Most of the methanol and ethyl acetate extracts had a high polyphenol content and high free radical scavenging activity. Almost all the plant extracts contained some amount of flavonoids. The plants with high polyphenol content (gallic acid equivalents over 50 mg/g of extract) were *B. ceylanica, C. calaba, C. championii, D. macrophylla, O. jabotapita* and *S. subpeltata* (Table 1). Plants with high flavonoid content (quercetin equivalents over 4 mg/g of extract) were *A. stenophylla, B. ceylanica, C. calaba C. championii M. rostratum O. jabotapita and V. copallifera* (Table 1). *B. ceylanica, C. championii, C. moonii, C. tomentosum, D. macrophylla, M. rostratum*, *N. distillatorya and O. jabotapita* had free radical scavenging activity greater than that of the positive control, L-Ascorbic acid (3.55 µg/mL) (Table 1).

3.3 Apoptotic Activity of Selected Extracts on MDA-MB-231 Breast Cancer Cell Line

Following 24 h exposure to active plant extracts, caspase 3/7 activity was determined in MDA-MB-231 cell line. Only the plant extracts cytotoxic to MDA-MB-231 as shown by SRB assay were used. Results are shown in Fig. 1. Caspase 3/7

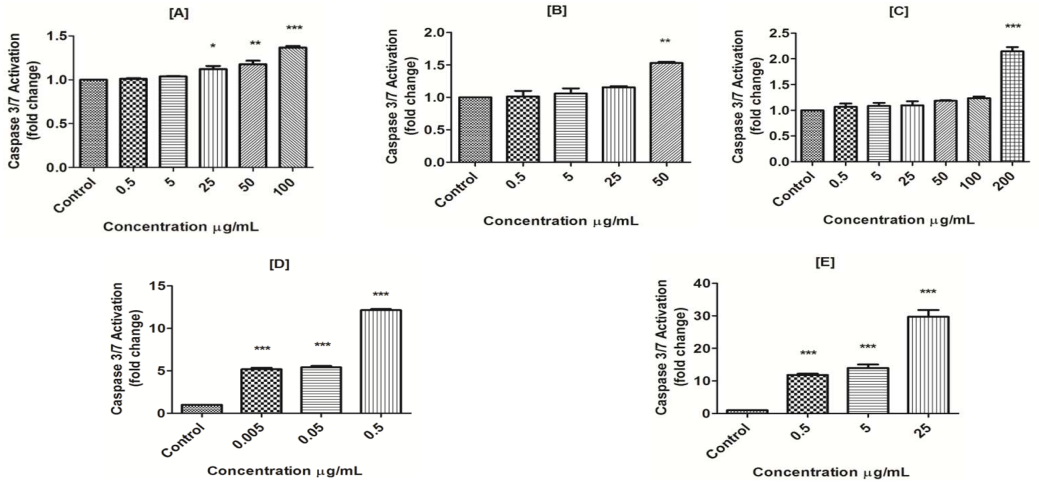

Fig. 1. Activity of caspase3/7 in MDA-MB-231 cells in response to selected plant extracts

*Cells were treated with plant extracts which were cytotoxic to MDA-MB-231 cells. Results are expressed as mean±SEM of three independent experiments [A] C. moonii leaves methanol extract [B] B. ceylanica bark ethyl acetate extract [C] C. zeylanica leaves hexane extract [D] C. zeylanica bark hexane extract [E] C. zeylanica bark chloroform extract. P<0.05 was considered significant. * P<0.05, ** P<0.01, *** P<0.001 compared to respective controls*

was significantly activated by the methanol extract of leaves of *C. moonii* and ethyl acetate extract of bark of *B. ceylanica* at 25 and 50 µg/mL respectively. Hexane extract of leaves of *C. zeylanica* induced caspase activation at 200 µg/mL, whereas the hexane and the chloroform extracts of the bark activated caspase 3/7 at 0.005 µg/mL and at 0.5 µg/mL respectively. The other extracts did not show any significant induction of caspase 3/7 when compared to the controls.

4. DISCUSSION

There is a continuous search for new chemotherapeutic drugs with fewer side effects for the treatment and prevention of cancer. Hence plants have been a prime source for new anticancer drug development [19]. Therefore this study provides evidence for the anti breast cancer potential of some Sri Lankan endemic plants.

By screening four solvent extracts each from leaves and bark of 20 endemic plants of Sri Lanka, we identified 35 extracts cytotoxic to breast cancer cells. In the present study extracts with IC_{50} values less than 100 µg/mL were considered as cytotoxic. Overall, in this screen of 156 plant extracts (80 leaf extracts and 76 bark extracts), 22 extracts (14.1%) showed a therapeutic effect (IC_{50}<100 µg/mL on cancer cells with IC_{50}>100 µg/mL on normal cells), while 12 extracts (7.7%) were clearly toxic being cytotoxic (IC_{50}<100 µg/mL) to normal cells. Toxicity was mostly seen with the bark extracts with 13.2% of the bark extracts and only 2.5% of the leaf extracts being toxic. In contrast, the therapeutic effect was mostly seen with the leaf extracts with 18.75% of the leaf extracts and only 10.5% of the bark extracts being therapeutic.

Of the, extracts with therapeutic value, fourteen were cytotoxic only to MCF-7 cells, four only to MDA-MB-231 cells and four to both MCF-7 and MDA-MB-231 cells. Hence, our results indicate that some plant extracts exert phenotype specific cytotoxicity to breast cancer cells. The plant extracts which had a therapeutic potential were, all active extracts of *C. nervosum, O. jabotapita, C. calaba, D. macrophylla, C. coriaceus, C. tomentosum, B. ceylanica* and *L. capitellata,* hexane, ethyl acetate and methanol extracts of leaves of *C. zeylanica* and *C. moonii,* ethyl acetate extract of leaves of *S. castaneifolia*, ethyl acetate extracts of the bark and leaves of *M. rostratum* and hexane extract of leaves of *C. championii*. Chloroform extracts of leaves of *C. championii* and *C. zeylanica,* all active extracts of bark of *C. zeylanica, S. castaneifolia, C. moonii*, and *G. crameri* and the hexane extract of bark of *M. rostratum* were equally or more cytotoxic to normal mammary epithelial cells rendering them with no potential therapeutic value.

Highly reactive free radicals and oxygen species can cause oxidative stress that can damage lipids, proteins and DNA resulting in cancer. Antioxidants and polyphenols derived from plants have the ability to neutralize the damaging effect of reactive oxygen species and combat oxidative stress [20]. Of all the extracts tested, the hexane extract of the bark of *C. zeylanica* showed the highest cytotoxicity to both MCF-7 and MDA-MB-231 cells. Interestingly total polyphenol content, flavonoid content and radical scavenging activity of the hexane extract of the bark of *C. zeylanica* was not the highest. In contrast to reports from some other investigators [21,22] our results did not show any consistent relationship between cytotoxicity and radical scavenging activity.

Apoptosis, the process of programmed cell death, is recognized as a dynamic process in the regulation of tissue development and homeostasis [23]. Development and maintenance of organisms require a tight regulation between cell proliferation and cell death. Disorders of either can lead to the development of cancer. Defects in the ability to activate the death signaling pathway renders tumor cells less susceptible to apoptosis. Caspase 3 is one of the key regulators of the apoptotic pathways [24,25]. Of the twenty plants studied, only some extracts appeared to mediate apoptosis by a caspase 3/7 dependent pathway. In the present study the plant extracts cytotoxic to MDA-MB-231 cells were used for ApoTox-Glo assay to screen for caspase activation as the MCF-7 cells do not express caspase 3. To capture the caspase activation of the hexane extract of bark of *C. zeylanica* which had an IC_{50} value of 0.5 µg/mL in the SRB assay, a concentration range from 0.005 µg/mL to 0.5 µg/mL was used to assess caspase activation. Highest effect on activation of caspases 3/7 in the MDA-MD-231 cells was exerted by the hexane and the chloroform extracts of the bark of *C. zeylanica,* however these extracts were highly cytotoxic to normal cells. Hexane extract of the leaves of the same plant which was less cytotoxic to normal cells also activated caspases 3/7 in MDA-MD-231 cells, whereas chloroform

and ethyl acetate extracts of *C. zeylanica leaves* perhaps caused apoptosis by caspase 3/7 independent pathways. A dose dependent activation of caspase 3/7 in MDA-MD-231 cells by the ethanol extract of the kernel of *Mangifera indica,* a plant from the family Anacardeaceae to which *C. zeylanica* belongs has been reported by others [26]. In the present study, besides hexane and chloroform extracts of *C. zeylanica,* methanol extract *C. moonii* leaves and ethyl acetate extract *B. ceylanica* bark also appeared to cause apoptosis by caspase dependent pathways. In contrast, besides the chloroform and ethyl acetate extracts of *C. zeylanica* leaves, several other extracts appeared to cause apoptosis by caspase independent pathways. These were the ethyl acetate extract of *S. castaneifolia* leaves, all active extracts *S. castaneifolia* bark, hexane extracts of *M. rostratum* bark and *C. moonii* leaves and, all active extracts of leaves of *C. championii and C. nervosum*. However, the possibility of caspase 3 and 7 activation if cells are exposed to these plant extracts for a longer duration cannot be excluded as we measured caspase 3/7 activity following a 24 h exposure to the extract.

Polyphenols which are secondary metabolites synthesized in plants include many chemical groups including flavonoids. Quercetin, which was used as the standard for detecting flavonoid content, is the most abundant dietary flavonol [27-29]. From the 20 plants studied, six plant extracts with high polyphenol content (gallic acid equivalents over 50 mg/g extract), eight plant extracts with high flavonoid content (quercetin equivalents over 4 mg/g extract) and ten plant extracts with high free radical scavenging activity (<3.55 µg/mL) were identified. However most of the plants with high polyphenol content, high flavonoid content and high free radical scavenging activity were not cytotoxic to any of the cell lines studied. For example, ethyl acetate extract of the bark of *S. subpeltata* showed the highest polyphenol content among all the extracts (68.68 mg GAE/g of extract), even though the cytotoxicity towards MCF-7 and MDA-MB-231 cells of the extract was over 1000 and 258.45 µg/mL respectively, chloroform extract of leaves of *V. copallifera* had the highest flavonoid content (7.55 mg QE/g of extract) and the cytotoxicity was 143.90 µg/mL and 461.90 µg/mL for MCF-7 and MDA-MB-231 cells respectively. The highest free radical scavenging activity was observed in the methanol extract of the bark of *B. ceylanica* 1.04 µg/mL) while its cytotoxicity (IC_{50}) on MCF-7 and MDA-MB-231 cell was 162.00 and 181.20 µg/mL respectively. However others have reported some plant extracts with a high free radical scavenging activity being also highly cytotoxic to cancer cells [30,31].

Only the methanol extract of the *D. macrophylla* bark*,* chloroform extract *O. jabotapita* leaves, ethyl acetate extracts of bark of *M. rostratum* and *C. calaba,* chloroform extract of *C. championii* leaves and hexane extract of *C. Moonii* leaves showed a high polyphenol content, high flavonoid content and high free radical scavenging activity while exhibiting cytotoxicity. Hence cytotoxicity did not always correlate with the polyphenol content, flavonoid content or with the free radical scavenging activity. Further investigations are needed to identify mechanisms of cytotoxicity exerted by the active compounds in the extracts studied.

5. CONCLUSIONS

By screening leaves and bark of 20 endemic plants from Sri Lanka, we have shown that some of these possess cytotoxic and apoptotic activity against breast cancer cells. Some extracts were specifically cytotoxic to estrogen receptor positive breast cancer and others to triple negative breast cancer. A few were cytotoxic to both cancer cell types. Plants that were found to be preferentially cytotoxic to cancer cells may provide strong leads for future drug discovery for combating breast cancer. In view of the higher toxicity and lower therapeutic effects shown by the bark extracts, limiting investigations to the leaf extracts in future screening studies is likely to be more cost effective in identifying active compounds with potential therapeutic activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

This work was supported by Ministry of Higher Education, Sri Lanka and constitutes part of the MPhil studies of PPJ.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. American Cancer Society. Cancer facts & figures 2015. Atlanta: American Cancer Society; 2014.
2. Zhang MH, Man HT, Zhao XD, Dong N, Ma SL. Estrogen receptor positive breast cancer molecular signatures and therapeutic potentials (Review). Biomed Rep. 2013;2(1):41-52.
3. Reddy KB. Triple-negative breast cancers: An updated review on treatment options. Curr Oncol. 2011;18(4):e173-179.
4. Hudis CA, Gianni L. Triple-negative breast cancer: An unmet medical need. Oncologist. 2011;16(suppl 1):1-11.
5. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neuro-degenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 2009;7(1):65-74.
6. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008;4(2):89-96.
7. Elmore S. Apoptosis: A review of programmed cell death. Toxicol Pathol. 2007;35(Suppl 4):495-516.
8. Ouyang L, Shi Z, Zhao S, Wang FT, Zhous TT, Liu B, Bao JK. Programmed cell death pathways in cancer: A review of apoptosis, autophagy and programmed necrosis. Cell Prolif. 2012;45(6):487-498.
9. Portt L, Norman G, Clapp C, Greenwood M, Greenwood MT. Anti-apoptosis and cell survival: A review. Biochem Biophys Acta. 2011;1813(1):238-259.
10. Campos KC, Rivera JH, Gutierrez JR, Rivera IO, Velev AC, Torres MP, Ortiz MP, Milan CAO. Biological screening of select Puerto Rican plants for cytotoxic and antitumor activities. P R Health Sci J. 2015;34(1):25-30.
11. George S, Bhalerao SV, Lidstone EA, Ahmad IS, Abbasi A, Cunningham BT, Watkin KL. Cytotoxicity screening of Bangladeshi medicinal plant extracts on pancreatic cancer cells. BMC Complement Altern Med. 2010;10(1):1.
12. Kumar S, Pandey AK. Medicinal attributes of *Solanum xanthocarpum* fruit consumed by several tribal communities as food: An *in vitro* antioxidant, anticancer and anti HIV perspective. BMC Complement Altern Med. 2014;14(1):112.
13. Phang CW, Malek SNA, Ibrahim H. Antioxidant potential, cytotoxic activity and total phenolic content of *Alpinia pahangensis* Rhizomes. BMC Complement Altern Med. 2013;13(1):243.
14. Tanih NF, Ndip RN. Evaluation of the acetone and aqueous extracts of mature stem bark of *Sclerocarya birrea* for antioxidant and antimicrobial properties. Evid Based Complement. Alternat Med. 2012;1-7.
15. Karunaratne V. The rich diversity and the potential medicinal value of the Sri Lankan flora. PHYTA. 2001;5(Suppl 1).
16. Samarakoon SR, Thabrew I, Galhena PB, Silva DD, Tennekoon KH. A comparison of the cytotoxic potential of standardized aqueous and ethanolic extracts of a polyherbal mixture comprised of *Nigella sativa* (seeds), *Hemidesmus indicus* (roots) and *Smilax glabra* (rhizome). Pharmacognosy Res. 2010;2(6):335-342.
17. Ediriweera MK, Tennekoon KH, Samarakoon SR, Thabrew I, Dilip De Silva E. A study of the potential anticancer activity of *Mangifera zeylanica* bark: Evaluation of cytotoxic and apoptotic effects of the hexane extract and bioassay-guided fractionation to identify phyto-chemical constituents. Oncol Lett. 2016;11(2):1335-44.
18. Samarakoon SR, Kotigala SB, Liyanage IG, Thabrew I, Tennekoon KH, Siriwardana A, Galhena PB. Cytotoxic and apoptotic effect of the decoction of the aerial parts of *Flueggea leucopyrus* on human endometrial carcinoma (AN3CA) cells. Trop J Pharm Res. 2014;13(6):873-880.
19. Pan L, Chai H, Kinghorn AD. The continuing search for antitumor agents from higher plants. Phytochemistry Letters. 2010;3(1):1-8.
20. Kumar S, Pandey AK. Medicinal attributes of *Solanum xanthocarpum*fruit consumed by several tribal communities as food: an in vitro antioxidant, anticancer and anti HIV perspective. BMC Complement Altern Med. 2014;14(1):112.
21. Abdullah AH, Mohammed AS, Rasedee A, Mirghani MES, Al-Qubaisi MS. Induction of apoptosis and oxidative stress in estrogen receptor-negative breast cancer, MDA-MB231 cells, by ethanolic mango seed extract. BMC Complement Altern Med. 2015;15(1):45.
22. Chaudhary S. Chandrashekar S, Pai KSR, Setty MM, Devkar RA, Reddy ND, Shoja MH. Evaluation of antioxidant and

anticancer activity of extract and fractions of *Nardostachys jatamansi* DC in breast carcinoma. BMC Complement Altern Med. 2015;15(1):50.
23. Ouyang L, Shi Z, Zhao S, Wang FT, Zhous TT, Liu B, Bao JK. Programmed cell death pathways in cancer: A review of apoptosis, autophagy and programmed necrosis. Cell Prolif. 2012;45(6):487-498.
24. Brentnall M, Menocal LR, De Guevara RL, Cepero E, Boise LH. Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. BMC Cell Biol. 2013;14(1):32.
25. Broker LE, Kruyt FAE, Giaccone G. Cell death independent of caspases: A review. Clin Cancer Res. 2005;11(9):3155-3162.
26. Abdullah AH, Mohammed AS, Rasedee A, Mirghani MES, Al-Qubaisi MS. Induction of apoptosis and oxidative stress in estrogen receptor-negative breast cancer, MDA-MB231 cells, by ethanolic mango seed extract. BMC Complement Altern Med. 2015;15(1):45.
27. Ahmad S, Sukari MA, Ismail N, Ismail IS, Abdul AB, Bakar MFA, Kifli N, Ee GCL Phytochemicals from *Mangifera pajang* Kosterm and their biological activities. BMC Complement Altern Med. 2015;15(1): 83.
28. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79(5):727-747.
29. Perez-Jimenez J, Neveu V, Vos F, Scalbert A. Identification of the 100 richest dietary sources of polyphenols: An application of the Phenol-Explorer database. Eur J Clin Nutr. 2010;64:S112-S20.
30. Abdullah AH, Mohammed AS, Rasedee A, Mirghani MES, Al-Qubaisi MS. Induction of apoptosis and oxidative stress in estrogen receptor-negative breast cancer, *MDA-MB231* cells, by ethanolic mango seed extract. BMC Complement Altern Med. 2015;15(1):45.
31. Apostolou A, Stagos D, Galitsiou E, Spyrou A, Haroutounian S, Portesis N, Trizoglou I, Wallace HA, Tsatsakis AM, Kouretas D. Assessment of polyphenolic content, antioxidant activity, protection against ROS-induced DNA damage and anticancer activity of *Vitis vinifera* stem extracts. Food Chem Toxicol. 2013;61:60-68.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14690