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Research Article

ESTROGENIC ACTIVITY OF SELECTED MYRSINACEAE SPECIES IN MCF-7 HUMAN BREAST CANCER CELLS

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ABSTRACT

Eight Myrsinaceae species (*Ardisia kunstleri* King & Gamble, *A. oxyphylla* Wall., *A. villosa* Roxb., *Embelia coriacea* Wall., *Labisia pumila* (Blume) Mez var. *alata* (purple-leafed), *L. pumila* (Blume) Mez var. *alata* (green-leafed), *Maesa ramentacea* Wall. and *Rapanea porteriana* (Wall. ex A.DC.) Mez) were investigated for their estrogenic activity in the estrogen-sensitive MCF-7 human breast cancer cells. A total of 30 extracts of different plant parts (leaves, twigs and roots) comprising of methanol (14) and water (16) extracts were assayed for cell proliferation activity at a concentration range of between 10^{-10} and 10^{-4} g/mL. 17β-Estradiol (10^{-9} M) was used as a positive control. The aqueous extract of *M. ramentaceae* leaves showed the highest cell proliferation activity (136.9±2.5% at 10^{-5} g/mL, $p<0.01$ compared to estrogen-free solvent control, 100%) with an RPE value of 121.6% comparative to 17β-estradiol (RPE = 100%), followed by the aqueous leaf extract of *L. pumila* var. *alata* (purple-leafed) (131.0±2.9% at 10^{-8} g/mL, $p<0.01$; RPE = 83.1%). The methanol extracts of *A. oxyphylla* roots and *M. ramentacea* leaves, and the aqueous leaf extracts of *R. porteriana* were found to significantly stimulate the proliferation of MCF-7 cells. The results may suggest that the Myrsinaceae species can potentially be explored for estrogenic activity and for sources of phytoestrogens.

Keywords: MCF-7 proliferation assay, Relative proliferative effect, *Maesa ramentacea, Labisia pumila* var. *alata*.

INTRODUCTION

Plant substances that exert estrogenic and/or anti-estrogenic effects on animals and humans are called phytoestrogens. Phytoestrogens are known to be diverse in their origins and have ligand binding affinities to mammalian estrogen receptors *in vitro* and *in vivo* to bring about the estrogenic responses.

Numerous reports and reviews are available, indicating the beneficial roles of phytoestrogens towards human health particularly in the prevention or treatment of menopausal-related problems including breast cancer, cardiovascular diseases such as hypertension, hyperchlolesterolemia, hyperglycemia, artherosclerosis and coronary heart disease, reduction in bone mineral density and bone and hip fracture, cognitive abilities and mood in postmenopausal women[1-11]. Several studies have also reported the phytoestrogens' values on the alleviation of menopausal symptoms in peri- and postmenopausal women, prolongation of the menstrual cycle of premenopausal women, improvement of exercise-induced body weight and body mass index; as well as antioxidant, anti-inflammatory, arterial vasodilation effects and on prostate cancer[1, 12-15]. A recent study revealed that the methanolic extract of *Justicia pectoralis* Jacq. (Acanthaceae), traditionally used in Costa Rica for the management of menopausal symptoms and dysmenorrhea, has estrogenic, progestagenic and anti-inflammatory properties[16].

A variety of estrogen-dependent assay systems have been established to ascertain estrogenic activity of phytoestrogens, including *in vitro* assays of ligand-receptor binding, and whole cell commonly ERα-positive human breast cancer cell lines such as MCF-7[17], human endometrial adenocarcinoma (Ishikawa Var-1) cells[18] and recombinant yeast transfected with ER-α[19].

Our previous preliminary studies demonstrated that the ethanolic extract of *Labisia pumila* var. *alata* (Myrsinaceae) root was weakly estrogenic when tested at low concentrations (10-50 μg/mL) in Ishikawa-Var I cells and recombinant yeast estrogen screen assays but was cytotoxic towards the Ishikawa cells at a higher concentration (100 μg/mL)[20-21], suggesting concentration-dependent biphasic estrogenic activity. In Malaysia, the water decoction of the root or whole plant of *L. pumila* var. *alata* is traditionally consumed by the Malay women for induction and facilitation of labour, as well as for the treatment of flatulence, dysentery, dysmenorrhoea, gonorrhoea and "sickness in the bones"[22]. *In vivo* investigation of the plant water extract suggested possible role in the modulation of postmenopausal weight gain[23-24], protection against cardiovascular risks[25] and osteoporosis[26], in a similar manner to that reported for estrogen. Based on the preliminary findings of the abovementioned species, this prompted us to conduct a screening of other plants belonging to the family Myrsinaceae for possible estrogen-like activity. A chemotaxonomic approach was adopted to select plant samples based on the close relativeness of the phytochemical content[27].

In this study, 30 extracts of 18 specimens of 8 Malaysian Myrsinaceae forest plant species from the *Ardisia*, *Embelia*, *Labisia*, *Maesa* and *Rapanea* genera were screened to assess their ability to produce estrogenic activity in ERα-positive human breast cancer cell line MCF-7. Results from this study show that six plant extracts significantly induce cell proliferation. To our knowledge, this is the first report of MCF-7 cell proliferative activity of the species.

MATERIALS AND METHODS

Collection of Plant Materials

Six plant species of *Ardisia kunstleri, A. oxyphylla, A. villosa*, *Embelia coriacea, Maesa ramentacea* and *Rapanea porteriana* were collected from the Malaysian forests (Angsi Mountain, Port Dickson, Hutan Simpan Gunung Berembun and Pasoh of Negeri Sembilan and Kuala Kubu of Selangor) between May and July 2007. *Labisia pumila* var. *alata,* purple-leafed and green-leafed species, were obtained from Taiping, Perak in September 2003. All voucher specimens were deposited in the Herbarium of Universiti Kebangsaan Malaysia and authenticated by Professor Dr. Abd. Latif Mohamad (Table 1).

Preparation of Extracts

Each plant species was separated according to the different parts, such as leaves, twigs and roots, to afford 18 specimens. The specimens were air-dried, ground to powder and then individually extracted to give 30 test extracts (Table 1). Methanolic extracts were prepared by maceration technique and concentration to dryness *in vacuo*, whereas the aqueous extracts were prepared by reflux extraction for 2 hours and then freeze-dried[28]. Extracts were individually dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 10^{-4} g/mL and then diluted with phenol red-free RPMI 1640 medium (Gibco, U.S.A) to afford six different concentrations (10^{-10}-10^{-5} g/mL), immediately before use. The final concentration of DMSO in the culture medium was ensured not to exceed 0.1% in order not to affect the cell responses[29].

Table 1: Information of Plant Species and Extracts Used in This Study

Species	Traditional Uses in Malaysia[22]	Collection Site	Voucher ID	Plant Part	Crude Extract
Ardisia kunstleri King & Gamble	NR	Pasoh, Negeri Sembilan	29817	Leaves	MeOH
					H_2O
				Twig	MeOH
					H_2O
				Roots	MeOH
					H_2O
Ardisia oxyphylla Wall.	Sore, swelling.	Gunung Angsi, Negeri Sembilan	AZ 13	Leaves	MeOH
					H_2O
				Twig	MeOH
					H_2O
				Roots	MeOH
					H_2O
Ardisia villosa Roxb.	Cough, dropsy, fever, hepatosis.	Gunung Angsi, Negeri Sembilan	AZ 45	Leaves	MeOH
				Twig	MeOH
Embelia coriacea Wall.	NR	Kuala Kubu, Selangor	29812	Leaves	MeOH
					H_2O
				Twig	MeOH
					H_2O
Labisia pumila (Blume) Mez var. *alata* (purple-leafed)	Parturition, flatulence, dysentery, dysmenorrhoea, gonorrhoea,	Taiping, Perak	SM 744	Leaves	H_2O
				Roots	H_2O
Labisia pumila (Blume) Mez var. *alata* (green-leafed)	'sickness in the bones'.	Taiping, Perak	SM745	Leaves	H_2O
				Roots	H_2O
Maesa ramentacea Wall.	Angina, dermatosis.	Gunung Berembun Forest Reserve, Pahang	29813	Leaves	MeOH
					H_2O
				Twig	MeOH
					H_2O
Rapanea porteriana (Wall. ex A.DC.) Mez	NR	Port Dickson, Negeri Sembilan	29810	Leaves	MeOH
					H_2O
				Twig	MeOH
					H_2O

NR=Not Reported.

MCF-7 Cell Proliferation Assay

Cells were maintained in RPMI 1640 medium (Gibco, U.S.A) supplemented with 10% (v/v) foetal bovine serum (FBS) (Sigma Chemicals Company, U.S.A) at 37°C, 80% humidity and 5% CO_2 atmosphere. Cells were sub-cultured in phenol red-free RPMI medium containing 5% (v/v) charcoal stripped FBS (CS-FBS) (Sigma Chemicals Company, U.S.A) one week prior to conducting the cell proliferation assay according to a previously described method[30] with slight modification. Cells in 180 µL medium were seeded at a density of 3,000 cells/well in flat-bottomed 96-well microtitre plates that were incubated overnight to allow cell attachment. Subsequently, into each well, 20 µL of each concentration of the various test samples were added and the plates were incubated for six days. The cell proliferation was quantitated by modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay[31] whereby 50 µL of MTT substrate solution (2 mg/mL) was added into each well and incubated for a further 4 hours. Formation of dark blue formazan product indicated viable cells and correlated with cell proliferation. Solution in all wells was discarded and DMSO (100 µL) was added to solubilize the MTT formazan. The resultant absorbance (A_{550}) was measured spectrophotometrically at a wavelength of 550 nm using a microplate reader. Percentage of cell viability was calculated as 100 × (A_{550} of test sample)/(A_{550} of estrogen-free solvent control). The 17β-estradiol of concentration 10^{-9} M in phenol red-free RPMI 1640 was used as a positive control.

Relative proliferative effect (RPE) of the extracts was determined from the following formula[29]:

$$\text{RPE (\%)} = \frac{(\text{PE of test extract} - 1)}{(\text{PE of } 17\beta\text{-estradiol} - 1)} \times 100$$

Where by proliferative effect (PE) was calculated as the ratio between cell proliferation obtained in the test samples and cell proliferation in the estrogen-free solvent control. The extract with RPE value of 100 is regarded as a full estrogen agonist comparative to 17β-estradiol, whereas a value of 0 suggests lack of estrogenicity, while intermediate values signify partial estrogen agonist property of the extracts[29].

Data Analysis

Results are expressed as the mean ± standard error of mean (SEM) of three separate experiments (n=3). Each concentration was tested in triplicate in each experiment. Statistical analysis was performed by one-way analysis of variance (ANOVA) with Dunnett's Multiple Comparison post test using Prism 5 (GraphPad Software) where $p<0.05$ value was considered statistically significant.

RESULTS AND DISCUSSION

Generally, the percentage yield of methanolic extracts (2.3-26.7%) was higher than that of the aqueous extracts (1.3-13.3%). Two extracts with the highest percentage yield were methanolic leaf extracts of *M. ramentaceae* (26.7%) and *A. villosa* (20.0%) (Fig. 1). When comparing the yield of extracts from different plant parts, majority of the leaves yielded highest percentage, followed by the roots and the twigs. The higher methanolic extractive values of the leaves compared to the respective aqueous extracts could suggest the possible high content of organic-soluble phytochemicals.

MCF-7 Cell Proliferation Activity

In this study, phenol red-free RPMI 1640 was used as the assay medium because phenol red has been shown to have estrogenic activity in hormone-sensitive breast cancer cell lines[32]. In addition, the use of charcoal-stripped foetal bovine serum eliminates serum steroids that are capable of stimulating proliferation of breast cancer cells. Thus, the assay used in the study ensured that proliferation of MCF-7 cells was solely due to the effects from the plant extracts. ERα is mainly involved in promoting cell proliferation[33] and MCF-7 cells are ERα predominant. 17β-estradiol was used as a positive control and is known to be more selective to ERα than ERβ[34-35].

The MCF-7 cell proliferative effects of methanolic and aqueous extracts of various plant parts of eight species are shown in Fig. 2 and Table 2. Six extracts (20%) of four Myrsinaceae species showed significant proliferative activity (Fig. 2). The aqueous extract of *M. ramentaceae* leaves showed the highest cell proliferation activity (136.9±2.5% at 10^{-5} g/mL, p<0.01) as compared to the estrogen-free solvent control (100%) with an RPE value of 121.6% (Table 3), followed by the aqueous leaf extract of *L. pumila* var. *alata* (purple-leafed) (131.0±2.9% at 10^{-8} g/mL, p<0.01; RPE = 83.1%). The others included methanol extracts of *A. oxyphylla* roots and *M. ramentacea* leaves, and the aqueous leaf extracts of *R. porteriana*.

The additional twenty-two extracts (73%) of eight Myrsinaceae species exhibited proliferative activity that was not significant compared to the control (p>0.05) (Table 2), having RPE values ranging from 70.3-6.2%. Two extracts (7%) were found not to induce MCF-7 cell proliferation, as represented by the zero value of RPE.

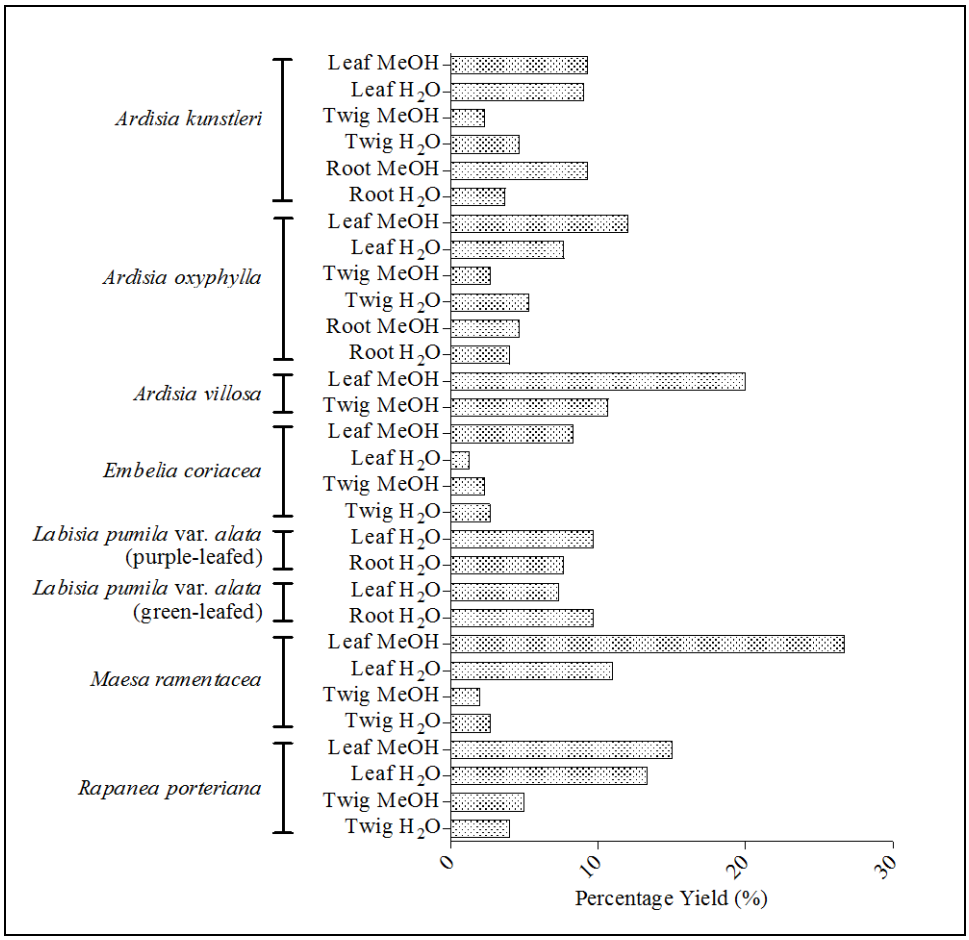

Fig. 1: Shows percentage yield of extracts

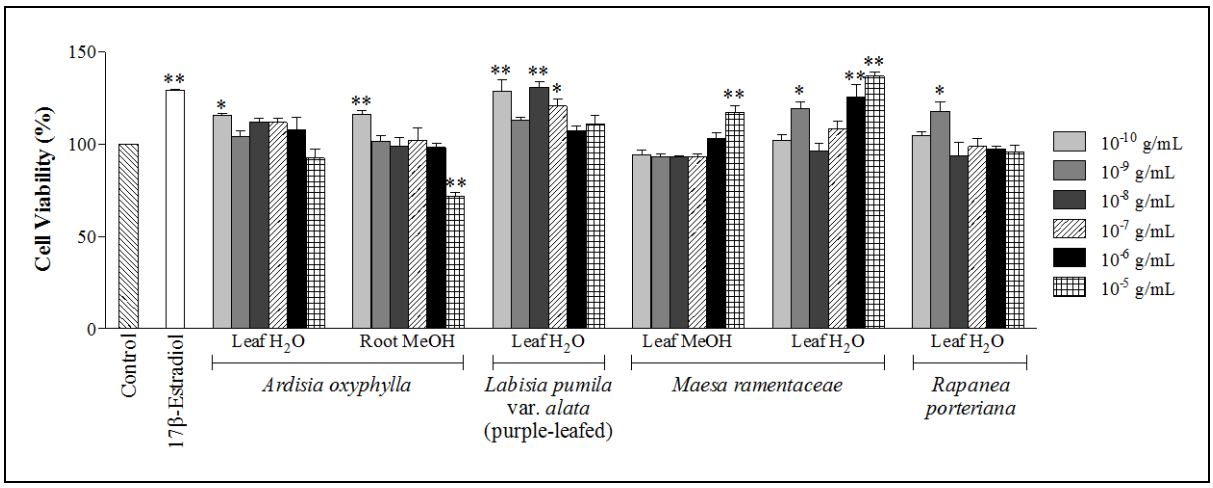

Fig. 2: Shows estrogenic effects of six extracts of four Myrsinaceae species on the MCF-7 cell proliferation, compared to control (100%) and 17β-estradiol (10^{-9} M)

Each value represents the mean ± SEM of three experiments (n=3). Asterisks denote significant differences from the control (ANOVA, *p<0.05, **p<0.01)

Table 2: Effects of Twenty-Four Extracts of Eight Myrsinaceae Species on the Proliferation of MCF-7 Cells

Species	Plant Part	Crude Extract	Percentage of cell proliferation ± SEM (%) at different concentrations (g/mL)[a]					
			10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Ardisia kunstleri	Leaf	MeOH	95.8±1.7	94.1±0.7	92.6±0.2	94.7±1.9	95.6±2.2	97.9±1.2
		H_2O	105.2±0.6	103.4±1.1	98.4±1.2	102.7±2.6	104.5±0.5	102.6±0.9
	Twig	MeOH	94.6±2.6	97.2±4.8	96.3±4.0	97.7±1.9	100.9±5.2	105.2±4.0
		H_2O	92.6±2.4	93.4±2.5	91.5±2.9	92.4±3.7	103.1±2.0	92.6±3.0
	Root	MeOH	97.5±2.4	97.4±2.3	95.2±3.4	98.4±3.2	99.3±1.4	107.5±2.3
		H_2O	100.8±3.3	103.9±1.6	98.6±3.0	93.2±6.5	99.3±2.4	99.7±4.4
Ardisia oxyphylla	Leaf	MeOH	108.6±5.5	106.8±1.0	101.6±3.3	105.4±0.8	113.3±6.8	98.4±10.2
	Twig	MeOH	102.9±3.2	103.0±5.0	97.7±2.7	107.7±5.9	98.3±4.1	97.9±4.8
		H_2O	102.6±1.8	104.9±1.1	102.5±3.5	115.0±1.5	114.7±6.4	108.9±7.0
	Root	H_2O	112.6±10.0	104.9±6.7	104.3±3.1	97.2±1.0	113.6±7.1	98.1±3.3
Ardisia villosa	Leaf	MeOH	112.4±0.7	109.0±1.0	95.0±6.0	96.3±4.1	95.0±1.7	96.4±3.1
	Twig	MeOH	105.5±4.0	102.1±2.3	100.1±4.5	100.4±4.8	94.2±9.3	8.2±0.9**
Embelia coriacea	Leaf	MeOH	101.6±1.6	100.5±2.3	100.5±2.9	104.2±0.6	96.7±2.7	80.4±4.7**
		H_2O	92.4±6.2	94.5±2.6	97.1±3.6	98.9±4.1	94.8±2.1	98.4±3.0
	Twig	MeOH	96.2±3.3	97.9±4.1	100.9±4.1	100.5±6.2	107.0±6.3	101.3±0.5
		H_2O	86.5±5.8	94.1±0.7	91.5±5.6	86.1±5.8	92.5±4.3	99.8±3.7
Labisia pumila var. *alata* (purple-leafed)	Root	H_2O	100.8±4.8	118.3±2.7	109.3±3.1	115.6±9.3	101.9±2.6	103.7±0.9
(green-leafed)	Leaf	H_2O	101.6±3.4	117.9±3.9	108.3±10.6	111.4±4.0	113.5±1.8	108.0±4.0
	Root	H_2O	98.8±0.8	122.8±6.8	98.5±2.0	114.0±4.9	105.1±10.9	119.5±5.9
Maesa ramentacea	Twig	MeOH	98.9±1.0	95.8±1.2	91.9±1.7	99.0±2.8	102.5±2.6	101.2±5.1
		H_2O	103.4±0.6	106.7±2.3	107.7±2.8	112.9±7.7	104.3±4.5	100.3±6.4
Rapanea porteriana	Leaf	MeOH	102.6±3.1	103.8±1.2	104.6±1.8	104.3±1.9	107.0±3.5	67.2±5.7**
	Twig	MeOH	100.1±0.9	96.0±5.7	99.6 ± 5.7	98.0±3.1	101.9±1.5	77.9±3.4**
		H_2O	102.0±5.0	100.5±4.1	95.6 ± 4.2	113.2±6.9	118.3±10.5	100.8±2.6
Control			100					
17β-Estradiol (10^{-9} M)			137.1±1.2**					

[a] Each value represents the mean ± SEM (n=3). Asterisks denote significant differences from the control (100%) (ANOVA, **p<0.01) .

Table 3: Relative Proliferative Effect of Extracts at the Concentration of Maximal Proliferative Effect

Test Samples			Concentration[a]	RPE (%)
17β-Estradiol			10^{-9} M	100.0
Ardisia kunstleri	Leaves	MeOH	10^{-5} g/mL	0
		H_2O	10^{-10} g/mL[b]	17.4
	Twig	MeOH	10^{-5} g/mL	14.6
		H_2O	10^{-6} g/mL	10.8
	Roots	MeOH	10^{-5} g/mL	25.2
		H_2O	10^{-9} g/mL	13.6
Ardisia oxyphylla	Leaves	MeOH	10^{-6} g/mL	47.1
		H_2O	10^{-10} g/mL[b]	53.1
	Twig	MeOH	10^{-7} g/mL	25.8
		H_2O	10^{-7} g/mL	52.1
	Roots	MeOH	10^{-10} g/mL[b]	70.3
		H_2O	10^{-6} g/mL	47.3
Ardisia villosa	Leaves	MeOH	10^{-10} g/mL[b]	34.6
	Twig	MeOH	10^{-10} g/mL[b]	18.5
Embelia coriacea	Leaves	MeOH	10^{-7} g/mL	15.3
		H_2O	10^{-4} g/mL[c]	34.6
	Twig	MeOH	10^{-6} g/mL	24.7
		H_2O	10^{-5} g/mL	0
Labisia pumila var. *alata* (purple-leafed)	Leaves	H_2O	10^{-8} g/mL	83.1
	Roots	H_2O	10^{-9} g/mL	50.7
Labisia pumila var. *alata* (green-leafed)	Leaves	H_2O	10^{-9} g/mL	50.5
	Roots	H_2O	10^{-9} g/mL	63.5
Maesa ramentacea	Leaves	MeOH	10^{-5} g/mL	58.6
		H_2O	10^{-5} g/mL	121.6
	Twig	MeOH	10^{-6} g/mL	8.3
		H_2O	10^{-7} g/mL	34.6
Rapanea porteriana	Leaves	MeOH	10^{-6} g/mL	23.0
		H_2O	10^{-9} g/mL	69.8
	Twig	MeOH	10^{-6} g/mL	6.2
		H_2O	10^{-6} g/mL	64.7

[a] Concentration of test sample with maximal proliferative effect.

[b] Lowest concentration of extract tested in the assay.

[c] Highest concentration of extract tested in the assay.

An earlier phytochemical study revealed that *M. ramentacea* had high content of triterpenoid saponins[36]. The leaf aqueous extract and saponin mixture were found to have antifungal activity on crops[37] and piscicidal activity[38], leading to the isolation of a bioactive triterpenoid saponin (saponin A)[39]. In our study, the aqueous leaf extract of *M. ramentacea* was found to significantly induce cell proliferation and considered to be a full estrogen agonist for MCF-7 cells with greater proliferative effect than 17β-estradiol. Although majority of the known phytoestrogens are phenolics, non-phenolic triterpenoid saponins such as ginsenosides[40] and soyasapogenol A[41] have been reported to possess estrogenic activity. Thus, it could be possible that the terpenoid saponin-rich content contributes to the estrogenicity of the Myrsinaceae species.

Previous studies reported the estrogenic activity of *L. pumila* var. *alata in vitro*[23-24] and *in vivo*[26,28]. In this study, two types of *L. pumila* var. *alata* purple-leafed (Fig. 4A) and green-leafed (Fig. 4B) were compared. The aqueous extracts of both leaves and roots were found to be partial agonist for MCF-7 cells. However, the purple-leafed leaf aqueous extract had higher proliferative effect compared to that of the green-leafed leaves, suggesting the potential medicinal use of the purple-leafed species. Several scientific studies revealed that extracts of *L. pumila* had various activities such as antibacterial[42-43], antifungal[43], antioxidant[44], platelet-activating factor receptor binding inhibition[45], immunomodulation[46], prevent skin photoaging[47] and anti-oedema *in vivo*[48]. Recent publications reported the presence of flavonoids, phenolic acids, alkyl phenols and saponins[49-50]. *L. pumila* var. *alata* have been reported to contain the known estrogenic flavonols such as apigenin, kaempferol, quercetin and myricetin[43, 49, 51-53]. Further work is underway to determine the bioactive component(s) responsible for the MCF-7 cell proliferative activity in order to justify the ethnobotanical uses.

(A) **(B)**

Fig. 4: Shows *Labisia pumila* var. *alata* of (A) purple-leafed and (B) green-leafed

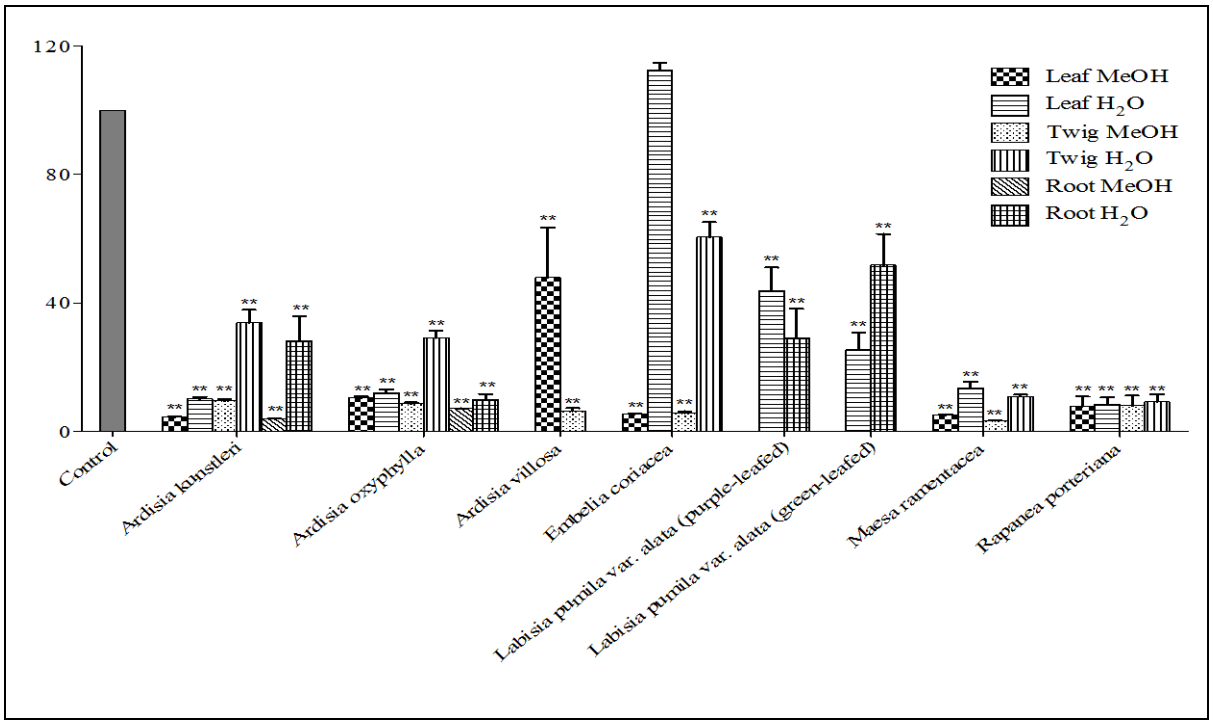

Fig. 3: Shows cytotoxic effect of extracts (10^{-4} g/mL) on the MCF-7 Cells

Each value represents the mean ± SEM (n=3). Asterisks denote significant differences from the control (100%) (ANOVA, **p<0.01)

Cytotoxic Effects of Extracts

With the exception of methanol extract of *A. villosa* twig (10^{-5} g/mL) and aqueous extract of *E. coriaceae* leaves (10^{-4} g/mL), all extracts were cytotoxic at a concentration of 10^{-4} g/mL (Fig. 3). While sixteen extracts significantly reduced ≥90% MCF-7 cell proliferation at 10^{-4} g/mL, five extracts significantly reduced cell proliferation from 10^{-5} g/mL (Table 2). The cytotoxicity effect could be preliminarily determined by viewing the morphological changes of MCF-7 cells under inverted microscope, whereby untreated cells would appear actively dividing and the treated cells would display uncharacterised bodies[54,55].

Our findings support the previous reports that many phytoestrogens demonstrate biphasic activity, that is, they are estrogenic at low concentrations ($<10^{-6}$ M) but exhibit cytotoxic effects at high concentrations ($>10^{-6}$ M) [56-57]. For example, genistein has biphasic activity in MCF-7 cells, that is, as a potent agonist and antiproliferative over a concentration range of 10 nM to 20 μM[58]. Ligand-binding and estrogen-responsive gene assays demonstrate the higher selectivity of genistein for ERβ than ERα[52] and this is evident by its selective effects towards bone (ERβ-positive) tissues[58].

From this study, further work needs to be carried out to elucidate the bioactive components and to define the possible ER pathways that these components act onto. Hypothetically, estrogenic effect of extracts or pure compounds in human breast cancer cells could be associated with the expression of endogenous estrogen-regulated and exogenous estrogen-responsive reporter genes[59]. Thus, up-regulation of endogenous estrogen-responsive genes, such as pS2, PR, and PTGES, in MCF-7 cells has been used to indicate the estrogenicity via ERα[60]. Such *in vitro* data could be utilised to predict potential estrogenic effect of these extracts or pure compounds, however, *in vivo* animal study will essentially determine the potential benefits to humans[61]. The proposed work is underway in our continuing effort to discover phytoestrogenic compounds from the Myrsinaceae species.

CONCLUSION

Aqueous leaf extracts of *M. ramentacea* and *L. pumila* var. *alata* (purple-leafed) demonstrated full agonist and partial agonist effects for MCF-7 cells, respectively, comparable to that for 17β-estradiol. Plant parts and extracts of other species such as *A. oxyphylla* and *R. porteriana* were also found to significantly enhance MCF-7 cell proliferation. The results of this study may suggest that some Malaysian Myrsinaceae species can potentially be explored for estrogenic activity and used as natural sources of dietary phytoestrogens.

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REFERENCES

1. Cornwell T, Cohick W, Raskin I. Dietary phytoestrogens and health. Phytochemistry 2004; 65: 995-1016.
2. Jamali B, Nickavar B. Phytoestrogens: recent developments. IJPR 2004; 3 Suppl 2: 86-87.
3. Shu XO, Zheng Y, Cai H, Gu K, Chen Z, Zheng W, et al. Soy food intake and breast cancer survival. JAMA 2009; 302: 2437-2443.
4. Liang YL, Teede H, Dalais F, McGrath BP. The effects of phytoestrogen on blood pressure and lipids in healthy volunteers. Zhonghua Xin Xue Guan Bing Za Zhi 2006; 34: 726-729.
5. Buck K, Zaineddin AK, Vrieling A, Linseisen J, Chang-Claude J. Meta-analyses of lignans and enterolignans in relation to breast cancer risk. Am. J. Clin. Nutr. 2010; 92: 141-153.
6. Cassidy A, Albertazzi P, Nielsen IL, Hall W, Williamson G, Tetens I, et al. Critical review of health effects of soybean phyto-oestrogens in postmenopausal women. Proc. Nutr. Soc. 2006; 65: 76-92.
7. Wu J, Oka J, Tabata I, Higuchi M, Toda T, Fuku N, et al. Effects of isoflavone and exercise on BMD and fat mass in postmenopausal Japanese women: A 1-year randomized placebo controlled trial. J. Bone Miner. Res. 2006; 21: 780-789.
8. Wu J, Oka J, Higuchi M, Tabata I, Toda T, Fujioka M, et al. Cooperative effects of isoflavones and exercise on bone and lipid metabolism in postmenopausal Japanese women: A randomized placebo-controlled trial. Metabolism 2006; 55: 423-433.
9. Zhang X, Shu X-O, Li H, Yang G, Li Q, Gao Y-T, et al. Prospective cohort study of soy food consumption and risk of bone fracture among postmenopausal women. Arch. Intern. Med. 2005; 165: 1890-1895.
10. Casini ML, Marelli G, Papaleo E, Ferrari A, D'Ambrosio F, Unfer V. Psychological assessment of the effects of treatment with phytoestrogens on postmenopausal women: A randomized, double-blind, crossover, placebo-controlled study. Fertil. Steril. 2006; 85: 972-978.
11. Aubertin-Leheudre M, Lord C, Khalil A, Dionne IJ. Effect of 6 months of exercise and isoflavone supplementation on clinical cardiovascular risk factors in obese postmenopausal women: A randomized, double-blind study. Menopause 2007; 14: 624-629.
12. Mahn K, Borras C, Knock GA, Taylor P, Khan IY, Sugden D, et al. Dietary soy isoflavone induced increases in antioxidant and eNOS gene expression lead to improved endothelial function and reduced blood pressure in vivo. FASEB J. 2005; 19: 1755-1757.
13. Mu H, Bai YH, Wang ST, Zhu ZM, Zhang YW. Research on antioxidant effects and estrogenic effect of formononetin from *Trifolium pratense* (red clover). Phytomedicine 2009; 16: 314-319.
14. Verdrengh M, Jonsson IM, Holmdahl R, Tarkowski A. Genistein as an anti-inflammatory agent. Inflamm. Res. 2003; 52: 341-346.
15. Zhang HT, Wang Y, Deng XL, Dong MQ, Zhao LM, Wang YW. Daidzein relaxes rat cerebral basilar artery via activation of large-conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle cells. Eur. J. Pharmacol. 2010; 630: 100-106.
16. Locklear TD, Huang Y, Frasor J, Doyle BJ, Perez A, Gomez-Laurito J, et al. Estrogenic and progestagenic effects of extracts of *Justicia pectoralis* Jacq., an herbal medicine from Costa Rica used for the treatment of menopause and PMS. Maturitas 2010; 66: 315-322.
17. Watanabe T, Inoue S, Ogawa S, Ishii Y, Hiroi H, Ikeda K, et al. Agonistic effect of tamoxifen is dependent on cell type, ERE-promoter context, and estrogen receptor subtype: functional difference between estrogen receptors α and β. Biochem. Biophys. Res. Comm. 1997; 236: 140-145.
18. Wober J, Weißwange I, Vollmer G. Stimulation of alkaline phosphatase activity in Ishikawa cells induced by various phytoestrogens and synthetic estrogens. J. Steroid Biochem. Mol. Biol. 2003; 83: 227-233.
19. Routedge E, Sumpter JP. Oestrogenic activity of surfanctants and some of their degradation products assessed using a recombinant yeast screen. Environ. Toxicol. Chem. 1996; 15: 241-248.
20. Jamal JA, Houghton PJ, Milligan SR. Testing of *Labisia pumila* for oestrogenic activity using a recombinant yeast screen. J. Pharm. Pharmacol. 1998; 50: 79.
21. Jamal JA, Houghton PJ, Milligan SR, Jantan I. The oestrogenic and cytotoxic effects of the extracts of *Labisia pumila* var. *alata* and *Labisia pumila* var. *pumila in vitro.* MJHS 2003; 1: 53-60.
22. Burkill IH. A dictionary of the economic products of the Malay Peninsula. London: Crown Agents; 1935.
23. Al-Wahaibi A, Nazaimoon WWM, Farihah HS, Azian AL. Effect of ovariectomy, *Labisia pumila* var. *alata* treatment and estrogen replacement therapy on the morphology of adipose tissue in ovariectomized Sprague Dawley rats. J. Med. 2007; 1. http://www.scientificjournals.org/journals2007/articles/1024.htm. (accessed October 29, 2011)
24. Mansor F, Nazaimoon WWM, Harvest GF, Claes-Goran O. *Labisia pumila* extract regulates body weight and adipokines in ovariectomized rats. Maturitas 2009; 62: 91-97.
25. Al-Wahaibi A, Nazaimoon WWM, Norsyam WN, Farihah HS, Azian AL. Effect of water extract of *Labisia pumila* var. *alata* on aorta of ovariectomized Sprague Dawley rats. PJN 2008; 7: 208-213.

26. Ahmad NS, Leong LP, Norliza M, Norazlina M, Ima NS. The effects of *Labisia pumila* var. *alata* on bone markers and bone calcium in a rat model of post-menopausal osteoporosis. J. Ethnopharmacol. 2011; 133: 538-542.
27. Jantan I. Medicinal plant research in Malaysia: scientific interests and advances. MJHS 2004; 2: 27-46.
28. Jamal JA, Sateri AA, Husain K, Jantan I, Mohd-Arshad MS. Pelbagai kaedah pengekstrakan air *Labisia pumila* var. *alata*. MJHS 2006; 4: 37-45.
29. Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ. Health Perspect. 1995; 103: 113-122.
30. Zauyah Y, Lope-Pihie AH, Noah RM, Stanslas J. Cytotoxicity of goniothalamin on hormone-dependent breast cancer cells. Malaysian J. Biochem. Mol. Biol. 1997; 2: 29-32.
31. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 1983; 65: 55-63.
32. Berthois Y, Katzenellenbogen JA, Katzenellenbogen BS. Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. PNAS 1986; 83: 2496-2500.
33. Vergote I, Neven P, van Dam P, Serreyn R, De Pins F, De Sutter P, et al. The estrogen receptor and its selective modulators in gynaecological and breast cancer. Eur. J. Cancer 2000; 36: S1-S9.
34. Kostelac D, Rechkemmer G, Briviba K. Phytoestrogens modulate binding response of estrogen receptors alpha and beta to the estrogen response element. J. Agric. Food Chem. 2003; 51: 7632-7635.
35. Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson JA, Nilsson S. Differential response of estrogen receptor α and estrogen receptor β to partial estrogen agonists/antagonists. Mol. Pharmacol. 1998; 54: 105-112.
36. Rahmani M, Kiew R, Lajis N, Othman R, Toia RF. A contribution to the phytochemical survey of Peninsular Malaysia. Pertanika 1985; 8: 347-357.
37. Phongpaichit S, Schneider EF, Picman AK, Tantiwachwuttikul P, Wiriyachitraii P, Arnason JT. Inhibition of fungal growth by an aqueous extract and saponins from leaves of *Maesa ramentacea* Wall. Biochem. Syst. Ecol. 1995; 23: 17-25.
38. Chiayvareesajja S, Rittibhonbhun N, Hongpromyart M, Wiriyachitra P. Toxicity of the Thai piscicidal plant, *Maesa ramentacea*, to freshwater fishes in ponds. Aquacult. 1997; 158: 229-234.
39. Tuntiwachwuttikul P, Pancharoen R, Mahabusarakam W, Wiriyachitra P, Taylor WC, Bubb WA, et al. A triterpenoid saponin from *Maesa ramentacea*. Phytochemistry 1997; 44: 491-95.
40. Dong S, Kiyama R. Characterisation of oestrogenic activity of ginsenosides in MCF-7 cells using a customised DNA microarray. Food Chem. 2009; 113: 672-678.
41. Rowlands JC, Berhow MA, Badger TM. Estrogenic and antiproliferative properties of soy sapogenols in human breast cancer cells *in vitro*. Food Chem. Toxicol. 2002; 40: 1767-1774.
42. Fasihuddin A, Rahman AH, Hasmah R. Medicinal plants used by Bajau community in Sabah. In: Chan KL, Abas H, Amirin S, Yuen KH, Mohd-Zaini A, Zhari I, editors. Trends in traditional medicine research. Penang: The School of Pharmaceutical Sciences, University of Science Malaysia; 1995. p. 493-504.
43. Karimi E, Jaafar HZE, Ahmad S. Phytochemical analysis and antimicrobial activities of methanolic extracts of leaf, stem and root from different varieties of *Labisia pumila* Benth. Molecules 2011; 16: 4438-4450.
44. Norhaiza M, Maziah M, Hakiman M. Antioxidative properties of leaf extracts of a popular Malaysian herb, *Labisia pumila.* J. Med. Plants Res. 2009; 3: 217-223.
45. Jantan I, Young-Hwa K, Dae-Yeon S, Byung HH. Inhibitory effects of Malaysian medicinal plants on the platelet-activating factor (PAF) receptor binding. Nat. Prod. Sci. 1996; 2: 86-89.
46. Pandey A, Bani S, Sangwan P, Koul S. Selective Th1 upregulation by ethyl acetate fraction of *Labisia pumila*. J. Ethnopharmacol. 2010; 132: 309-315.
47. Choi H-K, Kim D-H, Kim JW, Ngadiran S, Sarmidi MR, Park CS. *Labisia pumila* extract protects skin cells from photoaging caused by UVB irradiation. J. Biosci. Bioeng. 2010; 109: 291-296.
48. Rasadah MA, Nik-Musa'adah M, Aznie-Aida A, Mohd-Rizal AK. Inhibitory activity of some selected Malaysian medicinal plants on TPA induced mouse ear oedema. In: Hamzah AS, Ismail NH. Zainal-Ariffin Z, Hamzah Z, editors. Fine chemicals from natural resources. Selangor: University Publication Centre, University of Technology MARA; 2001. p. 371-376.
49. Chua LS, Latiff NA, Lee SY, Lee CT, Sarmidi MR, Aziz RA. Flavonoids and phenolic acids from *Labisia pumila* (Kacip Fatimah). Food Chem. 2011; 127: 1186-1192.
50. Ali Z, Khan IA. Alkyl phenols and saponins from the roots of *Labisia pumila* (Kacip Fatimah). Phytochemistry 2011; 72: 2075-2080.
51. Karimi E, Jaafar HZE. HPLC and GC-MS determination of bioactive compounds in microwave obtained extracts of three varieties of *Labisia pumila* Benth. Molecules 2011; 16: 6791-6805.
52. Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, Saag PTVD, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 1998; 139: 4252-4263.
53. Maggiolini M, Recchia AG, Bonofiglio D, Catalano S, Vivacqua A, Carpino A, et al. The red wine phenolics piceatannol and myricetin act as agonists for estrogen receptor α in human breast cancer cells. J. Mol. Endocrinol. 2005; 35: 269-281.
54. Ranjit PM, Krishna PM, Silpa P, Nagalakshmi V, Anjali M, Girish K, Chowdary YA. In vitro cytotoxicity activities of *Calotropis procera* latex and flower extracts against MCF-7 and HeLa cell line cultures. Int. J. Pharm. Pharm. Sci. 2012; 4(1): 66-70.
55. Kumala S, Septiseptiana EP, Meiyanto E. Cytotoxic effect of secondary metabolites produced by endophytic fungi 1.3.11, 1.1.6 and 1.2.6 isolated from the fruit of "tanaman buah makassar" (*Brucea janvanica* (L.) Merr) on *in vitro* T47D and MCF7 intact cells and identification of the fungus 1.3.11 by ribosomal DNA sequence analysis. Int. J. Pharm. Pharm. Sci. 2010; 2(2): 80-83.
56. Zava DT, Duwe G. Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells in vitro. Nutr. Cancer 1997; 27: 31-40.
57. Maggiolini M, Bonofiglio D, Marsico S, Panno ML, Cenni B, Picard D, et al. Estrogen receptor α mediates the proliferative but not the cytotoxic dose-dependent effects of two major phytoestrogens on human breast cancer cells. Mol. Pharmacol. 2001; 60: 595-602.
58. Cotter A, Cashman KD. Genistein appears to prevent early postmenopausal bone loss as effectively as hormone replacement therapy. Nutr. Rev. 2003; 61: 346-351.
59. Gehma BD, Levenson AS, Liu H, Lee E-J, Amundsenb BM, Cushman M, et al. Estrogenic effects of resveratrol in breast cancer cells expressing mutant and wild-type estrogen receptors: role of AF-1 and AF-2. J. Steroid Biochem. Mol. Biol. 2004; 88: 223-234.
60. Frasor J, Danes JM, Komm B, Chang KCN, Lyttle CR, Katzenellenbogen BS. Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype. Endocrinology 2003; 144: 4562-4574.
62. Doyle BJ, Frasor J, Bellows LE, Locklear TD, Perez A, Gomez-Laurito J, et al. Estrogenic effects of herbal medicines from Costa Rica used for the management of menopausal symptoms. Menopause 2009; 16:748-55.