

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. ramentacea* leaves showed the highest cell proliferation activity ($136.9 \pm 2.5\%$ 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) ($131.0 \pm 2.9\%$ 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, hypercholesterolemia, hyperglycemia, arteriosclerosis and coronary heart disease, reduction in bone mineral density and bone and hip fracture, cognitive abilities and mood in postmenopausal women¹⁻¹¹. 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 properties¹⁶.

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 ER α -positive human breast cancer cell lines such as MCF-7¹⁷, human endometrial adenocarcinoma (Ishikawa Var-1) cells¹⁸ 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 μ g/mL) in Ishikawa-Var I cells and recombinant yeast estrogen screen assays but was cytotoxic towards the Ishikawa cells at a higher concentration (100 μ g/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 gain²³⁻²⁴, protection against cardiovascular risks²⁵ and osteoporosis²⁶, 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 relatedness of the phytochemical content²⁷.

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 ER α -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-dried²⁸. 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 responses²⁹.

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
<i>Ardisia kunstleri</i> King & Gamble	NR	Pasoh, Negeri Sembilan	29817	Leaves	MeOH H ₂ O
				Twig	MeOH H ₂ O
				Roots	MeOH H ₂ O
<i>Ardisia oxyphylla</i> Wall.	Sore, swelling.	Gunung Angsi, Negeri Sembilan	AZ 13	Leaves	MeOH H ₂ O
				Twig	MeOH H ₂ O
				Roots	MeOH H ₂ O
<i>Ardisia villosa</i> Roxb.	Cough, dropsy, fever, hepatitis.	Gunung Angsi, Negeri Sembilan	AZ 45	Leaves Twig	MeOH MeOH
<i>Embelia coriacea</i> Wall.	NR	Kuala Kubu, Selangor	29812	Leaves Twig	MeOH MeOH H ₂ O
<i>Labisia pumila</i> (Blume) Mez var. <i>alata</i> (purple-leafed)	Parturition, flatulence, dysentery, dysmenorrhoea, gonorrhoea, 'sickness in the bones'.	Taiping, Perak	SM 744	Leaves	H ₂ O
				Roots	H ₂ O
<i>Labisia pumila</i> (Blume) Mez var. <i>alata</i> (green-leafed)		Taiping, Perak	SM745	Leaves	H ₂ O
				Roots	H ₂ O
<i>Maesa ramentacea</i> Wall.	Angina, dermatosis.	Gunung Berembun Forest Reserve, Pahang	29813	Leaves	MeOH H ₂ O
				Twig	MeOH H ₂ O
<i>Rapanea porteriana</i> (Wall. ex A.D.C.) Mez	NR	Port Dickson, Negeri Sembilan	29810	Leaves	MeOH H ₂ O
				Twig	MeOH H ₂ O

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% CO₂ 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 method³⁰ 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) assay³¹ 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 (A₅₅₀) was measured spectrophotometrically at a wavelength of 550 nm using a microplate reader. Percentage of cell viability was calculated as 100 × (A₅₅₀ of test sample)/(A₅₅₀ 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 formula²⁹:

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

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 extracts²⁹.

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 lines³². 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 proliferation³³ 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 ERβ³⁴⁻³⁵.

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. ramentacea* leaves showed the highest cell proliferation activity ($136.9 \pm 2.5\%$ 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) ($131.0 \pm 2.9\%$ 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. porteriiana*.

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.

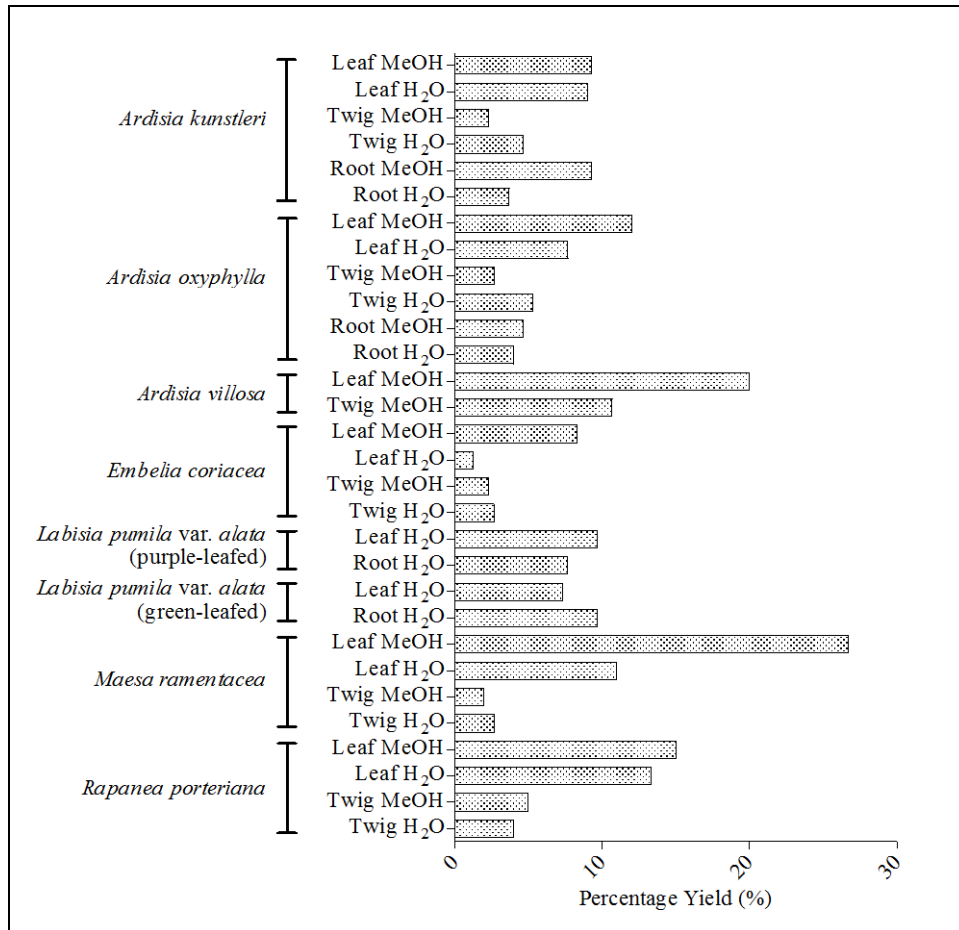


Fig. 1: Shows percentage yield of extracts

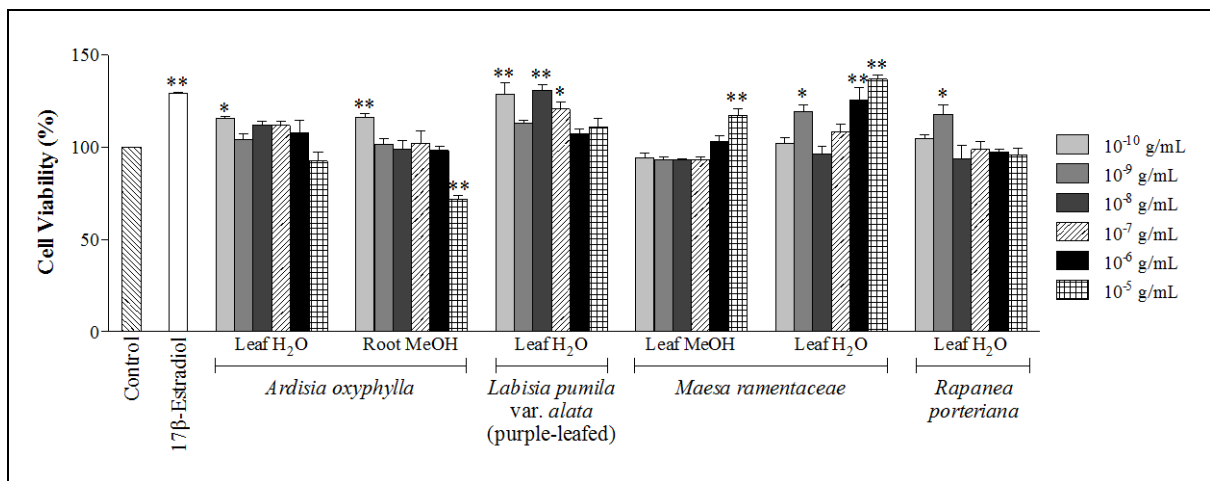


Fig. 2: Shows estrogenic effects of six extracts of four Myrsinaceae species on the MCF-7 cell proliferation, compared to control (100%) and 17β-estradiol (10^{-9} M)

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 \pm SEM (%) at different concentrations (g/mL) ^a					
			10 ⁻¹⁰	10 ⁻⁹	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵
<i>Ardisia kunstleri</i>	Leaf	MeOH	95.8 \pm 1.7	94.1 \pm 0.7	92.6 \pm 0.2	94.7 \pm 1.9	95.6 \pm 2.2	97.9 \pm 1.2
		H ₂ O	105.2 \pm 0.6	103.4 \pm 1.1	98.4 \pm 1.2	102.7 \pm 2.6	104.5 \pm 0.5	102.6 \pm 0.9
	Twig	MeOH	94.6 \pm 2.6	97.2 \pm 4.8	96.3 \pm 4.0	97.7 \pm 1.9	100.9 \pm 5.2	105.2 \pm 4.0
		H ₂ O	92.6 \pm 2.4	93.4 \pm 2.5	91.5 \pm 2.9	92.4 \pm 3.7	103.1 \pm 2.0	92.6 \pm 3.0
	Root	MeOH	97.5 \pm 2.4	97.4 \pm 2.3	95.2 \pm 3.4	98.4 \pm 3.2	99.3 \pm 1.4	107.5 \pm 2.3
		H ₂ O	100.8 \pm 3.3	103.9 \pm 1.6	98.6 \pm 3.0	93.2 \pm 6.5	99.3 \pm 2.4	99.7 \pm 4.4
<i>Ardisia oxyphylla</i>	Leaf	MeOH	108.6 \pm 5.5	106.8 \pm 1.0	101.6 \pm 3.3	105.4 \pm 0.8	113.3 \pm 6.8	98.4 \pm 10.2
	Twig	MeOH	102.9 \pm 3.2	103.0 \pm 5.0	97.7 \pm 2.7	107.7 \pm 5.9	98.3 \pm 4.1	97.9 \pm 4.8
		H ₂ O	102.6 \pm 1.8	104.9 \pm 1.1	102.5 \pm 3.5	115.0 \pm 1.5	114.7 \pm 6.4	108.9 \pm 7.0
<i>Ardisia villosa</i>	Root	H ₂ O	112.6 \pm 10.0	104.9 \pm 6.7	104.3 \pm 3.1	97.2 \pm 1.0	113.6 \pm 7.1	98.1 \pm 3.3
	Leaf	MeOH	112.4 \pm 0.7	109.0 \pm 1.0	95.0 \pm 6.0	96.3 \pm 4.1	95.0 \pm 1.7	96.4 \pm 3.1
<i>Embelia coriacea</i>	Twig	MeOH	105.5 \pm 4.0	102.1 \pm 2.3	100.1 \pm 4.5	100.4 \pm 4.8	94.2 \pm 9.3	8.2 \pm 0.9**
	Leaf	MeOH	101.6 \pm 1.6	100.5 \pm 2.3	100.5 \pm 2.9	104.2 \pm 0.6	96.7 \pm 2.7	80.4 \pm 4.7**
H ₂ O		92.4 \pm 6.2	94.5 \pm 2.6	97.1 \pm 3.6	98.9 \pm 4.1	94.8 \pm 2.1	98.4 \pm 3.0	
MeOH		96.2 \pm 3.3	97.9 \pm 4.1	100.9 \pm 4.1	100.5 \pm 6.2	107.0 \pm 6.3	101.3 \pm 0.5	
<i>Labisia pumila</i> var. <i>alata</i> (purple-leafed)	Root	H ₂ O	86.5 \pm 5.8	94.1 \pm 0.7	91.5 \pm 5.6	86.1 \pm 5.8	92.5 \pm 4.3	99.8 \pm 3.7
		H ₂ O	100.8 \pm 4.8	118.3 \pm 2.7	109.3 \pm 3.1	115.6 \pm 9.3	101.9 \pm 2.6	103.7 \pm 0.9
		H ₂ O	101.6 \pm 3.4	117.9 \pm 3.9	108.3 \pm 10.6	111.4 \pm 4.0	113.5 \pm 1.8	108.0 \pm 4.0
<i>Maesa ramentacea</i>	Twig	MeOH	98.9 \pm 1.0	95.8 \pm 1.2	91.9 \pm 1.7	99.0 \pm 2.8	102.5 \pm 2.6	101.2 \pm 5.1
		H ₂ O	103.4 \pm 0.6	106.7 \pm 2.3	107.7 \pm 2.8	112.9 \pm 7.7	104.3 \pm 4.5	100.3 \pm 6.4
<i>Rapanea porteriana</i>	Leaf	MeOH	102.6 \pm 3.1	103.8 \pm 1.2	104.6 \pm 1.8	104.3 \pm 1.9	107.0 \pm 3.5	67.2 \pm 5.7**
		MeOH	100.1 \pm 0.9	96.0 \pm 5.7	99.6 \pm 5.7	98.0 \pm 3.1	101.9 \pm 1.5	77.9 \pm 3.4**
	Twig	H ₂ O	102.0 \pm 5.0	100.5 \pm 4.1	95.6 \pm 4.2	113.2 \pm 6.9	118.3 \pm 10.5	100.8 \pm 2.6
Control			100					
17 β -Estradiol (10 ⁻⁹ M)			137.1 \pm 1.2**					

^a Each value represents the mean \pm 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 (%)
17 β -Estradiol			10 ⁻⁹ M	100.0
<i>Ardisia kunstleri</i>	Leaves	MeOH	10 ⁻⁵ g/mL	0
		H ₂ O	10 ⁻¹⁰ g/mL ^b	17.4
	Twig	MeOH	10 ⁻⁵ g/mL	14.6
		H ₂ O	10 ⁻⁶ g/mL	10.8
	Roots	MeOH	10 ⁻⁵ g/mL	25.2
		H ₂ O	10 ⁻⁹ g/mL	13.6
<i>Ardisia oxyphylla</i>	Leaves	MeOH	10 ⁻⁶ g/mL	47.1
		H ₂ O	10 ⁻¹⁰ g/mL ^b	53.1
	Twig	MeOH	10 ⁻⁷ g/mL	25.8
		H ₂ O	10 ⁻⁷ g/mL	52.1
	Roots	MeOH	10 ⁻¹⁰ g/mL ^b	70.3
		H ₂ O	10 ⁻⁶ g/mL	47.3
<i>Ardisia villosa</i>	Leaves	MeOH	10 ⁻¹⁰ g/mL ^b	34.6
	Twig	MeOH	10 ⁻¹⁰ g/mL ^b	18.5
<i>Embelia coriacea</i>	Leaves	MeOH	10 ⁻⁷ g/mL	15.3
		H ₂ O	10 ⁻⁴ g/mL ^c	34.6
	Twig	MeOH	10 ⁻⁶ g/mL	24.7
<i>Labisia pumila</i> var. <i>alata</i> (purple-leafed)	Leaves	H ₂ O	10 ⁻⁵ g/mL	0
		H ₂ O	10 ⁻⁸ g/mL	83.1
<i>Labisia pumila</i> var. <i>alata</i> (green-leafed)	Roots	H ₂ O	10 ⁻⁹ g/mL	50.7
	Leaves	H ₂ O	10 ⁻⁹ g/mL	50.5
<i>Maesa ramentacea</i>	Leaves	H ₂ O	10 ⁻⁹ g/mL	63.5
		MeOH	10 ⁻⁵ g/mL	58.6
	Twig	H ₂ O	10 ⁻⁵ g/mL	121.6
		MeOH	10 ⁻⁶ g/mL	8.3
<i>Rapanea porteriana</i>	Leaves	H ₂ O	10 ⁻⁷ g/mL	34.6
		MeOH	10 ⁻⁶ g/mL	23.0
	Twig	H ₂ O	10 ⁻⁹ g/mL	69.8
		MeOH	10 ⁻⁶ g/mL	6.2
		H ₂ O	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 saponins³⁶. The leaf aqueous extract and saponin mixture were found to have antifungal activity on crops³⁷ and piscicidal activity³⁸, 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 ginsenosides⁴⁰ and soyasapogenol A⁴¹ 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-leaved (Fig. 4A) and green-leaved (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-leaved leaf aqueous extract had higher proliferative effect compared to that of the green-leaved leaves, suggesting the potential medicinal use of the purple-leaved species. Several scientific studies revealed that extracts of *L. pumila* had various activities such as antibacterial⁴²⁻⁴³, antifungal⁴³, antioxidant⁴⁴, platelet-activating factor receptor binding inhibition⁴⁵, immunomodulation⁴⁶, prevent skin photoaging⁴⁷ and anti-oedema *in vivo*⁴⁸. Recent publications reported the presence of flavonoids, phenolic acids, alkyl phenols and saponins⁴⁹⁻⁵⁰. *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.



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

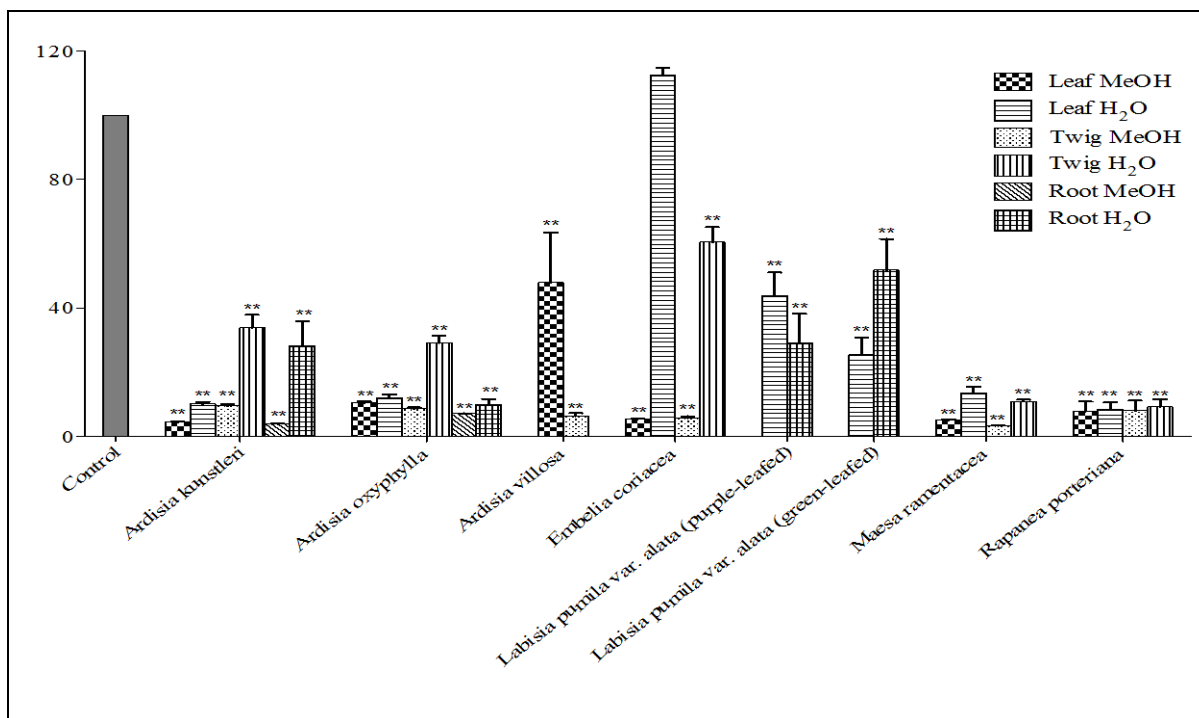


Fig. 3: Shows cytotoxic effect of extracts (10⁻⁴ g/mL) on the MCF-7 Cells

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. coriacea* leaves (10^{-4} g/mL), all extracts were cytotoxic at a concentration of 10^{-4} g/mL (Fig. 3). While sixteen extracts significantly reduced $\geq 90\%$ 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 μ M⁵⁸. Ligand-binding and estrogen-responsive gene assays demonstrate the higher selectivity of genistein for ER β than ER α ⁵² and this is evident by its selective effects towards bone (ER β -positive) tissues⁵⁸.

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 genes⁵⁹. 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 ER α 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 humans⁶¹. 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 17 β -estradiol. Plant parts and extracts of other species such as *A. oxyphylla* and *R. porteri*ana 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.

ACKNOWLEDGEMENT

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