Original Research Article

Cytotoxic and Antioxidant Activities of *Antidesma thwaitesianum* Müll Arg (Euphorbiaceae) Fruit and Fruit Waste Extracts

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Abstract

Purpose: To investigate the cytotoxic and antioxidant activities of the fruit and fruit waste (residue and marc) extracts of Antidesma thwaitesianum Müll. Arg., known as mamao in Thai, using chemical and cell-based assays.

Methods: The cytotoxicity of mamao fruit and fruit waste extracts obtained by expression, maceration and decoction against a panel of six human cancer cell lines (COR-L23, A549, LS174T, PC-3, MCF7 and HeLa) was determined by sulforhodamine B (SRB) assay. In addition, their antioxidant activities were measured by chemical methods: 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging assay and ferric ion reducing antioxidant power (FRAP) assay and cell-based methods: nitroblue tetrazolium (NBT) dye reduction assay and nitric oxide (NO) scavenging assay. The phenolic and flavonoid contents were assessed colorimetrically at 765 nm and 415 nm respectively.

Results: Among the test extracts, the ethanol extracts of fresh fruits (FME) and marc left after squeezing fresh fruits (MME) exhibited moderate cytotoxicity against human breast MCF7 cells while the extract obtained by decocting the residue left after maceration of dried fruits (RDW) was moderately cytotoxic to lung large cell carcinoma COR-L23 cells. In the chemical assays, the extract obtained by decocting the residue left after maceration of dried marc (RMW) displayed the strongest ABTS radical-scavenging and ferric-reducing activities among the extracts. In the cell-based assays, however, FME and DME exerted potent nitric oxide scavenging activity whereas the extract obtained by decocting the residue left after maceration of fresh fruits (RFW) showed moderate superoxide radical-scavenging activity relative to the test extracts. The ABTS radical-scavenging and ferric-reducing activities of these extracts strongly correlate with their phenolic and flavonoid contents, indicating their specific contributions to such activities.

Conclusion: The ethanol extracts of fresh and dried mamao fruits exhibit both cytotoxic and cellular antioxidant activities, and thus possess great potentials for application in the development of effective dietary supplements to prevent oxidative stress-induced diseases.

Keywords: Antidesma thwaitesianum Müll. Arg., Cytotoxicity, Lung cell carcinoma, Antioxidant activity, Cellular assays

INTRODUCTION

Oxidative stress reflects an imbalance between oxidants and antioxidants [1]. Oxidants that produce free radicals and reactive oxygen species (ROS) in particular via oxidation reactions are extremely reactive and easily able to react with biomolecules such as proteins, lipids and nucleic acids. However, these radicals can be neutralized by antioxidants that can slow or prevent such various reactions [2]. Due to insufficient or ineffective antioxidants, excess free radicals can cause oxidative damage implicated in many diseases including cancer [3]. In this regard, plant-derived antioxidants have attracted much attention because of their potential antioxidant activities and safety for consumption[4].

Antidesma thwaitesianum Müll. Arg., also called mamao in Thai, is a member of the Euphorbiaceae family. Mamao is a medium-sized evergreen tree with spade-shaped leaves and dioecious flowers that are small white and purple occurring in clusters. Its fruits are edible and concentrated around the base of flower clusters. Immature fruits of *Antidesma thwaitesianum* Müll. Arg. are yellowish white, and its ripe fruits become purplish red to dark purple depending on the level of ripeness [5]. Products such as mamao juice and wine have become more popular in Thailand. Previous studies have shown that extracts from mamao seeds and marc, waste products from the process of making mamao juice and wine, exhibited antioxidant potential similar to grape seed extracts [6] and also conferred anti-apoptotic and anti-inflammatory effects through specific molecular mechanisms [7]. Ripe fruits of *Antidesma thwaitesianum* Müll. Arg. are thought to be rich in natural antioxidants; however, no specific report regarding health benefits of *Antidesma thwaitesianum* fruits has been published to date. Thus, the present study is the first to determine the cytotoxic and antioxidant activities of mamao fruit and fruit waste extracts obtained by different extraction methods using chemical and cell-based assays.

EXPERIMENTAL

Plant materials

A. thwaitesianum (Euphorbiaceae) fruits were collected from five–year-old plants in Sakonnakhon province, Thailand in September 2009. The voucher specimen (BKF no. 173994) was deposited in the herbarium of Royal Forest Department, Bangkok, Thailand.

Extraction methods

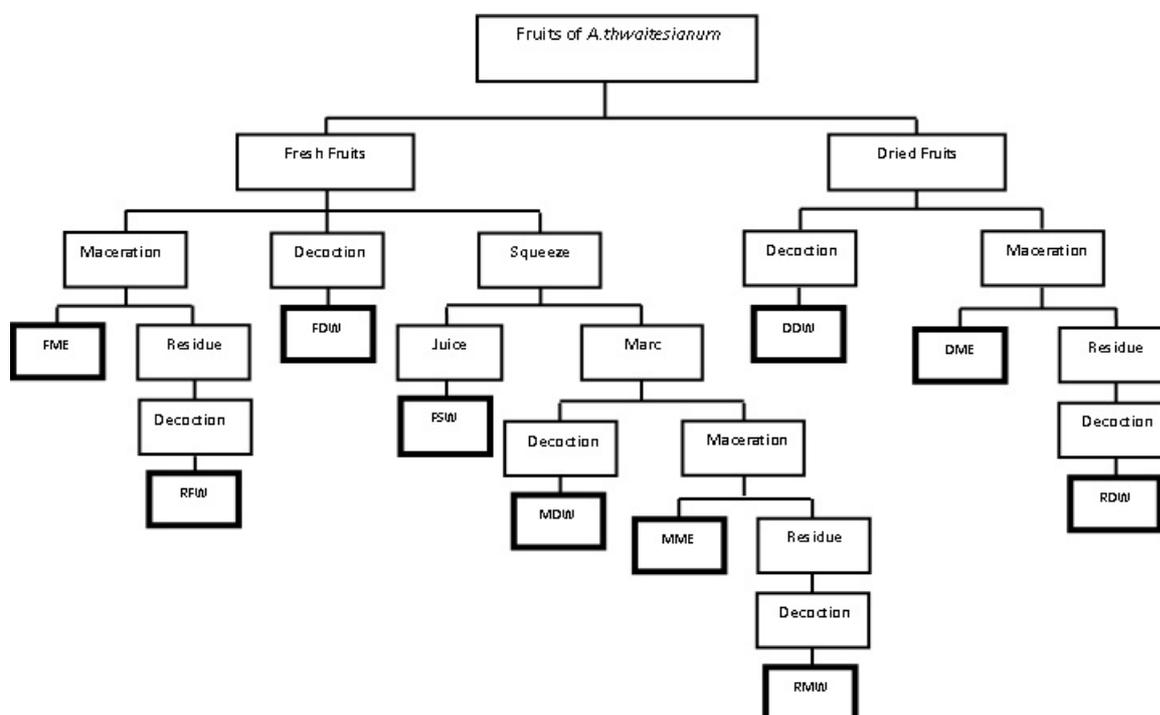*A. thwaitesianum* fruits were prepared by various extraction methods as shown in Figure 1.

Fig 1: A simplified flow chart for the different extraction methods used for the preparation of the *A.thwaitesianum* fruit extracts

Fresh fruits were hand-squeezed to yield juice (FSW), which was subsequently filtered through Whatman no. 1 filter paper. The solid residue of fresh fruits after pressing for juice was called marc.

Maceration of fruit

Fruits and marc were dried using a hot air oven at 50 °C. Fresh fruits, dried fruits and dried marc were separately macerated in 95 % ethanol for 3 days to obtain FME, DME and MME extracts, respectively.

Preparation of decoction

Fresh fruits, dried fruits and dried marc were boiled in distilled water for 15 min. The extracts were filtered through Whatman No. 1 filter paper and then dried by a lyophilizer to obtain FDW, DDW and MDW extracts, respectively.

The residue left after maceration of fresh fruits, dried fruits and dried marc were dried in a hot air oven at 50 °C. Each residue was then boiled in water for 15 min and the filtrate dried using a lyophilizer to obtain RFW, RDW and RMW extracts, respectively.

Cell culture

COR-L23 (human large cell lung carcinoma), A549 (human non-small cell lung adenocarcinoma), LS174T (human colon adenocarcinoma), PC-3 (human prostate adenocarcinoma), RAW 264.7 (mouse leukaemic monocyte macrophage), MCF7 (human breast adenocarcinoma), HeLa (human cervical cancer), and HL-60 (human promyelocytic leukemia) cell lines were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA).

COR-L23, A549, PC-3, HL-60 and RAW 264.7 cells were cultured in RPMI 1640 medium supplemented with 10 % FBS, 50 IU/ml penicillin, and 50 µg/mL streptomycin. LS174T cells were cultured in DMEM medium containing 10 % FBS, 50 IU/mL penicillin and 50 µg/mL streptomycin.

MCF-7 cells were cultured in MEM with Earle's salt (without glutamine) supplemented with 10 % FBS, 50 IU/mL penicillin, 50 µg/mL streptomycin and 1 % non-essential amino acid. HeLa cells were cultured in MEM with Earle's salt supplemented with 10 % FBS, 50 IU/mL penicillin and 50 µg/mL streptomycin. These cells were maintained at 37 °C with 5 % CO_2 and 95 % humidity.

Sulphorhodamine B (SRB) assay

Cytotoxicity of the extract was measured by SRB assay [8]. In the assay, COR-L23, A549 and PC-3 cells were seeded at 1×10^3 cells/well, LS174T, while MCF7 and HeLa cells were seeded at 3×10^3 cells/well. After 24 h, the extract at different concentrations was added to these cells and further incubated for 72 h. Cells were then fixed with ice-cold 40 % Trichloroacetic acid (TCA) (w/v). The plates were incubated for 1 h at 4 $^{\circ}C$, washed with water and air dried. Then 50 µL of 0.4 % (w/v) SRB solution in 1 % acetic acid was added to each well. After staining, unbound dye was removed with 1 % acetic acid, and plates were air dried. The SRB-stained cells were dissolved in 10 mM Tris base solution, and color intensity was measured at 492 nm using a microplate reader (PowerWave XS, BioTek). Growth inhibition at each extract concentration was calculated as in Eq 1.

$$\text{Inhibition (\%)} = \{(Ac - Ae)/Ac\}100 \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where Ac is the absorbance of the control (solution without extract), and Ae is the absorbance of the test extract. A dose-response curve was obtained by plotting the percent inhibition values versus extract concentrations. The concentration required for 50 % growth inhibition was determined by cubic spline interpolation and presented as IC_{50} (µg/mL).

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

Prior to performing the nitric oxide assay, the MTT assay was used to assess cytotoxicity of the extract on RAW 264.7 cells. The cells (1×10^5 cells/well) were incubated in 96-well microplates with the extract for 48 h. MTT solution was then added into these cells and further incubated for 4 h. The medium was removed, and isopropanol containing 0.04 M HCl was added to dissolve the formazan product in these cells. The absorbance (A) of formazan solution was measured with a microplate reader at 570 nm. Cell survival was calculated as in Eq 2.

$$\text{Cell survival (\%)} = \{(Ac - Ae)/Ac\}100 \quad \ldots\ldots\ldots (2)$$

where Ac is the absorbance of the control (solution without extract), and Ae is the absorbance of the test extract. A dose-response curve was obtained by plotting the percent inhibition values versus extract concentrations. The extracts were considered to be non-toxic at cell survival > 70 %.

3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay

Prior to performing nitroblue tetrazolium (NBT) assay, the MTS assay was used to determine cytotoxicity of fruit extracts on HL-60 cells. The cells (5×10^5 cells/ml) were incubated with the extracts in Hank's Buffered Salt Solution (HBSS) for 15 min and further incubated with 250 ng/mL PMA for 60 min. Thereafter, 100 µL of cells was seeded in 96-well microplates in triplicate wells, followed by addition of 20 µL of tetrazolium dye and further incubation for 4 h. The amount of formazan product was measured at 490 nm. Percent cell survival was calculated as in Eq 2 above.

Nitric oxide (NO) scavenging assay

NO concentrations were detected by indirectly measuring nitrite (NO_2^-), the stable end product of NO using Griess reagent [9]. RAW 264.7 cells were seeded at 1×10^5 cells/well in 96-well microplates and allowed to adhere for 12 h. The medium was then replaced with fresh medium containing 5 µg/mL lipopolysaccharide (LPS), followed by the addition of the extract and further incubation for 48 h. The percent inhibition for each concentration was calculated as in Eq 3.

Inhibition (%) = {(Ac − Ae)/Ac}100 …………… (3)

where Ac = difference between the absorbance of control with LPS and the one without LPS, and Ae = difference between the absorbance of extract with LPS and the one without LPS. A dose-response curve was obtained by plotting the percent inhibition values versus extract concentrations. The effective concentration of the extract required to scavenge NO by 50 % was determined by cubic spline interpolation and presented as EC_{50} (µg/mL).

Nitroblue tetrazolium (NBT) dye reduction assay

HL-60 cells were incubated for 6 days in PRMI 1640 medium supplemented with 10 % FBS and 1.3 % DMSO [10]. Intracellular superoxide formation (O_2^-) was quantified by the NBT dye reduction assay. Briefly, HL-60 cells (5×10^5 cells/ml) were incubated with various dilutions of the extracts and dissolved in 200 µL of HBSS for 15 min. Thereafter, they were incubated with 250 ng/mL PMA and 0.625 mg/mL NBT solution in HBSS for another 60 min. At the end of incubation, 2 mL of ice-cold 1 M HCl was added.

The precipitate of insoluble formazan product was dissolved in 300 µL of DMSO. The absorbance was measured at 572 nm using a microplate reader (PowerWave XS, BioTek). The $O_2^{\cdot-}$ scavenging activity for each concentration was calculated as in Eq 4.

Inhibition (%) = {(Ac − Ae) − (Ae − Ab)/(Ac − Ab)}100 …..… (3)

where Ac, Ae and Ab are the absorbance of a reaction mixture containing only PMA, both PMA and extract, and neither PMA nor extract, respectively. The scavenging activity of the extracts against superoxide was expressed as EC_{50} (µg/mL).

ABTS radical scavenging assay

Total antioxidant capacity of the extracts was assayed according to Re *et al* [11]. $ABTS^{\cdot+}$ solution was produced by reacting 7 mM ABTS stock solution in distilled water with 2.45 mM potassium persulfate. The mixture was allowed to stand in the dark at room temperature for 12 - 16 h. The $ABTS^{\cdot+}$ solution was diluted with distilled water to get the absorbance of 0.68 - 0.72 at 734 nm before use. The assay was performed in 96-well microplates by mixing $ABTS^{\cdot+}$ solution with 10 µl of the extracts or standard trolox. The reaction was carried out for 6 min, and then the absorbance was measured at 734 nm using a microplate reader (PowerWave XS, BioTek). The scavenging activity of the extracts against $ABTS^{\cdot+}$ was expressed as EC_{50} (µg/mL) and trolox equivalent antioxidant capacity (TEAC) (mg trolox equivalents/g extract).

Ferric reducing antioxidant power (FRAP) assay

The chelating activity of the extracts was measured using the procedure described by Benzie and Strain [12]. To prepare FRAP solution, 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride hexahydrate and 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl were mixed in a volume ratio of 10:1:1. In 96-well microplates, 20 µL of the extract was added in triplicate, followed by the addition of 180 µl of FRAP solution. The absorbance was measured at 593 nm using a microplate reader (PowerWave XS, BioTek). The ferric reducing ability is determined from a standard curve of ferrous sulfate in FRAP assay. A FRAP value was expressed as mg Fe (II) per 1 g of dry extract (mg Fe (II)/ g extract.

Determination of total phenolic content

The extract and a standard (20 µL) were transferred to 96-well microplates. Then 100 µL of Folin-Ciocalteau's reagent and 80 µL of 7.5 % (v/w) sodium carbonate solution were added and mixed. The plate was incubated for 30 min at room temperature 25 °C. Absorbance at 765 nm was measured using a microplate reader (PowerWave XS, BioTek). A calibration curve of gallic acid was constructed to determine the amount of total phenolic content, which was expressed as mg of gallic acid equivalent (GAE) per 1 g of dry extract [13].

Determination of total flavonoid content

The extract and a standard (20 µL) were transferred to 96-well microplates. Then 60 µL of methanol, 10 µl of 10 % (w/v) aluminium chloride, 10 µL of 1 M potassium acetate and 120 µL of distilled water were added, mixed and incubated at room temperature for 30 min. Absorbance was determined at 415 nm. A calibration curve of quercetin was constructed to determine the amount of total flavonoid content, which was expressed as mg of quercetin equivalent (QE) per 1 g of dry extract [14].

Statistical analysis

Data are expressed as mean ± SD (n = 3). IC_{50} and EC_{50} values were calculated using GraphPad Prism software (version 4.03). Student t-test and one-way ANOVA followed by Duncan's or Dunnett's T3 was used to compare all groups. Statistical analysis was performed using SPSS for Windows (version 16.0). Statistical significance was set at a level of $p < 0.05$. For correlation analysis, Pearson's correlation coefficient (r) with p-value between antioxidant activities and contents was identified. $P < 0.05$ was considered statistically significant.

RESULTS

Cytotoxicity against six human cancer cell lines

Among the extracts, the ethanol extracts of fresh fruits (FME) and marc left after squeezing fresh fruits (MME) exerted concentration-dependent inhibitory effects on human breast MCF7 cells with an $IC_{50} < 100$ µg/mL. In addition, the ethanol extracts of dried fruits (DME) and the extract obtained by decocting the residue left after maceration of dried fruits (RDW) conferred these inhibitory effects on lung large cell carcinoma COR-L23 cells with a similar range of IC_{50} values (Table 1). In contrast, the rest of the extracts caused < 50 % growth inhibition in all six tested cancer cell lines at the highest concentration tested (100 µg/mL) (Table 1).

Antioxidant activity based on chemical methods

All of the tested extracts displayed ABTS radical-scavenging and ferric-reducing activities in a dose-dependent manner with their specific IC_{50} value (Table 2). Among these extracts, the extract obtained by decocting the residue left after maceration of marc (RMW) showed the best activity in scavenging ABTS free radicals with an EC_{50} value of 38.93 µg/ml and a TEAC value of

Table 1: Cytotoxicity activity (IC_{50} µg/mL ± SD) of *A. Thwaitesianum* fruit and fruit waste (marc and residue) extracts against six cancer cell lines using the SRB assay (n = 3)

Fruit Types	Fruit and Fruit waste Extracts	Cytotoxicity (IC_{50} µg/mL)					
		A549	COR-L23	PC-3	HeLa	MCF-7	LS 174T
Fresh Fruits	FME	>100	>100	>100	>100	90.34±0.53[b]	>100
	FDW	>100	>100	>100	>100	>100	>100
	FSW	>100	>100	>100	>100	>100	>100
	MME	>100	>100	>100	>100	81.70±0.70[a]	>100
	MDW	>100	>100	>100	>100	>100	>100
	RFW	>100	>100	>100	>100	>100	>100
	RMW	>100	>100	>100	>100	>100	>100
Dried Fruits	DME	>100	94.15±0.80[b]	>100	>100	>100	>100
	DDW	>100	>100	>100	>100	>100	>100
	RDW	>100	86.06±0.81[a]	>100	>100	>100	>100

Different superscript letters (a, b) within the same column are significantly different from one another (p < 0.05)
A549 (human non-small cell lung adenocarcinoma), COR-L23 (human large cell lung carcinoma), PC-3 (human prostate adenocarcinoma), HeLa (human cervical cancer), MCF7 (human breast adenocarcinoma), LS174T (human colon adenocarcinoma)

142.04 mg trolox equivalents/g extract (Table 2). RMW also exhibited the strongest reducing power with a FRAP value of 264.77 mg Fe (II)/g extract.   In the two chemical methods, BHT was used as positive control for comparison.

Antioxidant activity by cell-based methods

Among the tested extracts, only the extract obtained by decocting the residue left after maceration of fresh fruits (RFW) exhibited superoxide radical-scavenging activity in a dose-dependent manner with an EC_{50} value of 196.25 µg/mL. The majority of the extracts caused < 50 % inhibition in superoxide production in HL-60 cells at the highest concentration tested (500 µg/mL). However, this scavenging activity of fresh fruit juice (FSW), the extract obtained by decocting dried marc (MDW) and the extract obtained by decocting the residue left after maceration of marc (RMW) was unable to be determined correctly by NBT assay. This is because these extracts at high concentrations of 250 and 500 µg/ml caused more than 30 % of dead cells, as detected by MTS assay (data not shown). In addition, only the ethanol extracts of fresh fruits (FME) and dried fruits (DME) exerted nitric oxide-scavenging activity with EC_{50} values of 76.68 and 90.49 µg/mL, respectively.   In contrast, the rest of the extracts showed < 50 % inhibition in NO production in RAW 264.7 cells at the highest concentration tested (100 µg/mL). The cytotoxicity of all the tested extracts was also determined by MTT assay to ensure that

there was no~ 30 % cell death, which would cause false-positive results of NO assay. No such cytotoxicity was observed (data not shown). In the two cell-based methods, propyl gallate and indomethacin were used as positive control for comparison in NBT and NO assays, respectively.

Total phenolic and flavonoid contents

The contents of total phenolics and flavonoids in *A. thwaitesianum* fruit and fruit waste extracts ranged from 22.67 - 85.77 mg GAE/g extract and 0.09 - 2.58 mg QE/g extract, respectively. Moreover, these contents in the extracts strongly correlate with their scavenging activity against ABTS free radicals (r = 0.855, *p* < 0.05) and reducing power (r = 0.977, *p* < 0.05), thus indicating specific contributions of phenolics and flavonoids to these two activities.

DISCUSSION

To the best of our knowledge, only the 12 % ethanol extracts of stems and branches of *A. thwaitesianum* has been shown to be cytotoxic to MDA-MB 435 human breast carcinoma cell line [15].

This is the first report of the cytotoxicity of *A. thwaitesianum* fruit and fruit waste (marc and residue) extracts against lung and breast cancer cells. Over the past few decades, plant extracts have been increasingly reported to exert different antioxidant activities through various

Table 2: Antioxidant activities as well as phenolic and flavonoid contents of *A. thwaitesianum* fruit and fruit waste extracts

Fruit & Fruit waste Extracts	DPPH scavenging activity		FRAP (mg Fe(II)/g)	O_2^- scavenging activity(EC_{50} µg/ml)	NO scavenging activity (EC_{50} µg/ml)	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
	EC_{50} (µg/ml)	TEAC (mg Trolox/g)					
FME	>100	31.69±0.58[g]	55.60±0.76[i]	>500	76.68±0.50[a]	36.17±0.68[e]	0.47±0.20[d]
FDW	88.02±0.49[f]	59.49±0.75[e]	117.81±0.21[f]	>500	>100	30.18±1.00[e,f,g]	0.09±0.01[f]
FSW	>100	36.65±0.64[f]	75.98±0.41[h]	ND	>100	22.67±0.06[g]	0.09±0.01[f]
MME	97.08±0.96[g]	60.38±0.95[e]	117.19±0.50[f]	>500	>100	33.63±0.35[e]	0.43±0.09[d,e]
MDW	47.72±0.95[c]	111.29±0.81[c]	221.56±0.11[c]	ND	>100	59.50±0.32[b]	1.22±0.02[b]
RFW	59.96±0.51[d]	100.63±0.92[c]	183.68±0.64[d]	196.25±0.47[b]	>100	48.88±0.10[c]	0.66±0.18[c]
RMW	38.93±0.85[b]	142.04±0.62[b]	264.77±0.31[b]	ND	>100	85.77±0.34[a]	2.58±0.13[a]
DME	>100	36.15±0.24[f]	54.81±0.78[i]	>500	90.49±0.44[b]	25.77±0.80[f,g]	0.18±0.05[f]
DDW	81.46±0.14[e]	74.71±0.40[d]	134.03±0.84[e]	>500	>100	40.91±0.52[d]	0.37±0.02[d,e]
RDW	98.76±0.56[h]	59.43±0.37[e]	101.66±0.59[g]	>500	>100	32.38±0.23[e,f]	0.25±0.03[e,f]
BHT	5.66±0.26[a]	395.99±0.99[a]	451.23±0.18[a]	-	-	-	-
Propyl gallate	-	-	-	21.98 ± 1.98[a]	-	-	-
Indomethacin	-	-	-	-	92.23±1.19[b]	-	-

ND = Not detected. Different superscript letters (a-i) within the same column are significantly different from one another (p < 0.05)

mechanisms of action [16]. For this reason, a method for measuring a specific mode of antioxidant action should not be used to determine their antioxidant activities. In this study, the two chemical methods for detecting different mechanisms of antioxidant action were used and demonstrated clearly that RMW conferred the greatest activity in scavenging ABTS free radicals and reducing ferric ions among the tested extracts.

Consistent with our previous results, RMW possessed the highest scavenging activity against DPPH free radicals [17]. Although chemical assays offer advantages of simplicity and cost efficiency, they do not reflect the cellular physiologic conditions [18]. Thus, cell-based assays are increasingly employed to evaluate potential antioxidant activity of plant extracts or compounds because they reflect more closely *in vivo* conditions [19]. In this study, the results obtained by the cell-based assays were different from those obtained by the two chemical assays. Moreover, total phenolic and flavonoid contents, which appeared to correlate strongly with only antioxidant activities in the chemical assays cannot be used to indicate intracellular antioxidant activities accurately. Thus, it is possible that different antioxidant mechanisms were attributed to not only contents but also specific subtypes of these compounds obtained by different extraction methods. Phenolics and flavonoids have been increasingly reported to exert beneficial effects on human health due to their potent antioxidant activities [20].

As cellular assays allow evaluation of antioxidant capacity of any particular compound or plant extract under biologically relevant conditions, aspects of membrane partitioning, bioavailability, distribution, and metabolism of such a compound or plant extract are also taken into account [21]. These factors contribute mainly to inconsistent data between chemical and cell-based assays [22]. More importantly, the antioxidant capacity involving such factors can extrapolate the performance of a compound or extract in vivo more precisely. Therefore, FME, DME and RFW are more likely to provide antioxidant health benefits despite exerting low antioxidant capacity against synthetic radicals in the chemical assays. Overall, the present study suggests that the extracts of *A. thwaitesianum* fruits and fruit waste (marc and residue) obtained by different extraction methods may contain various mixtures of antioxidants, especially phenolics and flavonoids, which result in their diverse antioxidant activities against synthetic and/or natural free radicals.

CONCLUSION

This is the first study to show that the ethanol extracts of *A. thwaitesianum* fresh and dried fruits conferred both cytotoxic and antioxidant activities using the cell-based assays. Thus, the findings indicate that these fruit extracts possess great potentials for use in the development of natural nutritional supplements that are safe, effective and of high quality to prevent oxidative stress-induced diseases.

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