IJPSR (2017), Vol. 8, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 July, 2016; received in revised form, 19 August, 2016; accepted, 02 September, 2016; published 01 January, 2017

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF PEELS, PULPS AND SEED KERNELS OF THREE COMMON MANGO (*MANGIFERA INDICAL* L.) VARIETIES IN SRI LANKA

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Keywords:

Mango; Peel, Pulp, Seed Kernel, Phenolic Content, Flavonoid Content, Antioxidant Activity, Anti-Inflammatory

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ABSTRACT: Mango products are well demanded by consumers due to their unique flavor and mango pulps are developed as various products while mango peels and seeds are the major agro-industrial wastes in canned fruit manufacture and fruit juice processing. Hence the aim of the present study was to investigate on their bioactive phenolic compounds in mango byproducts. Extracts of pulps, seed kernels and peels were prepared using ethyl acetate. Total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity and the anti-inflammatory activity were evaluated. The higher TPC was observed in the peels (52.67-275.61 mg GAE/ g of extract) and the seed kernels (132.95-270.56 mg GAE/ g of extract) than that of the pulp while the highest TFC was present in the pulps (120.20-479.80 mg QE/g of extract). The extracts of mango seed kernels and peels exhibited greater antioxidant activity on DPPH, ABTS, NO, FRAP assays and higher anti-inflammatory activity with IC_{50} values of seed kernel and peel extracts were found in the range of 7.73-222.10 µg/mL and 11.86 to 197.29 µg/mL respectively. The present study demonstrates significantly high antioxidant and anti-inflammatory potential uses of mango byproducts as sources of bioactive compounds.

INTRODUCTION: Phenolic compounds including flavonoids and other pigments are known to produce as secondary metabolites in fruits. Several studies have shown that the content of phenolic compounds is higher in byproducts mainly peel and seeds with respect to the edible tissue of several fruits [1]. The utilization of fruit byproducts are becoming popular as many researchers have shown that the peels and seeds contained more biologically active compounds than that of the main consumer fractions.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(1).70-78

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (1).70-78

Those compounds are mainly phenolic compounds responsible for their antioxidant, anti-inflammatory, anti-carcinogenic and other health promoting activities [2, 3]. It is evident that many synthetic compounds used in food industries as food additives have some side effects such as toxicity and carcinogenicity [4, 5].

There is a growing interest for natural sources of antioxidants and anti-inflammatory compounds due to the increased potential health risk associated with synthetic antioxidants and anti-inflammatory compounds. Therefore there is an increasing demand for bioactive compounds in fruits and vegetable, herbs and agro waste among the scientific community in order to search for alternatives to replace toxic synthetic compounds in food and cosmetic industry and pharmaceutical industries [6].

Mango is one of the important tropical, seasonal fruits in the world with excellent exotic flavors and health promoting activities. Mango fruits are rich source of phenolic compounds, ascorbic acid, carotenoids, exhibit good antioxidant and anti-proliferating activity [1, 7, 8]. It is reported that mango is produced commercially in more than 87 countries [9]. Asia is the main mango fruit producer in the world with 76.9 % of the total world production [10]. Mango is one of the most cultivated fruits in Sri Lanka and a considerable amount of foreign exchange is earned by exporting raw and processed mango products due to strong aroma, intense peel coloration, delicious taste and high nutritional value [10].

Presently in Sri Lanka, mangoes have been cultivated in about 26,000 ha of the whole land area and total mango production is around 96,500 tons per annum [10] and there are wide ranges of mango cultivation in Sri Lanka. Fruits of different varieties have their own characteristic flavor and taste. According to the data available in Department of Agriculture, 10 types of mango varieties are commonly grownin Sri Lanka. They are Karuthacolomban, Willard, Vellaicolomban, Ambalavi, Chembatan, Malwana, Bettiamba, Gira-amba, Peterpras and Dampara. Among those varieties, Karuthacolomban, Willard, Vellaicolomban, Ambalavi and Malwana mangoes are native to Sri Lanka. Mangoes are grown on three different agro-ecological region; dry zone, wet zone and intermediate zone. Dry zone mangoes have unique color, flavor, taste and aroma which attain high consumer demand.

Mangoes are commonly available during period from October to December and April to July in Sri Lanka. Mango fruits are processed for various products such as puree, nectar, leather, pickles, ice-cream and canned slices. Peels and seeds are major agro-industrial waste during mango processing manufacture and at present they are discarded and cause negative impact on the environment. The major objective of this study was to investigate bioactivity of ethyl acetate extracts of mango seed kernels and peels obtained from Karuthacolomban, Willard and Vellaicolomban against antioxidant and anti-inflammatory activities and compared those values with that of mango pulps.

MATERIALS AND METHODS:

Plant material:

Three mango (*Mangifera indica* L.) varieties, Willard (**Fig. 1**), Karuthacolomban (**Fig. 2**) and Vellaicolomban (**Fig. 3**) were selected and collected from Jaffna district in Sri Lanka during April to July, 2015. Mango fruits were harvested at maturity stage and kept two to three days to ripe at room temperature. Mangoes were selected according to color and free of physical defects in a maturity stage (**Table 1**).

FIG. 1: WILLARD

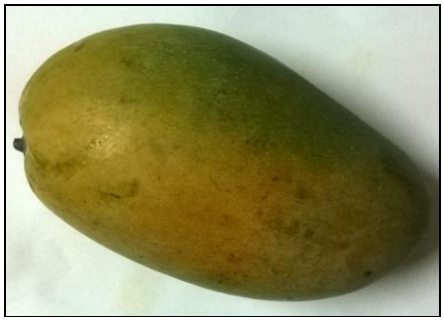

FIG. 2: KARUTHACOLOMBAN

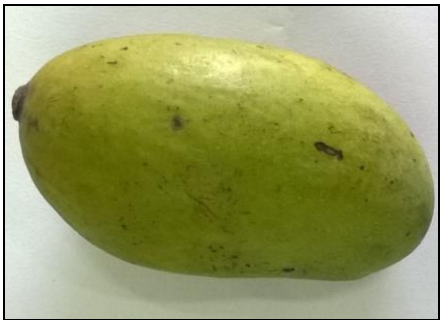

FIG. 3: VELLAICOLOMBAN

TABLE 1: PHYSICAL PROPERTIES OF WILLARD, KARUTHACOLOMBAN AND VELLAIOLOMBAN MANGO VERITIES

Physicochemical Properties	Willard	Karuhacolomban	Vellaicolomban
Firmness (N)	12.25±1.29	12.50±1.91	8.79±2.64
pH	4.54±0.27	4.57±0.01	4.08±0.57

All data are presented as mean± SD of the three replicates

Sample preparation: All fruits were washed with distilled water and air dried at room temperature. Peels and seed kernels of ripe mangoes were removed from the fruits manually. Pulp was cut into small cubes and seeds were dried at room temperature in order to obtain kernel from the seed.

Byproducts (peel and seed kernel) and pulp were used for the extraction of phenolic compounds separately.

Ethyl acetate (250 mL) was used to prepare extracts of pulp (100 g), peel (150 g) and seed kernel (150 g) separately to obtain secondary metabolites. The solvent was evaporated under reduce pressure using a rotary evaporator (RV 8V IKA –werke GmbH - Germany) at 40^{o}C and the semisolids obtained were carefully transferred into small vials. The remaining solvent in each vial was evaporated by passing nitrogen gas.

Determination of Total phenolic content (TPC):
The total phenolic content of extracts of peel, pulp and seed kernel of three mango varieties were determined by Folin Ciocalteu method with slight modification[5].The test mango sample (0.5 mL) was mixed with 0.5 mL of Folin-Ciocalteu reagent (1: 1). After 5 min, 0.5 mL Na_2CO_3 (6% w/v) followed by 2.0 mL of distilled water was added to the mixture. The mixture was kept in dark for 60 min, after which its absorbance was read at 765 nm. A gallic acid (GA) standard curve (R^2= 0.99) was used to measure the phenolic content and total phenolic content was express as mg GAE / g of dry weight of extract.

Determination of Total flavonoid content (TFC):
The flavonoid content was measured by the aluminum chloride colorimetry assay[11]. Calibration curve was constructed using Quercetine (QE) standard and total flavonoid content was express as mg QE/g of dry weight of extract.

***In vitro* antioxidant activity:**
DPPH free radical scavenging assay: This assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH$^{\bullet}$ [5]. A dilution series of each extract was (0.001-0.03 mg/mL) prepared. DPPH reagent prepared in methanol (5 mg/100 mL, 2.0 mL) was added to each test sample (1.5 mL) and mixed with 0.5 mL of methanol. The mixture was allowed to stand for 10 min in the dark and absorbance was measured at 517 nm. L- Ascorbic acid, BHT and Trolax were used as reference standards. The capacity to scavenge DPPH radical was calculated by following equation 1:

$$\text{Scavenging activity (\%)} = [1-(A_s/A_0)] \times 100$$

Where A_0 is absorbance of the control and *As* is the absorbance in presence of extracts or standard. The results were plotted as the % of scavenging activity against concentration of the sample and scavenging activity express in IC_{50}.

ABTS radical scavenging assay: This assay is based on the ability of the antioxidants to scavenge the blue-green $ABTS^{\bullet+}$radical compared to the scavenging ability [12]. The $ABTS^{\bullet+}$ radical was produced by the reaction between 1.8 mM ABTS and 0.63 mM $K_2S_2O_8$ solution, stored in the dark at room temperature for 16 hrs. Before measure the absorbance of the test fruit samples, the $ABTS^{\bullet+}$ solution was diluted with methanol to obtain the absorbance of 0.900±0.020. The test fruit extracts (50 µL) or standards (L- Ascorbic acid, BHT and Trolax) were allowed to react with 950 µL of the $ABTS^{\cdot+}$ solution. Absorbance was taken after 6 min at 734 nm. Scavenging activity of $ABTS^{\cdot+}$ was calculated using equation 1. The percentage of scavenging activity was plotted against the concentration of extracts and scavenging activity express in IC_{50}.

Nitric oxide radical ($NO^{\cdot}$) scavenging assay: Nitric oxide scavenging activity was measured using the modified method [15]. Sodium nitroprusside (SNP, 5 mM) in phosphate buffer saline (PBS) was mixed with different concentration of extracts and incubated at 25˚C for 30 min. After incubation the sample from the above were reacted with Griess reagent a (0.33% sulphonic acid in 20% glacial acetic acid). After 5 min Griess reagent B (0.1% w/v naphthyl ethylenediaminedichloride) was mixed with test sample. The absorbance of the pink chromphore formed during the diazation of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylenediaminedichloride was read at 546 nm. L-Ascorbic acid, BHT and trolax were used as positive control. The percentage of scavenging activity of $NO^{\cdot}$ for each extract and standard L- Ascorbic acid BHT and Trolax were calculated according to equation 1.

Ferric ion reducing power assay (FRAP): FRAP was conducted using the method [16]. Phosphate buffer pH 6.6 and potassium ferricyanide (1%, w/v) was added to the sample. The reaction mixture was incubated in water bath at 50˚C for 20 min. Reaction mixture was rapidly cooled and trichloro

acidic acid (10 %, w/v) was added to stop the reaction and 1 % ferric chloride solution was added. The mixture was allowed to stand for 20 min and absorbance was measured at 700 nm. L-Ascorbic acid, BHT and Trolax were used as the standard.

Anti-inflammatory assay:

Human Red Blood Cell (HRBC) Membrane stabilization assay: The blood was collected from healthy human volunteer who had not taken any anti-inflammatory drugs for 2 weeks prior to the experiment and transferred to the heparinized centrifuge tubes and centrifuged at 3,000 rpm. The blood was washed three times with equal volume of normal saline and reconstituted as 10 % v/v suspension with normal saline. The reaction mixture was prepared with 1.0 mL of test solution and 0.1 mL of 10 % RBC suspension. Aspirin was used as standard. All the centrifuge tubes containing reaction mixtures were incubated in a water bath at 56^{o}C for 30 minutes and the tubes were cooled. The reaction mixture was centrifuged at 3000 rpm for 10 minutes and the absorbance of the supernatants was measured at 540 nm using UV-Visible spectrophotometer[15]. The percentage inhibition of hemolysis was calculated using the equation 2 given below.

$$\text{Inhibition of hemolysis (\%)} = [1-(A_t/A_c)]\times 100$$

Where, A_t is absorbance at 540 nm of the control and *Ac* is the absorbance in presence of extracts or standard. The percentage stability versus test concentrations was plotted in order to compare the anti-inflammatory activity of extract with the standard compound separately and inhibition of hemolysis express in IC_{50} value.

Statistical analysis: All analyses were performed in triplicate and data were reported as mean±SD. One way ANOVA was used to determine the difference of means, and $p \leq 0.05$was considered to be statistically significant. This statistical analysis were carried out using Microsoft Excel. Minitab15 was used to calculate IC_{50} values.

RESULTS AND DISCUSSION: Previous studies demonstrated that fresh-cut mango byproducts (peel and seed) signified 40 % of the total weight of fruit [16]. In 2005, it has been reported that large percentage of byproducts, 33 % - 85 % of unused pulp, 7 % - 24 % of peel and 9 % - 40 % of seed have been generated during industrial production of processed mangoes [17]. The byproducts of mangoes in Sri Lanka is wasted and considerable amounts of mango byproducts generates during production of processed mangoes. In the present study, bioactivity of byproducts of selected Sri Lankan mango varieties were tested in order to investigate whether they can be used to develop value added products as these byproducts have no further use in Sri Lanka.

Ethyl acetate extracts were prepared from peels, seed kernels and pulp of three mango varieties, Willard, Karuthacolomban and Vellaicolomban in Sri Lanka separately and the percentage weights of the extracts are presented in **Table 2**. The results reveal that extract obtained from peels of Karuthacolomban has the highest weight percentage and these extracts were used to evaluate TPC, TFC, antioxidant activities and anti-inflammatory activity as parameters for bioactive components in the ethyl acetate extracts.

TABLE 2: THE WEIGHT PERCENTAGES OF THE ETHYL ACETATE EXTRACTS OBTAINED FROM THREE MANGO VARIETIES.

Extracts	Willard (%)	Karuthacolomban (%)	Vellaicolomban (%)
Peel	3.11	5.47	2.52
Pulp	0.18	0.22	0.11
Seed kernel	6.83	4.98	4.34

Total phenolic content (TPC) and total flavonoid content (TFC): Plant polyphenolics have ability to act as hydrogen atom donor, reducing agent and singlet oxygen scavenger [5, 18]. Phenolic compounds are some of the most important biologically active compounds present in mango peels and seeds. It has been reported that high antioxidant activity of mango byproducts are associated with some of these phenolic compounds. Phenolic compounds have the ability to scavenge free radicals by hydrogen donation or electron donation and against oxidative damages. TPC of the ethyl acetate extracts of peels, seed kernels and pulp of the three mango varieties were determined using Folin-

Ciocalteu (FC) colorimetric method. The TPC of respective extracts is shown in **Table 3**. The TPC of peel, seed kernels and pulp were ranged from 52 to 275 mg GAE/g, 132 to 270 mg GAE/g and 16 to 110 mg GAE/g respectively. Willard peel and seed kernels showed the highest TPC and these values were not significantly different from each other ($p \geq 0.05$). Nevertheless, there is a significant difference between TPC of peel extracts obtained from the three mango Varieties ($p \leq 0.05$) and the lowest TPC was observed in the Vellaicolomban peel extract (52.67 ±2.43 mg GAE/g) (**Table 3**).

Flavonoids are another group of phenolic compounds present in fruits and vegetables with higher antioxidant activity [11]. TFC in ethylacetate extract of peels, seed kernels and pulps were in the range of 140 to187 mg QE/ g of extract, 98 to 110 mg QE/g of extract and 120 to 479 mg QE/g of extract respectively. As shown in **Table 3**, pulp of Vellaicolomban showed the highest TFC 479.80±15.30 mg QE/g extract and this value is significantly different from TFC of other two test mango varieties ($p \leq 0.05$). The results also reveal that TFC content in Vellaicolomban was approximately four fold higher than seed extracts and two fold higher than peel extract. Our results showed that mango peel contained more phenolics and flavonoids than mango pulp which is consistence with previously reported data [1].

Although bioactivity of byproducts of mango has not been studied in Sri Lanka before, the results obtained for TPC and TFC from the present study were compared with previously reported data. TPC and TFC of 80% ethanol extract of ripe mango peel collected in Korea ranged from 55 to 100 mg/g and 26.9±3.76 mg GAE/g of the extract respectively[1]. A study carried out in Mexico indicated that TPC and TFC in 80 % acetone extract of ripe mango peels ranged from 145 to 168 mg GAE/g and 55 to 91 mg QE/g respectively [18]. Hence the highest phenolic compound in mango peel was reported from the peels obtained from Malaysia (537.70 ± 10.01mg GAE/g extract weight) [19].

In our study, the results revealed that mango seed kernels contained high phenolic compounds than that of peels except for Willard. Similar results were indicating total phenolic content of the mango seeds and peels as 153 mg/g and 123.80 mg/g respectively [20]. Nevertheless the highest phenolic content in the seeds were reported as 346 mg GAE/g [18]. Which is significantly higher value than that of the present study ($p \leq 0.05$).

TABLE 3: THE TOTAL PHENOLIC AND FLAVONOID CONTENTS OBTAINED FOR ETHYL ACETATE EXTRACT OF THREE MANGO VARIETIES IN SRI LANKA.

Test samples		TPC mg GAE/g of extract	TFC mg QE/g of extract
Peel	Willard	275.61±5.24^{a,b}	140.56±14.23^{a}
	Vellaicolomban	52.67±2.43^{c}	161.92±27.10^{b}
	Karuthacolomban	86.69±3.25^{d}	187.65±17.05^{c}
Pulp	Willard	110.88±6.62^{e}	120.20±10.29^{d,h}
	Vellaicolomban	16.67±3.34^{f}	479.80±15.30^{e}
	Karuthacolomban	31.36±3.84^{g}	176.70±40.97^{d}
Seed kernel	Willard	270.56±4.89^{a,g}	108.07±16.53^{f,g,d}
	Vellaicolomban	132.95±3.59^{g,h}	98.34±11.60^{f}
	Karuthacolomban	187.63±11.18^{b,h}	110.11± 7.22^{g,h}

All data are presented as mean± SD of the three replicates. GAE- gallic acid equivalent, QE- Quercetin equivalent. Mean followed by different letter in the same column differs significantly ($p \leq 0.05$).

Antioxidant capacity of the fresh-cut mango byproducts extracts: Antioxidant activities of extracts of the three mango varieties were determined using DPPH, ABTS, NO and FRAP assays. **Table 4** show the antioxidant capacity of the extracts obtained from peels, seed kernels and pulps of three mango varieties. A significant effect of the three evaluated mango varieties was found ($p \leq 0.05$).

DPPH radical scavenging activity: DPPH radical is one of the few commercially available stable organic nitrogen radical. DPPH radical scavenging model is widely used common method to evaluate antioxidant activity. This assay is based on hydrogen donating ability or radical scavenging ability of extract in alcoholic medium and it yields color change from purple to yellow [20, 22]. Among the standards, highest DPPH radical scavenging activity was shown by Trolax. In the present study, the results revealed that the ethyl acetate extracts of seed kernel and peel exhibited effective free radical scavenging activity than that of pulp extracts (**Table 4**).

Except extracts of pulp of Karuthacolomban and Vellaicolamban, all other seven extracts showed significantly ($p > 0.05$) higher activity than that of the standard, BHT (32.26±2.41µg/mL). Karuthacolomban seed kernel gave the lowest IC_{50} value (7.73±0.26 µg/mL) indicating the highest radical scavenging activity among the test extracts and the IC_{50} value obtained was significantly lower than that of standard L-Ascorbic acid (10.97 ±0.22 µg/mL) ($p \leq 0.05$).

A significantly lower DPPH radical scavenging activity was observed for pulp of the three mango varieties ($p > 0.05$) than that of the peel and the seed kernels. DPPH radical scavenging activity of 80 % acetone extracts obtained from peels of two mango varieties, Rasapuri and Badami were significantly different from each other ($p \leq 0.05$) with IC_{50} values 1.83± 0.02 µg of GAE and 3.67±0.06 µg of GAE respectively [4]. The radical scavenging activity of ripe mango peels increased with increasing concentrations, with 3.99%, 6.67%, and 81.86% scavenging activity for 12.5 µg/ml, 25 µg/ml, and 50 µg/ml extracts, respectively [1].

ABTS radical cation- scavenging assay: ABTS assay based on electron transfer ability of the test sample with long life and reactive radical anion 2,2-azinobis (3 - ethylbenzothiazoline - 6-sulfonic acid). $ABTS^{\cdot+}$ is applicable for both liophilic and hydrophilic compounds analysis. In this assay, ABTS oxidize by persullfate and form green intense color radical cation $ABTS^{\cdot+}$ which has a characteristic spectrum at λ_{max} 734 nm [12, 22]. The ABTS radical cation-scavenging activities of mango extracts obtained from peel, pulp and seed kernel of the test mango varieties are shown in **Table 4**. Mango peel extracts and seed kernel extracts exhibit good scavenging activity than that of pulp extracts. Peel extract of Vellaicolomban and seed extracts of all three verities showed higher ABTS radical cation- scavenging activity compared to the standard, Trolax. IC_{50} value obtained for Willard peel and Karuthacolomban peels were 186.68 ± 13.63 µg/mL and 186.07±11.36 µg/mL respectively (**Table 4**). These results suggest that there are no significant different between ABTS radical cation scavenging between Willard peel and Karuthacolomban peel ($p>0.05$). The $ABTS^{\cdot+}$ assay indicates that seed kernel of Karuthacolomban (46.22±1.82 µg/mL) showed significantly highest radical scavenging activity as IC_{50} of the positive control, trolax was 136.92±3.29 µg/mL ($p \leq 0.05$).

Our results were compared with previously reported ABTS radical cation- scavenging activity of mango byproducts and it was reported that ABTS radical cation- scavenging activity of ripe mango peel increased in a dose – dependent manner and it was indicated that ripe mango peel showed more radical scavenging activity than ripe mango pulp of 80% ethanol extract obtained from Irwin variety [1]. Our results also consistent with the previously reported results.

Nitric oxide radical ($NO^{\cdot}$) scavenging assay:
Excess production of nitric oxide free radical in body cause several diseases such as inflammation, cancer and other pathogenic conditions. Therefore natural products that can inhibit the nitric oxide formation is important to maintain good health condition of the human body [21, 23]. In this assay, Nitric oxide generated reacts with sodium nitroprusside under aerobic condition to produce stable product through intermediate NO_2, N_2O_4 and N_3O_4 [13]. The results obtained in the present study indicate that pulp extract of Vellaicolomban did not show inhibitory effect against nitric oxide radical scavenging assay at the range of 100 to 500 µg/mL (**Table 4**).

Karuthacolomban peel and Vellaicolomban peels extracts showed the NO scavenging activity and no significant differences were observed between two extracts ($p \geq 0.05$). When compared the NO antioxidant activity in peel extracts of the three mango varieties, a significant difference was

observed ($p < 0.05$) and the highest NO scavenging activity was given in the Vellaicolomban peel extract (47.99 ± 2.56 µg/mL). A through literature survey indicated that NO scavenging activity has not been tested to evaluate antioxidant capacity of mango byproducts.

Ferric ion reducing power assay: Ferric Reducing power assay (FRAP) was used to measure the direct electron donating ability of extracts [19]. The result visualized by measuring absorbance of blue–green color complex formed at 700 nm. Mango peel extracts and seed kernel extracts exhibit strong scavenging activity than that of pulp extracts. The peel extracts and seed extracts from the three mango verities showed significantly different ($p > 0.05$) IC_{50} values of ferric reducing ability, ranging from 24 µg/mL to 88 µg/mL and 23 µg/mL to 52 µg/mL respectively (**Table 4**). IC_{50} values obtained for positive controls, BHT, L-Ascorbic acid and Trolax were 47.66±2.59 µg/mL 51.83±0.94 µg/mL and 49.7±3.81 µg/mL respectively (**Table 4**) and these values were not significantly different from each other ($p \geq 0.05$). The FRAP assay indicates that seed kernel of Karuthacolomban (23.30±1.00 µg/mL) showed a significantly higher ferric reducing ability than the standard, L-Ascorbic acid (51.83±0.94 µg/mL) ($p < 0.05$).

In previous studies, FRAP values for mango peels have been reported as 10.13 mM/100 g [24] and 37.51 ± 0.621 mM/100g [19]. The results of the present study revealed that FRAP values of mango peels were significantly higher than the previously reported data except for Willard variety (23.61±0.56 µg /mL).

TABLE 4: ANTIOXIDANT ACTIVITY OF MANGO PEEL, PULP AND SEED KERNEL OF WILLARD, VELLAICOLOMBAN AND KARUTHACOLOMBAN

Test samples		Anti-oxidant activity (IC_{50} µg / mL)			
		DPPH	ABTS	NO	FRAP
Peel	Willard	11.86 ± 0.33^{a}	188.29 ± 8.42^{a}	132.65 ± 0.06^{a}	23.61 ± 0.56^{a}
	Vellaicolomban	$18.91 \pm 0.43^{b,d}$	121.22 ± 4.63^{b}	47.99 ± 2.56^{b}	88.31 ± 1.66^{b}
	Karuthacolomban	$14.86 \pm 2.70^{b,a,c}$	187.53 ± 9.73^{a}	52.21 ± 1.35^{b}	73.19 ± 3.25^{c}
Pulp	Willard	24.67 ± 0.88^{e}	228.76 ± 14.80^{d}	$>500^{d}$	118.66 ± 2.78^{d}
	Vellaicolomban	ND	$>500^{e}$	ND	211.27 ± 3.11^{e}
	Karuthacolomban	$>500^{f}$	242.09 ± 3.21^{d}	$>500^{e}$	$>500^{f}$
Seed kernel	Willard	12.23 ± 0.18^{d}	81.06 ± 1.29^{f}	140.05 ± 1.45^{f}	52.10 ± 0.61^{g}
	Vellaicolomban	13.68 ± 0.31^{g}	101.60 ± 0.86^{g}	324.33 ± 15.60^{g}	44.94 ± 1.05^{h}
	Karuthacolomban	7.73 ± 0.26^{h}	46.22 ± 1.82^{h}	60.31 ± 2.12^{h}	23.30 ± 1.00^{a}
Standard	BHT	32.26 ± 2.41^{i}	114.83 ± 5.46^{b}	ND	$47.66 \pm 2.59^{g,h,i}$
	L-Ascorbic acid	10.97 ± 0.22^{c}	156.70 ± 6.86^{i}	22.23 ± 1.61^{i}	$51.83 \pm 0.94^{g,i}$
	Trolax	7.67 ± 0.09^{h}	136.57 ± 5.16^{j}	38.67 ± 3.94^{j}	$49.7 \pm 3.81^{g,h,i}$

All data are presented as mean± SD of the three replicates. Mean followed by different letter in the same column differs significantly ($p \leq 0.05$). Note- ND –Not Detected.

Human Red Blood Cell (HRBC) Membrane stabilization assay: Human Red Blood Cell membrane stabilization by heat-induced hemolysis method was used to assess anti-inflammatory activity of the peel, seed and pulp extracts as HRBC membrane is analogous to the lysosomal membrane. Since the erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysomal membrane [25, 26, 27]. Aspirin, anti-inflammatory drug was used as a standard to compare the anti-inflammatory properties of mango peel, pulp and seed kernel extracts. The extract obtained from peel of Karuthacolomban showed the highest anti-inflammatory activity (151.08 ± 3.57 µg/mL) when compared with other test samples and the lowest activity was observed in the extract of Willard pulp. The peel extracts from the three mango verities showed significantly different values of inhibition of hemolysis, ranging from (IC_{50}) 151 µg/mL to 197 µg/mL (**Table 5**). Except Willard pulp all eight tested extracts showed significantly ($p > 0.05$) higher anti-inflammatory activity than that of the standard, aspirin (481.39 ± 8.11 µg/mL).

TABLE 5: THE IC $_{50}$ RESULTS OBTAINED FROM ANTI-INFLAMMATORY ASSAY FOR MANGO PEEL, PULP AND SEED KERNEL OF WILLARD, VELLAICOLOMBAN AND KARUTHACOLOMBAN

Test samples		Anti-inflammatory IC_{50} (µg/ mL)
Peel	Willard	151.08 ±3.57 [a]
	Vellaicolomban	169.53±5.23 [b]
	Karuthacolomban	197.29±4.71 [c]
Pulp	Willard	>1000[d]
	Vellaicolomban	364.14 ± 11.93[e]
	Karuthacolomban	370.81±44.09 [e]
Seed kernel	Willard	248.61±33.74 [f]
	Vellaicolomban	222.10±23.09 [f]
	Karuthacolomban	128.19±5.27 [g]
Standard	Aspirin	481.39 ± 8.11 [h]

All data are presented as mean± SD of the three replicates. Mean followed by different letter in the same column differs significantly ($p \leq 0.05$).

CONCLUSIONS: In summary, the investigation of three mango varieties has demonstrated that the secondary metabolites content varies with the variety. Ethyl acetate extracts of peel and seed kernel contained more polyphenolos than pulp and exhibited good antioxidant activity by scavenge varies free radicals such as DPPH radical, ABTS radical cation, Nitric oxide radicals and FRAP different antioxidant system. In addition, it has been explain that mango peel and seed act as good anti-inflammatory reagent. When compare the peel and seed kernel, mango seed kernel exhibit more antioxidant and anti-inflammatory activity. Thus, the high phenolic content, antioxidant and anti-inflammatory activity of the mango peel and seed kernel makes them as value added product. Thus, mango seed kernel and peel, by-product of mango processing industries, can be used to develop potential value added ingredient.

ACKNOWLEDGEMENTS: The authors are grateful to the College of Chemical Sciences, Institute of Chemistry Ceylon (Grant number 14/01), Rajagiriya, Sri Lanka and University of Kelaniya (RP/03/02/06/02/2015) for financial supports for this research work.

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How to cite this article:
Kuganesan A, Thiripuranathar G, Navaratne AN and Paranagama PA: Antioxidant and anti-inflammatory activities of peels, pulps and seed kernels of three common Mango (*Mangifera indica* L.) Varieties in Sri Lanka. Int J Pharm Sci Res 2017; 8(1): 70-78.doi: 10.13040/IJPSR.0975-8232.8(1).70-78.