Evaluation of antioxidant activity and protein denaturation inhibition of selected underutilized fruits grown in Sri Lanka

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Summary

Exploiting underutilized food crops may help to strengthen food security and opens new avenues for food and pharmaceutical industries, employment and novel research concepts. Methanolic extracts of seed, peel and the mesocarp of selected fruits were analysed for their antioxidant activities and protein denaturation inhibition properties. Among the studied fruits, mesocarp extract of *Phyllanthus emblica* showed significantly highest ($p < 0.05$) levels of phenolics (1512.01 mg·kg^{-1}, expressed as gallic acid equivalents), total antioxidant capacity (7.90 g·kg^{-1}, expressed as ascorbic acid equivalents) and reducing power (362.91 g·kg^{-1}, expressed as ascorbic acid equivalents). Mesocarp extracts of *Elaeocarpus serratus* and *Phyllanthus emblica* showed significantly higher flavonoids content comparatively. Among three *Annona* spp. studied, extracts of *Annona squamosa* showed significant antioxidant properties compared to the extracts of *Annona reticulata* and *Annona muricata*. Concentration-dependent inhibition of protein denaturation was observed in all fruit extracts. Except for the extracts of *Phyllanthus emblica*, all other fruit extracts showed above 50% protein denaturation inhibition potential at the concentration of 1.0 g·l^{-1}. In conclusion, the study evidenced that seed, peel and the mesocarp parts of studied locally available underutilized fruit crops showed significant antioxidant and protein denaturation inhibition properties.

Keywords

flavonoid; reducing power; anti-inflammatory; underutilized; antioxidant activity; protein denaturation

A highly diverse variety of naturally occurring compounds in plants that exert non-nutritional benefits and are often produced in smaller quantities from primary and secondary metabolism are referred to as phytochemicals. They can be categorized as glycosides, flavonoids, proanthocyanidins, tannins, lignans, alkaloids, peptides, proteins and terpenoids [1]. The fruit, bark, leaves, stem, root, twig and sap of many fruit crops are identified for their therapeutic effects in traditional medicine since early civilizations, to treat many health complications [2]. According to BANERJEE et al. [3], the contents of bioactives varies among the different parts of fruits and, often, the discarded parts are reported to have bioactive compounds in significantly higher contents than edible parts. The therapeutic effects including antioxidant, anti-inflammatory, antiulcer, antihypertensive, anti-thrombotic, anti-diabetic, anti-obesity, anti-tumor, anti-Alzheimer's disease and many others are attributed to phytochemicals found in crops. These attract a growing attention of the world scientific community [4]. Being a tropical country, Sri Lanka has many fruit crops that are underutilized including *Atalantia ceylanica*, *Phyllanthus acidus* and *Phyllanthus emblica*. These food crops have not been extensively studied for their phytochemicals, food-based applications and many industrial uses, due to their seasonality and low availability. Many *Citrus* species in Sri Lanka contain phytochemical compounds namely alkaloids, tannins, phenols and saponins [5], which have a remarkable ability to reduce low-density lipoprotein cholesterol, tumor initiation, platelet aggregation and eicosanoid synthesis. According to BENT et al. [6], based on the efficacy and safety of *Citrus aurantium*, it can be used as a therapeutic agent for weight loss. Further, FERNANDO and SOYSA [7] showed that the leaf extracts of *Atalantia ceylanica* possess hepatoprotective activities against ethanol-induced liver toxicity of porcine liver slices. This may be due to the antioxidant properties of the plant materials.

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CORIA-TÉLLEZ et al. [8] reported that *Annona muricata* contained several phytochemicals that could fight inflammation. As reported by CHAVAN et al. [9], *Annona squamosa* showed analgesic, anti-inflammatory, antipyretic, antiulcer and abortifacient activities. *Elaeocarpus serratus* has been reported to contain many significant phytochemicals including saponins, tannins, cardiac glycosides, flavonoids and steroids [10]. In the literature, it was mentioned that *Pouteria campechiana* fruit extracts contained several carotenoid compounds, namely β-carotene, zeaxanthin and violaxanthin [11], as well as many biologically active polyphenols such as gallic acid, catechin and myricitrinin. *Phyllanthus acidus* is known to contain many phenolic compounds, have high contents of vitamin C and medicinal properties such as antipyretic, analgesic, anti-inflammatory, anti-hepatotoxic and antiviral properties [12]. Identification and characterization of bioactive compounds and bioactivities in these underutilized dietary sources for developing functional food and nutraceuticals are trending topics nowadays. There are various in vitro methods that can be used to evaluate the antioxidant properties of commodities. Single electron-transfer methods such as evaluating the total polyphenols (Folin-Ciocalteu method), 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity and reducing power of a potential antioxidant are used to evaluate the antioxidant capacity of the various plant extracts.

Denaturation of protein molecules is well described during the inflammation process such as arthritis [13]. Inhibition of protein denaturation may play an important role in the anti-inflammatory activity of various non-steroid anti-inflammatory drugs (NSAID). Effect on protein denaturation by phytochemicals available in fruit extracts compared to the effect of commonly administered steroidal and NSAID was evaluated by DHARMADEVA et al. [14]. Besides using the anti-inflammatory synthetic compounds having side effects, the community nowadays demands natural ailments with less or no side effects. Therefore, this research aimed to evaluate seed, peel and mesocarp of selected underutilized fruit species for their potential antioxidant and protein denaturation inhibition properties, which may exert therapeutic effects when exploited in functional food formulations.

MATERIALS AND METHODS

Chemicals

Rutin, gallic acid, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, phosphate buffered saline and methanol were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals used in this study were of analytical grade.

Sample preparation

Elaeocarpus serratus (Ceylon olive), *Pouteria campechiana* (Lawulu), *Annona squamosa* (Weli Aatha custard apple), *Annona muricata* (Katu Anoda soursop), *Annona reticulata* (Bullock's heart), *Citrus aurantium* (sour orange), *Atalantia ceylanica* (Yaki naran), *Phyllanthus acidus* (star gooseberry) and *Phyllanthus emblica* (Indian gooseberry) were collected at well matured and ripened stage from local home gardens at Kurunegala, Kandy and Gampaha areas of Sri Lanka. Fruits were washed and peel, flesh and seeds were separated. Each part was lyophilized using Alpha 1-2 L Dplus (Martin Christ, Osterode, Germany), ground to a fine powder and stored in amber-coloured bottles at –18 °C.

Preparation of hydro-methanolic extract

The powdered samples were weighed, 0.50 g of each sample was mixed with 20 ml methanol/water (80 %, v/v). vortexed at high speed for 5 min and then centrifuged at 2600 ×*g* for 10 min using EBA 20 centrifuge (Hettich, Tuttlingen, Germany). The extracts were filtered through filter paper Whatman No. 42 (Whatman, Maidstone, United Kingdom) and the prepared extracts were stored at –18 °C until further analysis within 4 weeks.

Total polyphenol content

The total polyphenol content of the methanolic extracts was assessed using the Folin-Ciocalteu method of SINGLETON et al. [15] with some modifications as described by JANARNY and GUNATHILAKE [16]. Folin-Ciocalteu reagent (0.5 $mol \cdot l^{-1}$, 0.2 ml) was added to 1 ml of the sample and incubated in the dark for 15 min. Then, 5 ml of 7.5% Na_2CO_3 were added to each mixture and incubated in the dark for 2 h. The absorbance was measured at 760 nm using a UV/VIS spectrophotometer 840-210800 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the content of total polyphenols was expressed as milligrams of gallic acid equivalents (GAE) per kilogram of dry weight (DW) of fruit.

Total flavonoid content

Total flavonoid content was analysed spectrophotometrically by the method reported by ZHISHEN et al. [17], with some modifications. Briefly, 1.5 ml of methanolic extract or rutin standard solution was added to 3.5 ml of distilled

water. Then, 0.3 ml of 5% $NaNO_2$ was added and incubated for 5 min at room temperature. Approximately 0.3 ml of 10% $AlCl_3$ was added into each solution and again incubated for 6 min at room temperature. Then, 2 ml of 1.0 mol·l^{-1} NaOH was added and the solutions were immediately made up to 10 ml by adding distilled water. The absorbance of the fruit extracts was measured at 510 nm. Total flavonoid content was expressed as grams of rutin equivalents (RE) per kilogram DW of the sample.

Antioxidant activity

DPPH radical scavenging ability

The assay of radical scavenging ability (*RSA*) was conducted according to Hatano et al. [18] with some modifications as mentioned by Janarny and Gunathilake [16], to assess the ability of the prepared methanolic extracts to scavenge the stable free radical DPPH. Methanolic solution of DPPH (1 mmol·l^{-1}, 3.9 ml), which was prepared freshly, was added to 0.1 ml of sample and mixed by vortex for 15 s. Then, it was incubated at room temperature for 30 min in the dark. DPPH solution, without a sample, was used as the control. The absorbance of the samples was measured at 517 nm and the percentage scavenging ability was calculated as follows.

$$RSA = \frac{A_0 - A}{A_0} \times 100 \quad (1)$$

where A_0 is the absorbance of the control and A is the absorbance of the sample.

Total antioxidant capacity

The total antioxidant capacity (*TAC*) of the methanolic extracts of fruits was evaluated as reported by Prieto et al. [19] with some modifications as mentioned by Kumari and Gunathilake [20]. In this method, 0.3 ml of the fruit extract was mixed with 3 ml of phosphomolybdenum reagent (4 mmol·l^{-1} ammonium molybdate, 0.6 mmol·l^{-1} H_2SO_4 and 28 mmol·l^{-1} Na_3PO_4) and incubated at 90 °C for 90 min. Subsequently, absorbance was measured at 695 nm. Ascorbic acid was used as the standard and the antioxidant capacity was expressed as grams of ascorbic acid equivalents (AAE) per kilogram DW of fruit sample.

Reducing power assay

Reducing power was determined using the method described by Oyaizu [21] with slight modifications as explained by Hettiarachchi et al. [22]. Briefly, 1 ml of methanolic extract was mixed with 2.5 ml of phosphate buffer (0.2 mol·l^{-1}, pH 6.6) and 2.5 ml of 10 g·l^{-1} potassium ferricyanide. The mixture was incubated at 50 °C in a water bath for 20 min. Then, approximately 2.5 ml of trichloroacetic acid solution (100 g·l^{-1}) was added and the mixture was centrifuged at 1 200 ×g for 10 min. Finally, 2.5 ml of the upper layer was mixed with distilled water (2.5 ml) and 0.5 ml of 1 g·l^{-1} ferric chloride solution. The absorbance of the mixtures was measured at the wavelength of 700 nm. Reducing power was expressed as grams of AAE per kilogram DW of fruit sample.

Anti-inflammatory properties

Effect on protein denaturation was assessed according to the method described by Dharmadeva et al. [14] and Gunathilake et al. [13], with minor modifications as follows. Reaction mixtures were prepared using 2.8 ml of phosphate-buffered saline (pH 6.4) and 0.2 ml of egg albumin (from fresh hen's egg). Then, 2 ml of methanolic extract from each sample (with concentrations of 0.25 g·l^{-1}, 0.50 g·l^{-1}, 0.75 g·l^{-1} or 1.0 g·l^{-1}) was mixed gently with the reaction mixtures. The solutions were incubated at 37 ± 2 °C in a water bath for 15–20 min. Later, it was heated at 70 °C for approximately 5 min and then the reaction mixture was cooled down to room temperature. The absorbance of the reaction mixture was measured before and after denaturation at 660 nm. Each test was repeated thrice and the mean absorbance was calculated. Phosphate-buffered saline without sample was used as the control. The percentage of protein inhibition (*PI*) was determined on a percentage basis concerning control using the following formula:

$$PI = \frac{A_0 - A}{A_0} \times 100 \quad (2)$$

where A_0 is the absorbance of the control and A is the absorbance of the sample.

Statistical analysis

Significant differences between the results were calculated using ANOVA (General linear model and Tukey's test) with the help of Minitab 18 (Minitab, State College, Pennsylvania, USA). Differences of means at $p < 0.05$ were considered significant. All the data collected were presented as the mean ± standard deviation for all assays where samples were analysed in triplicate.

RESULTS AND DISCUSSION

Total phenolic content

Polyphenols are the widest group of compounds among the phytochemicals. Their mole-

cules consist of an aromatic ring and one or more phenol groups. Flavonoids are a sub-group of phenolic compounds that have a structure of C_6-C_3-C_6 [23]. According to Tab. 1, among the fruits studied, the highest phenolic content (expressed as GAE) of 1512.01 mg·kg^{-1} was found in the mesocarp of *P. emblica* fruit. However, the seed fraction of the fruit was very much lower in phenolic content (12.64 mg·kg^{-1}). Compared to the mesocarp of *P. emblica*, mesocarp of *P. acidus* had much lower phenolic content (217.59 ± 20.80 mg·kg^{-1}). It has been proven that the antioxidant activity of *P. emblica* fruit is mainly due to high phenolic content [24]. Among the *Annona* species studied, mesocarp extract of *A. muricata* showed the highest total phenolic content (234.12 ± 35.40 mg·kg^{-1}) compared to that of *A. squamosa* (172.71 ± 28.00 mg·kg^{-1}) and *A. reticulate* (61.45 ± 4.80 mg·kg^{-1}). The obtained data in the present study did not show a correlation between *RSA* and phenolic content. As reported by Silva and Sirasa [25], mesocarp extracts of *A. reticulata*, *A. muricata* and *A. squamosa* showed the phenolic contents of 1991.0 mg·kg^{-1}, 865.0 mg·kg^{-1} and 7470.0 mg·kg^{-1} in fresh weight basis, respectively. The results of the latter study may differ from those of the present study due to the cultivar selected, geographical and other physicochemical differences of the samples used in the two studies. The highest total phenolic content (463.51 ± 70.10 mg·kg^{-1}) was recorded for seed extract of *A. reticulata*. The highest total phenolic content and total flavonoid content were recorded for the peel extract of *A. squamosa*, indicating that the peel is a significant source of phenolic compounds among *Annona* species studied. Both mesocarp and seed extracts of *E. serratus* showed a significantly higher ($p < 0.05$) content of total phenolics and flavonoids (Tab. 1). Peel extracts of *C. aurantium* was significantly higher ($p < 0.05$) in flavonoids compared with all *Annona* species.

Flavonoids are the most available and diverse group of phenolic compounds. They are categorized into several classes according to the degree of instauration and the degree of oxidation of the carbon segment [26]. The total flavonoid content was evaluated using aluminum chloride method and expressed as RE. Rutin is a flavonol, which is a subclass of flavonoids that is abundant in citrus fruits, berries, apples, peaches and green tea. Among the studied underutilized fruits, both *P. emblica* (28.76 ± 1.30 g·kg^{-1}) and *E. serratus* (30.42 ± 0.01 g·kg^{-1}) had the highest flavonoid contents (expressed as RE) in the mesocarp. However, *P. acidus* showed a significantly lower ($p < 0.05$) flavonoid content when compared with mesocarp of *P. emblica*. The total flavonoid content of mesocarp extracts of three *Annona* species varied according to the order: *A. squamosa* > *A. muricata* > *A. reticulata*. The total flavonoid content was much lower in *A. reticulata* and *A. muricata* seed extracts compared to *A. squamosa*. The total phenolic content in seed ranged from 7.59 ± 1.90 mg·kg^{-1} to 463.51 ± 70.10 mg·kg^{-1} (expressed as GAE). Peel extract of *A. squamosa* showed significantly higher ($p < 0.05$) flavonoid content compared with the peel extracts of the other two *Annona* species studied. Generally, peels of *Annona* species showed comparatively higher flavonoid contents than their mesocarp and seeds.

Antioxidant activity

The antioxidant activity of mesocarp, seed and peel extracts of selected fruits was evaluated with DPPH radical scavenging assay, total antioxidant capacity assay and reducing power assay, the results are shown in Tab. 2.

RSA due to fruit extracts ranged from 5.8 % to 95.9 %. *RSA* more than 95 % were observed for the mesocarp and seed extracts of *P. emblica*, whereas the extracts of *P. acidus* showed *RSA* only 12.4 % and 5.8 %, respectively. Therefore, it appears certain that *P. emblica* fruit mesocarp is superior in antioxidant activity compared to its seed and *P. acidus* fruit flesh. There was a correlation between *RSA* and the flavonoid content ($r = 0.592$). According to Nandhakumar and Indumathi [27], the presence of higher total polyphenol levels will lead to higher *RSA*. DPPH is a stable free radical. When the radical is scavenged by compounds that can donate hydrogen to form stable DPPH molecules, which are known as antioxidants, the solution colour changes from purple to yellow, which is measured using spectrophotometry [27]. Since phenolic and flavonoid compounds possess antioxidant activities, they directly scavenge the DPPH radicals. However, the present study did not show a significant positive correlation between phenolic and flavonoid contents and the DPPH radical scavenging. However, the lack of a direct relationship between *RSA* and the presence of phenolic and flavonoid compounds is in agreement with previous findings by Kainama et al. [28]. Due to structural changes in molecules, particularly when hydroxyl groups are not positioned in a way that they are freely able to donate protons and act as radical scavengers, phenolic compounds and flavonoids do not contribute to antioxidant activity [28]. Among the *Annona* species tested, mesocarp extracts of *A. squamosa* and *A. muricata* showed *RSA* nearly

Tab. 1. Total phenolic content and total flavonoid content of methanolic extracts of selected underutilized fruit species.

Common name	Scientific name	Total phenolic content [mg·kg^{-1}]			Total flavonoid content [g·kg^{-1}]		
		Mesocarp	Seed	Peel	Mesocarp	Seed	Peel
Custard apple	*Annona squamosa*	172.71 ± 28.00^{g}	281.28 ± 24.50^{d}	528.89 ± 11.40^{a}	25.62 ± 1.38^{b}	7.37 ± 0.12^{a}	59.00 ± 3.85^{a}
Soursop	*Annona muricata*	234.12 ± 35.40^{e}	7.59 ± 1.90^{e}	6.87 ± 1.10^{c}	13.57 ± 0.28^{c}	1.95 ± 0.24^{e}	15.91 ± 0.62^{b}
Bullock's heart	*Annona reticulate*	61.45 ± 4.80^{h}	463.51 ± 70.10^{c}	180.72 ± 18.00^{b}	6.56 ± 0.77^{d}	0.92 ± 0.19^{g}	14.02 ± 1.71^{b}
Ceylon Olive	*Elaeocarpus serratus*	796.94 ± 11.30^{b*}	849.75 ± 145.40^{a}	*	30.42 ± 0.01^{a*}	3.57 ± 0.70^{d}	*
Canistel	*Pouteria campechiana*	480.74 ± 23.20^{d*}	5.67 ± 1.90^{e}	*	0.30 ± 0.06^{g*}	5.79 ± 0.87^{c}	*
Indian gooseberry	*Phyllanthus emblica*	1512.01 ± 47.20^{a*}	12.64 ± 5.80^{e}	*	28.76 ± 1.30^{a*}	5.81 ± 0.06^{c}	*
Star gooseberry	*Phyllanthus acidus*	217.59 ± 20.80^{f*}	481.63 ± 31.40^{c}	*	1.39 ± 0.01^{f*}	0.36 ± 0.09^{h}	*
Sour orange	*Citrus aurantium*	755.41 ± 6.70^{c}	650.85 ± 13.60^{b}	775.36 ± 2.90^{c}	4.20 ± 0.57^{e*}	1.97 ± 0.19^{e}	7.91 ± 0.11^{c}
Yakinaran	*Atalantia ceylanica*	976.75 ± 53.50**			6.55 ± 0.07**		

Means with different superscript letters in individual columns are significantly ($p < 0.05$) different from each other. Values are presented as mean ± standard deviation, $n = 3$.
Total phenolic content is expressed as milligrams of gallic acid equivalents per kilogram of sample dry weight. Total flavonoid content is expressed as grams of rutin equivalents per kilogram of sample dry weight.
* – mesocarp and peel together, ** – whole fruit.

Tab. 2. DPPH radical scavenging ability, total antioxidant capacity and reducing power of methanolic extracts of selected underutilized fruit species.

Common name	Scientific name	DPPH radical scavenging ability [%]			Total antioxidant capacity [g·kg^{-1}]			Reducing power [g·kg^{-1}]		
		Mesocarp	Seed	Peel	Mesocarp	Seed	Peel	Mesocarp	Seed	Peel
Custard apple	*Annona squamosa*	95.2 ± 0.6^{a}	47.1 ± 0.4^{b}	95.2 ± 0.4^{a}	0.25 ± 0.02^{g}	1.56 ± 0.05^{e}	8.13 ± 0.07^{b}	3.05 ± 0.43^{f}	1.20 ± 0.04^{e}	120.15 ± 1.09^{a}
Soursop	*Annona muricata*	94.0 ± 0.7^{a}	21.6 ± 3.5^{c}	94.1 ± 0.1^{b}	0.83 ± 0.01^{e}	0.44 ± 0.04^{h}	0.74 ± 0.02^{d}	10.34 ± 1.40^{c}	5.13 ± 0.14^{d}	0.65 ± 0.07^{d}
Bullock's heart	*Annona reticulate*	74.5 ± 0.3^{b}	2.6 ± 0.9^{f}	93.6 ± 0.8^{b}	11.14 ± 0.09^{a}	1.01 ± 0.04^{f}	8.57 ± 0.05^{a}	3.56 ± 0.18^{e}	0.44 ± 0.05^{g}	8.24 ± 0.41^{c}
Ceylon Olive	*Elaeocarpus serratus*	95.2 ± 0.2^{a*}	95.2 ± 0.6^{a}	*	0.62 ± 0.02^{f*}	2.65 ± 0.03^{c}	*	79.17 ± 0.05^{b*}	6.56 ± 1.32^{c}	*
Canistel	*Pouteria campechiana*	37.2 ± 3.3^{c*}	95.1 ± 0.3^{a}	*	0.83 ± 0.03^{e*}	2.57 ± 0.05^{d}	*	2.20 ± 0.00^{g*}	13.37 ± 2.21^{b}	*
Indian gooseberry	*Phyllanthus emblica*	95.9 ± 0.6^{a*}	95.4 ± 0.3^{a}	*	7.90 ± 0.02^{b*}	3.00 ± 0.01^{b}	*	362.91 ± 2.73^{a*}	23.78 ± 0.68^{a}	*
Star gooseberry	*Phyllanthus acidus*	12.4 ± 0.1^{e*}	5.8 ± 0.8^{e}	*	2.31 ± 0.02^{c*}	0.64 ± 0.02^{g}	*	4.10 ± 0.14^{d*}	0.32 ± 0.05^{h}	*
Sour orange	*Citrus aurantium*	23.9 ± 0.5^{d}	8.1 ± 3.3^{d}	44.5 ± 3.1^{c}	1.30 ± 0.01^{d}	3.91 ± 0.04^{a}	3.85 ± 0.01^{c}	1.29 ± 0.09^{h}	0.83 ± 0.39^{f}	11.99 ± 0.18^{b}
Yakinaran	*Atalantia ceylanica*	9.1 ± 1.2**			5.04 ± 0.01**			0.20 ± 0.09**		

Means with different superscript letters in individual columns are significantly ($p < 0.05$) different from each other. Values are presented as mean ± standard deviation, $n = 3$.
Total antioxidant capacity is expressed as milligrams of ascorbic acid equivalents per kilogram of sample dry weight. Reducing power is expressed as milligrams of ascorbic acid equivalents per kilogram of sample dry weight.
* – mesocarp and peel together, ** – whole fruit.

95 % but the mesocarp extract of *A. reticulate* showed *RSA* only 74.5 %. Peel extracts of all three *Annona* species used for the study showed *RSA* nearly 95 % and the values were highly comparable with the *RSA* by peel extract of *C. aurantium* (44.5 %). *RSA* of the seed extracts of selected *Annona* fruit varieties was within the range of 2.7 % to 47.2 %. Significantly higher ($p < 0.05$) *RSA* (47.2 ± 0.4 %) was observed in the seed extract of *A. squamosa*. *RSA* by seed extracts varied according to the order: *A. reticulate* < *A. muricata* < *A. squamosa*. The *RSA* of mesocarp and seed extracts of *E. serratus* were nearly 95 % and they proved that both mesocarp and seed are very potent radical scavengers. Though the phenolic content (expressed as GAE) of *P. campechiana* mesocarp extract (480.74 mg·kg^{-1}) was higher than its seed extract (5.67 mg·kg^{-1}), *RSA* (95.1 %) was higher in its seed extract than the mesocarp extract. *A. ceylanica* whole fruit extract showed a higher total phenolic content (976.75 mg·kg^{-1}). However, relatively lower *RSA* (9.14 %) was observed in the fruit extract of *A. ceylanica*.

Fruits with high antioxidant capacities are useful in the prevention of oxidative stress-related diseases and other issues including aging in humans [2, 12]. *TAC* of the fruit extracts was evaluated using the phosphomolybdenum method. This method is based on the reduction of Mo(VI) to Mo(V) by the antioxidants and subsequent formation of a green phosphate/Mo(V) complex in an acidic pH [19]. *TAC* (expressed as AAE) of the fruit extracts was within the range of 0.25–11.14 g·kg^{-1}. Mesocarp extracts of *A. reticulate* showed significantly higher *TAC* values (11.14 g·kg^{-1}) than other fruits' mesocarp extracts. Mesocarp extracts of *P. emblica, P. acidus, C. aurantium* and *A. ceylanica* also showed high *TAC* values (Tab. 2). It can be realized that the high content of ascorbic acid available in these fruits may have contributed to high *TAC*. The qualitative tests performed by KUMARI et al. [29] revealed that *P. emblica* and *P. acidus* fruit extracts contained compounds such as flavonoids, tannins, phenolic compounds, terpenoids, alkaloids, saponins and phytosterols. Mesocarp, seed and peel extracts of three *Annona* species showed varying antioxidant capacities. MOGHADAMTOUSI et al. [30] also reported the availability of alkaloids, phenolics and flavonoids in mesocarp, seed and peel extracts of *Annona* species, which may have contributed to *TAC*. Peel extracts of *A. squamosa* (8.13 g·kg^{-1}) and *A. reticulate* (8.57 g·kg^{-1}) showed a higher *TAC* values compared with peel extracts of *A. muricata* and *C. aurantium*. The present study showed that the mesocarp extracts of *E. serratus* were lower in *TAC* comparatively. However, its seed extracts showed higher *TAC* values. According to DE LIMA et al. [31], *E. serratus* fruit extracts have high contents of ascorbic acid and carotenoids. Because of this, it is considered a phytomedicine for the prevention of atherosclerosis plaque formation. *P. campechiana*, a golden yellow-coloured fruit that is a rich source of carotenoids [14], may have an antioxidant capacity owing to ascorbic acid since the present study reported an antioxidant capacity of 0.83 g·kg^{-1} for its mesocarp extract and 2.57 g·kg^{-1} for its seed extract.

The ability to reduce any compound can be a significant indicator of potential antioxidant activity and it may represent the reducing action of phytochemical compounds upon radicals inside the human body. The assay is based on the conversion of potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}), by reducing compounds and reacting potassium ferrocyanide with ferric chloride to form a ferric-ferrous complex. Fe^{2+} is directly proportional to the reducing ability of the test sample. Among the studied underutilized fruits, the highest reducing power of 362.91 g·kg^{-1} (expressed as AAE) was reported for *P. emblica* mesocarp extract and the second-highest reducing potential (120.15 g·kg^{-1}) was shown by *A. squamosa* peel extract. However, when compared to the reducing potential of the mesocarp extract of *P. emblica*, mesocarp extracts of *P. acidus* showed significantly lower ($p < 0.05$) reducing potentials. This indicates that *P. emblica* fruit may be superior in antioxidant potential. JAMUNA et al. [32] reported a reducing potential of 615.00 g·kg^{-1}, expressed as AAE, in the aqueous extract of *P. emblica*. As reported by BASU and MAIER [33], kiwi fruit, grapes and berry fruits such as blueberry, blackberry, raspberry or strawberry, have very high antioxidant capacities and are ranked among the best antioxidant fruits in the world. However, the studied underutilized fruits have a remarkable reducing potential not less than that of the aforementioned. Among the studied peel extracts of fruits, peel extracts of *A. squamosa* showed significantly higher reducing potential than the peel extracts of *A. muricata, A. reticulata* and *C. aurantium*.

The reduction potential of the fruit extract of *E. serratus* (79.17 g·kg^{-1}) was remarkable as recorded in this study and may be a further proof of the high antioxidant content. Compared to its flesh extract, *P. campechiana* seed extract showed a significantly higher reducing potential (13.37 g·kg^{-1}). According to the present study, fruit extracts of *C. aurantium* and *A. ceylanica* did not show remarkable reduction potentials, though they were

found to contain numerous reducing compounds as reported in the literature. In the majority of the studied fruits, mesocarp and peel extract were reported to have significant antioxidant activity, higher than the seed extract, and to have contents of bioactive compounds. It may be due to the fact that all essential metabolic pathways, through which these plant secondary metabolites are produced, take place in the flesh cells of the fruit. Nevertheless, the peel is more prone to damage due to foreign substances and get attacked by insects and viruses. Often, these phytochemicals are produced as a result of defensive reactions. Hence, the peel is contains more phytochemicals than seeds. On the other hand, the seed, even covered with flesh not only with the peel, is supposed to carry essential macronutrients for reproduction.

Anti-inflammatory properties

Inflammation occurs as a response to tissue injury, infectious conditions caused by pathogens or chemical irritation. Reactive oxygen and nitrogen species (ROS and RNS) contribute in triggering immune responses and causing damage to live and healthy tissues [13]. When there is no potential for bodily antioxidant defense systems to scavenge free radicals, many risk conditions like cancer, cardiovascular diseases may occur. Proteins lose their ability to fulfill biological function when they lose their tertiary structure due to denaturation induced by heat, stress and the effect of other harmful compounds [34]. Therefore, denaturation of tissue proteins is also a cause for inflammatory reactions that occur in the body. Denaturation of proteins may induce the production of autoantigens during some disease conditions [35]. Most phytochemicals have the ability to prevent protein denaturation and thereby reduce inflammation. Hence, the effect on protein denaturation inhibition is a measure of how potent the extracts to inhibit the protein denaturation induced by heat. Fig. 1 shows the effect of methanolic extract of fruits at different concentrations on protein denaturation and it shows a concentration-dependent effect. Almost all fruit extracts showed *PI* above 50 % at 1.0 $g \cdot l^{-1}$ concentrations. However, *P. emblica* flesh extract showed significantly lower inhibition rates at selected methanolic extract concentrations compared with others. Almost all other fruit extracts showed *PI* more than 25 % at 0.25 $g \cdot l^{-1}$ concentration. None of the extracts showed *PI* more than 80 % at any concentration selected. However, these *PI* should be compared with reference standards like pharmaceutical drugs that are commonly used to prevent inflammation. Such drugs include steroidal anti-inflammatory drugs (SAID) like prednisolone and non-steroidal anti-inflammatory drugs (NSAID) like aspirin, diclofenac or ibuprofen [3, 35].

CONCLUSION

The study sheds new light on the fact that, among other studied fractions of underutilized fruits, the seed extracts of selected *Annona* species carry significant antioxidant properties which may be deployed in functional food product develop-

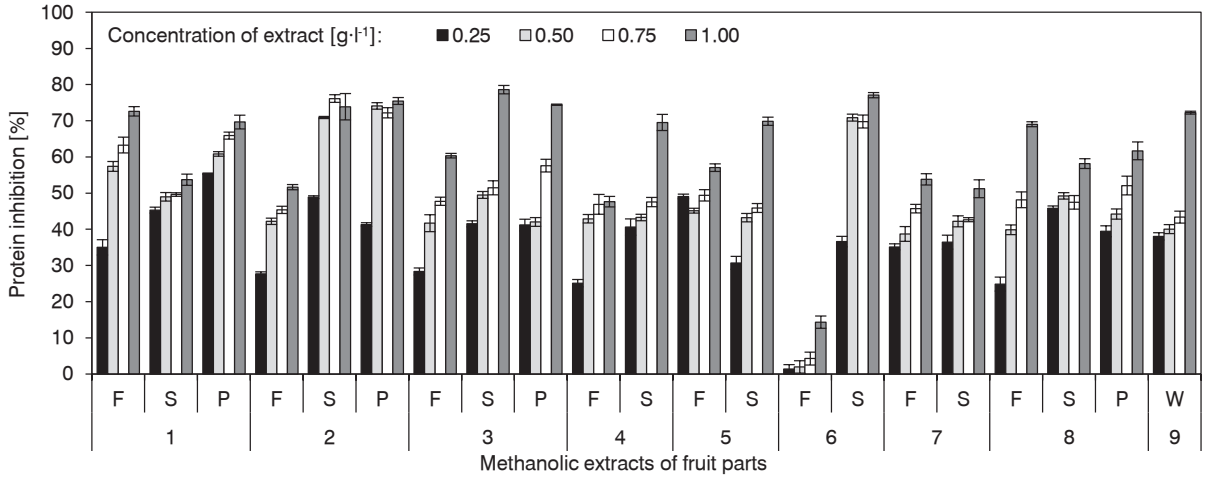

Fig. 1. Inhibition of protein denaturation by methanolic extracts at various concentrations.

1 – *Annona squamosa*, 2 – *Annona muricata*, 3 – *Annona reticulata*, 4 – *Elaeocarpus serratus*, 5 – *Pouteria campechiana*, 6 – *Phyllanthus emblica*, 7 – *Phyllanthus acidus*, 8 – *Citrus aurantium*, 9 – *Atalantia ceylanica*.
Fruit parts: F – flesh, S – seed, P – peel, W – whole fruit.

ment. *A. squamosa* flesh, seed and peel extracts have significant antioxidant properties when compared with the other two *Annona* species. *P. emblica* fruit flesh is a significant source of polyphenols, flavonoids, ascorbic acid and other phytochemicals carrying high antioxidant potential. *P. emblica* has superior antioxidant powers to *P. acidus*. In the same vein, both flesh and the seed extracts of *E. serratus* show remarkable antioxidant properties according to the present study. Except for *P. emblica* fruit extracts, almost all other fruit extracts presented in the study showed good anti-inflammatory properties at low concentrations. Nevertheless, this study has evinced that locally available underutilized fruit crops are valuable sources of polyphenols. The potential phytochemical compounds should be further investigated using novel techniques before utilization for product development.

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