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Original Article

Screening of Selected Sri Lankan Seasonal Fruit Extracts for Total Antioxidant Activity in Vitro

Amarosige Shanoli Kavindya Fernando¹, Anoja Priyadarshani Attanayake², Kamani Ayoma Perera Wijewardena Jayatilaka²

¹Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka.
²Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

ABSTRACT

The present study aimed to determine the total polyphenol content and total antioxidant activities of ten selected seasonal fruit extracts grown in Sri Lanka. The aqueous refluxed fruit extracts (4h) were used at the initial concentration of 0.05 g/mL. The total polyphenol content was determined according to the Folin-Ciocalteu method. The total antioxidant activity was evaluated by DPPH (2, 2-diphenyl-2-picrylhydrazyl), FRAP (ferric reducing power) and TBA (thiobarbituric acid assay) assays with L-ascorbic acid as the reference compound. The total polyphenol content of the fruit extracts varied from 0.81±0.02 to 17.54±0.50 mg GAE (gallic acid equivalents) per gram of dry weight. The antioxidant activities ranged in IC₅₀ of 46.60±0.60 to 367.90±4.90 μg/mL, 3.41±0.02 to 50.46±1.02 μM, 30.62±0.50 to 182.64±0.90 mg AAE (L-ascorbic acid equivalents) per gram of dry weight for DPPH, FRAP, TBA assays, respectively. All aqueous fruit extracts exerted dose dependent in vitro antioxidant activities in different degrees. Among the selected fruit extracts S. carophyllatum, P. zeylanica, A. comosus exert relatively high total antioxidant activity together with high total polyphenol content. Hence, the selected fruit extracts are deserved to be as potent sources for antioxidant lead compounds in the development of nutraceuticals using the above edible fruits.

Key words: Antioxidant assays, FRAP assay, DPPH assay, nutraceuticals, Sri Lankan fruit extracts, TBA assay

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*Address for Correspondence
Anoja Priyadarshani Attanayake, Department of Biochemistry Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka

INTRODUCTION

Fruits as plant based food have been deserved a particular interest over the last two decades. In fact, fruits possess a multitude of health benefits, widely known since pre-historic era. Notwithstanding, due to the absence of scientific validation, awareness on nutritional benefits, fruits were poorly consumed by general public, and/or recommended as dietary supplements for different disease conditions. In recent years, it is evident that the consumption of plant based foods is related to reduction in mortality in several oxidative stress related diseases, including mortality from cardio vascular disease and cancer¹. This may be due in part to the high amounts of beneficial components present in plant food such as dietary fiber, folate, antioxidants, vitamins, polyphenols, and potassium, which are associated with lower risk of cardio vascular disease. Accordingly, five daily portions of fruits and vegetables (approximately 400 g/day) have been recommended as part of a public health strategy for the prevention of chronic diseases².

Medical and nutritional research has for decades, emphasized the health benefits of consuming fruits and their role in the management/ prevention of chronic diseases/conditions such as cardiovascular disease, certain cancers, type II diabetes mellitus, obesity, chronic kidney disease, chronic lung disease, chronic obstructive pulmonary disease etc³. Further, the World Health Organization estimation data has revealed that, approximately 1.7 million (2.8%) deaths from diet related chronic diseases per annum are linked with low consumption of fruits and vegetables⁴. The importance of consumption of fruits and their involvement in the protection of cells from oxidative stress has become a debatable topic in this era⁵. In particular, phytochemicals with antioxidant potential are well-reported to play a role in reducing the consequences of oxidative stress in disease development and in aging process, thus contribute to overall health-protective effects⁶. Antioxidants are molecules which are capable of delaying or inhibiting the action of free radicals. Indeed,
antioxidants from exogenous sources are considered as important in such oxidative stress conditions, in order to prevent or slow down the oxidative stress induced by excessive production of free radicals. Although there are synthetic antioxidants, certain physical properties related to these such as high volatility, instability and carcinogenicity have shifted scientists’ attention to isolate natural antioxidants specifically from plant origin. Accordingly, fruits contain a wide variety of phytochemicals that may help to protect cellular systems from oxidative damage and to reduce the occurrence of chronic diseases. Among the identified phytochemicals, polyphenolic compounds including tannins and flavonoids are important groups of antioxidants, targeting nutritional and medicinal applications.

Fruits are considered as important components of the Sri Lankan diet for decades. Recent study shows that, there is an upward trend in the consumption of fruits by Sri Lankans for the past 30 years. With consideration of the great value of fruits as dietary sources of antioxidants and development of nutraceuticals with potent antioxidant potentials for patients with oxidative stress related diseases, the determination of antioxidant activity and identification of related antioxidants/phytochemicals of fruits seems an important area of ethno-pharmacology research. Fruits have historically held a place in dietary guidance because of their concentrations of vitamins, especially vitamins C and A; minerals, especially electrolytes; and more recently phytochemicals, especially antioxidants.

However, many of the Sri Lankan fruit extracts have not been subjected to determine the antioxidant activity. Despite the evidence put forward by studies in the recent past, there is still a need for the determination of total antioxidant activities of seasonal fruit extracts of Sri Lankan origin. The selected fruits for the investigation are as follows. Durian- Durio zibethinus L. (Bombacaceae), Pineapple- Ananas comosus L. Merr. (Bromeliaceae), Namnam- Cynometra cauliflora L. (Fabaceae), Santol- Sadoricum koefiipe L. (Meliaceae), Heen dan- Syzygium caryophyllatum L. Alston (Myrtaceae), Whild date palm- Phoenix zeylanica L. (Palmae), Strawberry- Fragaria vesca L. (Rosaceae), Rambutan- Nephelium lappaceum L. ( Sapindaceae), Kirala- Sonneratia alba Sm. (Sonneratiaeae) and Grapes- Vitis venefera (Vitaceae). The objectives of the present study were to determine the total polyphenol content of the selected aseptic fruit extracts of Sri Lankan origin and to determine the total antioxidant activities of the aseptic fruit extracts using standard assay methods.

**MATERIALS AND METHODS**

**Chemicals and reagents**

All chemicals and solvents were of analytical grade and were used without any purification.

**Instruments**

A UV visible spectrophotometer (Gallenkamp PLC, UK) and a pH meter (EUTEC, Singapore) were used for spectrophotometric measurements and for the measurement of pH respectively.

**Collection and identification of fruits**

The selected fruits were collected from Galle, Deniyaya, Negombo, Kalpitiya and from Jaffna, Sri Lanka during July to September 2016. The botanical identification of the fruits/plants was done with the use of description given by Jayaweera, 1982.

**Preparation of fruit extract**

Aqueous refluxed extracts of fruits were used in experiments. The edible fruit part (100 g) was oven dried at 40°C to a constant weight and ground to obtain the dried powder. Powdered fruit material (5.0 g) was dissolved in 400 mL distilled water and refluxed for 4 hr. The mixture was strained and the final volume was adjusted to 100.0 mL. The initial concentration of each of the fruit extract was 0.05 g/mL. A graded series of dilutions was prepared (1-500 μg/mL) for the DPPH assay.

**Estimation of total polyphenol content**

Total polyphenol content was estimated using Folin-Ciocalteu method using gallic acid as the reference compound. Briefly, fruit extract (1.0 mL) was mixed with 95% EtOH (1.0 mL), distilled water (5.0 mL) and of 50% Folin-Ciocalteu (0.5 mL) reagent. The mixture was allowed to react for 5 min and 5% Na2CO3 (1.0 mL) was added. Thereafter, it was thoroughly mixed and placed in dark at room temperature (25°C) for 1 hr and the absorbance was measured spectrophotometrically at 725 nm. Quantification was done with respect to the standard curve of gallic acid (0-50 μg/mL). The results were expressed in mg gallic acid equivalents mg GAE/g of the dry weight.

**Determination of total antioxidant activity**

Total antioxidant activity was measured by DPPH radical scavenging assay method. EtOH was used as the sample blank. A graded series of a concentration (1-500 μg/mL) of fruit extract (1.0 mL) were added to 0.004% ethanol DPPH (3.0 mL) solution. The mixture was shaken vigorously, allowed to stand at 25°C in dark for 30 min. The decrease in absorbance of the resultant solution was measured spectrophotometrically at the same wave length (Acontrol). L-Ascorbic acid was used as the reference compound. The antioxidant activity is expressed in terms of IC50 (concentration of the extract/reference compound required to inhibit DPPH radical formation by 50%). % DPPH radical scavenging activity= (Acontrol-Asample)/Acontrol*100% where, Acontrol represents the absorbance of the control without the plant/reference compound.

**Ferric reducing antioxidant potential (FRAP) assay**

FRAP assay was used as described by Benzie and Strain, 1999. The FRAP working reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl and 20 mM FeCl3 at 10:1:1 (v/v/v). The FRAP reagent (3.0 mL) and sample solution
(100 µL) were mixed and the absorbance of the resulting solution was measured (t=0) at 593 nm spectrophotometrically (A_sample t=0) against a sample blank (FRAP working reagent).

Thereafter the sample was placed in a water bath (37°C) and the absorption was measured at the same wave length after 4 min. (A_sample t=4). L-ascorbic acid (1000 µM) was used as the standard compound and preceded as in the same way.

FRAP value of the plant extract (µM) = (A_sample t=0-4)/A_standard t=0-4) x FRAP value of 1000 µM ascorbic acid.

where, (A_sample t=0-4) represents the change in absorbance in a sample from 0 to 4 min, (A_standard t=0-4) is the change in absorbance in the standard from 0 to 4 min. FRAP value of ascorbic acid is 2.

Thiobarbituric acid assay (TBA)

TBA assay was performed as described by Ottolenghi, 1959 15. Briefly, 20% trichloroacetic acid (2.0 mL) was mixed with 0.67% thiobarbituric acid (2.0 mL) and sample solution (1.0 mL) was added to the above acidic media. This mixture was boiled (100°C) in a water bath for10 min. It was allowed to cool and filtered. The absorbance of the sample was measured at 552nm using a water blank. L-ascorbic acid (100 – 500 µg/mL) was used as the reference compound. The results were expressed in ascorbic acid equivalents mg AAE/g of the dry weight.

Phytochemical screening of fruits

Preliminary qualitative phytochemical screening for the presence of saponins(a), cardenolide glycosides(b), phenols(c), alkaloids (d), flavonoids (e), tannins(f), reducing sugars(g), and proteins (h) was carried out by the reported protocol 16.16

Statistical data analysis

The triplicates of each sample were used for statistical analysis and the values were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The present study was carried out to determine the total polyphenol content and antioxidant activity of selected fruit extracts using standard assay methods. The selection of fruits for the investigation was mainly based on the availability of them during the period of July to September of the year. Most of the fruits selected for the investigation was mainly based on the availability of them during the period of July to September of the year. Most of the fruits selected for the investigation were widely consumed by the general public in the selected areas. Some of the selected fruits also have been used as a dietary supplement for the management of a number of oxidative stress related chronic disease conditions. Examples include A. comosus juice is used for the management of diarrhoea and jaundice, S. carophylatum is prescribed for diabetes mellitus, V. venefera is useful for cough etc. 11. The antioxidant potential of the most of the fruit extracts is documented in literature however, it has not scientifically proven up to date.

is measured at ambient temperature, therefore that the risk of thermal degradation of the molecules tested is

In the present study, the total polyphenol content of the fruit extracts was determined by classical Folin-Ciocalteu reagent method. Three well established antioxidant assay methods were selected to determine total antioxidant activities accordingly DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) radical scavenging assay, FRAP (ferric reducing power) assay, and TBA (Thiobarbituric assay) assay, considering the wide diversity in the antioxidants’ chemical structure 16. In addition this may optimize the antioxidant properties of a single fruit extract to a maximum possible extent.

Polyphenols are considered as a group of secondary metabolites that are mostly related to the antioxidant activity. In addition, polyphenolic compounds (including tannins and flavonoids) are the most important groups of exogenous antioxidants that have been used as nutritional and medicinal therapeutics. Polyphenols react with an oxidizing agent phosphomolybdate in Folin-Ciocalteu reagent under alkaline conditions and result in the formation of a blue coloured complex, the molybdenum blue which is measured at 650 nm spectrophotometrically. The total polyphenolic content of the selected aqueous fruit extracts were calculated by using regression analysis of gallic acid standard curve (y=0.01017x+0.004810) and the total polyphenol values of the fruit extracts were ranged from 0.81±0 to 17.54±0.50 mg GAE/g. The highest and the lowest total polyphenol content were shown in the fruit extracts of S. carophylatum and S. koeluple respectively. The total polyphenol content was increased in the ascending order of extracts of S. alba, P. zeylanica, V. venefera, F. vesca, S. carophylatum as shown in Table 1. The polyphenol content of most of the reported fruit extracts were comparable with the results obtained in the present investigation. As examples, the extracts of Dialium guineense (Leguminosae, velvet tamarind), Flacourtia inermis (Flacouriaceae, Lovi), Phyllanthus emblica (Euphorbiaceae, Nelli) the total polyphenol content was in a range of 0.77 to 12.8 mg GAE/g 17, 18, 19 as the results of this study is ranged within 0.8 to 17.5 mg GAE/g.

It’s a well-known fact that antioxidant activity varies according to the extraction method and on the solvent used for the extraction because of the presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent 9. However, in the present study, water extracts were used since the water extracts are more nutritionally and medicinally relevant for human consumption 20. Indeed, the aqueous extracts are more relevant and are being able to simulate the way of consumption since fruits are eaten in raw or used as fresh juice. In this study fruit extracts were refluxed for four hours for the maximum extraction of active phytochemicals to the relevant extract 21.

DPPH assay method is the most widely mentioned radical scavenging assay method to determine the antioxidant activities in most available recent research. It is an accurate, easy, rapid and simple method to evaluate antioxidant activity in vitro. The antioxidant efficiency eliminated. The advantage of this method is that DPPH is allowed to react with the whole sample and sufficient
time is given in the method which allows DPPH to react slowly even with weak free radical scavengers. However, it has some limitations due to DPPH radical interaction with other radicals and the time response curve to reach the steady state is not linear with different ratios of antioxidant/DPPH. The results of the present investigation demonstrated that the selected fruit extracts exert relatively high antioxidant activity as evident by the values of IC50 in DPPH assay (46.60±0.60 to 367.90 ± 4.90 µg/mL). However, the IC50 values estimated for DPPH assay in the study is not comparable mostly with the previously published reports. As example Caryota urens (Areccacea, Kithul) IC50 ranged from 6.02 to 3.73 µg/mL and for Punica granatum L (Punicaceae, Delum) IC50 is 0.182 mg/mL. The differences in IC50 values may due to the differences between methods of extraction, time of extraction, regions where the fruits were collected. In this study, L-ascorbic acid was used as the reference compound and showed the lowest IC50 value thus exhibiting highest antioxidant activity. Folin Ciocalteu method can be interfered by other oxidation substrate in a given extract in an inhibitory, additive or enhancing manner. All extracts and standard compounds exhibited concentration dependent radical scavenging activities in DPPH assay and it was ranged from IC50 values of 46.60±0.60 to 367.90 ± 4.90 µg/mL. P. zeylanica has the lowest IC50 value expressing the highest radical scavenging activity. S. koefjape has the highest IC50 value expressing the lowest radical scavenging activity in the ascending order of S. alba, D. zibebinus, S. carophyllatum, A. comosus, P. zeylanica (Table 1).

The FRAP assay is inexpensive and reagents are simple to prepare. The results are highly reproducible, and the procedure is straightforward and relatively speedy. FRAP values are obtained by comparing the change of absorbance at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Changes in the absorption are linear over a wide concentration range of natural extracts. Reducing power of each fruit extract is expressed as FRAP value which is ranged from 3.41±0.02 to 50.46±1.02 μM as shown in Table 1.

### Table 1: Total polyphenol content and antioxidant activity of ten selected fruit extracts

<table>
<thead>
<tr>
<th>Fruit extract</th>
<th>Total Polyphenol content (mgGAE/g DW)</th>
<th>DPPH radical scavenging assay IC50 (µg/mL IC50)</th>
<th>FRAP assay FRAP value (µM)</th>
<th>TBA assay (AAE/gDW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. zibethinus</td>
<td>1.46 ± 0</td>
<td>116.7 ± 1.3</td>
<td>3.5 ± 0.04</td>
<td>30.6 ± 0.5</td>
</tr>
<tr>
<td>A. comosus</td>
<td>2.48 ± 0.12</td>
<td>87.8 ± 0.6</td>
<td>4.7 ± 0.04</td>
<td>133.4 ± 0.5</td>
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<tr>
<td>C. cauliflora</td>
<td>1.30 ± 0.10</td>
<td>328.0 ± 9.0</td>
<td>24.4 ± 2.05</td>
<td>42.0 ± 0.6</td>
</tr>
<tr>
<td>S. koefjape</td>
<td>0.81 ± 0</td>
<td>367.9 ± 4.9</td>
<td>4.6 ± 0.03</td>
<td>114.8 ± 1.1</td>
</tr>
<tr>
<td>S. carophyllatum</td>
<td>17.54 ± 0.50</td>
<td>98.2 ± 1.1</td>
<td>50.5 ± 1.02</td>
<td>104.3 ± 0.5</td>
</tr>
<tr>
<td>P. zeylanica</td>
<td>3.07 ± 0.20</td>
<td>46.6 ± 0.6</td>
<td>33.3 ± 1.02</td>
<td>182.6 ± 0.9</td>
</tr>
<tr>
<td>F. vesca</td>
<td>9.181 ± 0.15</td>
<td>218.4 ± 13.2</td>
<td>25.0 ± 0.36</td>
<td>87.9 ± 0.4</td>
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<tr>
<td>N. lappaceum</td>
<td>1.26 ± 0.01</td>
<td>116.7 ± 1.2</td>
<td>3.4 ± 0.02</td>
<td>68.6 ± 0.2</td>
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<tr>
<td>S. alba</td>
<td>2.50 ± 0.10</td>
<td>142.8 ± 0.9</td>
<td>29.2 ± 0.58</td>
<td>58.9 ± 0.1</td>
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<tr>
<td>V. venefera</td>
<td>8.46 ± 0.10</td>
<td>192.5 ± 0.6</td>
<td>22.9 ± 0.00</td>
<td>72.2 ± 0.5</td>
</tr>
</tbody>
</table>

All values are the mean of three measurements and expressed as mean ± SD.

The FRAP value of the fruit extracts in the decreasing order of S. carophyllatum, P. zeylanica, S. alba, F. vesca and C. cauliflora. TBA values are ranged from 30.62±0.50 to 182.64±0.90 mg AAE/ g of dry weight. The highest and the lowest values in the TBA assay in the extracts of P. zeylanica and D. zibethinus respectively (Table 1). The TBA assay method is an easy and inexpensive method, but the use of TBARS test has received wide criticism over the years. The main problem is the lack of sensitivity and specificity, since TBA reacts with a variety of compounds in medicinal plant extracts.

The phytochemicals present in selected fruit species are mentioned in Table 2. The mentioned phytochemicals mainly polyphenols and flavonoids may be attributed to the total antioxidant activities of the selected fruit extracts.
Cyanogenic glycosides were not detected. - sign indicates the absence, + sign indicates the presence

CONCLUSIONS
All aqueous fruit extracts exert in vitro antioxidant activities in different degrees. Among the selected fruit extracts S. carophyllatum, P. zeylanica, A. comosus extracts exert relatively high total antioxidant activity together with high total polyphenol content. Hence, the selected fruit extracts are deserved to be as potent sources of antioxidant lead compounds in the development of nutraceuticals using fruits. This is the first ever report on the total antioxidant activity of the selected seasonal fruit extracts grown in Sri Lanka.

RESEARCH FUNDING
The financial assistance by National Research Foundation is greatly appreciated (NSF/RG/2007/HS/10).

CONFLICT OF INTEREST
Authors declare no conflict of interest.

REFERENCES

Table 2: Phytochemicals present in selected fruit extracts

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<th>(a)</th>
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