IJPSR (2017), Vol. 8, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 November, 2016; received in revised form, 11 January, 2017; accepted, 14 January, 2017; published 01 June, 2017

DETERMINATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *BORASSUS FLABELIFFER* LINN. FRUIT PULP COLLECTED FROM SEVERAL PARTS OF SRI LANKA

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Keywords:

Palmyra fruit pulp, Antioxidants, DPPH, Phosphmolybdenum, Phenolic compounds

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ABSTRACT: *Borassus flabeliffer* Linn. (Palmyra Palm) is widely distributed in the Northern parts of Sri Lanka. Its leaves, fruits and pith have various uses. Palmyra based industries provide living for many families in that region. Palmyra pulp obtained from the ripe fruit is used in many traditional food items in the Jaffna Peninsula and surrounding areas. It is also being used in folk medicine to cure various diseases. In this study, total phenolic content and antioxidant activity of aqueous palmyra fruit pulp extracted from fruits of Kilinochchi, Anamaduwa and Batticalo districts of Sri Lanka were evaluated. The total phenolic content of the pulp extracts of fruits obtained from Anamaduwa district showed the highest value (9.297 ±0.018 mg GAE/mg extract). DPPH Radical scavenging activity was seen highest in the Anamaduwa samples (3.065 ±0.176 mg ml^{-1}) and lowest in Batticalo sample (7.466 ±0.156 mg ml^{-1}). The total antioxidant capacity based on the phosphomolybdenum assay also indicated the highest value in the Anamaduwa sample (68.171± 2.068 mg AE/g extract) and lower value in Batticalo sample (31.134 ±0.470 mg AE/g extract).

INTRODUCTION: Oxidative metabolism is important for the survival of life [1]. Due to both exogenous and endogenous factors, reactive oxygen species (ROS) and free radicals are formed within the body. An imbalance between the production and removal of ROS causes oxidative stress [2]. The formed ROS can attack DNA, lipids and other proteins and disrupt normal cellular activities, leading to diseases such as arthritis, cancer, diabetes and neurodegenerative disorders [3]. Oxidation does not only affect human body, but also food stuffs.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(6).2701-05

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

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (6).2701-05

It is found out that oxidation is one of the main reasons leading to food spoilage, which results in rancidity, deterioration of nutritional quality, colour, falvour and texture [1, 4].

Defense mechanisms against ROS are provided by antioxidants, which inhibit the initiation or propagation of oxidative chain reactions [5]. There are several antioxidants within the body itself, which includes enzymes, hormones etc. But, these are not efficient to remove large amounts of ROS [6]. Thus dietary intake of antioxidants are important. Epidemiologic studies have shown that there is an inverse relationship between the intake of dietary antioxidant rich food and incidence of human diseases [2]. Antioxidants are explained based on their activity and capacity. Antioxidant capacity refers to the thermodynamic conversion efficiency of an oxidant, upon its reaction with an antioxidant

and antioxidant activity refers to the measure of kinetics of a reaction between the prooxidant/ radical scavenged and the antioxidant [7]. Antioxidants are categorized into primary (Chain breaking) antioxidants such as vitamins, flavonoids etc and secondary (preventive) antioxidants such as butylated hydroxy toluene (BHT), propyl gallate etc. [1, 4]

Dietary antioxidants include both natural and synthetic compounds such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA). Though these are included in processed food, they are said to have harmful effects on human health [8]. Therefore, the potential use of natural antioxidants and its isolation from herbs, spices, seeds, fruits and vegetables by extraction, fractionation and purification are widely studied [9, 10].

The palmyra palm, belongs to the family Aracaceae [11]. These palms are remarkably widespread in the drier topical regions such as Africa, South and Southeast Asia. The Asian species is identified as *Borassus flabeliffer* Linn. *Borassus flabeliffer* is cultivated for its economic uses and less commonly for its ornamental features [11]. The palm usually grows to a height of 20- 30 m and has an erect, straight, unbranched trunk, which is almost black. The trunk is broader at the base and tapers above the breast height. The diameter of the trunk is more or less uniform up to the crown. The terminal crown forms a rosette of about 30-40 fan shaped leaves, each being attached to the stem by a long fibrous petiole of 90- 120 cm in length. The palmyra palm is dioceous and the two trees cannot be distinguished from each other until it produces the inflorescence, which occurs after 15-20 years of growth [12]. The fruits of *B.flabeliffer* are usually spherical or hemispherical in shape, with a diameter between 8-15 cm. It has a smooth shiny pericarp, which is greenish when young and violet-brown when mature (**Fig. 1**). There may be 2-3 nutlets in a fruit, with a thick yellow, fibrous mesocarp [13] (**Fig. 2**).

This palm is widely spread in the Northern parts of Sri Lanka. Fruits mature during the month of August and falls off the tree within September to October. There is very few use for the ripe fruit in countries such as India, Thailand and Indonesia. It is only in Jaffna, Sri Lanka, the ripe fruit has an appreciable value [13]. The palmyra fruit pulp (PFP) extracted from the fruit is said to contain 0.42 g of amino acids (where lysine, phenyl alanine and glutamate dominate) per 100 g of pulp. The PFP is a rich source of carotenoids. Although PFP is included in several recipes, the use is limited due to the presence of a saponin named *flabelifferin* II [14]. When considering the medicinal uses, it has been widely studied to reduce blood glucose levels and as an antibacterial agent, thus utilized to produce ointments for tropical applications of ulcers and sores.[15]

As a result of such benefits, in this present study palmyra fruit pulp extracts which were collected from different areas of the Northern and North Eastern parts of Sri Lanka have been compared for their total phenolic content and antioxidant activity.

FIG. 1: MATURE *B.FLABELIFFER* FRUIT

FIG. 2: PALMYRA FRUIT SEPARATED INTO 3 NUTLETS

MATERIALS AND METHODS: *Borassus flabeliffer* (Palmyra palm) fruit samples were collected from three different districts of Sri Lanka, namely, Anamaduwa, Kilinochchi and Batticalo, during the months of August to September 2014.

Sample Preparation: The fruit samples were separated into nutlets and each nutlet was weighed. The pulp was squeezed from its fibers into a bowl using minimal amount of distilled water. The pulp was filtered through a muslin cloth to remove excess fibers. The filtered pulp was then transferred to a weighed glass beaker (**Fig. 3**) and was freeze dried. The resultant powder was named as "Aqueous extract" of palmyra fruit pulp (PFP) **(Fig. 4**), which was used for analysis. For each of the assays mentioned, a specific amount of the freeze dried product was dissolved in water, to obtain a solution.

FIG. 2: EXTRACTED PALMYRA FRUIT PULP

FIG. 4: FREEZE DRIED PRODUCT

Determination of total phenolic content: The total soluble phenols in the samples were determined using the "Folin Ciocalteu" reagent, with slight modifications. The sample/ extract (0.5ml) was mixed with 0.5 ml of Folin Ciocleteu reagent (1:1) and was kept in dark for 5 minutes at room temperature, followed by the addition of 0.5ml of 6% sodium carbonate and 2 ml of distilled water. This mixture was kept in dark for 1 hour at room temperature and the absorbance was measured at 765 nm using a UV visible spectrophotometer (Hitachi U-2910 UV/Vis). Gallic acid was used as the standard (0.01-0.06 mg ml^{-1}). The results were expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g) [8]. All the samples were analysed in triplicates.

DPPH Radical Scavenging Activity: The radical scavenging activity was determined using DPPH (1,1 diphenyl-2-picrylhydrazyl) free radical. A DPPH solution (0.006% w/v) was prepared in methanol. Different concentrations of sample/ standard (1 ml) was mixed with 2 ml of DPPH solution. This was incubated in dark at room temperature for 30 minutes and absorbance was measured at 517 nm using the UV- visible spectrophotometer.

Ascorbic acid (0.008-0.3 mg ml^{-1}) was used as the standard. A control was prepared using 1 ml of methanol and 2 ml of DPPH [17]. The results were interpreted based on their IC_{50} values.

Phosphomolybdenum assay: The total antioxidant capacity of the fruit pulp was assessed by phosphomolybdenum assay. Sample/ standard (0.3 ml) was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate with a ratio of 1:1:1). The reaction mixture was incubated at a temperature of 95 ^{0}C for 90 minutes. Afterwards, the absorbance of the resulting green colour was measured at 695nm. Ascorbic acid was used as the standard. (0.06- 0.14 mg ml^{-1}). The antioxidant capacity was expressed as "ascorbic acid equivalents" (mg AE/ g extract) [18]. All the samples were analyzed in triplicates.

Statistical analysis: The results of assays performed in triplicates were reported as mean ± SD. One way ANOVA was performed and $p \leq 0.05$ was considered to be significant [19].

RESULTS AND DISCUSSION: The results obtained for weights of each extract are given in **Table 1.**

TABLE 1: YIELD OF PULP EXTRACTS OBTAINED FROM PALMYRA FRUIT

Sample	Weight of nutlet used (g)	Weight of pulp extracted (g)	Percentage of pulp extracted (%)	Weigh of freeze dried product (g)	Percentage of freeze dried product (%)
Anamaduwa	412.33	63.57	15.42	5.26	1.28
Kilinochchi	417.76	141.29	33.82	2.07	0.49
Batticalo	413.63	150.82	36.46	6.63	1.60

N=03

Determination of Total Phenolic content: The assessment of total phenolic content performed using the Folin ciocalteu reagent, measures the sample's reducing capacity. This is an electron transfer assay, where the general reaction is as follows-

Mo (VI) (yellow) + e^- (from AH) → Mo (V) (blue)

According to the results, which are summerised in table 2 below, Anamaduwa PFP extracts showed the highest phenolic content out of the three samples tested (8.297 ± 0.017 mg GAE/g), which is significantly different from the results obtained for Batticalo and Kilinochchi samples ($p \leq 0.05$). The lowest total phenolic content is observed in Batticalo sample. The respective values for the total phenolic content was calculated using the gallic acid standard curve

$$[y= 10.89x +0.1 (R^2= 0.99)]$$

Phosphomolybdenum assay: In this assay, the antioxidants reduce Mo (VI) to Mo (V), which is green coloured. The total antioxidant capacity was assessed using the equation represented by the standard curve of ascorbic acid [$y= 3.66x +0.006$ ($R^2= 0.982$)]. The highest capacity is shown again by Anamaduwa PFP samples (68.171 ± 2.07 mg AE/ g). These are significantly different from the results obtained for the other two samples ($p \leq 0.05$). But there is no significant difference between the antioxidant capacity of Kilinochchi and Batticalo samples ($p > 0.05$). The results are summerised in **Table 2** below.

DPPH radical scavenging activity: The reduction of DPPH free radical by the sample was measured by the decrease in purple colour at 700nm. DPPH reacts with the hydrogen donors of the plant extract, yielding a stable product, 1,1-Diphenyl-2-picrylhydrazine.(yellow colour) [20]. The ability to scavenge DPPH radical was found by the following [21].

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of test}} x\ 100$$

The percentage inhibition is proportional to the concentration of the antioxidant. By using this, IC_{50} (the concentration of the antioxidant that reduces 50% of the initial concentration of DPPH) values were determined using Minitab software. According to the results summerised in **Table 3**, ascorbic acid showed a higher radical scavenging activity than the PFP samples ($p \leq 0.05$). But, upon comparison amongst PFP samples, Anamaduwa PFP showed a higher scavenging activity (3.065 ±0.003 mg/ml), which is significant than the Kilinochchi and Batticalo samples ($p \leq 0.05$).

TABLE 2: TOTAL PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF PFP SAMPLES

Sample	Total phenolic content (mg GAE/g of extract) (±SD)	Antioxidant capacity (mg AE/g of extract) (±SD)
Anamaduwa	9.297 (± 0.018)[a]	68.171 (±2.068) [a]
Kilinochchi	7.332 (±0.199)[b]	32.358 (±1.667) [b, d]
Batticalo	6.448 (±0.098)[c]	31.134 (±0.470) [c, d]

All data are presented as mean ± SD of three replicates. Gallic acid and ascorbic acid were used as the controls. Mean followed by different letters in the same column differs significantly. ($p \leq 0.05$)

TABLE 1: RADICAL SCAVENGING ACTIVITY AND REDUCING ACTIVITY OF PFP

Sample	IC_{50} (mg ml^{-1}) (±SD)
Ascorbic	0.047 (±0.003) [a]
Anamaduwa	3.065 (±0.176) [b]
Kilinochchi	3.916 (±0.049) [c]
Batticalo	7.466 (±0.156) [d]

All data are presented as mean ± SD of three replicates. Gallic acid and ascorbic acid were used as the controls. Mean followed by different letters in the same column differs significantly. ($p \leq 0.05$)

CONCLUSION: The results obtained through the mentioned assays indicate that the increase in total phenolic compounds resulted in the increasing antioxidant capacity of the extracted aqueous PFP samples. Thereby, it can be concluded that the phenolic compounds present in the pulp samples are partly responsible in removing reactive oxygen species. The antioxidant activity of the three districts are in the order of Anamaduwa > Kilinochchi> Batticalo. This study also suggests that PFP could be employed as a natural antioixidant, which could lead to the production of more value added products from the ripe *Borassus flabeliffer* Linn. pulp.

ACKNOWLEDGEMENTS: We thank the College of Chemical Sciences, Institute if Chemistry Ceylon, for providing the financial assistance for this research and the Institute of Indigenous medicine, Rajagiriya, Sri Lanka for freeze drying the *Borassus flabeliffer* Linn. samples.

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How to cite this article:
Kurian A, Thiripuranathar G and Paranagama PA: Determination of total phenolic content and antioxidant activity of *Borassus flabeliffer* linn. Fruit pulp collected from several parts of Sri Lanka. Int J Pharm Sci Res 2017; 8(6): 2701-05.doi: 10.13040/IJPSR.0975-8232.8(6).2701-05.