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Research Paper

Antioxidant Activity and Nutritional Properties of Freshly Tapped Palmyra (*Borassus flabellifer*) Sap

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Abstract

This study aims to analyze the antioxidant activity and the nutritional profile of the freshly tapped palmyra sap. Samples were collected in pre-sterile sample collection bottles from different parts of Jaffna peninsula and immediately transferred into an ice box. Pooled samples were centrifuged (1000rpm, 5 minutes) and the supernatant is stored at the refrigerator (4°C) for nutritional, and antioxidant analysis. The results exhibit that fresh sap consists of Na (15.3±0.14 mg/100g) and K (22.6±0.12 mg/100g), while the total ash content was 0.62±0.45 (g/100g). The total sugar content of the sap was 16.43±0.07(g/100g) and the reducing sugar and non-reducing sugar content were 2.16±0.32 (g/100g), 14.27±0.04 (g/100g) respectively. Sap exhibited a relatively low amount of fat 0.02±0.01 (mg/100g). DPPH scavenging activity with regard to IC 50 value was 1.36±0.35 mg/mL, and the total phenolic content and ascorbic acid content were recorded as 186±12.27(mg GAE/100g), 12.16±0.31 (mg/100g) respectively. It can be concluded that the fresh sap of palmyra is a good source of antioxidant properties and nutritional value.

Keywords: *Antioxidant activity, palmyra fresh sap, total phenolic content*

Introduction

Palmyra (*Borassus flabellifer*) is a potential resource abundantly available in the Northern part of Sri Lanka. Palmyra has been known for its versatile usage and each part from the palm results in a value-added product. One of the economically important substances yields from palmyra is the sap commonly known as 'neera' or '*pathaneer*'. Palmyra sap can be obtained from both the male and female inflorescence of the palm via the process of 'tapping' [1]. Freshly tapped sap is known as sweet toddy and it is susceptible for the spontaneous fermentation by the yeast present in the environment [2] and results in the alcoholic beverage 'Toddy'. Addition of Calcium hydroxide (lime) or adding '*Hul'* bark will preserve the fresh sap [3] from the fermentation. Fresh sap is rich in nutrients, sugars,

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and minerals [3] and poses several medicinal properties [4] as mentioned in the traditional medicine of Sri Lanka. Fresh sap is the raw material used to produce value-added products such as Palmyra jaggery, treacle, and sugar candy. However, the research on antioxidant potential and the nutrient profile of fresh palmyra sap is not yet analyzed. Therefore this study aims to analyze the fresh palmyra sap with a specific objective to provide scientific information about the antioxidant activity and nutritional properties of the fresh sap which would help to promote the sap-based value-added products and are the base for further development of research on fresh sap of the palmyra.

Materials and Method

Analytical grade reagents used for the analysis were obtained from Sigma Aldrich. All glassware used for the study was certified by ISO and all the equipment was calibrated before the analysis.

Sample Preparation

Borassus flabellifer (Palmyra) palm sap was collected from the same age palmyra trees in 'Chavakacheri', 'Ariyalai', 'Uduvil' and 'Chankanai' tapping locations of Jaffna peninsula as a pooled sap sample. Freshly tapped sap was collected in a pre-sterilized sample collecting bottle and immediately transferred into an icebox to prevent it from fermentation. The fresh sap was stored at refrigerator 4°C for further analysis. Part of a pooled sap sample was centrifuged at 1000rpm for 5 minutes [5] under sterile conditions and the supernatant was stored at the temperature of 4°C for antioxidant activity analysis. Each test was performed in triplicates.

Determination of pH

A homogenized sample (20 mL) was taken in a clean beaker (25 mL) and the pH of the sample was measured by using a digital-pH meter (Sension PH 31-Spain) [6]

Determination of Ash Content

Ash content of the sample was estimated by the method explained in SLS 772:1987 [7].

Estimation of Protein Content

Protein content was estimated by using Bicinchonic acid (BCA) described by Smith and others [8]. The standard bovine solution of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 up to 2.0 mL was measured into ten labeled test tubes and each test tube was volume up to 2 ml with distilled water. Protein standard solution of 50 mL was mixed with BCA/$CuSO_4$ solution of 1 mL and all the tubes were incubated at the temperature of 37°C for 40 minutes to obtain the color development after that the color developed samples were measured in a

spectrophotometer ((Spectronic 21D) against the reagent blank at the wavelength of 562 nm. The blank reagent was made up of 50 mL distilled water with 1 mL of BCA/$CuSO_4$. a standard curve was obtained from the readings and the protein content was calculated.

Determination of Fat Content

The fat content of the fresh sap was estimated by the solvent extraction method described in the AOAC manual [6].

Determination of Total Sugars

Lane and Eyon constant titer method [9] was used to determine the total sugar content of the sap. Standard dextrose solution, methyl blue indicator, and felings'solution were prepared and the prepared feling's solution was standardized before the analysis [9].

Preparation of Solution for Reduction Sugar

Sample (1.5 g) was weighed and transferred to a 250 mL volumetric flask and the distilled was used to make up the volume. An incremental method of titration was done. The standard method of titration was done [9].

Preparation of Solution for Non-Reducing Sugar

Sample (1.5g) was dissolved in 250 mL distilled water and 2.25 mL of above-prepared solution was taken in a conical flask then 2.5 mL of con HCl and distilled water(10 mL) were added. The flask was kept at 60 - 70°C for 10 min in a water bath. It was immediately cooled and neutralized with 30 % NaOH. Distilled water was added to volume up the solution to 250 mL, incremental method of titration was done & standard method of titration was done [9]. Total sugar was calculated from the results.

Estimation of Total Phenolic Content (TPC)

Estimation was done by the method described by Singleton and others [10]. A stock saponin solution was prepared (dissolving 0.1g of Gallic acid in 1000 mL of distilled water). The standard solution of different concentrations (0.0 to 0.1 g/l) of Gallic acid was prepared. Then 0.5mL standard and sample were taken into test tubes and mixed with 2.5mL of diluted Folin- Ciocalteau reagent (15%) and 2mL of sodium carbonate (7.5%). Parafilm was used to cover the tubes and the tubes were allowed to stand for 45 minutes at room temperature and then the absorbance was measured at the wavelength of 730 nm. Then the total phenolic content of fresh palmyra sap was calculated.

Estimation of DPPH Radical Scavenging Activity

DPPH radical scavenging activity was estimated by the method described by Blois [11].

Preparation of standard

The standard working solution (0.01 to 0.05 mg /mL) was prepared and 1mL of each standard solution was reacted with 1.5 mL methanol and 2 mL of DPPH solution. The tubes were covered with parafilm and kept for 30 minutes in a dark place. A control sample was prepared to contain the same volume without ascorbic acid. Methanol and ascorbic acid solution were used as the blank. Absorbance readings were taken by UV/ Visible Spectrophotometer at 516 nm.

Determination of Antioxidant Activity for Samples

One mL of each different concentration of test solution was reacted with 1.5 mL methanol and 2 mL of DPPH solution. The tubes were covered using a parafilm and allowed for 30 minutes in a dark place. A control sample was prepared to contain the same volume without ascorbic acid. Methanol and test solution were used as the blank. Absorbance readings are taken by a UV/ Visible Spectrophotometer at 516 nm.

Estimation of Ascorbic Acid Content

Ascorbic acid content was estimated by redox titration using iodine solution [12]. An aliquot of the sample (20 mL) was pipette out into a 250 mL conical flask and distilled water (150 mL) and starch solution (1 mL) were added. The sample was titrated with iodine solution (0.005 mol/l). The first permanent trace of a dark blue-black color was identified as an endpoint.

Determination of Na and K Content

Na and K content were estimated using a flame photometric technique as explained by Luh and Niketic [13].

Estimation of Total Plate Count

Total plate count was determined based on the SLS microbiological analysis method of, SLS 516: Part 1 [14].

Statistical Analysis

Data collected from each experiment were statistically analyzed using MINITAB 17 software using the ONE-WAY ANOVA test.

Results and Discussion

Table 1: DPPH radical scavenging activity of fresh Palmyra sap

Sample	IC50 value (mg/mL)
Fresh palmyra sap	1.36±0.35
Ascorbic acid standard	4.64±0.31

Table 2: Total phenolic content and ascorbic acid content of fresh Palmyrah sap

Parameters	Amount present in the fresh sap
Total phenolic content	186±12.27(mg GAE/100g)
Ascorbic acid content	12.16±0.31 (mg/100g)

IC 50 value, total phenolic content, and ascorbic acid content are the parameters to determine the antioxidant activity. Palmyra fresh sap poses a good antioxidant activity (Table 1, Table 2), which is known to be a functional property. Similar research on Kithul sap done by Ranasinghe and others concluded that the IC 50 values of kithul sap range between 2.07 to 3.4mg/mL and the total phenolic content ranges between 120 to 161 mg GAE/100g with the ascorbic acid content ranges between 24 to 30 mg/100g [5]. Antioxidant activity promotes health benefits as mentioned in traditional medicine for instance controlling cell damage and helps to prevent oxidative stress[5]. It can be expected that the products made from palmyra sap will be enriched in antioxidant properties.

Table 3: Nutritional profile of freshly tapped palmyra sap

Nutritional profile	Amount present in the fresh sap
Protein	3.12±0.42 (mg/100g)
Fat	0.02±0.01(mg/100g)
Total sugars	16.43±0.07(g/100g)
Non reducing sugars	14.27±0.04 (g/100g)
Reducing sugars	2.16±0.32 (g/100g)
Na	15.3±0.14 (mg/100g)
K	22.6±0.12 (mg/100g)

Fresh palmyra sap is a nutritious beverage (Table 1) and when considering the sugar content, 86 % of the total sugar is non-reducing sugar (14.27g), and the rest 14 % is

reducing sugars (2.16 g). These results were in agreement with the study done by Ronald Valder [15]. Furthermore, the fresh palmyra sap is rich in minerals such as Na and K. Fat content of the fresh sap is reportedly low compared to other parameters which will be an advantage to produce healthier palm sugar and other value-added products with low-fat content.

Table 4: pH and Ash content of freshly tapped Palmyra sap

Parameters	Amount present in the fresh sap
pH	6.42±0.24
Ash content	0.62±0.45 (g/100g)

the pH of the fresh sap is 6.42 and it is nearly the neutral value exhibits the low acidic nature of the sap. Ash content reports as 620 mg per 100g of sap and it indicates the considerable amount of minerals present in the fresh sap which may lead to producing palm sugar with minerals (Table 3). Studies show that the pH of the fresh Kithul sap is 5.7-5.8 [5] whereas the pH of coconut sap is in the range of 5.71 [16].

Total Plate Count of Freshly Tapped Palmyra Sap

Table 5: Total bacterial colony count of Palmyra sap

sample	*10-1	*10-2	*10-3	*10-4
Palmyra sap	1	1	-	-

Total plate count shows the presence of microorganisms but less than 4 x10 $^{-2}$ colonies which indicates the sap is of good quality without fermentation. Collection of sap in sterilized bottles and immediate refrigeration might be the reason behind the microbial quality of the sap.

Conclusions

The fresh sap of Palmyra is mineral-rich, low acidic, nutritious, low-fat sap with antioxidant potentials like radical scavenging activity and total phenolic content. This can be a good source to produce palm sugar, jaggery, and treacle with antioxidant properties and health benefits. Processing methods to preserve the fresh sap without eliminating the antioxidant and inherent properties need to be analyzed in the future.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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