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Antioxidant and anti-diabetic properties of *Caryota urens* (*Kithul*) flour

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Abstract

Starch extracted from pith of *Caryota urens* L. (Family: Arecaceae) palm is known as "*Kithul* flour" and is claimed to have health benefits according to folklore and Ayurveda. Antioxidants are believed to possess numerous health benefits. However, as yet, health benefits of *C. urens* flour have not been scientifically investigated. Antioxidant properties of *C. urens* flour were tested using different *in vitro* assays namely, 2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^+$), ferric reducing antioxidant power, oxygen radical absorbance capacity and ferrous ion chelating assays. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were also evaluated. Anti-diabetic properties were estimated using alpha amylase and alpha glucosidase enzyme inhibition assays. Dried methanolic extracts of both boiled and raw samples were used in all assays. Results of the study showed that *C. urens* flour possess free radical scavenging activity(raw 0.02± 0.01 and boiled 0.04±0.01mg trolox equivalent (TE)/ g flour), electron donating reducing power (raw 0.10±0.03 and boiled 0.36±0.11 mg TE/g flour), oxygen radical absorbance capacity (raw 2.29± 0.71 and boiled 192.3 ± 57.71 mg TE/1g flour) and metal ion chelating capacity (raw 0.03±0.01 and boiled 0.14±0.04 mg EDTA equivalents /g flour) exhibiting its antioxidant potential. TPC (raw 1.1±0.3 and boiled 5.12 ± 1.89 mg GAE/g flour) and TFC (raw 1.65 ± 0.47 and boiled 6.69 ± 1.27 µg QE/g flour) which are said to be contributed to antioxidant activities were also found. In addition, boiled flour which is generally consumed as a food has shown higher antioxidant activity. *Caryota urens* flour did not contain marked anti-diabetic properties. It is evident from results that *Caryota urens* flour has moderate antioxidant property which may associate with its traditional health claims.

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Keywords: antioxidants; free radicals; *in vitro* assay

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1. Introduction

Caryota urens L. (Family Arecaceae) is an underutilized palm species which is native to low land forests of tropical Asia including India, Malaysia and Indonesia and is popular as *Kithul* in Sri Lanka. Jaggery, treacle and toddy (fermented beverage) are produced from sap of the young inflorescence of *C. urens*. It is a multipurpose palm which provides strong fibre, woody stems for building materials and healthy food from starch which is extracted from the pith of the stem. *Caryota* flour is mixed with *Cryota* syrup to produced locally esteemed porridge and sweetmeats, which are commonly used in festival days in Sri Lanka. In India, stem starch of *C. urens* represents a food source among some tribal peoples.

Inflorescence sap of *Caryota urens* and sap based products possess health promoting properties, according to folkloric knowledge and are used in treatments in the ayurvedic medical system practiced in Sri Lanka. Several studies have been done on analysis of *C. urens* sap and sap based products[1].Free radicals are molecules or molecular fragments which contain one or more unpaired electrons and involve in the pathogenesis of certain human diseases including Alzheimer’s disease, Parkinson’s disease, Cancer, Down’s syndrome, atherosclerosis and ageing.

Protection against free radicals can be enhanced by improving the dietary intake of antioxidants. Antioxidants are capable of slowing or preventing the oxidation of other molecules, thereby protecting cells from damages caused by exposure to reactive oxygen species (ROS) which are produced during normal oxidation reactions in biological cells.

Traditionally claimed health benefits of *C. Urens* flour may be contributed with its antioxidants and anti-diabetic properties. However, these bioactive properties of *C. Urens* flour have not reported. Therefore, in this study antioxidant and anti-diabetic properties *Caryota urens* flour was evaluated.

2. Methodology

2.1 Collection and storage of Caryota urens flour samples

Pure *Caryota urens* flour samples were collected from seven different locations around UvaParanagama area (6.95 ^{0}N, 80.88 ^{0}E and 1100 m above MSL) in Badulla district, Sri Lanka. Samples were sealed, labelled and stored at - 20 ^{0}C temperature.

2.2 Treatments

Raw and boiled *Caryot aurens* flour samples were used as two treatments.

2.3 Preparation of extracts

In first treatment (T_1), dried flour samples were extracted by weighing samples of 10 grams of finely ground flour and extracting with 100 mL of methanol GR (99.8% Assay GC). In second treatment (T_2), the same amount from each sample was boiled with 100 mL of hot distilled water and then extracted with 100 mL of methanol.

Samples were sonicated for 2 hours in a digital heated ultrasonic bath at 50 ^{0}C followed by concentration using rotavapor.Extracts of T_1 were dried using sample concentrator at 45 ^{0}C. Extracts of T_2 were frozen in - 40 ^{0}C and dried overnight using freeze dryer. From each extracts, 5 mg/mL concentrated solutions were prepared and stored at - 40 ^{0}C.

2.4 Antioxidant and anti-diabetic assays

Antioxidant properties of *C. urens* flour were tested using different *in vitro* assays. Free radical scavenging activity was measured using 2,2-azinobis-3-ethylbenzothiozoline-6-sulfonic ($ABTS^+$) radicals based on the methods described by Re *et al.* (1999)[2] Five concentrations of *C. Urens* flour extract (31.25, 62.5, 125.0, 250.0 and 500.0 µg/mL) were used to calculate IC_{50} value.Trolox was used to construct the standard curve.Ferric reducing antioxidant power[3]and oxygen radical absorbance capacity[4]were measured using trolox as the standard.The ferrous iron chelation assay was performed based on method developed by Carter (1971)[5]. IC_{550} values were measured using five different concentrations of flour extract (0.31, 0.62, 1.25, 2.5 and 5.0 mg/mL) and Ethylenediaminetetraacetic acid (EDTA) was the standard.The Folin-Ciocalteumethod[6] was adopted to determine total phenolic contents in *C. urens* flour using Gallic acid standard curve. Total flavonoid content of the flour was determined by Aluminium chloride method[7]. Quercetin was used to construct the standard curve.Anti-diabetic properties were estimated using alpha amylase and alpha glucosidase enzyme inhibition assays. Alpha amylase enzyme inhibition activity was determined according to the method described by Bernfeld (1955)[8]and the method developed by Matsui *et al.* (2001)[9] was adopted for alpha glucosidase enzyme inhibition assay.

2.5 Data analysis

Softmax Pro 5.2 v software of the micro plate reader (SPECTRAmaxPLUS^{384} Molecular Devices, California, USA) was used to calculate *in vitro* antioxidant and ant-diabetic values of all *Caryota urens* flour samples. Data of each experiment were statistically analysed using SAS^{TM} software (Version No. 9.00 - 2002) followed by comparison of means using Duncan's Multiple Range Test (DMRT). All *in vitro* assays were performed in triplicate. All the results presented are mean of samples with a standard error of the mean.

3. Results and Discussion

Dry weight of extractable of raw *C. urens* flour was 2.13 ± 0.43 mg per one gram of flour while boiled flour was 4.07 ± 0.96 mg per gram of flour. The dry weight of extractable of raw flour was not significantly ($P>0.05$) different to boiled flour. Even though it was not significantly different, extractable dry weight of boiled flour was noticeably higher than the raw flour.

When starch is heated with excess water, an individual starch granule absorb water, swells and increases its volume. Mechanical disruption of these structures may lead to an increased release of compounds and nutrient availability [10].

Caryota urens flour extract showed $ABTS^+$ radical scavenging activity at all tested concentrations regardless the treatment. $ABTS^+$ radical scavenging activity was increased with the increase of the *C. urens* flour concentration in both treatments. Flour which was boiled in water showed significantly higher ($P<0.05$) Trolox equivalent antioxidant concentration (TEAC) value (Table 1). $ABTS^+$ radical scavenging activity estimated as inhibitory concentration 50% (IC_{50}) showed significantly higher ($P<0.05$) value in raw *C. urens* flour (Table 1).

Raw *C. Urens* flour was extracted directly by methanol and molecules that are soluble only in methanol were responsible for the scavenging activity. In boiled flour, compounds which soluble in both water and methanol were responsible for radical scavenging activity. So, values increased significantly.

Reduction of metal ions in *C. urens* flour was measured using Ferric Reducing Antioxidant Power (FRAP) assay. EDTA equivalent antioxidant concentration of boiled flour was significantly higher ($P<0.05$) than raw flour (Table 1). Metal ion chelating capacity estimated as inhibitory concentration 50% (IC_{50}) showed significantly higher ($P<0.05$) value in raw *C. urens* flour (Table 1).

Iron is capable of oxidizing a wide range of substances and cause biological damage. But it is extensively used in biological systems and several reactions are controlled or mediated. Hence, any natural occurring substances which have high reducing power of metal ions are very important. *Caryota.urens* flour possess considerable amount of ferric reducing antioxidant power.

Caryota urens flour extracts showed ferrous ion chelating activity at all tested concentrations and the activity was increased with the increment of the *C. urens* flour concentration in both treatments. Ferrous ion chelating activity of *C. urens* flour which was estimated as inhibitory concentration 50% (IC_{50}) showed significantly higher ($P<0.05$) value in raw flour whereas EDTA equivalent antioxidant concentration showed a significantly ($P<0.05$) higher value in boiled flour (Table 1).*Caryota urens* flour contains considerable amount of ferrous ion chelating capacity. Since there are several types of chelating agents in nature, further studies needed to identify these substances in *C. urens* flour.

ORAC value of boiled *C. urens* flour was significantly ($P<0.05$) higher than raw flour (Table 1). Interestingly, boiled *C. urens* flour showed very high oxygen radical absorbance capacity when compare with raw flour. Reasons for astonishingly higher ORAC values in boiled flour should be investigated. There is a highly positive relationship between total phenolics and antioxidant activity in many plant species[11]. Both TPC and TFC values of boiled *C. urens* flour were significantly ($P<0.05$) higher than raw flour (Table 1).

When compare with previous studies of Ranasinghe *et al*., (2012)[1]antioxidant activity of *C. urens* flour was lower than *C. urens* sap in means of $ABTS^+$ radical scavenging activity, chelation and reducing power of metal ions, TPC and TFC.

In anti-diabetic assays, percentage inhibition of alpha amylase enzyme for 5 mg/mL concentrated raw *C. urens* flour was 8.42 ± 0.97 % while for boiled flour was 10.77 ± 2.64 %. The percentage inhibition of raw flour was not significantly ($P>0.05$) different to boiled flour. Both raw and boiled *C. urens* flour did not show marked alpha glucosidase enzyme inhibition activity up to 5 mg/mL concentrated *C. Urens* flour.

Table 1.*In vitro* antioxidant activity, total phenolic and flavonoid contents of raw and boiled *Caryota urens* flour

In vitro assay	Unit	Flour	
		Raw	Boiled
$ABTS^+$ radical scavenging activity	IC_{50} (mg/mL) TEAC (mg TE/g)	32.04±7.41 0.02± 0.01	29.77±7.31 0.04±0.01
Ferrous ion chelating capacity	IC_{50} (mg/mL) (mg EDTAE/g)	18.91±10.4 0.03±0.01	7.39±4.78 0.14±0.04
FRAP value	(mg TE/g)	0.10±0.03	0.36±0.11
Oxygen Radical Absorbance Capacity	(mg trolox/g)	2.29±0.71	192.3.±57.71
Total Phenolic Content	(mg GAE/g)	1.1±0.3	5.1±1.89
Total Flavonoid Content	(µg QE/g)	1.65±0.47	6.69±1.27

Values are presented as mean ± SEM of 7 independent replicates

4. Conclusions

The study showed antioxidants activity of *Caryota urens* flour, estimated as free radical scavenging activity, metal ion chelating capacity, electron donating reducing power and oxygen radical absorbance capacity providing some scientific information for traditionally claimed health benefits. It also contains phenolic and flavonoid compounds. *Caryota urens* flour contains very low anti-diabetic properties. Further, it was found that there’s a marked difference in antioxidant property in boiled *Caryota urens* flour.

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