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Variation in phenolic compounds and antioxidant potential in arecanut of different selections and processing methods

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Arecanut (*Areca catechu* L.) is a perennial palm that belongs to the family Arecaceae. The crop is tropical, with Southeast Asia as its centre of origin. The economic part is the kernel/seed nut, mainly used as a masticatory. India ranks first in the world in the production of arecanuts. It is cultivated in 14 states of India, covering 4,72,360 ha with a production of 7,35,860 MT. Karnataka covers an area of 271.4 thousand hectares with a production of 5,77,900 MT with 2129 kg ha^{-1} yield potential (Anonymous, 2020).

There are many varieties/selections of arecanut in India that are developed depending on the cultivation location. Although several studies on the medicinal properties of the arecanut are reported, particularly on its cancer-causing properties, the antioxidant properties of different selections and types of processed nuts are not estimated. Therefore, the present work was conducted to assess the status of antioxidant content in various collections and types of processed arecanut.

The antioxidants can impede or interrupt the oxidation of an oxidizable substrate in a chain reaction and hence play a vital role in the management of many diseases (Halliwell *et al*., 1992). Habitually, arecanut is used to cure several complaints because of its therapeutic properties such as antibacterial, anti-hypertension, anti-heartburn, anti-ulcer, anti-diarrheal, digestive, laxative, antimalarial, carminative, diuretic, anthelmintic, hypoglycemic properties (Jaiswal *et al*., 2011; Amudhan *et al*., 2012; Rashid *et al*., 2015). Alkaloids like arecoline, arecaidine, guvacine, guvacoline, isoguvacine, arecolidine and homoarecoline existing in arecanut possess curative properties (Peng *et al*., 2015). Extract of areca has defensive effects versus damage caused by peroxidase and has anti-inflammatory properties (Anthikat and Michael, 2012). The antioxidant contents in the processed nuts of commercially grown types are not reported so far. The present study was undertaken with this objective.

Fourteen different processed arecanut types from Uttara Kannada district from four selections/ varieties [Sirsi selection (SAS-1)] grown in Sirsi and Tamil Nadu areas (Sagar type, Mohitnagar and Mangala varieties) were used for estimation of total anthocyanins, phenols, flavonoids (Chun *et al.,* 2003), total antioxidant properties (Ferric reducing the ability of plasma, FRAP) (Benzie and Strain, 1996) and radical scavenging ability (1,1-diphenyl-2-picrylhydrazyl, DPPH). The analysis was done in Completely Randomized Design (CRD), replicated thrice.

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Types of arecanut

Totally 14 processed types were used for the study. They comprised of two groups (of four selections); five processed types from tender nuts (*Kali pak*) and three types from ripe nuts (*Chali*). The details are given in Table 1 and Figure 1.

Total phenol content was estimated by the spectrophotometric method using the Folin-Ciocalteu reagent, according to Singleton and Rossi (1965) and is expressed in mg gallic acid equivalents 100 g^{-1}. At alkaline pH, the brick red colour is developed by flavonoids when reacted with $AlCl_3$

Table 1. Types of arecanut used for the study

A. Kalipak (tender/green nut de-husked, boiled and dried) includes *raashi*, *bette*, *aapi*, *muri*, *chooru*, *kempu gotu*	
1. *Bette*	The type of *kalipak* with smooth surface having a bit hard nature of nut while cutting
2. *Raashi*	The *kalipak* type with more constrictions on the surface of the nut, which are a bit soft/smooth than that of *bette*
3. *Aapi*	The flat type with more of constrictions and high amount of stickiness/gumminess on its surface. More tender in nature of all types
4. *Muri*	Flat type variety with constrictions on the surface of the nuts
5. *Kempu gotu*	The variety in which nuts are with the outer coat/portion of the husk intact
B. Chali (ripe nuts dried under sun and de-husked) or *kotta pak* includes *bili gotu*, *chali kempu*, *koka*	
1. *Bili gotu*	After dehusking process in *chali*, the portion of the husk remains intact- those types are *bili gotu*
2. *Chali kempu*	During dehusking process, some reddish or brown nuts found, those are *chali kempu*
3. *Koka*	In de-husked *chali*, blackish, hard surfaced, weak nuts are *koka*

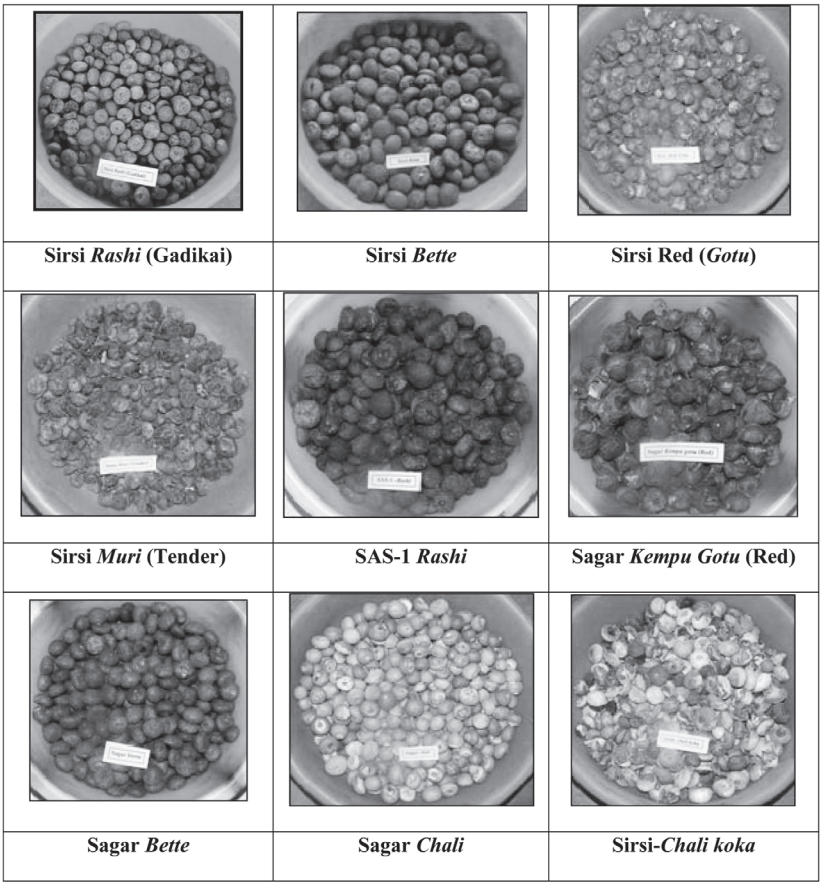

Fig. 1. Different types of processed arecanut used for analysis

and $NaNO_2$. The absorbance of the reaction complex was read at 510 nm using catechin or quercetin as standard (Chun *et al.,* 2003). Ferric reducing antioxidant potential (FRAP) was calculated by measuring the change in absorption of the blue colour solution at 593 nm as the sign of the reduction of ferric tripyridyltriazine (Fe^{3+} - TPTZ) complex to the ferrous form at low pH. Any half-reaction that has a lower redox potential than that of the ferric/ferrous half-reaction under reaction conditions will drive the ferric (Fe^{3+}) to ferrous (Fe^{2+}) reaction. Then the reaction is nonspecific. Therefore, the change in absorbance is directly related to the combined or 'total' reducing the power of the electron-donating antioxidants present in the reaction mixture. Results are expressed as ascorbic acid equivalent antioxidant capacity (Benzie and Strain, 1996). The estimation of antioxidant activities in a relatively short time is done with the help of DPPH, a stable free radical. The assay was based on reducing DPPH radicals in methanol by the antioxidants, which cause a reduction of absorbance at 517 nm (Kang and Saltveit, 2002). Anthocyanins are water-soluble flavonoid compounds present mainly in dark coloured fruits. Anthocyanins are sensitive to pH showing purple colour in acidic medium and turning green to blue as the pH increases. Cyanidin hydrochloride is taken as standard, and the amount of anthocyanin in an unknown sample was calculated and expressed as mg per 100 g fresh weight.

Anthocyanins are reported to have a high level of antioxidant ability (Miguel, 2011). Among the 14 types studied (Table 2), maximum total anthocyanin was observed in cultivar Sagar *bette* (3.51 mg g^{-1} dw), which was on par with cv. Sagar *kempu gotu* (Red) (3.50 mg^{-1} g dw) and minimum total anthocyanin were recorded in cv. Sirsi - Red *gotu* (1.83 mg g^{-1} dw).

Phenolic or polyphenolic compounds are said to possess anti-oxidative properties, and a direct relationship is established between the content of total phenols and the antioxidant capacity of plants (Ferreira *et al*., 2007; Robards *et al*., 1999).

Table 2. Antioxidant properties of arecanut collections

Sl. No.	Sample name	Total anthocyanins mg g^{-1} dw	Total phenols mg g^{-1} dw	Total flavonoids mg g^{-1} dw	FRAP mg g^{-1} dw	DPPH mg g^{-1} dw
1.	SAS-1-*Rashi*	2.03 ^{h}	233.9 ^{g}	88.9 ^{ef}	91.1 ^{fg}	97.2 ^{f}
2.	Mangala- *Rashi*	1.99 ^{hi}	221.0 ^{ghi}	79.6 ^{i}	90.5 ^{efgh}	83.5 ^{hi}
3.	Sirsi *bette*	1.88 ^{ijk}	290.9 ^{ab}	107.1 ^{ab}	103.7 ^{a}	82.3 ^{hij}
4.	Sirsi-Red *Gotu*	1.83 ^{kl}	233.3 ^{gh}	86.0 ^{efg}	99.4 ^{abcd}	45.7 ^{m}
5.	Sirsi *Rashi* (Gadikai)	3.07 ^{c}	2717 ^{cd}	96.6 ^{d}	94.2 ^{def}	109.8 ^{abcd}
6.	Sirsi-*Muri* (Tender)	2.55 ^{d}	204.9 ^{ijk}	70.6 ^{jkl}	89.7 ^{fghi}	73.3 ^{k}
7.	Sirsi- *chali-koka*	1.96 ^{hij}	283.7 ^{bc}	103.2 ^{bc}	102.0 ^{abc}	68.9 ^{kl}
8.	Sagar (Kyasnur-*Rashi*)	1.68 ^{m}	211.3 ^{ij}	73.4 ^{ij}	89.2 ^{fghi}	30.9 ^{n}
9.	Sagar *bette*	3.51 ^{a}	304.6 ^{a}	108.9 ^{a}	88.3 ^{fghijk}	114.8 ^{a}
10.	Sagar *kempugotu* (Red)	3.50 ^{ab}	265.3 ^{de}	91.2 ^{de}	86.6 ^{hijkl}	91.1 ^{fg}
11.	Sagar *chali*	2.38 ^{e}	263.4 ^{def}	88.5 ^{efg}	86.0 ^{ghijklm}	87.9 ^{gh}
12.	Mohitnagar (TN)	2.38 ^{e}	204.5 ^{ijkl}	71.3 ^{jk}	102.4 ^{ab}	114.2 ^{ab}
13.	Sirsi SAS-1 (TN)	2.36 ^{ef}	197.2 ^{jklm}	67.2 ^{klm}	96.7 ^{bcde}	112.7 ^{abc}
14.	Mangala (TN)	2.22 ^{g}	191.1 ^{klmn}	63.9 ^{mn}	85.8 ^{hijklmn}	108.2 ^{abcde}
C. D. (5%)		0.13	17.7	5.6	6.3	6.9
S. Em+		0.04	6.1	1.9	2.1	2.4
S. E(d)		0.06	8.6	2.7	3.0	3.4
C. V. (%)		3.22	4.4	3.9	3.9	4.8

S. E (d): Standard error of the difference between means
TN: Nuts collected from Tamil Nadu plantation

Table 3. Effect of processing type on the antioxidant content in arecanut

Sl. No.	Character	Red type	Ripe type
1.	Total Anthocyanin (mg g^{-1} dw)	1.87-3.51	1.96-2.38
2.	Total Phenols (mg g^{-1} dw)	204.99-304.58	191.13-283.71
3.	Total Flavonoids (mg g^{-1} dw)	70.63-108.89	63.89-103.17
4.	FRAP (mg g^{-1} dw)	86.57-103.65	85.76-102.46
5.	DPPH (mg g^{-1} dw)	45.7-114.79	68.92-114.17

Total phenol content was also maximum in cv. Sagar *bette* (304.6 mg g^{-1} dw), but was on par with cv. Sirsi *bette* (290.9 mg g^{-1} dw) and minimum total phenol content was recorded in Mangala (191.1 mg g^{-1} dw).

Areca flower, husk, and seeds contain flavonoids, the main components of the total phenols (Zhang *et al*., 2009). Flavonoids have chelating properties, due to which they chelate or bind to metal ions in the human body and prevent them from being accessible for oxidation. Another possible mechanism of flavonoids may be the induction of internal antioxidant enzymes (Banjarnahor and Artanti, 2015). Total flavonoid content was also maximum in Sagar *bette* (108.9 mg g^{-1} dw), which was on par with Sirsi *bette* (107.1 mg g^{-1} dw) and was minimum in var. Mangala (63.9 mg g^{-1} dw).

DPPH radical-scavenging assay is the best and most widely used method to evaluate the antioxidant capacity in a short time (Blois, 1958). It was maximum in Sagar *bette* (114.8 mg g^{-1} dw) which was on par with Mohitnagar (TN) (114.2 mg g^{-1} dw), Sirsi SAS-1 (TN) (112.7 mg g^{-1} dw), Sirsi *Rashi* (Gadikai) (109.8 mg g^{-1} dw) and var. Mangala (108.2 mg g^{-1} dw). The minimum DPPH scavenging activity was observed in Sagar (Kyasnur-*Rashi*) (30.9 mg g^{-1} dw). Most of the compounds out of the 11 isolated from arecanut exhibited considerable scavenging activity on the DPPH assay (Zhang Xing *et al*., 2010).

The reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex can be measured with the aid of FRAP assay (Nilima and Hande, 2011). FRAP analysis showed maximum antioxidant activity in Sirsi *bette* (103.7 mg g^{-1} dw), which was on par with var. Mohitnagar (TN) (102.5 mg g^{-1} dw), Sirsi-*chali-koka* (102.0 mg g^{-1} dw) and Sirsi -Red *Gotu* (99.4 mg g^{-1} dw). Minimum ferrous reducing antioxidant power was observed in var. Mangala (85.8 mg g^{-1} dw).

Overall, except for the FRAP assay, Sagar *bette* showed maximum antioxidant activity in all other antioxidant properties. In comparison, Sirsi *bette* showed maximum antioxidant activity in all other antioxidant estimation properties except for total anthocyanin and DPPH analysis.

In the case of two processing types (red and ripe nuts), the values of antioxidant contents were over-lapping (Table 3) except for the phenol contents. The higher phenolic contents (204.9 to 304.6 mg g^{-1} dw) were recorded in the red nut processing type compared to ripe nut (*chali*) processing. This is quite evident that the tender arecanuts will have a higher content of phenols than ripe nuts. These results indicated that there was little effect of the method of processing on the content of antioxidant properties.

This study focuses on the antioxidant status of 14 types of arecanut. Based on the study, it may be concluded that selection Sagar *bette* has the maximum antioxidant activity compared to the other 13 collections, followed by Sirsi *bette*. There was little difference in the antioxidant contents between the types of processing of nuts except for the content of phenols which recorded a higher amount in tender nut processing. The present study indicated that the processed arecanuts have a higher potential in diversifying their usage.

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