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Research article

Phytochemical composition and antioxidant activity of coconut cotyledon

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ABSTRACT

Coconut tree (*Cocos nucifera* L.), a perennial, monocot tree, belonging to the family Arecaceae, is distributed through the tropics. Bioactivities of coconut water, husk fiber, oil, flowers, spadix and mesocarp of coconut fruit are widely reported. However, there is no study on cotyledon of coconut. In this study, carbohydrates, proteins, lipids, phenols, flavonoids, tannins, alkaloids and antioxidants were quantified in hot and cold percolated extracts of coconut cotyledon. Further, the antioxidant activity was studied using 2,2-diphenyl-1-picrylhydrazyl (DPPH); ferric reducing antioxidant power (FRAP); ferric thiocyanate (FTC); thiobarbituric acid (TBA); nitric oxide (NO) radical scavenging and β-carotene bleaching assays. Among the secondary metabolites, only cardiac glycosides were detected. Methanolic extraction by cold percolation extracted high content of secondary metabolites and exhibited significant antioxidant activity in DPPH, FRAP, NO and β-carotene bleaching assays, with EC_{50} of 0.12, 6.43, 16.21 and 8.09 mg/ml respectively. The chloroform extracts recorded high lipid content and scavenged the radicals in FTC (EC_{50} 13.31 mg/ml) and TBA (EC_{50} 9.21 mg/ml) assays. The study recommends extraction of compounds using methanol through cold percolation. The cotyledon of coconut is found to be a potent nutritive source equivalent to the endosperm.

1. Introduction

Cocos nucifera L., popularly called as coconut tree, is a perennial, monocot tree, belonging to the family Arecaceae. The tree, native to Southeast Asia and Melanesia, is distributed throughout the tropics and sub-tropics of the world (Chan and Elevitch, 2006). Nearly 60.77 million metric tons of coconuts were produced during the year 2017 globally (Shahbandeh, 2019). *C. nucifera* has wide range of uses, which include making of brooms and thatched roofs using leaves; ropes and mats using coir; furniture using lumber; fuel using shell and husk; use of coconut oil & milk for cooking, and coconut water as a refreshment in sports drink & alcoholic beverages (Toddy). The importance of different parts of *C. nucifera* in different industries has been documented (Roopan and Elango, 2015). Nutritionists claim that coconuts contain vitamins, electrolytes, fibre and few minerals like potassium, phosphorus & manganese.

The bioactivities viz., antiulcerogenic (Nneli and Woyike, 2008), wound healing (Srivastava and Durgaprasad, 2008), antimicrobial, anti-inflammatory, anti-diabetic, anti-neoplastic, anti-parasitic, insecticidal, leishmanicidal (Roopan, 2016), antioxidant (Muritala et al., 2018) and cell proliferation (Dhanyakrishnan et al., 2018) of one or more parts, such as coconut water, husk fiber, oil, flowers, spadix and mesocarp of coconut fruit are documented. However, the bioactivity of *C. nucifera* cotyledon has not been studied so far.

The cotyledon of coconut, also known as coconut apple, sprout and pearl, is a white, off-white or creamy, spongy structure, formed during the germination of zygotic embryo. They form the basis of nutrition for the developing plant. Cotyledons are found to possess parenchyma cells with few vascular tissues. Though the cotyledons of coconut are being consumed by people at large, they have been explored only for their role in clonal propagation (Nguyen et al., 2015). The present study is focused on the quantification of the primary and secondary metabolites present in cotyledons, and evaluation of their free radical scavenging activity.

2. Materials and methods

2.1. Plant source and solvent extraction

Germinated coconut (*Cocos nucifera*) (Figure 1a) was procured from local markets of Chennai, Tamil Nadu, India. The cotyledons were separated from the shell (Figure 1b), sliced into pieces (0.5 cm^3) and

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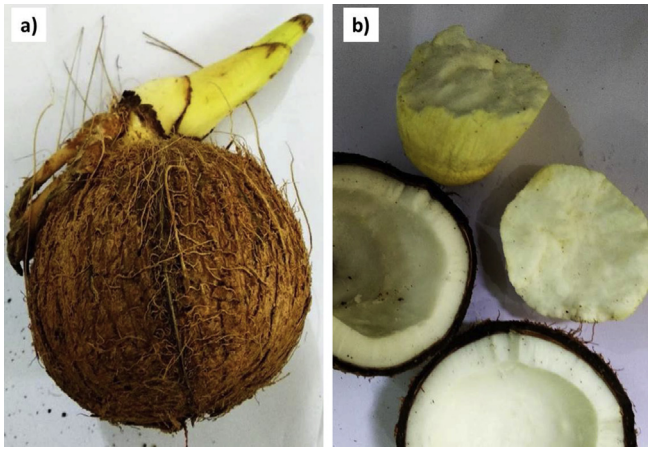

Figure 1. a. Germinated coconut. b. Cotyledon of *C. nucifera*.

subjected to extraction. Solvent extraction of the cotyledons was performed by hot and cold percolation methods, using the solvents methanol and chloroform. Soxhlet apparatus was used for the hot percolation method, with the cotyledon-solvent ratio being 1:10 (w/v). The temperature was set at 70 °C and the apparatus was operated for 5 cycles. Extraction by cold percolation was carried out by the addition of 100 ml of solvent to 10 g of the sliced cotyledon. The extracts were maintained at 30 ± 2 °C in a temperature controlled shaker for 48 h and then filtered. The extracts thus obtained through hot and cold percolation were concentrated to obtain the crude. The crude was diluted with respective solvents for further analysis. The methanol extract obtained through hot percolation is abbreviated as HPME and that of chloroform extract as HPCE. The respective solvent extracts obtained by cold percolation are presented as CPME and CPCE.

2.2. Analysis of plant metabolites

2.2.1. Primary metabolites

The carbohydrate content in the extracts of *C. nucifera* cotyledons was quantified by DNSA (Dinitrosalicyic acid) method (Saqib and Whitney, 2011). To 1 ml of the extract, 1 ml of DNSA reagent was added and incubated in a boiling water bath at 100 °C for 10 min. The volume was made upto 10 ml using distilled water and the absorbance was measured at 540 nm, with glucose as the standard.

Lowry's method was employed for estimating the protein content of *C. nucifera* cotyledons (Thangaraj, 2016). To 100 µl of the extract, 100 µl of 2N NaOH was added and maintained at 100 °C for 10 min in a boiling water bath. Upon cooling to room temperature, 1 ml of Lowry's reagent was added and incubated at room temperature for 10 min. To this, 100 µl of Folin reagent was added and maintained at room temperature for 30 min, after which the absorbance was measured at 680 nm in UV-Vis spectrophotometer. Standard plot of bovine serum albumin was used to quantify the protein content.

The total lipid content was estimated according to Bligh & dyer method (Thangaraj, 2016). The extract (10 ml) was added to a pre-weighed petridish and the solvent was allowed to evaporate in an oven at 100 ± 2 °C. The total lipid content was measured from the difference of weight between the petridish prior to heating and after heating.

2.2.2. Secondary metabolites

2.2.2.1. Qualitative analysis. The presence of secondary metabolites such as tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides, steroids, alkaloids, quinones and coumarins was studied according to Evans (2009) & Udayaprakash et al. (2013).

Tannins: A few drops of 0.1% ferric chloride were added to 5 ml of the extract. Brownish green or blue black colour indicated the presence of tannins.

Phlobatannins: Upon boiling the extract (10 ml) with 1% HCl, development of red precipitate indicated the presence of phlobatannins.

Saponins: Water (3 ml) was added to 10 ml of the extract and shaken well. A few drops of olive oil were then added. The presence of a stable emulsion denotes saponins.

Flavonoids: Yellow coloration of the solution, upon addition of a few drops of 1% liquor ammonia to the extract confirms the presence of flavonoids.

Terpenoids: To 5 ml of the extract, 2 ml of chloroform and 3 ml of concentrated sulphuric acid were added. The presence of terpenoids was indicated by a reddish brown interface.

Cardiac glycosides: Two ml of glacial acetic acid containing a drop of ferric chloride and concentrated sulphuric acid (1 ml) were added consecutively to 5 ml of the extract. Development of a brown ring indicated the presence of cardiac glycosides.

Steroids: To the extract (2 ml), acetic anhydride (2 ml) and a few drops of concentrated sulphuric acid were added. The presence of steroids was indicated by a blue green ring.

Alkaloids: The presence of reddish brown precipitate, upon addition of Wagner's reagent (2 ml) to the plant extract (1 ml) indicated alkaloids.

Quinones: Sodium hydroxide (10%) was added in drops to 1 ml of the extract. The presence of quinones was indicated by blue green or red color in the solution.

Coumarins: To the extract, NaOH (10%) and chloroform were added at equal proportions. An yellow colored solution indicated the presence of Coumarins.

2.2.2.2. Quantitative analysis. Total phenolic content (TPC): According to Folin-Ciocalteu method, 500 µl of distilled water and 100 µl of Folin-Ciocalteu reagent were added to 100 µl of the extract and incubated for 6 min at room temperature. The volume was made to 3 ml using water, after adding 1.25 ml of 7% sodium carbonate. After incubation for 90 min, the absorbance was recorded at 760 nm, and the result expressed as mg Tannic acid equivalents (TAE) per gram dry weight (DW) of the plant material (Udayaprakash et al., 2015).

Total flavonoid content (TFC): The solvent in 200 µl of the extract was evaporated and 5 ml of aluminium chloride (0.1 M) was added to the residue. After incubation for 40 min, the absorbance was measured at 415 nm. The TFC was expressed with reference to quercetin equivalents (QE) per gram dry weight (DW) of the plant material (Udayaprakash et al., 2015).

Total tannin content (TTC): The extract (100 µl), water (8.4 ml), Folin-Ciocalteau reagent (500 µl) and sodium bicarbonate (1 ml) were mixed together and incubated for 30 min. The results are expressed as mg Tannic acid equivalents (TAE) per gram dry weight (DW) of the plant material (Tambe and Bhambar, 2014).

Total alkaloid content (TAlC): One ml of 2 N HCl was added to the extract and filtered. Boromocresol green (5 ml) and phosphate buffer (5 ml) were added to the filtrate. To this, chloroform was added in successive volumes (1–4 ml) in a separating funnel and mixed vigorously. The chloroform layer was then separated and the volume made up to 10 ml. The absorbance was measured at 470 nm and expressed as caffeine equivalents (CE) per gram dry weight (DW) of the plant material (John et al., 2014).

Total antioxidant content (TAC): The TAC was studied by phosphomolybdenum method. To the extract (0.5 ml), 4.5 ml of reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added. The solution was maintained at 95 °C for 90 min in a boiling water bath. Subsequently, the absorbance was measured at 695 nm and the TAC was expressed as mg Tannic acid equivalents (TAE) per gram dry weight (DW) of the plant material (Udayaprakash et al., 2015).

2.3. Free radical scavenging assays

2.3.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Varying concentrations of *C. nucifera* extracts (2, 4, 6, 8 & 10 mg/ml) were made upto 1 ml using the respective solvents. To this, 1 ml of 0.01 mM DPPH was added and incubated in the dark for 30 min, after which the absorbance was measured at 517 nm (Udayaprakash et al., 2014).

2.3.2. Ferric reducing antioxidant power (FRAP) assay

To different concentrations of the extract (2, 4, 6, 8 & 10 mg/ml), 2.5 ml of 0.2 M phosphate buffer (pH 7) and 2.5 ml of 1% potassium ferricyanide were added and maintained at 50 °C for 30 min. Further, 10% trichloroacetic acid (2.5 ml) was added and centrifuged for 10 min at 6500 rpm. To the supernatant, 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride were added and the absorbance was measured at 700 nm (Udayaprakash et al., 2015).

2.3.3. Thiobarbituric acid (TBA) assay

One ml of 2.51% linoleic acid, 20% trichloroacetic acid (200 μl) and 0.67% thiobarbituric acid (200 μl) were added to varying concentrations of *C. nucifera* extracts (2, 4, 6, 8 & 10 mg/ml) and maintained for 10 min in boiling water bath. After centrifugation at 3000 rpm, the absorbance of the supernatant was measured at 532 nm (Udayaprakash et al., 2015).

2.3.4. Ferric thiocyanate (FTC)

To the extracts of *C. nucifera* (2, 4, 6, 8 & 10 mg/ml), 120 μl of 98% ethanol, 100 μl of 2.51% linoleic acid and 9 ml of 40 mM phosphate buffer (pH 7) were added. Further, the solution was maintained at 40 °C in dark for 40 min. To this solution (100 μl), 75% ethanol (9.7 ml), 30% ammonium thiocyanate (100 μl) and 20 mM $FeCl_3$ in 3.5% HCl (100 μl) were added successively. The absorbance was measured at 500 nm (Udayaprakash et al., 2015).

2.3.5. Nitric oxide (NO) radical scavenging assay

Two ml of sodium nitroprusside (5 mM) was added to different concentrations of *C. nucifera* extracts (2, 4, 6, 8 & 10 mg/ml). The solutions were incubated at room temperature for 1 h, to which 5 ml of Griess reagent was added. The absorbance was then measured at 546 nm (Jagetia et al., 2004).

2.3.6. β-carotene bleaching assay

An emulsion was prepared by mixing 11 μl of β-carotene (8.2 μM), 4.4 μl of linoleic acid (628 μM) and 22 μl of Tween 40 (0.2 g/ml). On removal of the solvent from the emulsion, 2.4 ml of phosphate buffer (0.02 M, pH 7) and varying concentrations of *C. nucifera* extracts (2, 4, 6, 8 & 10 mg/ml) were added. The solutions were maintained at 50 °C for 10 min and the absorbance was measured at 460 nm (Lelono et al., 2009).

The percent of inhibition exhibited by different concentrations of the extracts in all the free radical scavenging assays was calculated as:

$$Percent\ inhibition = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control} \times 100$$

where control refers to the solution containing all the reagents as applicable for each assay, and does not possess the sample (extract). The concentration at which 50% of the radicals were scavenged i.e., EC_{50} was calculated from the percent inhibition values, by regression analysis.

2.4. Statistical analysis

The EC_{50} values for the free radical scavenging assays were calculated by the best fit as applicable for each assay (logarithmic regression for DPPH assay and linear trend for all other radical scavenging assays). Correlation and Principal Component analysis (PCA) were performed to correlate the mean values of the phytochemicals quantified with the EC_{50} of the free radical scavenging assays. Correlation was performed at 95% confidence interval and the Pearson correlation coefficient (R^2) was recorded. Regression, correlation and PCA were carried out using Minitab, LLC (2019).

3. Results

3.1. Primary metabolites

The total protein content was the highest in HPME (1.93 ± 0.04 mg/g) followed by that in cold percolation method, i.e., CPME (1.62 ± 0.15 mg/g). The chloroform extract possessed protein content of 1.27 ± 0.10 mg/g and 1.14 ± 0.05 mg/g in hot and cold percolation methods respectively. Similarly, the total carbohydrate content was highest in HPME (12.72 ± 0.15 mg/g), followed by CPME (10.39 ± 0.16 mg/g). However, the total lipid content was found to differ, with higher quantity in the chloroform extracts when compared to methanol. The total content of proteins, carbohydrates and lipids of coconut cotyledons extracted using hot and cold percolation method with respective solvents is presented in Table 1.

3.2. Secondary metabolites

Preliminary screening for the detection of different secondary metabolites in the cotyledon of *C. nucifera* revealed the presence of cardiac glycosides alone in the chloroform extracts obtained through hot and cold percolation methods. However, cardiac glycosides were not detected in methanol extract either through hot or through cold percolation method. All other secondary metabolites analysed were not detected in the preliminary screening.

The yield of total phenolic content was found to be high (30.35 ± 0.94 mg TAE/g dw) in CPME, followed by HPME (29.58 ± 0.82 mg TAE/g dw). The total flavonoids were in trace amounts, in both the extraction methods. There was no difference in the total tannins content irrespective of the extraction method or solvent, in the range 1.85 ± 0.03 mg TAE/g dw to 1.94 ± 0.04 mg TAE/g dw.

The total alkaloid content of coconut cotyledon was high in chloroform extracts, with the values 19.7 ± 0.32 and 16.68 ± 0.42 mg CE/g dw respectively, in cold and hot percolation methods. The TAlC of the methanol extracts was recorded as 5.74 ± 0.37 mg CE/g dw through cold percolation and 5.01 ± 0.57 mg CE/g dw through hot percolation. Pertaining to total antioxidant content, the yield of CPME was the highest (155.87 ± 0.39 mg TAE/g dw), followed by HPME (143.28 ± 0.79 mg TAE/g dw). However, the solvent chloroform extracted nearly 20 fold lower quantities in both, hot and cold percolation methods. The total phenolics, flavonoids, tannins, alkaloids and antioxidant contents extracted from cotyledons of coconut using hot and cold percolation methods using methanol and chloroform as the solvents are presented in Table 1.

3.3. Free radical scavenging activity

C. nucifera extracts had high scavenging activity against DPPH radicals. The methanol extract of coconut cotyledon showed nearly 88% of inhibition at the concentration of 10 mg/ml. There was no difference in percent inhibition of DPPH at higher concentration of the extracts. However, significant difference was noticed at lower concentration (2 mg/ml) of the extract. Nearly 75% of inhibition was recorded for the CPME and only 55% for HPCE. The EC_{50} values were recorded as 0.44, 1.52, 0.12 and 1.47 mg/ml, respectively for HPME, HPCE, CPME and CPCE. The inhibition percent of DPPH radicals at different concentrations of the solvent extracts is presented in Figure 2a.

Similar trend was observed in the ferric reducing antioxidant power (FRAP) assay. The methanolic extract by cold percolation scavenged the radicals by 66.67%, while hot percolation resulted in 51.9%. The chloroform extracts of cold and hot percolation showed 48.67 and 16.67%

Table 1. Quantification of primary and secondary metabolites in *C. nucifera* cotyledons.

	Hot percolation		Cold percolation	
	Methanol	Chloroform	Methanol	Chloroform
Total protein content (mg/g)	1.93 ± 0.04	1.27 ± 0.1	1.62 ± 0.15	1.14 ± 0.05
Total carbohydrate content (mg/g)	12.72 ± 0.15	1.84 ± 0.14	10.39 ± 0.16	1.33 ± 0.12
Total lipid content (mg/g)	14 ± 0.05	15.4 ± 0.04	13.8 ± 0.04	16 ± 0.03
TPC (mg TAE/g)	29.58 ± 0.82	6.44 ± 0.72	30.35 ± 0.94	15.82 ± 0.81
TFC (μg QE/g)	23.67 ± 2.61	11.97 ± 5.17	24.3 ± 2.88	16.3 ± 4.94
TTC (mg TAE/g)	1.94 ± 0.04	1.85 ± 0.03	1.94 ± 0.01	1.85 ± 0.05
TAlC (mg CE/g)	5.01 ± 0.57	16.68 ± 0.42	5.74 ± 0.37	19.7 ± 0.32
TAC (mg TAE/g)	143.28 ± 0.79	7.05 ± 0.66	155.87 ± 0.39	8.17 ± 0.88

TAE: Tannic acid equivalent; QE: Quercetin equivalent; CE: Caffeic acid equivalent.

respectively at the concentration of 10 mg/ml (Figure 2b). The EC_{50} values of HPME, HPCE, CPME and CPCE were calculated as 9.36, 37.99, 6.43 and 10.41 mg/ml respectively.

Scavenging of radicals generated in lipid peroxidation is studied through FTC and TBA assays. In both the assays, the chloroform extract of cold percolation yielded higher inhibition percent, followed by that through hot percolation. In the first stage of lipid peroxidation as studied through FTC assay, upto 37% radicals were inhibited by the chloroform extract (cold percolation), which further increased to 51.31% in the secondary stage. The percent inhibition of the CPME ranged between 6 and 24.47% in FTC assay, while it was 22.47–40.26% in TBA assay (Figure 2c-d). Consequently, the EC_{50} values were lower in TBA assay when compared to FTC assay. The respective EC_{50} values of HPME, HPCE, CPME and CPCE in FTC assay were 22.2, 16.86, 21.78 and 13.31 mg/ml; which further decreased to 16.27, 10.25, 14.53 and 9.21 mg/ml in TBA assay.

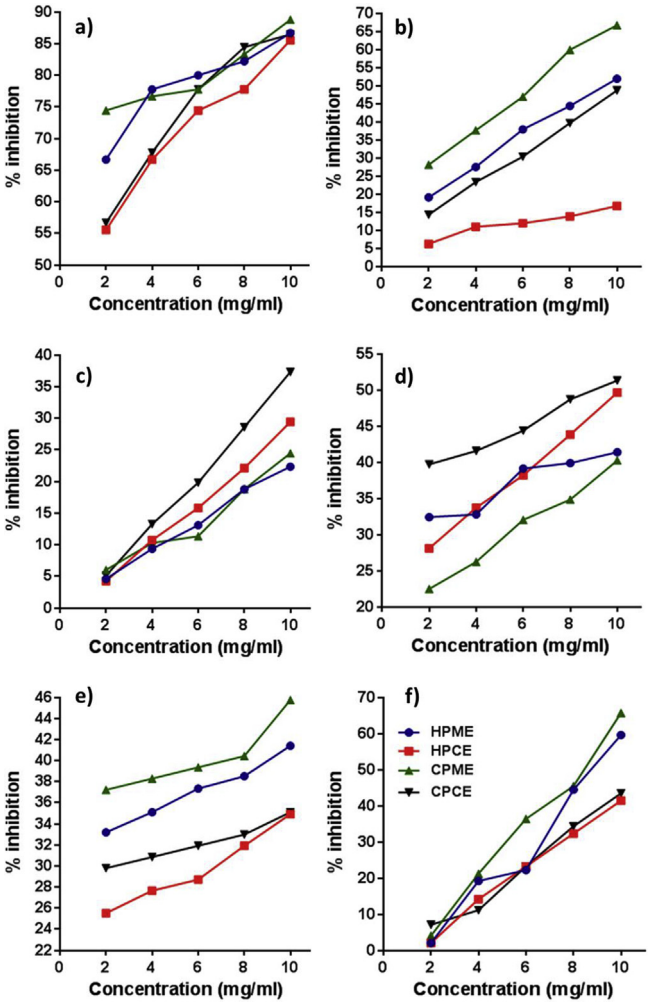

Figure 2. Percent inhibition of radicals recorded by *C. nucifera* cotyledon in a) DPPH radical scavenging assay, b) FRAP assay, c) FTC assay, d) TBA assay, e) NO radical scavenging assay, f) β-carotene bleaching assay.

Analogous to DPPH and FRAP assays, the methanolic extract of the coconut cotyledon extracted through cold percolation exhibited high percentage in scavenging NO radicals, with EC_{50} value of 16.21 mg/ml. About 45.74 and 41.4% of NO radicals were scavenged respectively by CPME and HPME (EC_{50} = 18.98 mg/ml). Not much difference in percent inhibition was observed between the chloroforms extracts obtained by hot (34.91%) and cold percolation (35.11%). However, the corresponding EC_{50} were 23.58 and 33.99 mg/ml.

Similarly, CPME and HPME exhibited 65.66% and 59.6% respectively in β-carotene bleaching assay, with respective EC_{50} of 8.1 and 8.92 mg/ml. The chloroform extracts obtained by cold and hot percolation revealed 43.43% and 41.41% of radical inhibition and EC_{50} of 11.45 and 11.65 mg/ml. The percent inhibition recorded in NO free radical scavenging assay and β-carotene bleaching assay are presented in Figure 2e and 2f respectively.

The loading plot (Figure 3.) and the correlation matrix (Table 2) represent the relation between the quantification of metabolites and the EC_{50} values of the free radical scavenging assays. TPC, TFC, TTC and TAC are found to reside in the same cluster, with high positive value in the first principal component. Lipids and alkaloids were the only metabolites which correlated positively with DPPH, NO radical scavenging and β-Carotene bleaching assays, while all other metabolites exhibited strong negative correlation. Similarly, total carbohydrates, proteins, tannins and total antioxidant contents positively correlated with FTC and TBA assays. However, no significant positive correlation was observed for FRAP assay with any of the metabolites, while TPC (R^2 = -0.859) and TFC (R^2 = -0.841) showed strong negative correlation.

4. Discussion

Coconut tree is an important subsistence crop in the tropics and almost every part of the plant is explored for application in different fields. Although many studies are available on the application of different plant parts (Lima et al., 2015), the same related to cotyledons of the tree has not been reported so far. Thus, the present study was conducted to know the presence of primary & secondary metabolites in the cotyledons of coconut and evaluate their free radical scavenging potency.

The cotyledon of coconut is described as a haustorial like tissue developed during embryogenesis (Buffard-Morel et al., 1995). As the embryo develops, the cotyledon becomes the dominant tissue, overwhelming the growth of root and shoot primordium (Haccius and Philip, 1979). Cotyledon is a significant part of the embryo and is hypogeal in germination, which remains below the ground. The cotyledons are not

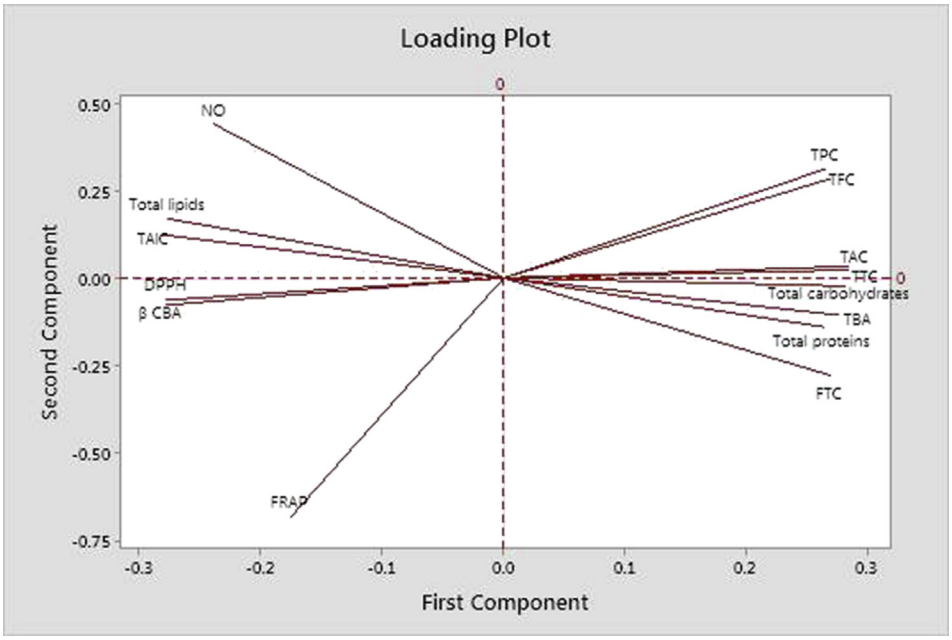

Figure 3. Loading plot recorded in principal component analysis.

Table 2. Correlation of primary and secondary metabolites in *C. nucifera* cotyledons with EC_{50} recorded in free radical scavenging assays.

	DPPH	FRAP	FTC	TBA	NO	β CBA
TP	-0.84	-0.447	0.932	0.988	-0.792	-0.832
TC	-0.94	-0.59	0.945	0.995	-0.811	-0.935
TL	0.961	0.452	-0.986	-0.954	0.938	0.956
TPC	-0.942	-0.859	0.771	0.866	-0.603	-0.945
TFC	-0.954	-0.841	0.792	0.878	-0.633	-0.957
TTC	-0.984	-0.639	0.939	0.969	-0.827	-0.981
TAlC	0.958	0.501	-0.983	-0.985	0.898	0.952
TAC	-0.993	-0.647	0.933	0.954	-0.831	-0.991

TP: Total protein content.
TC: Total carbohydrate content.
TL: Total lipid content.
TPC: Total phenolic content.
TFC: Total flavonoid content.
TTC: Total tannin content.
TAlC: Total alkaloid content.
TAC: Total antioxidant content.
DPPH: 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay.
FRAP: Ferric reducing antioxidant power.
FTC: Ferric thiocyanate assay.
TBA: Thiobarbituric acid assay.
NO: Nitric oxide radical scavenging assay.
β CBA: β-carotene bleaching assay.

photosynthetic and simply act as an organ to store and supply food for a developing embryo. This favours our study on the primary and secondary metabolites in highly nutritive tissues, i.e. cotyledons. Further, as they are widely consumed, their antioxidant potency was examined.

The study on the primary metabolites revealed the carbohydrate content of the cotyledon as 12.72 ± 0.15 mg/g, the protein content as 1.93 ± 0.04 mg/g and lipid content as 16 ± 0.03 mg/g. The protein content of coconut water or the endosperm of the coconut with different age groups of 7–10 months was reported to be in the range of 0.02%–0.13% and fat content as 0.5%–1.8% (Jackson et al., 2004). It is noticed that there is no significant difference between the nutritive content of cotyledon and the liquid endosperm of coconut. However, the difference is only attributable to the solvents used in the study.

Screening for the presence of secondary phytoconstituents revealed the presence of only cardiac glycosides. The medical significance of cardiac glycosides includes treatment of cardiac arrhythmias, congestive heart failure and as anticancer agents (Riganti et al., 2011). This favours the utilization of cotyledon of coconut as a good nutritive source to prevent heart ailments. Absence of other phytochemicals in preliminary screening may be due to their presence in trace quantities. The quantification of the total phenolics, flavonoids, tannins and alkaloids revealed their presence and further confirms that they are at meagre level. Further, certain phytochemicals, though present in the plant extracts, may provide negative results in the qualitative screening assays, owing to structural idiosyncrasies (Jones and Kinghorn, 2006).

Phytoconstituents such as proteins, carbohydrates, polyphenols, flavonoids and tannins are known to be responsible for antioxidant activity (Hu et al., 2016; Kim et al., 2003). Mahayothee et al. (2016) reported the TPC of coconut water in the range of 5.18–7.17 mg GAE/100 ml while the coconut meat possessed 6.28–10.01 mg GAE/100 g. The results of the present study reveal that the cotyledons of coconut possess high phenolic content when compared with coconut water and coconut meat. The TFC in the extracts varied between 12-24 μg QE/g dw. The TTC was almost the same, irrespective of the solvent and the extraction method. The total alkaloid contents were high in the chloroform extracts when compared with the methanolic extracts of *C. nucifera* cotyledons.

The studies on quantification of secondary metabolites, i.e. total phenols, flavonoids, tannins, alkaloids and antioxidants from the cotyledons of coconut revealed that their yield is higher in cold percolation method than hot percolation. Among the solvents studied, all the secondary metabolites except for alkaloids were extracted highly in methanol when compared with chloroform. Yields of different phytochemicals from plant extracts are found to vary depending on the method of extraction and the solvent used (Udayaprakash et al., 2019).

Different radicals generated during the metabolism of biomolecules in the biological system have the potency to hinder normal activities and lead to various diseases (Phaniendra et al., 2015). Hence, a balance between oxidants and antioxidants is necessary. Thus, using cotyledons of coconut as a nutritive source balances the ratio between oxidants and antioxidants. The EC_{50} value against the DPPH radicals was observed to be less than 2 mg/g of the extract of coconut cotyledon. The EC_{50} was recorded as >1 μg/μl for coconut water (Fonseca et al., 2009). Chakraborty and Mitra (2008) conducted a study on the potency of coconut meal in scavenging of radicals in DPPH, FRAP and TBA assays. They reported the efficiency of scavenging DPPH radicals in concentration range of 22.7–90.2 μg/ml. The EC_{50} recorded in FRAP assay was 6.434 mg/ml by the cotyledons, whereas that of coconut meal ranged from 768 to 1292 mM. In TBA assay, inhibition percent of 51.31% was recorded at the concentration of 10 mg/g of coconut cotyledon. The same was reported as 40.6% in coconut meal. The NO scavenging activity recorded for the cotyledon was 45.74%. Singla et al. (2011) reported the EC_{50} of coconut endosperm against NO radicals to be 538.44 μg/ml. The potency of radical scavenging by coconut through ferric thiocyanate assay and β-carotene bleaching assay is reported here for the first time, among any of the coconut parts studied so far. Antioxidants are classified as very strong (<50 μg/ml), strong (50–100 μg/ml), medium (101–150 μg/ml), weak (>150 μg/ml). Based on this, cotyledons of coconut are categorised as medium antioxidants against DPPH radicals and weak antioxidants against radicals in FRAP, FTC, TBA, NO & β-carotene bleaching assays.

In general, correlation coefficient values near 1 or -1 shows a strong relationship between the variables (Schober and Schwarte, 2018). From the correlation analysis of metabolite yield and EC_{50} of the free radical scavenging assays, it is evident that all the metabolites correlated strongly either positively or negatively with DPPH, FTC, TBA, NO radical scavenging and β-Carotene bleaching assays. However, only TPC and TFC showed strong negative correlation to FRAP assay.

In the present study, radicals generated in the DPPH, FRAP, NO and β-carotene leaching assays are predominantly scavenged by methanolic extracts than chloroform. This is similar to previous studies that suggest the use of methanolic extracts to assess the free radical scavenging ability of plants (Sharma and Bhat, 2009).

The significant findings of the study include (i) first study to report the phytochemistry and free radical scavenging activity of cotyledon of coconut; (ii) The presence of cardiac glycoside as the secondary metabolite proves that this particular part of coconut can be recommended for people ailing with cancer and cardiac diseases; (iii) the coconut cotyledon is analogous to the endosperm of coconut, in phytochemical constitution and antioxidant activity. Thus, consumption of cotyledon of coconut is recommended, similar to the endosperm of coconut.

5. Conclusion

The study reports the quantification of primary metabolites, detection and quantification of secondary metabolites and free radical scavenging activity of the cotyledon of coconut for the first time. Among the primary metabolites, high carbohydrate and lipid contents were recorded in the methanolic and chloroform extracts respectively. The detection of secondary metabolites showed the presence of cardiac glycosides which favours the use of cotyledon as a nutritive source for people ailing with cancer and heart disease. The total phenolics, tannins and alkaloids from the cotyledons proved that they are nutritionally equivalent to the endosperm of coconut. The free radical scavenging assays also proved that they act as medium/weak antioxidants. The percent inhibition and EC_{50} values indicate that the antioxidant activity of the cotyledons is equivalent to that of coconut water or the endosperm. Antioxidant activity by ferric thiocyanate radical scavenging and β-carotene bleaching assays is reported for the first time from coconut. Lipids and alkaloids were the only metabolites which positively correlated with DPPH, NO radical scavenging and β-Carotene bleaching assays; while carbohydrates, proteins and tannins correlated with FTC and TBA assays. Further, it is recommended to extract compounds through cold percolation, with methanol as the preferred solvent. The study concludes the cotyledon of coconut to be a potent nutritive source equivalent to the endosperm, i.e., either coconut meal or coconut water.

Declarations

Author contribution statement

Udaya Prakash Nyayiru Kannaian: Conceived and designed the experiments; Wrote the paper.

Jasmine Brighty Edwin: Performed the experiments.

Vidhya Rajagopal: Analyzed and interpreted the data.

Sripriya Nannu Shankar: Performed the experiments; Wrote the paper.

Bhuvaneswari Srinivasan: Conceived and designed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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