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ORIGINAL ARTICLE

Comparative Evaluation of Antioxidant Activity and Liquid Chromatography–Mass Spectrometry-based Phytochemical Profiling of Various Biological Parts of *Caryota urens*

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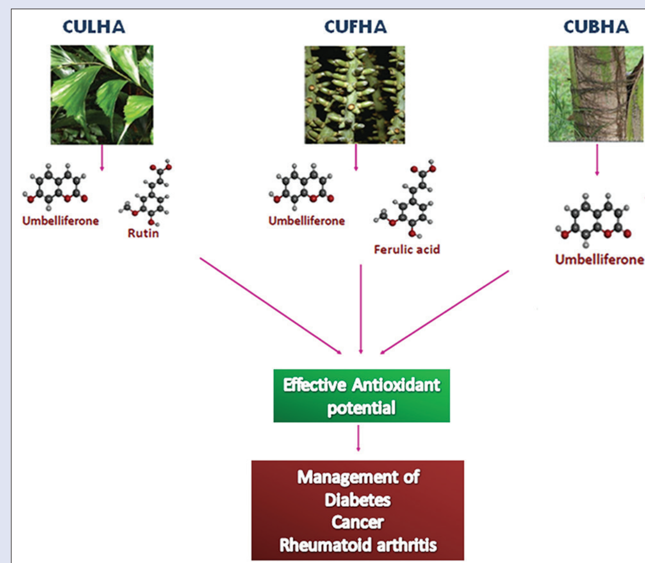
ABSTRACT

Introduction: *Caryota urens* belongs to the palm family and is widely distributed in Asia. Conventionally, it is used to treat gastric ulcers, snake bites, migraine, and rheumatic swellings. The objectives of this study were to determine the phytochemical content and antioxidant activities of hydroalcoholic and aqueous (Aq) extracts of various parts of *C. urens*. **Materials and Methods:** Extractions of various parts of *C. urens* were performed by cold maceration using different solvents such as 70% ethanol and distilled water. The extracts were subjected to assessment of their antioxidant potential using various *in vitro* systems such as 1,1-diphenyl-2-picrylhydrazyl radical, hydroxyl radical, superoxide radical, lipid peroxidation, and phosphomolybdenum reduction. The extract was subjected to Fourier-transform infrared spectroscopy, liquid chromatography–mass spectrometry (LC-MS), and high-performance liquid chromatography (HPLC) analysis to detect the phytoconstituents. **Results:** The total flavonoid and total phenol content was found to be highest in leaf hydroalcoholic extract (*C. urens* leaf hydroalcoholic extract [CULHA]), 43.84 ± 3.59 mg/g quercetin equivalent, and 41.68 ± 3.30 mg/g gallic acid equivalent, respectively. Hydroalcoholic extracts of *C. urens* (CULHA, *C. urens* fruit hydroalcoholic extract and *C. urens* bark hydroalcoholic extract) had the highest antioxidant activity when compared to Aq extracts. Phenolic acids, coumarins, carboxylic acids, and flavonoids were characterized by LC-MS. HPLC analysis further confirmed the presence of rutin, umbelliferone, and ferulic acid. **Conclusion:** A direct association between the high content of flavonoid rutin and antioxidant activity in leaf hydroalcoholic extract was noted. The fact that *C. urens* is rich in coumarins and rutin seems to explain the high antioxidant potential of this plant extract.

Key words: Antioxidant activity, *Caryota urens*, Fourier-transform infrared spectroscopy, liquid chromatography–mass spectrometry, rutin, umbelliferone

SUMMARY

- Among the different extracts of various parts of *Caryota urens*, the hydroalcoholic extracts exhibited significantly high antioxidant activity on the *in vitro* free radical systems such as 1,1-diphenyl-2-picrylhydrazyl, superoxide, lipid peroxidation, and hydroxyl radicals
- Further phytochemical profiling of hydroalcoholic extracts of leaf, fruit, and bark identified the presence of rutin, umbelliferone, 4-methylumbelliferone, ferulic acid, and phaseolin
- The presence of these phytochemicals along with the antioxidant potential suggests that the *C. urens* may also have a role in the treatment of cancer, diabetes, and rheumatoid arthritis.



Abbreviations Used: DPPH: 1,1-diphenyl-2-picrylhydrazyl; LPO: Lipid peroxidation; SO: Superoxide; NBT: Nitroblue tetrazolium; PMS: Phenazine methosulfate; CULHA: *C. urens* leaf hydroalcoholic extract; CULAq: *C. urens* leaf aqueous extract; CUFHA: *C. urens* fruit hydroalcoholic extract; CUFAq: *C. urens* fruit aqueous extract; CUBHA: *C. urens* bark hydroalcoholic extract; CUBAq: *C. urens* bark aqueous extract; ROS: Reactive oxygen species; %: Percent; °C: Celsius; µg: Microgram; µl: Microliter; mg: Milligram; ml: Milliliter; OD: Optical density; IC50: Concentration yielding 50% inhibition; FTIR: Fourier-transform infrared spectroscopy; LC-MS: Liquid chromatography–mass spectrometry; GAE: Gallic acid equivalent; QE: Quercetin equivalent; AE: Ascorbic acid equivalent; TPC: Total phenolic content; TFC: Total flavonoid content; TAC: Total antioxidant activity.

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INTRODUCTION

Human body produces a substantial amount of free radicals as a result of regular physiological functions. However, the production rate of free radicals can increase due to pollution, radiation, and industrial chemicals causing damage to lipids, proteins, and DNA.[1] Reactive oxygen species (ROS) generation can lead to

lifestyle diseases such as inflammatory disorders, diabetes, cancer, arthritis, and others.[2] Inflammation is a defense mechanism which the body uses to combat foreign agents. Controlled inflammatory response is beneficial for the body. If unregulated, inflammation can lead to acute and chronic diseases.[3] During inflammation, enzymic antioxidants such as superoxide dismutase and catalase protect the body from the oxidative stress generated by free radicals.[4] Deficiency in antioxidant level is accumulated in a number of diseases. Hence, a proper balance between antioxidants and free radicals are required to lead a healthy life.

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and SAIDs such as dexamethasone not only have been widely used to treat inflammation but also suffer significant side effects for instance, the development of gastrointestinal disorders.[5] Antioxidants as a supplement can scavenge the free radicals and are useful for the prevention of lifestyle diseases.[6]

Caryota urens belongs to the palm family and contains about 13 species which are widely distributed in Asia. It is also commonly known as wine palm/toddy palm. Conventionally, it is used to treat gastric ulcers, snake bites, migraine, and rheumatic swellings and has attained scientific interest for pharmaceutical purposes because of its documented health properties.

In this context, the aim of the study is to evaluate and compare the antioxidant activity of hydroalcoholic and aqueous (Aq) extracts of leaf, fruit, and bark of *C. urens*.

MATERIALS AND METHODS

Plant source and identification

C. urens used in this study was collected from Vels Institute of Science, Technology and Advanced Studies Campus, Chennai, in December 2016. Young fruits, leaves, and bark of the plant were collected for the study. It was authenticated (PARC/2016/3315) by plant taxonomist Dr. J. Jeyaraman, PARC, Tambaram, Chennai - 600045.

Preparation of plant extract

Plant material was washed to remove any dirt and dried under shade for 15 days. The dried plant material was made into coarse powder using a mixer grinder and was further subjected to extraction procedures.

Two hundred grams of each powered plant part sample was soaked in 70% ethanol (1600 ml) and distilled water for 72 h with intermittent stirring.[7] At the end of the process, the extract was filtered through Whatman filter paper No. 1 (Whatman Ltd., England). The filtrate was concentrated by evaporating to dryness using distillation at 50°C and stored at 4°C until when needed. The extracts were designated as follows:

- *C. urens* leaf hydroalcoholic extract (CULHA)
- *C. urens* leaf Aq extract (CULAq)
- *C. urens* fruit hydroalcoholic extract (CUFHA)
- *C. urens* fruit Aq extract (CUFAq)
- *C. urens* bark hydroalcoholic extract (CUBHA)
- *C. urens* bark Aq extract (CUBAq).

Their respective yields were calculated and then stored at 4°C until further use.

Determination of total phenols

The total phenolic content (TPC) was determined using Folin's Ciocalteu method.[8] Gallic acid was used as a standard. About 0.5 ml of plant extract or standard was aliquoted into each test tube. About 1 ml of the Folin's Ciocalteu reagent was added and mixed well. After 5 min, 3 ml of Na_2CO_3 was added to the mixture. The final volume was made up using distilled water. After incubating the samples for 2 h at room temperature, TPC was measured at 750 nm using an ultraviolet–visible spectrophotometer. The TPC was expressed as milligram gallic acid equivalent (GAE)/gram dry weight.

Determination of total flavonoids

Total flavonoid content (TFC) was measured by the method of Zhishen *et al*., 1999.[9] About 1 ml of each of the extracts and varying concentrations of standard quercetin solution was added to each test tube. To this, 0.3 ml of 5% $NaNO_2$ and 0.3 ml 10% $AlCl_3$ were added. After 1 min, 2 ml of 1 M NaOH was added, and the total volume was made up to 5 ml with distilled water. The solution was vortexed well, and the absorbance was read at 510 nm. TFC of extracts was expressed as milligram quercetin equivalent (QE)/100 g dry weight.

Determination of total antioxidant activity by phosphomolybdenum assay

Total antioxidant activity of the samples was determined according to the method of Prieto *et al*.[10] The assay is based on the reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V). In brief, 1.0 ml of extract at different concentrations was mixed with 1.0 ml of reagent solution. The presence of antioxidant components in the extract is assessed by recording the absorbance at 695 nm. The results of the assay were represented as ascorbic acid equivalent/gram of dry weight of the sample.

1,1-diphenyl-2-picrylhydrazyl radical scavenging assay

The method described by Mensor *et al*.[11] was used to determine 1,1-diphenyl-2-picrylhydrazyl (DPPH)-scavenging activity of the plant extract. The solution of 0.135-mM DPPH was prepared in methanol. Different concentrations of the extract (0.5 ml) were mixed with 2.5 ml of DPPH solution. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference. The ability of plant extract to scavenge DPPH radicals was calculated from the following formula:

%DPPH inhibition = ([optical density (OD) of control − OD of test]/[OD of control]) × 100

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the extracts was determined according to the method reported by Halliwell and Gutteridge.[12] The reaction mixture contained 0.1 ml of deoxyribose, 0.1 ml of $FeCl_3$, 0.1 ml of EDTA, 0.1 ml of H_2O_2, 0.1 ml of ascorbate, 0.1 ml of Phosphate buffer, and various concentrations of plant extracts in a final volume of 1.0 ml. The tubes were capped tightly and heated in a water bath at 37°C for 60 min; the reaction was terminated by adding 1.0 ml of ice-cold trichloroacetic acid (TCA) (10%). To the above reaction mixture, 1.0 ml of thiobarbituric acid (TBA) (0.5%) was added and incubated at room temperature for 15 min for color development. The intensity of the pink color formed was measured at 532 nm against a reagent blank. Mannitol was used as standard.

% inhibition = ([absorbance of control − absorbance of test sample]/absorbance of control) × 100

Lipid peroxidation assay

Lipid peroxidation (LPO), a well-established method, is used as an indicator of oxidative stress.

Inhibitions of LPO in the egg of hen were determined by TBA reactive species (TBARS) assay as previously described.[13] Egg homogenate (0.4 ml,

10% in distilled water, v/v), and various concentrations of each extract were mixed separately in a test tube, and the volume was made up to 1 ml, by adding distilled water. Finally, 0.05 ml $FeSO_4$ (0.07 M) was added to the above mixture and incubated for 60 min at 37°C, to induce LPO. After incubation, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA (w/v) in 1.1% sodium dodecyl sulfate and 0.05 ml 20% TCA were added, vortexed, and then heated in a boiling water bath for 60 min. After cooling, 5.0 ml of butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of supernatant was read at 532 nm.

% inhibition = ([absorbance of control − absorbance of test sample]/absorbance of control) × 100

Determination of superoxide anion radical scavenging activity

The assay for superoxide radical scavenging activity was based on a riboflavin light nitroblue tetrazolium (NBT) system.[14] The reaction mixture contained 0.5 ml of phosphate buffer (50 mM, pH 7.6), 0.3 ml riboflavin (50 mM), 0.25 ml phenazine methosulfate (20 mM), and 0.1 ml NBT (0.5 mM). Varying concentrations of 1 ml of plant extracts were added to this mixture. Reaction was initiated using a fluorescent lamp to illuminate the reaction mixtures containing the different concentrations of extracts. After 20 min of incubation, the absorbance was measured at 560 nm. The absorbance of the control was determined by replacing the sample with methanol.

Ascorbic acid was used as a positive control. The percentage inhibition of superoxide generation was calculated using the formula:

% inhibition = ([absorbance of control − absorbance of test sample]/absorbance of control) × 100

Phytochemical profiling of *Caryota urens* by Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) analysis was used to determine the possible biomolecules responsible for the antioxidant activity. The samples were analyzed using a JASCO FT-IR 4100 spectrometer. Spectra were collected from 50 scans at a resolution of 4/cm in the transmission mode of 4000–440/cm.

Phytochemical profiling of *Caryota urens* by liquid chromatography–mass spectrometry

Preparation of samples for mass spectrometry was performed immediately before analysis and after calibration of the mass spectrometer with the standard solution. For the preparation, samples were resuspended in methanol. Samples (20 µl) were introduced into ESI source at a flow rate of 100 µl/min. Samples were analyzed using Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with direct infusion electrospray ionization assembly. Electrospray ionization conditions were as follows: sheath gas flow rate (arb) – 8, ion spray voltage – 5 kV, spray current (µA) – 0.38, and capillary temperature (°C) – 275 controlled by Tune Plus software. Positive ion mode electrospray ionization mass spectra were obtained using Orbitrap Elite mass spectrometer, method took 3 min, and scan window was scanned only once. Molecular masses and associated peak abundance for each sample were extracted from the raw files and directly imported as simple PDF files containing m/z values and abundance of the measured mass features. Compound discoverer 2.0 was used to identify putative list of compounds.

High-performance liquid chromatography analysis

To prepare the standard solutions, rutin, umbelliferone, and ferulic acid were dissolved in methanol, at a concentration of 1 mg/m1. About 100 mg of finely powdered plant extracts, i.e., CULHA, CUFHA, and CUBHA were accurately weighed, dissolved in 5 ml methanol, and sonicated for 30 minutes. The solutions were filtered through a 0.45-mm membrane filter before further analysis.

The analyses were carried out using an Agilent high-performance liquid chromatography (HPLC) system and equipped with a 205-nm detector and a 4.6 mm × 25 cm column that contains packing L1. The flow rate is about 1.5 ml/min. The separation was carried out in a gradient system using a mixture of methanol, water, and phosphoric acid (100:100:1). The sample injection volume was 20 µl. Results are expressed as milligram/100 g dry weight of the plant extract.

RESULTS AND DISCUSSION

Plants are potential sources of natural antioxidants. There are several studies elucidating the antioxidant properties of fruits and vegetables against chronic diseases such as cancer, cardiovascular disease, and immune dysfunction.[15] Phenols and flavonoids are known to play a protective role in preventing lipids from peroxidation and inhibiting various antioxidant enzymes.[16,17] In the present study, various parts of *C. urens* were found to be rich in flavonoids and phenols, thereby illustrating their antioxidant property.

Evaluation of total phenolic content

The total phenol contents of the crude extracts as determined by established method are reported as GAEs. TPC was evaluated in two different solvent extracts of leaves, fruit, and bark. TPC of the six extracts ranged from 8 to 42 mg GAE/g dry weight. Among the extracts, CULHA contained the highest amount of TPC 41.68 ± 3.30 mg/g followed by the order CULAq > CUBHA > CUFHA > CUBAq > CUFAq. Studies by Arul Ananth *et al.*[18] reported that *C. urens* fruit and leaf extracts had near similar phenolic content, but in our investigation, leaves had significantly higher phenolic content as compared to fruits. This difference in the results may be due to different extraction procedures and different polarity of solvents used. In our current investigation, TPC of hydroalcoholic extracts was significantly higher than Aq extracts [Table 1]. Results are represented in Figure 1a.

Total flavonoid content

In the present study, the total flavonoid contents of the different crude plant extracts are reported as milligram QE per gram dry weight of extract. TFC of the six extracts ranged from 8 to 44 mg/g.

Among the different crude extracts from *C. urens*, CULHA was containing the highest TFC 43.84 ± 3.59 mg/g content followed by CUBHA > CULAq > CUFHA > CUBAq = CUFAq crude extract. In our current investigation, TFC of hydroalcoholic extracts was significantly higher than Aq extracts [Table 1]. Results are represented in Figure 1b. The results obtained in our study were concordant with the already reported data,[18] indicating that leaves have higher flavonoid content than fruits.

Table 1: Contents of total phenol, flavonoid, and total antioxidant capacity of *Caryota urens*

	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (mg AE/g)
CULAq	28.08±1.70	11.69±1.36	5.61±0.14
CULHA	41.68±3.30	43.84±3.59	21.25±4.51
CUFAq	8.67±2.38	8.55±1.36	5.44±0.06
CUFHA	16.79±2.06	10.90±1.36	11.2±0.89
CUBAq	9.40±0.81	8.55±1.36	7.10±0.33
CUBHA	20.06±0.87	16.39±2.72	14.75±1.90

Results are expressed as mean±SD of three determinations. TPC: Total phenol content; TFC: Total flavonoid content; TAC: Total antioxidant capacity; GAE: Gallic acid equivalents; QE: Quercetin equivalents; AE: Ascorbic acid equivalent; SD: Standard deviation

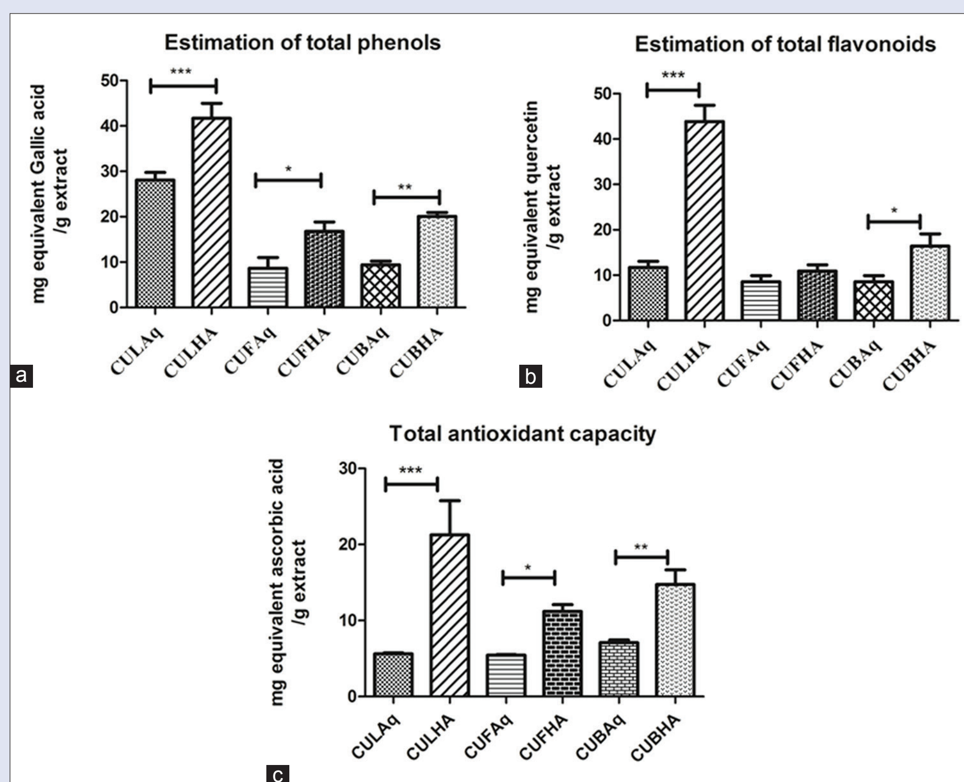

Figure 1: Histogram showing (a) total phenolic content, (b) total flavonoid content, and (c) total antioxidant activity content in hydroalcoholic and aqueous extract of leaf, fruit, and bark of *Caryota urens*. ***$P < 0.001$; **$P < 0.01$; *$P < 0.1$

Total antioxidant activity by phosphomolybdenum assay

Antioxidant capacity of various extracts of *C. urens* was determined as per the method of Prieto *et al*. The formation of the complex was measured by the intensity of absorbance in extracts. Principle is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex with the maximal absorption at 695 nm. In the ranking of the antioxidant capacity obtained by this method, the hydroalcoholic extract of *C. urens* showed higher phosphomolybdenum reduction followed by Aq extract. Sahab *et al*.[19] reported higher antioxidant activity of leaf extracts of *C. urens* by phosphomolybdenum assay. Figure 1c presents the total antioxidant capacity obtained through the phosphomolybdenum assay for each extract in comparison with that of ascorbic acid standard. High antioxidant activity of leaf hydroalcoholic extract (CULHA) 21.25 ± 4.51 mg/g similar to that of ascorbic acid indicates antioxidants in this fraction, and these could be attributable to the presence of phenolic compounds [Table 1]. Total antioxidant capacity can be ranked as CULHA > CUBHA > CUFHA > CUBAq > CULAq > CUFAq.

1,1-diphenyl-2-picrylhydrazyl radical scavenging assay

The DPPH is a stable radical that can readily undergo scavenging by antioxidants.[20] It has been widely used to evaluate the antioxidant activity of plant extracts and foods.[21] As presented in Figure 2a, the scavenging abilities of different solvent extracts of *C. urens* were expressed as IC_{50} values. Concentration of the sample required to scavenge the initial concentration of DPPH radical by 50% (IC_{50}) under the experimental condition was calculated. Therefore, a lower IC_{50} value indicates a higher antioxidant activity. CUFHA had the highest scavenging activity, 12.86 μg/ml. The order of inhibition is as follows: CUFHA > CULHA > CULAq > CUBHA > CUBAq > CUFAq. The results of the present study are in parallel with those reported in related previous studies.[18,22] Samples extracted in hydroalcohol had significantly higher radical scavenging activity than those extracted with water [Table 2]. This indicates hydroalcohol to be a better solvent for extracting antioxidants from *C. urens.*

Hydroxyl radical scavenging activity

Hydroxyl radical (HO•) is one of the most powerful free radical directly involved in the irreversible damage caused by oxidative stress. It is generated mainly through Fenton reaction, and the overall effects of hydroxyl radicals have the inclination of causing mutagenesis, carcinogenesis, and aging.[23] CUFHA had the highest scavenging activity, with an IC_{50} of 5.22 μg/ml. The order of inhibition is as follows: CUFHA > CULHA > CUBHA > CULAq > CUFAq > CUBAq. Results are represented in Figure 2b.

Our results show that the hydroxyl radical scavenging activities of the plant hydroalcoholic extracts significantly exceeded those of the standard compound mannitol at the concentrations used in this study [Table 2]. Similar to our results, Sahab *et al*.[19] showed the higher antioxidant activity of ethanol extract of *C. urens* leaf by hydroxyl radical scavenging assay. It is crucial to note that the extract displayed a significantly high level of potency. This fact still promisingly indicates the presence of potent phytoconstituents in *C. urens* that has the capability to scavenge hydroxyl radicals.

Lipid peroxidation assay

LPO is a primary toxicological event, caused by the generation of free radicals from a variety of sources including organic hydrogen peroxides

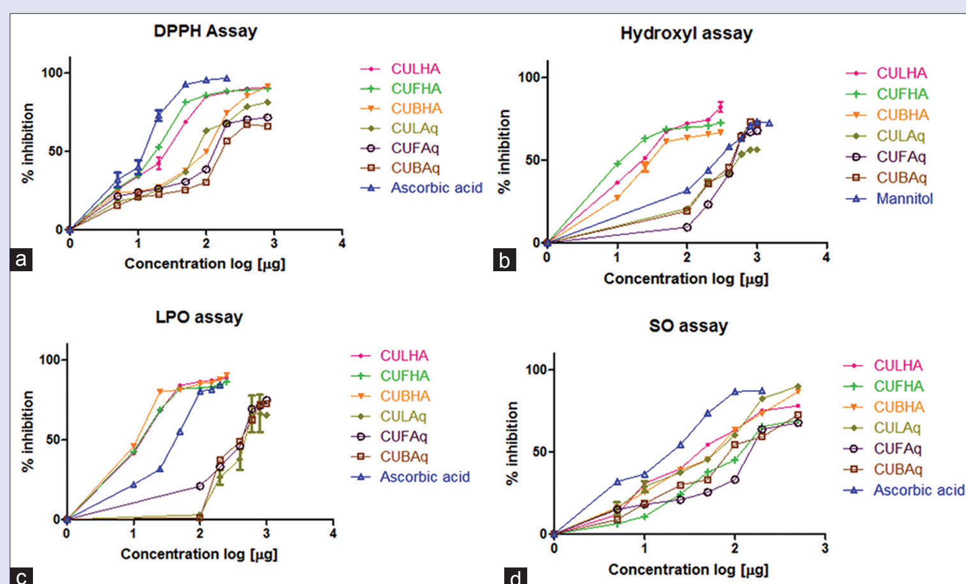

Figure 2: Determination of antioxidant activity of *Caryota urens* hydroalcoholic and aqueous extracts. (a) DPPH assay, (b) hydroxyl radical scavenging assay, (c) lipid peroxidation assay, (d) superoxide radical scavenging assay. DPPH: 1,1-diphenyl-2-picrylhydrazyl; LPO: Lipid peroxidation; SO: Superoxide

Table 2: Antioxidant activity of *Caryota urens* by *in vitro* assays

Extracts	DPPH assay IC_{50} (µg/ml)	Hydroxyl assay IC_{50} (µg/ml)	LPO assay IC_{50} (µg/ml)	SO assay IC_{50} (µg/ml)
CULHA	17.41±1.70	10.31±0.77	10.75±0.09	20.89±0.23
CUFHA	12.86±0.16	5.22±0.08	9.83±0.30	57.44±1.81
CUBHA	471.97±42.80	13.15±0.51	9.42±0.17	94.01±9.22
CULAq	59.0±0.67	196.47±17.57	483.50±24.17	173.43±16.73
CUFAq	540.33±34.10	338.63±25.97	507.57±26.14	389.80±13.26
CUBAq	523.60±47.12	477.27±11.78	242.73±11.30	79.26±4.75
Ascorbic acid	10.34±3.18	-	40.55±0.29	11.74±0.27
Mannitol	-	170.47±3.56	-	-

Results are expressed as mean IC_{50}±SD of three determinations. DPPH: 2, 2-diphenyl-1-picrylhydrazyl; LPO: Lipid peroxidation assay; SO: Superoxide radical scavenging assay; IC_{50}: 50% mean inhibition concentration

and iron-containing compounds. The TBARS assay has been used to measure the degree of LPO. TBA reacts with malondialdehyde (MDA), a secondary product of LPO to give a pink color, which may then be determined spectrophotometrically. In this study, hydroalcoholic extracts of leaf, fruit, and bark were capable of preventing the formation of MDA in a dose-dependent manner. CUBHA had the highest scavenging activity, with an IC_{50} of 9.4 µg/ml [Table 2]. The order of inhibition is as follows: CUBHA > CUFHA > CULHA > CUBAq > CULAq > CUFAq. Results are represented in Figure 2c.

Superoxide radical scavenging activity

Superoxide anions have an important role in the formation of ROS including lipid peroxides and hydrogen peroxide which can cause damage to lipids, DNA, and protein. Superoxide radical reduced NBT to a blue-colored formation that was measured at 560 nm. CULHA had the highest scavenging activity, 20.89 µg/ml [Table 2]. The order of inhibition is as follows: CULHA > CUFHA > CUBAq > CUBHA > CULAq > CUFAq. Thus, the extract seems to be a good scavenger of ROS, thus reducing rate of chain reaction. A high positive correlation was observed between the TFC content and the superoxide radical scavenging activity. Shyamala *et al.*[24] reported that leafy vegetables have a proton radical scavenging action, which is an important mechanism of antioxidants. Results are represented in Figure 2d. The superoxide-scavenging activity of *C. urens* investigated clearly indicates that *C. urens* is a potent scavenger of superoxide radicals.

Previous studies[18,19,22] have reported antioxidant properties and antimicrobial properties of leaf and fruit extracts of *C. urens*. In this study, antioxidant activity of *C. urens* leaf, fruit, and bark extracted in hydroalcoholic and Aq extracts was compared. There were marked differences in antioxidant activity and phenolic and flavonoid content between Aq and hydroalcoholic extracts, with leaf and fruit hydroalcoholic extract having the highest antioxidant activity.

Phytochemical analysis of *Caryota urens*

Overall, all the Aq extracts showed a much lower antioxidant potential than other extracts and fractions as well as standards. This result is consistent with the lowest antiradical effect of Aq extract determined by the phosphomolybdenum assay. Owing to the higher antioxidant activity, only hydroalcoholic extracts were taken for further phytochemical analysis.

Fourier-transform infrared spectroscopy

FTIR spectroscopy identified the potential functional groups responsible for antioxidant activity of *C. urens.* Carboxylic acids, lipids, alcohol group, esters, aliphatic and aromatic nitro compounds, and alkenes are seen. Our results confirmed the findings of Kavitha.[25]

Among the functional groups observed in the extracts, OH and O-C group was found to be present in all the hydroalcoholic extracts of

C. urens. The presence of OH and O-C group suggests the presence of flavonoids/phenols and carboxylic acids, respectively. Higher antioxidant potential of hydroalcoholic extracts could be probably attributed to the presence of these compounds. The peaks generated in FTIR spectroscopy were depicted in Table 3a-c and Figure 3a-c.

Liquid chromatography–mass spectrometry

The results showed the presence of rutin in CULHA which could be the reason for its higher antioxidant potential related to other extracts. The previous study conducted by other group showed the presence of rutin in fruit extract,[26] but in our current investigation, we have identified in leaf extract. This difference in results can be due to different methods of extraction or the time of collection of the plant material.

Coumarins, umbelliferone, and 4-methylumbelliferone are present in all the three plant parts and ferulic acid in fruit and bark alone. The presence of these compounds further describes the antioxidant potential of these plant extracts. The peaks generated in liquid chromatography–mass spectrometry (LC-MS) have been depicted in Table 4a-c and Figure 4a-c.

High-performance liquid chromatography analysis

Our HPLC method allowed the detection and quantification of 3 compounds (rutin, umbelliferone, and ferulic acid) from the extract obtained from the leaf, fruit, and bark of *C. urens*. CUBHA had the highest quantity of umbelliferone followed by CULHA and CUFHA. Rutin identified in CULHA by LC-MS was confirmed by HPLC. Ferulic acid in CUBHA was identified by LC-MS, while the same was not detected by HPLC. This is attributed to the very smaller quantity of ferulic acid present in CUBHA which is undetected by HPLC. To our knowledge, this is the first report on the extraction of umbelliferone from this species. Figure 5a-f represents the HPLC chromatogram of standards/extracts, and Table 5 summarizes the results of the quantification.

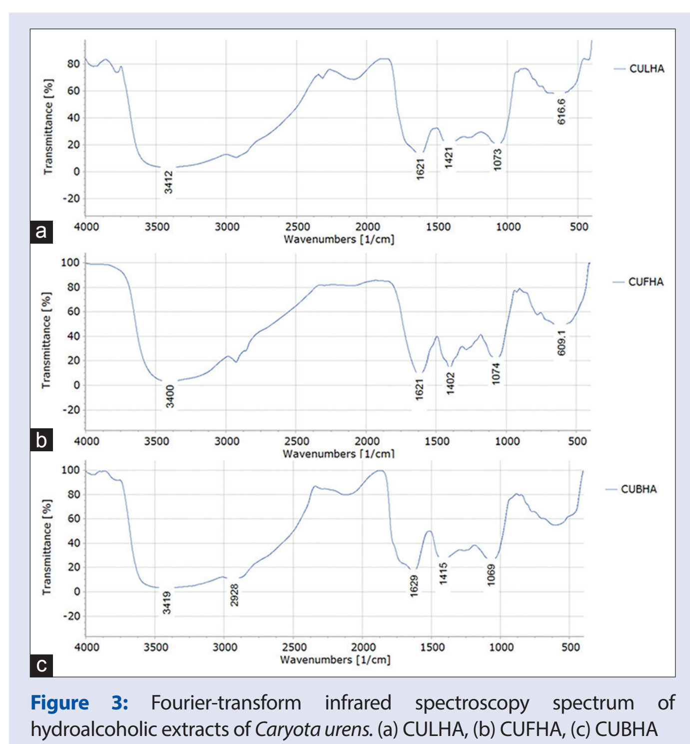

Figure 3: Fourier-transform infrared spectroscopy spectrum of hydroalcoholic extracts of *Caryota urens.* (a) CULHA, (b) CUFHA, (c) CUBHA

CONCLUSION

The antioxidant activity of *C. urens* was evaluated by various antioxidant assays, and the results indicated that hydroalcoholic extracts have high antioxidant potential. Further phytochemical profiling of hydroalcoholic extracts of leaf, fruit, and bark shows the presence of flavonoids, phenols, and coumarins, which is descriptive of its antioxidant potential.

Table 3a: Fourier-transform infrared spectral peak values and functional groups obtained for leaf hydroalcoholic extract of *Caryota urens*

CULHA absorption peak (/cm)	Functional group	Assignment
3412	O-H stretch/N-H stretch	Alcohol or phenol/amine
1621	C=C stretch	Alkenyl
1421	α-CH_2 bending	Aldehydes and ketones
1073	O-C stretch	Carboxylic acid and derivatives
616	C-H deformation	Alkynes

Table 3b: Fourier-transform infrared spectral peak values and functional groups obtained for fruit hydroalcoholic extract of *Caryota urens*

Absorption peak (/cm)	Functional group	Assignment
3400	O-H stretch/N-H stretch	Alcohol or phenol/amine
1621	C=C stretch	Alkenyl
1402	α-CH_2 bending	Aldehydes and ketones
1074	O-C stretch	Carboxylic acid and derivatives
609	C-H deformation	Alkynes

Table 3c: Fourier-transform infrared spectral peak values and functional groups obtained for bark hydroalcoholic extract of *Caryota urens*

CUBHA Absorption peak (/cm)	Functional group	Assignment
3419	O-H stretch/N-H stretch	Alcohol or phenol/amine
2928	C-H stretch	Alkyl
1629	C=C stretch	Alkenyl
1415	α-CH_2 bending	Aldehydes and ketones
1069	O-C stretch	Carboxylic acid and derivatives

Table 4a: Liquid chromatography-mass spectrometry identification of major constituents of leaf hydroalcoholic extract of *Caryota urens*

CULHA	Formula	Molecular weight	RT (min)
Flavonoids			
Rutin	$C_{27}H_{30}O_{16}$	610.15224	0.563
Lipids			
Cealysin	$C_6H_{12}N_6O_3$	216.09717	0.564
Erucamide	$C_{22}H_{43}NO$	337.33366	0.571
Ricinoleic acid	$C_{19}H_{36}O_3$	312.26595	0.561
Coumarins			
Umbelliferone	$C_9H_6O_3$	162.03185	1.46
4-methylumbelliferone	$C_{10}H_8O_3$	176.04758	0.076
Carboxylic acid			
Furoic acid	$C_3H_7NO_5S$	169.00506	0.601
Amino acids			
DL-arginine	$C_6H_{14}N_4O_2$	174.11133	0.603
Quinolones			
4-quinolinecarbaldehyde	$C_{10}H_7NO$	157.05315	0.404

CULHA: *Caryota urens* leaf hydroalcoholic extract; RT: Retention time

To our knowledge, this is the first report on LC-MS identification of phytochemicals in *C. urens,* which may explain and help in further *in vitro* and *in vivo* studies on free radicals and antioxidant activity with purified compounds. Our findings provide evidence to the spectrum of pharmacological properties supporting their use in traditional medicine.

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Conflicts of interest

There are no conflicts of interest.

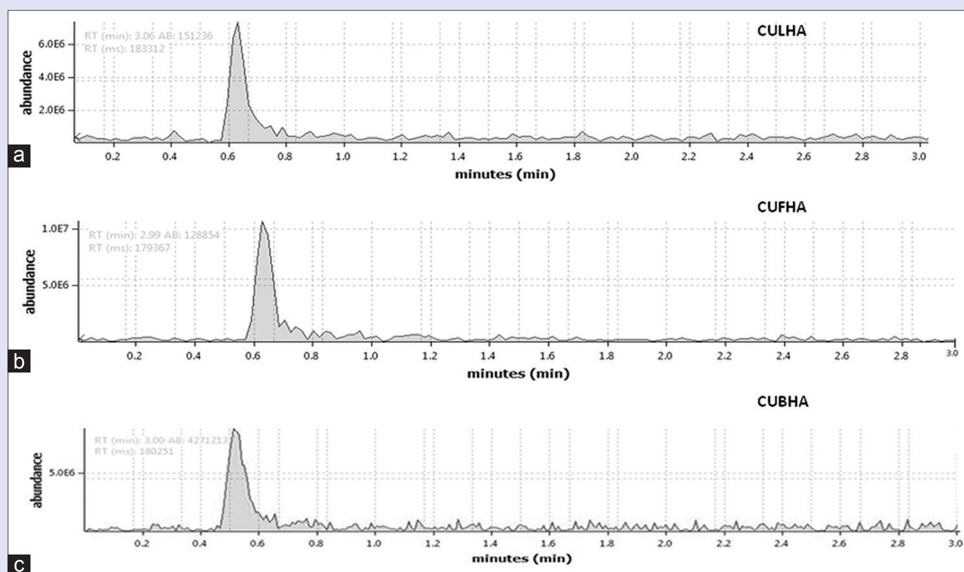

Figure 4: Liquid chromatography–mass spectrometry spectrum of hydroalcoholic extracts of *Caryota urens*. (a) CULHA, (b) CUFHA, (c) CUBHA

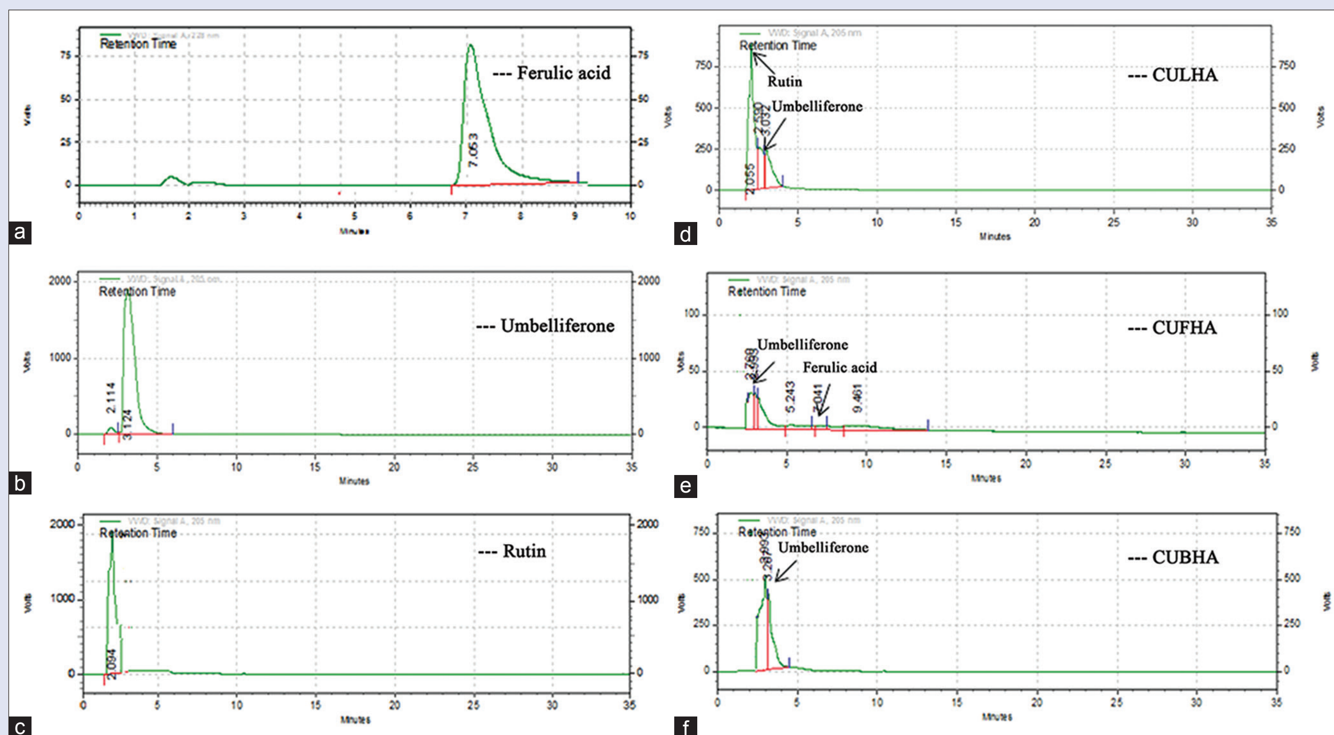

Figure 5: High-performance liquid chromatography chromatogram of standards/hydroalcoholic extracts of *Caryota urens*. (a) Ferulic acid, (b) umbelliferone, (c) rutin, (d) CULHA, (e) CUFHA, (f) CUBHA

Table 4b: Liquid chromatography-mass spectrometry identification of major constituents of fruit hydroalcoholic extract of *Caryota urens*

CUFHA	Formula	Molecular weight	RT (min)
Phenols			
(E)-ferulic acid	$C_{10}H_{10}O_4$	194.05802	1.138
Lipids			
Daucosterol	$C_{35}H_{60}O_6$	576.43712	0.632
Trilinolein	$C_{57}H_{98}O_6$	878.7368	0.649
Cealysin	$C_6H_{12}N_6O_3$	216.09741	0.18
Stigmasterol	$C_{29}H_{48}O$	412.37062	0.623
1-monolinolein	$C_{21}H_{38}O_4$	354.27702	0.626
Coumarins			
Umbelliferone	$C_9H_6O_3$	162.03179	2.085
Umbelliferyl oleate	$C_{28}H_{40}O_4$	440.29376	0.635
4-methylumbelliferone	$C_{10}H_8O_3$	176.0475	0.51
Carboxylic acid			
Phenylglyoxylic acid	$C_8H_6O_3$	150.03162	0.646
Amino acids			
DL-arginine	$C_6H_{14}N_4O_2$	174.11189	0.654
Galactosamine	$C_6H_{13}NO_5$	179.07964	0.619

RT: Retention time; CUFHA: *Caryota urens* fruit hydroalcoholic extract

Table 4c: Liquid chromatography–mass spectrometry identification of major constituents of bark hydroalcoholic extract of *Caryota urens*

CUBHA	Formula	Molecular weight	RT (min)
Flavonoid derivatives			
Phaseolin	$C_{20}H_{18}O_4$	322.12029	0.511
Apigetrin	$C_{21}H_{20}O_{10}$	432.10545	0.502
Lipids			
(E)-ferulic acid	$C_{10}H_{10}O_4$	194.05814	1.031
Cealysin	$C_6H_{12}N_6O_3$	216.09776	0.083
Trilinolein	$C_{57}H_{98}O_6$	878.73596	0.534
Daucosterol	$C_{35}H_{60}O_6$	576.43682	0.515
Ricinolein	$C_{57}H_{104}O_9$	932.76897	0.543
Coumarins			
4-methylumbelliferone	$C_{10}H_8O_3$	176.04753	0.31
Umbelliferone	$C_9H_6O_3$	162.03205	2.936
Carboxylic acid			
Phenylglyoxylic acid	$C_8H_6O_3$	150.03156	0.539
3-sulfopyruvic acid	$C_3H_4O_6S$	167.97313	0.092
Amino acids			
DL-arginine	$C_6H_{14}N_4O_2$	174.112	0.55

RT: Retention time; CUBHA: *Caryota urens* bark hydroalcoholic extract

Table 5: High-performance liquid chromatography quantification of major constituents of *Caryota urens*

	CULHA (mg/100 g)	CUFHA (mg/100 g)	CUBHA (mg/100 g)
Rutin (RT - 2.0)	70.5	ND	ND
Umbelliferone (RT - 3.2)	19.8	7.8	20.8
Ferulic acid (RT - 7.0)	ND	3.0	0

RT: Retention time; ND: Not determined

REFERENCES

1. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008;4:89-96.
2. Görlach A, Dimova EY, Petry A, Martínez-Ruiz A, Hernansanz-Agustín P, Rolo AP, *et al.* Reactive oxygen species, nutrition, hypoxia and diseases: Problems solved? Redox Biol 2015;6:372-85.
3. Khanna RD, Karki K. Inflammation, free radical damage, oxidative stress and cancer. Interdiscip J Microinflammation 2014;1:1-5.
4. Mateen S, Moin S, Khan AQ, Zafar A, Fatima N. Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis. PLoS One 2016;11:e0152925.
5. Huang ES, Strate LL, Ho WW, Lee SS, Chan AT. Long-term use of aspirin and the risk of gastrointestinal bleeding. Am J Med 2011;124:426-33.
6. Leopold JA. Antioxidants and coronary artery disease: From pathophysiology to preventive therapy. Coron Artery Dis 2015;26:176-83.
7. Othman A, Ismail A, Abdul Ghani N, Adenan I. Antioxidant capacity and phenolic content of cocoa beans. Food Chem 2007;100:1523-30.
8. Singleton VL, Rossi JA Jr. Colorimetry of total phenolics with phosphomoybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-58.
9. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;64:555-9.
10. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of Vitamin E. Anal Biochem 1999;269:337-41.
11. Mensor LL, Boylan F, Menezes S, Leita GG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2015;130:127-30.
12. Halliwell B, Gutteridge JM. Formation of thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. FEBS Lett 1981;128:347-52.
13. Ruberto G, Baratta MT. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem 2000;69:167-74.
14. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971;44:276-87.
15. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A 1993;90:7915-22.
16. Laughton MJ, Evans PJ, Moroney MA, Hoult JR, Halliwell B. Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. Biochem Pharmacol 1991;42:1673-81.
17. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, *et al.* Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J Nat Prod 1998;61:71-6.
18. Arul Ananth D, Sivasudha T, Rameshkumar A, Jeyadevi R, Aseervatham SB. Chemical constituents, *in vitro* antioxidant and antimicrobial potential of *Caryota urens* L. Free Radic Antioxid 2013;3:107-12.
19. Uddin S, Al Mamun A, Begum Y. Analysis of in vitro antioxidant activity of *Caryota urens* L. leaves: A traditional natural remedy. Journal of Coastal Life Medicine 2016;4:483-9.
20. Sowndhararajan K, Kang SC. Free radical scavenging activity from different extracts of leaves of Bauhinia vahlii wight and arn. Saudi J Biol Sci 2013;20:319-25.
21. Da Porto C, Calligaris S, Celotti E, Nicoli MC. Antiradical properties of commercial cognacs assessed by the DPPH test. J Agric Food Chem 2000;48:4241-5.
22. Azam S, Mahmud MK, Naquib MH, Hossain SM, Alam MN, Uddin MJ, *et al. In vitro* anti-oxidant and anti-microbial potentiality investigation of different fractions of *Caryota urens* leaves. Biomedicines 2016;4. pii: E17.
23. Halliwell B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol 2006;141:312-22.
24. Shyamala BN, Gupta S, Lakshmi AJ, Prakash J. Leafy vegetable extracts – Antioxidant activity and effect on storage stability of heated oils. Innov Food Sci Emerg Technol 2005;6:239-45.
25. Kavitha DS. Study on the bioefficacy of *Caryota urens* L. World J Pharm Res 2017;6:1381-98.
26. Srivastav AK, Singh R, Manimegala S, Rajeswari VD. Identification of flavonoids in methanolic extract of *Caryota urens* (Fish Tail Palm): A phytochemical screening involving structure analysis by FTIR spectroscopy. Res J Phytochem 2015;9:127-36.