Short communication

Chemical constituents, in vitro antioxidant and antimicrobial potential of Caryota urens L.

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C. urens
Antioxidant activity
Antimicrobial activity

Abstract

Objective: To evaluate chemical constituents, in vitro antioxidant and antimicrobial potential of Caryota urens L.

Methods: Qualitative analysis of crude extract of C. urens was carried out by gas chromatography mass spectrometry (GC–MS) method and their in vitro antioxidant activity was evaluated using DPPH, ABTS, reducing power and nitric oxide scavenging assays. Antimicrobial activity was determined by disc diffusion method.

Results: GC–MS analysis showed the presence of fatty acids, aliphatic, aromatic and phenolic acids. The antioxidant activity of immature fruit and leaf extracts yielded high activity when compared to the fruit skin. The fruit skin and immature fruit of C. urens exhibited strong antibacterial activity against the tested pathogens (Escherichia coli, Vibrio cholerae, Salmonella typhi, Staphylococcus aureus and Shigella flexneri) when compared to leaf.

Conclusion: In our results, we suggest that C. urens extracts have strong antioxidant and antimicrobial potential. The identified bioactive compounds of C. urens could be attributed to antioxidant and antimicrobial property.

Keyword: C. urens; antioxidant activity; antimicrobial activity; ABTS; GC–MS.

1. Introduction

Free radicals are reactive oxygen and nitrogen species (ROS/RNS) that are continuously produced in human body as a byproduct of cellular aerobic respiration. Over production of ROS/RNS leads an exposure to external oxidant substances, failure in the defense mechanism or damage to biomolecules such as DNA, lipids or proteins.1 Antioxidants are the agents capable of effectively neutralizing these free radicals by interfering with oxidation process, chelating catalytic metals and also by acting as oxygen scavengers.2 Previous reports suggest that the natural plant phytocompounds are source of excellent antioxidant agent to prevent oxidative damage caused by free radicals and thereby reduce the risk of several disorders including cancer, atherosclerosis, arthritis, central nervous system injury and degenerative disorders.3 Since the currently used synthetic antioxidants butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) were suspected to be carcinogen. Moreover, the currently available synthetic antibiotics exhibit certain demerits and side effects. It has been demonstrated that the antimicrobial effects of the plant derived compounds cause structural or functional damage to the bacterial cell membrane through various mechanisms of actions that attack the phospholipid bilayer of the cell membrane and disrupt enzyme systems.4 Therefore interest has been considerably increased in finding naturally occurring antioxidant and antimicrobial compounds suitable for use in food and medicine.5–7

Caryota urens belongs to the family Areaceae (Palmae) and the genus Caryota consist of 27 species widely distributed throughout Asia.8,9 C. urens was commonly known as wild coconut and wine palm. The plant products have admirable medicinal properties, and are used for the treatment of various ailments in the ayurvedic medical system practiced in Sri Lanka. The palm juices are used to treat seminal weakness and urinary disorders. In traditional medicine, porridge prepared from C. urens flower is used to treat gastric ulcer; migraine headaches, root bark and the cabbage or terminal bud of the palm to treat rheumatic swellings and snake bite.10 Bark and seed used to treat boils and the root is used for tooth ailments. Palm sap collected from the inflorescence is fermented with mixed
inoculum of yeast to obtain toddy. However, no reports have been concerning the antioxidant and antimicrobial activity of *C. urens*. Therefore, the aim of the present study was to compare and investigate chemical constituents and antimicrobial and antioxidant activity of the leaves, immature fruits and mature fruit skin of *C. urens*.

2. Materials and methods

2.1. Plant sample collection and extraction

Fresh *C. urens* plant material (leaf, immature fruits and fruit skin) were collected from Bodi mettu (Western Ghats), Tamil Nadu, India, which was authenticated by Dr. D. Stephen, Taxonomist, The American College, Madurai, Tamil Nadu, India. The plant samples were washed with distilled water and shade dried for three weeks (Fig. 1). After shade drying, the samples were ground to fine powder. 10 g of each sample powder extracted with 200 mL of methanol using Soxhlet apparatus for 14–16 h at solvent boiling temperature. Then the extract was concentrated using rotary evaporator at 50°C. Extracts were stored at −20°C until further use.

2.2. GC–MS analysis

GC/MS analysis of the methanol extracts was performed using a Shimadzu QP-2010 Plus with Thermal Desorption System TD 20. For MS detection, the electron ionization mode with ionization energy of 70 eV was used, with a mass range at m/z 50–550. An HP-5MS capillary column was used for GC/MS. Helium was used as carrier gas. Essential compounds were identified by their retention times and mass fragmentation patterns using data of standards at WILEY8.LIB.

2.3. Total phenolics content

Plant total phenolics content was estimated using Singleton and Rossi's standard protocol with some modifications. Folin–Ciocalteu reagent (0.5 mL) was added to 0.1 mL of the methanol extract prior to the addition of 7 mL of distilled water. The mixture was incubated in dark for 5 min at room temperature. To this mixture, 1.5 mL of sodium carbonate solution was added and incubated for 2 h at room temperature. The absorbance of blue color was read at 765 nm using UV-Spectrophotometer. Total phenolics content of the methanol extract was calculated as gallic acid equivalents (mg of GAE/g of extract).

2.4. Total flavonoid content

The total flavonoid content of plant extract was determined by Liu et al method with slight modifications. Plant methanolic extract (0.5 mL) was added with 2.5 mL of distilled water followed by addition of sodium nitrite (0.150 mL, 5%). This solution mixture was incubated for 6 min at room temperature then aluminum chloride (10%, 0.3 mL) was added and allowed to stand for 5 min. Finally, 1 mL of 1 M sodium hydroxide was added and made up the volume to 5 mL with distilled water. The absorbance was read at 510 nm using UV-Spectrophotometer. Total flavonoid content was expressed as milligram of rutin equivalents (mg of RE/g of extract).

2.5. In vitro antioxidant activity

2.5.1. DPPH radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was estimated using the method of Liana-Pathirana and Shahidi. Appropriate dilutions of the extract (1 mg/mL) was mixed with, 1 mL of 0.135 mM methanolic solution of DPPH radical. Absorbance was measured at 517 nm after 30 min of reaction. BHT was used as reference standard and the inhibition percentage was calculated using the following formula:

\[
\text{Percentage of inhibition} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100
\]

2.5.2. ABTS radical scavenging activity

Determination of 2, 2-azino-bis (3-ethylbenzthiazoline)-6-sulfonic (ABTS) radical scavenging ability of plant extracts was carried out by the method of Re et al. Previously, 7 mM ABTS solution and 2.4 mM potassium persulphate solution were prepared separately. Equal amount of two stock solutions were mixed and allowed to stand for 12 h in dark at room temperature. About 1 mL of diluted ABTS solution react with plant extract (5–25 μg/mL) after 10 min the absorbance was measured UV-spectrophotometrically at 734 nm against the blank solution. ABTS free radical inhibition was calculated by following equation:

\[
\text{Percentage of inhibition} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100
\]
Percentage of inhibition = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs control}} \times 100

2.5.3. Nitric oxide scavenging activity

Garratt,\(^\text{15}\) method was adopted to determine the nitric oxide scavenging activity of plant extract. The reaction mixture contained 2 mL of 10 mM Sodium nitroprusside, 0.5 mL of phosphate buffered saline (pH-7.4) and plant extract (100–500 μg/mL). The final volume of the solution was made up to 3 mL with distilled water. After incubation for 150 min at 25 °C, 0.5 mL of the incubated solution was taken and 1 mL sulphamic acid (0.33% in 20% glacial acetic acid) was added and allowed to stand for 5 min. Then 1 mL of 0.1% of naphthylethylenediamine dihydrochloride (w/v) was added and the mixture was incubated for 30 min at room temperature. The pink color chromophore formed was measured at 540 nm and the results were expressed as percentage of inhibition.

2.5.4. Reducing power activity

Oyaizu\(^\text{16}\) standard method was used for determination of reducing power capacity of plant extract. The reaction mixture contains 2.5 mL of 0.2 M Phosphate buffered saline (pH-6.6) and 2.5 mL of 1% potassium ferricyanide. To this mixture, plant extract (100 μg/mL) was added and incubated for 20 min at 50 °C. Then, 2.5 mL of 10% trichloro acetic acid was added. This mixture was centrifuged for 10 min at 3000 rpm. The upper layer (2.5 mL) contains 2.5 mL of 0.2 M Phosphate buffered saline (pH- 7.4) and plant extract (100 μg/mL) was added and incubated for 20 min at 50 °C. Then, 2.5 mL of 0.1% ferric chloride was added and absorbance was measured UV-Spectrophotometrically at 700 nm.

2.6. Antimicrobial activity

2.6.1. Test microorganisms

Bacterial cultures such as Escherichia coli (MTCC 1687), Vibrio cholerae (MTCC 3906), Salmonella typhi (MTCC 531), Staphylococcus aureus (MTCC 96) and Shigella flexneri (MTCC 1457) were obtained from microbial type culture collection (MTCC), Indian Institute of Microbial Technology, Chandigarh, India.

2.6.2. Disc diffusion method

The antimicrobial activity of C. urens methanol extract was assessed by the disc diffusion technique.\(^\text{17}\) Mueller Hinton agar plates were prepared and individually swabbed with pathogenic microorganisms. The sterile discs (6 mm) were placed over surface of the agar plates. Plant extract (10 mg/mL) was added on the discs at various concentrations (300 and 500 μg/mL). Disc containing standard concentrations of the antibiotic Kanamycin (30 mcg/disc) was used as positive control. The agar plates were incubated for 24 h at 37 °C, and the inhibition zones were measured in millimeter in diameter.

2.7. Statistical analysis

Experimental results were expressed as mean ± SD of three parallel measurements. The results were processed using Microsoft Excel 2007 and Origin 6.0.
3. Results and discussion

The chemical compounds of methanol extract of *C. urens* were analyzed by GC–MS method and they were represented in Table 2. By comparing the mass spectra of the plant extract with the WILEY8.LIB library, 23 compounds were identified in methanolic extract of *C. urens* leaf, 18 compounds in immature fruit and 25 compounds in fruit skin extract. Of the 66 compounds identified, the most prevailing compounds were stearic acid (octadeconoic acid) (28.51%), 10-undecenoic acid (12.86%), 2E,6E-farnesol (20.01%), hendecynoic acid (18.09%) and caffeine (22.12%).

Previous reports suggest that the similar compounds were also present in date palm leaf extracts. Catechins and 10-undecenoic acid have been reported to have an anti microbial effect, as well as anti carcinogenic, antioxidant, chemopreventive activities and antiviral antidiabetic activities. In addition, 10-undecenoic acid has effective fungicidal activity against *Candida albicans*, thus helps achieve a healthy balance of normal intestinal and vaginal flora.

Phenolic compounds were considered to be the major contributors to the antioxidant capacity of the plants. The total phenolics content of the *C. urens* methanolic extract was found to be $10.23 \pm 0.40$, $15.00 \pm 0.40$ and $17.63 \pm 0.51 \text{mg of GAE/g}$ in the fruit skin, leaf and immature fruit respectively (Table 1). The highest amount of phenolic content was found in immature fruit $(17.63 \pm 0.51 \text{mg GAE/g})$ while the lowest was in fruit skin $(10.23 \pm 0.40 \text{mg GAE/g})$. On the other hand, the amount of flavonoids was found to be higher in leaf $(15.35 \pm 0.41 \text{mg RE/g})$ when compared to immature fruit $(11.29 \pm 0.54 \text{mg RE/g})$ and fruit skin $(13.58 \pm 0.40 \text{mg RE/g})$ respectively. The phenolics and flavonoids naturally originate in medicinal plant extracts and have been reported to have major biological activities such as antioxidant and antimicrobial properties.

A highly positive relationship between total phenolics and antioxidant activity appears to be the trend in many aromatic and medicinal plant species with reference to Oktay et al. Phenolic compounds of medicinal as well as aromatic plants might be responsible to cure some inflammatory disease, like rheumatoid arthritis and nephrotoxicity.

Fig. 2a shows the dose response curve of DPPH radical scavenging activity of the methanol extracts of *C. urens* compared with anti

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**Table 2 (continued)**

<table>
<thead>
<tr>
<th>Peak</th>
<th>R.T$^a$</th>
<th>Area $^b$</th>
<th>MW$^c$</th>
<th>MF$^d$</th>
<th>Compound name</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>41.484</td>
<td>22.12</td>
<td>194</td>
<td>C$<em>{8}$H$</em>{10}$N$<em>{4}$O$</em>{2}$</td>
<td>Caffeine</td>
</tr>
<tr>
<td>24</td>
<td>42.415</td>
<td>10.86</td>
<td>254</td>
<td>C$<em>{16}$H$</em>{30}$O$_{2}$</td>
<td>Cyclopentaneundecanoic acid</td>
</tr>
<tr>
<td>25</td>
<td>44.672</td>
<td>10.96</td>
<td>182</td>
<td>C$<em>{11}$H$</em>{18}$O$_{2}$</td>
<td>10-Undecynoic acid</td>
</tr>
</tbody>
</table>

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$a$ R.T – retention time.  
$b$ Area %  
$c$ MW – molecular weight.  
$d$ MF – molecular formula.
The free radical DPPH possesses a characteristic absorption at 517 nm, which decreases significantly on exposure to plant extract by providing hydrogen atom or electron donation. It was observed that the leaf showed higher activity than that of the immature fruit and fruit skin extract. The percentage of inhibition of DPPH radicals by methanol extract of the leaves reached up to 88.27% at the concentration of 100 mg/mL, whereas the immature fruit and fruit skin showed 66.19% and 62.67% at the same concentration respectively. Although the DPPH and ABTS radical scavenging are based on the same principle, the data obtained from DPPH are lower than those obtained from the ABTS activity. Interestingly, the inhibition percentage of ABTS radicals by immature fruit was found to be higher (96.12%) than the standard reference BHT (87.74%), leaf (48%) and fruit skin (76.45%) at the concentration of 25 μg/mL (Fig. 2b). Reducing power assay revealed that assayed plant samples were able to reduce the ferric ions (Fe$^{3+}$) to ferrous ions (Fe$^{2+}$) in concentration dependent manner. The leaf and immature fruit methanol extracts show moderate reducing power capacity when compared to that of fruit skin and comparable with that of the standard BHT Fig. 2d. Nitric oxide scavenging ability assay revealed that all the extracts of different plant parts exhibited scavenging potential. Immature fruit showed better activity than leaf and fruit skin (Fig. 2c). At the concentration of 500 μg/mL, the methanol extract of immature fruit exhibited 62.77% nitric oxide scavenging ability while comparing to that of leaves and fruit skin (52.59% and 60.74%) respectively.

Previous reports suggest that phenolic and flavonoid compounds (gallic acid, caffeic acid, p-coumaric acid, quercetin, rutin and catechin) in plants possess strong antioxidant activity that might contribute to antimicrobial potential. The disc diffusion method was employed for the determination of antimicrobial activity of plant extracts against human pathogens. The zone of inhibition of the plant extract was found to be in the range from 7 to 18 mm (Fig. 2) against the entire tested microorganisms. Methanolic extract of fruit skin of C. urens showed maximum activity of about 18 mm against E. coli and immature fruit showed about 16 mm diameter zone against V. cholerae. Plant based antibiotic drugs have enormous therapeutic potential and have been proven effective in the treatment of infectious diseases with less or no side effects which are often associated with synthetic antibiotics. The terpene alcohols damage the cell membranes of E. coli, S. aureus, and Listeria monocytogenes, resulting in leakage of potassium ions from cells, which cause death of the organism. Recent reports suggest that the sesquiterpene alcohol like farnesol has been confirmed to reduce the growth of S. aureus and Streptococcus mutans. From these results, we have identified the major antimicrobial compounds such as 10-undecenoic acid, caffeine and 2E,6E-farnesol which may have many pharmacological activities and the results were supported by Zhonghui et al. More over, it is also reported that compounds in lower quantities might be involved in some type of synergism with the active compound which might be the reason behind the high activity of antioxidant and antimicrobial in immature fruits when compared to the leaves and fruit skin of C. urens.

4. Conclusion

The present work clearly demonstrated the antioxidant potential and antimicrobial activity of C. urens. The GC–MS analysis of methanolic extract of C. urens reveals the presence of major bioactive compounds such as phenolics, fatty acids, carboxylic acids, terpenoids and caffeine. The observed strong antioxidant and antimicrobial activity confirms the effectiveness of traditional use of this herbal drug against microbes which act as source of natural antioxidant with potential use in pharmaceutical industry. Antioxidants are reported to possess antimicrobial activity. Hence, we suggest that antimicrobial activity exhibited by the extract might be due to the presence of antioxidants. However, isolation of individual phytochemical constituents and subjecting it to the biological activity is in progress which will definitely give fruitful results.

Conflicts of interest

All authors have none to declare.
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References