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Short communication

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

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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 typhii*, *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.

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 Arecaceae (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

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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[11] 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[12] 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.[13] 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:

$$\text{Percentage of inhibition} = [(\text{Abs control} - \text{Abs sample}/\text{Abs control})] \times 100$$

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.[14] 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 $ABTS^{\cdot+}$ solution react with plant extract (5–25 μg/mL) after 10 min the absorbance was measured UV-spectrophotomatrically at 734 nm against the blank solution. ABTS free radical inhibition was calculated by following equation:

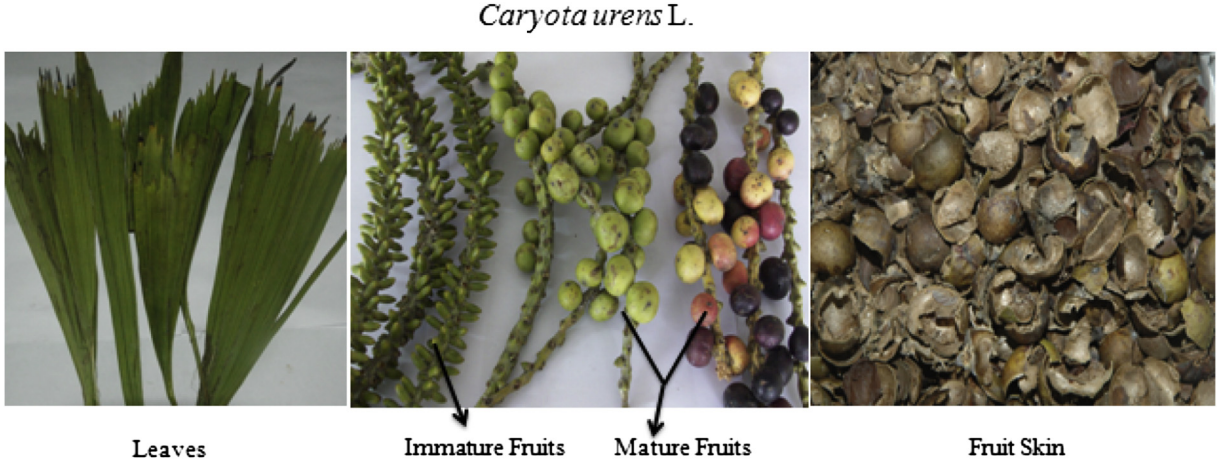

Fig. 1. *C. urens* (Wine palm) Plant.

Table 1
Total phenolics and flavonoid content of methanolic extract of *C. urens*.

Plant extracts	Total phenols (mg GAE/g dw)[a]	Total flavonoids (mg RE/g dw)[a]
Leaf	15.00 ± 0.40	15.35 ± 0.41
Immature fruit	17.63 ± 0.51	11.29 ± 0.54
Fruit skin	10.23 ± 0.40	13.58 ± 0.40

GAE/g dw – gallic acid equivalents/g dry weight; RE/g dw – rutin equivalents/g dry weight.
[a] Data are presented as the mean ± standard deviation of three determinations.

$$\text{Percentage of inhibition} = [(\text{Abs control} - \text{Abs sample}/\text{Abs control})] \times 100$$

2.5.3. Nitric oxide scavenging activity

Garratt,[15] method was adopted to determine the nitric oxide radical 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 sulphanilic acid (0.33% in 20% glacial acetic acid) was added and allowed to stand for 5 min. Then 1 mL of 0.1% of napthylethylenediamine 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[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 (100–500 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) was collected and dissolved in 2.5 mL of distilled water. Finally, 0.5 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 typhii* (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.[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.

Table 2
Profiling chemical constituents of *C. urens* through GC–MS analysis.

Peak	R.T[a]	%[b]	MW[c]	MF[d]	Compound name
(a) Leaf					
1	11.160	0.19	112	$C_7H_{12}O$	4-Hexen-2-one, 3-Methyl-
2	12.147	0.84	114	$C_6H_{10}O_2$	Hexanoic acid
3	15.499	3.15	273	$C_{13}H_{23}NO_5$	Dodecanoic acid
4	16.648	0.49	129	$C_6H_{11}NO_2$	Nitrous acid
5	19.315	0.81	99	C_5H_9NO	N-Ethyl acrylamide
6	20.683	0.34	188	$C_9H_{16}O_4$	Butyl propyl ester
7	20.952	4.78	270	$C_{17}H_{34}O_2$	Pentadecanoic acid
8	23.693	0.88	164	$C_9H_8O_3$	Benzoic acid
9	25.096	1.39	382	$C_{25}H_{50}O_2$	Lignoceric acid
10	25.577	1.25	296	C19H36O2	11-Octadecenoic acid
11	26.836	0.59	294	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid
12	28.512	1.79	292	$C_{19}H_{32}O_2$	Linolenic acid
13	29.187	1.38	143	$C_6H_9NO_3$	Pyroglutamic acid
14	29.549	10.27	398	$C_{26}H_{54}O_2$	Palmitaldehyde
15	30.508	0.23	116	$C_6H_{12}D_2N_2$	1,3-Cyclohexane-1,3-D_2-diamine
16	31.186	0.66	146	$C_8H_{18}O_2$	3,4-Hexanediol
17	31.792	0.53	83	C_5H_9N	Aziridine
18	35.098	28.51	284	$C_{18}H_{36}O_2$	Stearic acid
19	38.709	1.24	126	$C_7H_{10}O_2$	2-Oxabicyclo[3.2.1]octan-3-one
20	41.344	10.81	282	$C_{18}H_{34}O_2$	Cyclohexyl ester
21	42.448	12.00	282	$C_{18}H_{34}O_2$	Oleic acid
22	44.773	12.86	182	$C_{11}H_{18}O_2$	10-Undecenoic acid
23	48.284	5.01	154	$C_9H_{14}O_2$	8-Nonynoic acid
(b) Immature fruit					
1	12.109	1.50	98	$C_5H_6O_2$	Cyclopentanone
2	13.959	0.58	140	$C_9H_{16}O$	1,8-Nonadien-4-Ol
3	16.795	0.39	94	C_6H_6O	Phenol, Acide carbolique
4	20.418	0.50	150	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol
5	20.949	1.21	270	$C_{17}H_{34}O_2$	Pentadecanoic acid
6	21.656	0.38	154	$C_8H_{10}O_3$	Pyrogallol 1,3-dimethyl ether
7	23.688	0.79	222	$C_{12}H_{14}O_4$	Phthalic acid
8	25.577	1.50	296	$C_{19}H_{36}O_2$	11-Octadecenoic acid
9	26.844	0.97	156	$C_{10}H_{20}O$	Decylenic alcohol
10	29.179	0.71	143	$C_6H_9NO_3$	Pyroglutamic acid
11	31.144	20.01	222	$C_{15}H_{26}O$	(2E,6E)-Farnesol
12	33.501	0.42	138	$C_{10}H_{18}$	1,5-Heptadiene
13	35.026	28.26	284	$C_{18}H_{36}O_2$	Stearic acid
14	36.020	1.23	254	$C_{16}H_{30}O_2$	Cyclopentaneundecanoic acid
15	37.956	0.36	114	$C_6H_{10}O_2$	3-Butenoic acid
16	41.251	7.14	256	$C_{16}H_{32}O_2$	Palmitic acid
17	42.318	15.96	282	$C_{18}H_{34}O_2$	Oleic acid
18	44.571	18.09	182	$C_{11}H_{18}O_2$	Hendecynoic acid
(c) Fruit skin					
1	9.877	1.75	298	$C_{18}H_{19}FOSi$	4-Fluorobenzoyl
2	12.182	0.36	70	C_5H_{10}	Cyclobutane
3	13.318	0.98	73	C_3H_7NO	Isoxazolidine
4	13.943	1.12	103	$C_4H_9NO_2$	Nitrous acid
5	16.442	0.74	146	$C_7H_{18}Osi$	1-Propanol
6	17.972	0.59	326	$C_{12}H_{23}IO_2$	Dodecanoic acid
7	19.384	0.74	404	$C_9H_{18}Br_2O_4Si_2$	Propanedioic acid
8	19.698	0.40	200	$C_{10}H_{16}O_4$	Oxalic acid
9	20.966	1.39	130	$C_7H_{14}O_2$	Hexanoic acid
10	21.745	0.72	58	C_4H_{10}	Isobutane
11	22.147	0.64	404	$C_9H_{18}Br_2O_4Si_2$	Bis(trimethylsilyl) ester
12	25.121	0.33	89	$C_3H_7NO_2$	Isonitropropane
13	25.609	0.71	100	$C_6H_{12}O$	Oxetane
14	26.869	0.81	182	$C_{11}H_{18}O_2$	8-Nonynoic acid
15	29.217	0.34	146	$C_5H_{10}N_2O_3$	Levoglutamide
16	29.668	0.90	264	$C_{14}H_{24}OSi_2$	2,2-Bis(trimethylsilyl)styrene oxide
17	31.094	0.93	117	$C_5H_{11}NO_2$	Amyl nitrite
18	31.841	2.66	83	C_5H_9N	Aziridine
19	31.941	1.27	165	$C_6H_{18}B_3N_3$	Borazine
20	32.944	0.54	88	$C_5H_{12}O$	1-Butanol
21	35.076	34.68	298	$C_{19}H_{38}O_2$	Nonadecanoic acid
22	38.779	3.45	83	C_5H_9N	1-Propenyl-aziridine

(*continued on next page*)

Table 2 (*continued*)

Peak	R.T[a]	%[b]	MW[c]	MF[d]	Compound name
23	41.484	22.12	194	$C_8H_{10}N_4O_2$	Caffeine
24	42.415	10.86	254	$C_{16}H_{30}O_2$	Cyclopentaneundecanoic acid
25	44.672	10.96	182	$C_{11}H_{18}O_2$	10-Undecynoic acid

[a] R.T – retention time.
[b] Area %
[c] MW – molecular weight.
[d] MF – molecular formula.

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 2a–c). 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.[18] 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.[19] In addition, 10-undecenoic acid has effective fungicidal activity against *Candida albicans*, thus helps achieve a healthy balance of normal intestinal and vaginal flora.[20]

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 ± 0.40, 15.00 ± 0.40 and 17.63 ± 0.51 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 ± 0.51 mg GAE/g) while the lowest was in fruit skin (10.23 ± 0.40 mg GAE/g). On the other hand, the amount of flavonoids was found to be higher in leaf (15.35 ± 0.41 mg RE/g) when compared to immature fruit (11.29 ± 0.54 mg RE/g) and fruit skin (13.58 ± 0.40 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.[21] 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[22] Phenolic compounds of medicinal as well as aromatic plants might be responsible to cure some inflammatory disease, like rheumatoid arthritis and nephrotoxicity.[23,24]

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

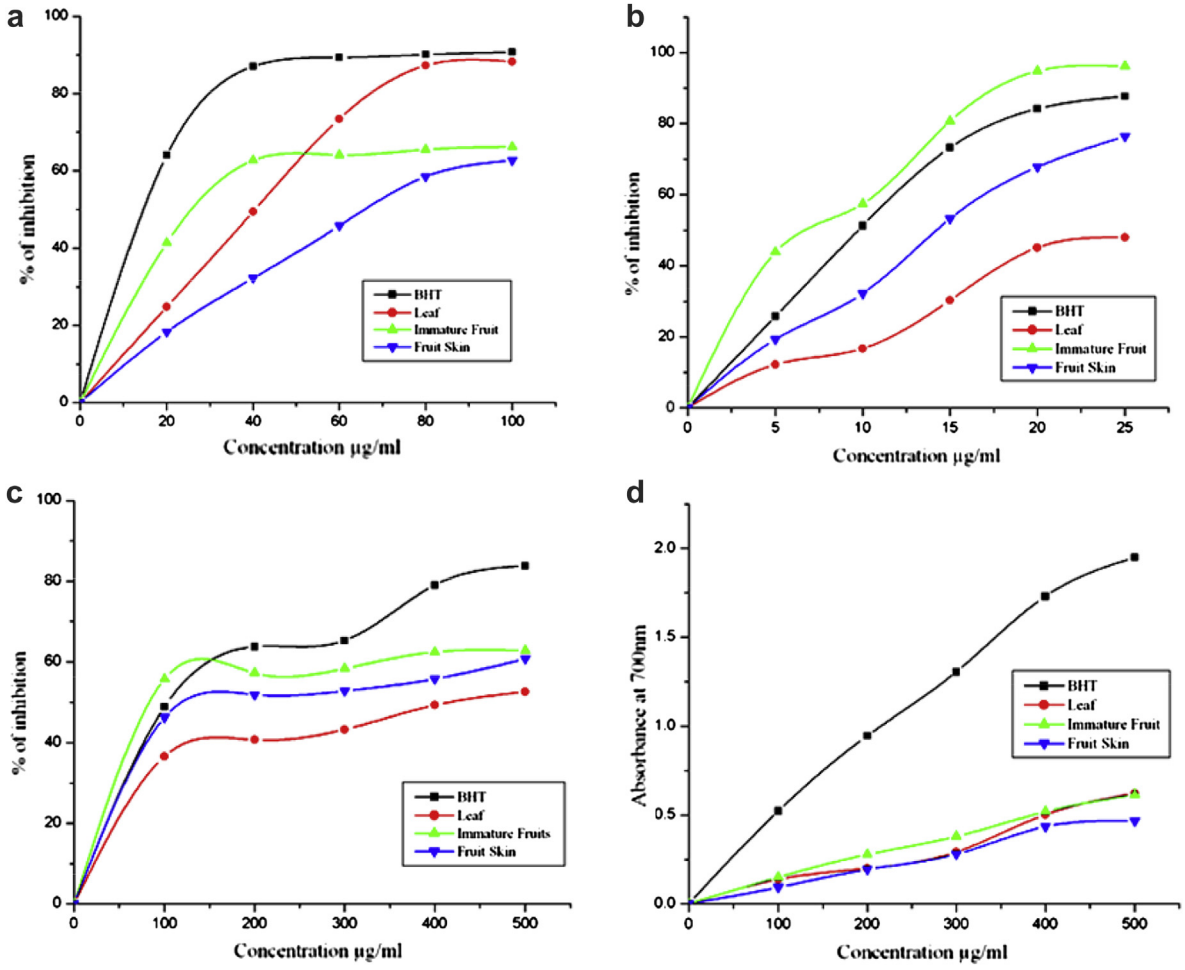

Fig. 2. Determination of antioxidant and reducing power activity of *C. urens* methanol extracts. (a) DPPH radical scavenging activity, (b) ABTS radical scavenging activity, (c) Nitric oxide scavenging activity, (d) Reducing power activity.

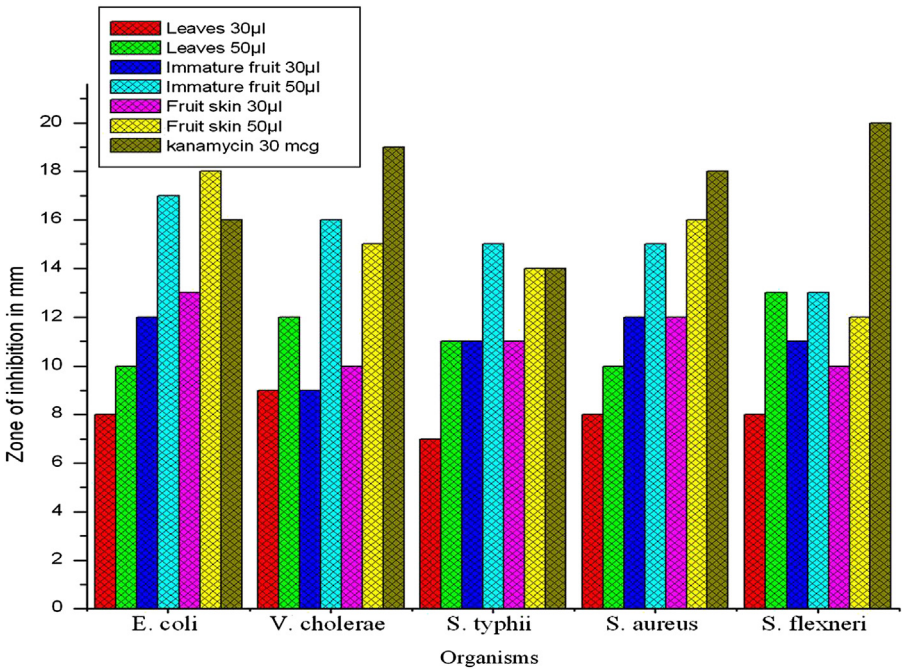

Fig. 3. Antimicrobial activity of *C. urens* methanol extracts. Zone of inhibition (mm).

BHT. 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.[25] 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 µg/mL, where as 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.[23] The leaf and immature fruit methanol extracts shows 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.[26] 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. 3) 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.[27] 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*.[28,29] 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[30] 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

1. Oh J, Jo H, Cho AR, Kim SJ, Han J. Antioxidant and antimicrobial activities of various leafy herbal teas. *Food Cont*. 2013;31:403–409.
2. Jayaprakash GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed extracts on peroxidation models *in-vitro*. *J Agric Food Chem*. 2001;55:1018–1022.
3. Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. *J Nutr Biochem*. 1996;7:66–76.
4. Yoo S, Murata RM, Duarte S. Antimicrobial traits of tea- and cranberry derived polyphenols against *Streptococcus mutans*. *Caries Res*. 2011;45:327–335.
5. Barlow SM. Toxicological aspects of antioxidants used as food additives. In: Hudson BJF, ed. *Food Antioxidants*. Amsterdam: Elsevier; 1990:23.
6. Branen AL. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *J Am Oil Chem Soc*. 1975;52:59–63.
7. Seong STT, Scog CC, Yong WC, Jin ITK, Scung B. Antioxidant extract of crude extract and pure components of *Acces ginnala* Max. *Bull Korean Chem Soc*. 2004;25:381–391.
8. Lotschert Wilhelm. *Palmen*. Stuttgart: Eugen Ulmer Verlag; 1985.
9. Everet Y. The *kitul* palm: ethnobotany of *Caryota urens* l. in highland sri lanka. *J Ethnobiol*. 1995;15:161–176.
10. Jayaweera DMA. *Medicinal Plants Used in Ceylon*. vol. IV. Colombo: National science council of Sri Lanka (NARESA); 1982.
11. Singleton VL, Rossi Jr JA. Colorimetry of total phenolics with phosphomolibdic–phosphotungtic acid reagents. *Am J Enol Viticult*. 1965;16:144–158.
12. Liu M, Li XQ, Weber C, Lee CY, Brown J, Liu RH. Antioxidant and antiproliferative activities of raspberries. *J Agric Food Chem*. 2002;50:2926–2930.
13. Liyana-Pathirana CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *J Agric Food Chem*. 2005;53:2433–2440.
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorizing assay. *Free Radic Biol Med*. 1999;26:1231–1237.
15. Garratt DC. *The Quantitative Analysis of Drugs*. vol. 3. Japan: Chapman and Hall Ltd; 1964:456–458.
16. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr*. 1986;44:307–315.
17. Rameshkumar A, Sivasudha T. *In vitro* antioxidant and antibacterial activity of aqueous and methanolic extract of *Mollugo nudicaulis* Lam. leaves. *Asian Pac J Trop Biomed*; 2012:S895–S900.
18. Azmat S, Ifzal R, Rasheed M, Mohammad FV, Ahmad VV. GC–MS analysis of n-hexane extract from seeds and leaves of *Phoenix dactylifera* L. *J Chem Soc Pak*. 2010;32:672–676.
19. Bourne N, Ireland J, Stanberry LR, Bernstein DI. Effect of undecylenic acid as a topical microbicide against genital herpes infection in mice and guinea pigs. *Antiviral Res*. 1999;40:139–144.
20. Mc Lain N, Ascanio R, Baker C. Undecylenic acid inhibits morphogenesis of *Candida albicans*. *Antimicrob Agents Chemo*. 2000;44:2873–2875.
21. Stevenson DE, Hurst RD. Polyphenolic phytochemicals – just antioxidants or much more? *Cell Mol Life Sci*. 2007;64:2900–2916.
22. Oktay M, Gulcin I, Kufrevioglu OI. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *Lebens-Wiss Technol*. 2003;36:263–271.
23. Rameshkumar A, Sivasudha T, Jeyadevi R, Sangeetha B, Smilin Bell Aseervatham G, Maheshwari M. Profiling of phenolic compounds using UPLC-Q-TOF-MS/MS and nephroprotective activity of Indian green leafy vegetable *Merremia emarginata* (Burm. f). *Food Res Int*. 2013;50:94–101.
24. Jeyadevi R, Sivasudha T, Rameshkumar A, Kumar LD. Anti-arthritic activity of the Indian leafy vegetable *Cardiospermum halicacabum* in Wistar rats and UPLC–QTOF–MS/MS identification of the putative active phenolic components. *Inflamm Res*. 2013;62:115–126.
25. Baumann J, Wurn G, Bruchlausen FV. Prostaglandin synthetaseinhibiting O_2 radical scavenging properties of some flavonoids and related phenolic compounds. Deutsche Pharmakologische Gesellschaft Abstracts of the 20th spring meeting, Naunyn–Schmiedebergs Abstract No, R27 cited in. *Arch Pharmacol*. 1979;307:R1–R77.
26. Maddox CE, Laur LM, Tain L. Antibacterial activity of phenolic compounds against the phytopathoger *Xylella fastidiosa*. *Curr Miccrobiol*. 2010;60:53–58.
27. Jeyadevi R, Sivasudha T, Ilavarasi A, Thajuddin N. Chemical Constituents and Antimicrobial activity of Indian Green Leafy Vegetable *Cardiospermum halicacabum*. *Indian J Microbiol*. 2013;53:208–213.
28. Kiyama H, Oono T, Huh WK, et al. Actions of farnesol and xylitol against *Staphylococcus aureus*. *Chemother*. 2012;48:122–128.
29. Jabra-Rizk MA, Johnson JK, Forrest G, Mankes K, Meiller TF, Venezia RA. Prevalence of *Candida dubliniensis* Fungemia at a large teaching hospital. *Clin Infect Dis*. 2005;41:1064–1067.
30. Zhonghui PU, Zhang Y, Yin ZQ, Jiao XU, Ren-yong JIA, Yang LU, Fan YANG. Antibacterial activity of 9-Octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3,4-diyl ester from neem oil. *Agric Sci China*. 2010;9:1236–1240.