ORIGINAL ARTICLE

CHEMICAL COMPOSITION AND ANTIOXIDANT, ANTIMICROBIAL AND HAEMOLYTIC ACTIVITIES OF *CRAMBE CORDIFOLIA* ROOTS

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Manuscript received: January 2017

Abstract

In the current study, we investigated the chemical composition, *in vitro* antioxidant, antimicrobial and haemolytic activities of the crude (methanolic) and n-hexane, chloroform, and ethyl acetate extracts of roots of *Crambe cordifolia* Steven. The GC-MS analysis led to identification of erucic acid (20.8%), p-cymene (20.9%), palmitic acid (19.2%) and vitamin E (13.8 %) as major components of methanol, n-hexane, chloroform and ethyl acetate extracts respectively**.** The results of spectrophotometric assays showed that the methanol extract had the highest total phenolic contents (210.3 ± 0.74 µg GAE/mg of dry plant material), total flavonoid contents (75.5 ± 0.32 µg QE/mg of dry plant material) and antioxidant potential. It was established by disc diffusion and minimum inhibitory concentration methods that, overall, the methanol extract exhibited the highest antimicrobial activity against most of the tested microbial strains (*Escherichia coli*, *Bacillus subtilis*, *Pasteurella multocida*, *Staphylococcus aureus*, *Aspergillus niger* and *Fusarium solani*) as compared with the n-hexane, chloroform, and ethyl acetate extracts. The outcome of the haemolytic assay showed that the tested extract and different solvent fractions were safe to human erythrocyte membrane.

Rezumat

În acest studiul, am investigat compoziția chimică, activitățile antioxidante, antimicrobiene și hemolitice *in vitro* ale extractelor brute (metanolice) și în n-hexan, cloroform și acetat de etil din rădăcinile de *Crambe cordifolia* Steven. Analiza GC-MS a condus la identificarea acidului erucic (20,8%), a p-cimenului (20,9%), a acidului palmitic (19,2%) și a vitaminei E (13,8%) ca și componente principale ale extractelor. Rezultatele testelor spectrofotometrice au arătat că extractul metanolic a avut cel mai mare conținut total de fenoli (210,3 ± 0,74 µg GAE/mg material vegetal uscat), precum și conținutul total de flavonoide (75,5 ± 0,32 µg QE/mg material vegetal uscat) și potențialul antioxidant. S-a stabilit prin metoda difuzimetrică și prin determinarea concentrației minime inhibitoare faptul că extractul metanolic a prezentat cea mai importantă activitate antimicrobiană împotriva majorității tulpinilor testate (*Escherichia coli*, *Bacillus subtilis*, *Pasteurella multocida*, *Staphylococcus aureus*, *Aspergillus niger* și *Fusarium solani*). Rezultatul testului hemolitic a arătat faptul că extractele testate au fost sigure pentru membrana eritrocitelor umane.

Keywords: GC-MS, *Crambe cordifolia*, total phenolic contents, antimicrobial, cytotoxic

Introduction

Plants have been recognized as a form of folk medicines due to their extensive therapeutic potential. Medicinal plants play a prime role in the development of traditional medicine as well as modern pharmaceuticals [1, 27]. Baluchistan region of Pakistan is has diversified medicinal flora due to the temperate agro-climatic and topographic conditions [2].

Crambe cordifolia Steven (*Brassiceae* family) is a perennial herb that may reach the height of 182 cm. It has lobed leaves with small white flowers. It contains many chemical compounds including amino acids, quercetin and glycosides of kaempferol [13, 15, 28]. Bukhari *et al*. reported the antioxidant potential of *C. cordifolia* plant parts [5]. But there was a complete gap about the chemical composition and biological attributes of different solvent extracts of *C. cordifolia*. Therefore, in the current study, an effort was made to investigate and compare the chemical components, *in vitro* antioxidant, antimicrobial and haemolytic attributes of different solvent (methanol, n-hexane, chloroform, and ethyl acetate) extracts of *C. cordifolia* roots, so as to explore their potential uses as natural antioxidative and antimicrobial agents for nutraceutical and pharmaceutical industries.

Materials and Methods

Plant material. The plant material was collected from Queta, Province Baluchistan, Pakistan. The plant was identified by Dr. Rasool Bakhsh Tareen (Botanist), Department of Botany, University of Baluchistan Quetta, Pakistan, where a voucher specimen (No. CC-RBT-05) has been deposited.

Extraction and fractionation. The shade-dried ground roots of *C. cordifolia* (4 kg) were exhaustively extracted with methanol (4 kg × 3 L × 72 h), accompanied by occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman No.1 filter paper. The extract was evaporated using rotary evaporator to yield a residue (95 g). The methanol crude extract was suspended in distilled water and fractionated with n-hexane (13 g), chloroform (22 g) and ethyl acetate (30 g). All the fractions (n-hexane, chloroform and ethyl acetate) were completely dried with rotavap.

GC-MS analysis. The chemical composition of various extracts (methanol, n-hexane, chloroform and ethyl acetate extracts) analysed by gas chromatography-mass spectrometry (GC-MS) using a gas chromatograph (Model Agilent 7890A) directly coupled to a mass spectrometer system (Model Agilent 5975C inert XL MSD) with a Triple-Axis Detector. A fused silica capillary column HB-5MS (5% phenyl methyl siloxane; length 30 m x inner diameter 0.25 mm x film thickness 0.25 µm) was employed with helium as the carrier gas at a constant flow rate of 1.0 mL/min. The column temperature was programmed as follows: at 50°C for 2 min; then increased from 50°C to 280°C at the rate of 5°C/min and held at 280°C for 2 min. For GC-MS detection, electron ionization (EI) system was used with ionization energy of 70 eV. The MS scan parameters included a mass range of 50 to 550 amu, a scan interval of 0.5 s, a scan speed of 1000 amu/s and a detector voltage of 1.5 kV. The MSD Chemstation was used to find all the peaks in the raw GC chromatogram. The percentage composition of the extract was computed from GC peak areas without correction factors. The qualitative analysis was based on a comparison of retention times and mass spectra with computer mass spectra libraries (NIST/EPA/NIH version 5.0).

Evaluation of the antioxidant activity

Total phenolic compounds. The total phenolic contents (TPC) of different extracts of *C. cordifolia* were determined using the Folin-Ciocalteu method [14]. Briefly, 1 mL of each extract solution was added to deionized water (10 mL) and Folin-Ciocalteu reagent (1.0 mL). After 5 minutes, 20% sodium carbonate (2.0 mL) was added to the reaction mixture and the absorbance was measured at 750 nm using a spectrophotometer (Shimadzu, Japan). The total phenolic contents were expressed as microgram gallic acid equivalent (GAE) *per* milligram of dry plant material.

The total flavonoid contents. The total flavonoid contents of *C. cordifolia* extracts were assessed by the $AlCl_3$ method, reported earlier [4]. Briefly, each extract solution (2 mL) solution was mixed with 2 mL of aqueous $AlCl_3$ x $6H_2O$ (0.1 mol/L). After incubation at room temperature for 10 minutes, the absorbance was measured using UV-Vis spectrophotometer at 417 nm. The total flavonoid contents in each sample were estimated using a standard curve for quercetin (1 µg/mL to 40 µg/mL). The total flavonoid contents were expressed as µg quercetin equivalents (QEs) *per* mg of plant extract.

Determination of reducing power. The reducing power of each extract solution was determined according to the procedure described by Chan *et al*. [7]. Different dilutions of each extract (methanol, chloroform, ethyl acetate and hexane) were mixed with to 2.5 mL of phosphate buffer and potassium ferricyanide (2.5 mL, 1% w/v). The reaction mixture was incubated at 50°C for 25 min. 2.5 mL of trichloro-acetic acid solution was added to stop the reaction. Then 2.5 mL of this reaction mixture was mixed with 2.5 mL of water and 500 µL of ferric chloride solution. Absorbance was recorded at 700 nm after 30 min spectrophotometrically.

The evaluation of antimicrobial activity

Disc diffusion assay. The agar disc diffusion method (NCCLS, 1997) [20] was used for the determination of inhibition zones diameters made by the dried crude methanol extract and different fractions of *C. cordifolia* against tested bacterial and fungal strains. Sterile nutrient agar was inoculated with 100 µL suspension of tested bacteria and sterile potato dextrose agar was inoculated with 100 µL of the tested fungi. The inoculated nutrient agar and potato dextrose agar were then poured into sterilized Petri plates individually. Sample solutions were prepared by dissolving dried extracts into dimethyl sulfoxide. Sterile filter discs impregnated with 50 µL of sample solution were placed in inoculated Petri plates with the help of sterile forceps. Rifampicin and terbinafine were used as positive control in bacterial and fungal inoculated plates, respectively. The plates were incubated at 37°C for 24 hrs and at 27°C for 48 hrs for maximum bacterial and fungal growth, respectively. Antibacterial and antifungal activities were evaluated by measuring the diameter (millimetres) of the inhibition zones with the help of Scan® 4000 inhibition zone reader.

The minimum inhibitory concentration (MIC) assay. The antimicrobial activity of crude methanol extract and different solvent fractions of *C. cordifolia* was estimated by measuring the minimum inhibitory concentration (MIC). Broth micro-dilution method (NCCLS, 1999) [21] was followed for determination of MIC values. Briefly, dilution series were prepared

by dissolving various dried extracts in dimethyl sulfoxide (DMSO) in a 96-well microtiter plate. 50 µL of nutrient broth (NB) and Sabouraud dextrose broth (SDB) were added for bacterial and fungal strains, respectively, onto microtiter plates. Then two fold serial dilutions were done using a micropipette so that each well carried 50 µL of extract solution in a serially decreasing concentration. Afterwards 10 µL inoculum of tested microbial strain was added to each well. Dimethyl sulfoxide in NB and SDB was used as negative control, while NB containing rifampicin and SDB containing terbinafine were used as positive controls for bacterial and fungal strains, respectively. The plates were incubated at 37°C for 24 hrs and at 27°C for 48 hrs for bacteria and fungi, respectively. Each extract sample was assayed in triplicate against each tested microbial strain.

The haemolytic assay. The haemolytic activity of *C. cordifolia* root extracts (methanol, chloroform, ethyl acetate and hexane) was estimated by the method described previously [23]. Each plant extract at the concentration of 10 mg/mL in 10% DMSO were prepared. Three mL of freshly obtained human blood was added in heparinized tubes to avoid coagulation and gently mixed, poured into a sterile 15 mL Falcon tube and centrifuged for 5 min at 850xg. The supernatant was poured off and RBCs were washed three times with 5 mL of chilled (4°C) sterile isotonic phosphate buffer saline (PBS) solution, adjusted to pH = 7.4. The washed RBCs were suspended in the 20 mL chilled PBS. Erythrocytes were counted on haemocytometer. The RBCs count was maintained to 7.068 x 108 cells *per* mL for each assay. The 20 µL of each extract solution was taken in 2 mL Eppendorf and then added 180 µL diluted blood cell suspension. The samples were incubated for 35 minutes at 37°C. Sample tubes were placed on ice for 5 minutes and centrifuged at 1310xg. After centrifugation, 100 µL supernatant was taken from the tubes and diluted with 900 µL chilled PBS. All Eppendorf were maintained on ice after dilution. After this 200 µL mixture from each Eppendorf was added into 96 well plates. For each assay, 0.1% Triton X-100 was considered as a positive control and phosphate buffer saline (PBS) was taken for each assay as a negative control. The absorbance was measured at 576 nm with a BioTek, µ Quant (BioTek, Winooski, VT, USA). The % lysis of RBCs was calculated by the following formula:

$$\% \text{ RBCs lysis} = A_{sample}/A_{Triton\ X\text{-}100} \times 100,$$

where A_{sample} is the absorbance of reaction mixture carrying plant extract and $A_{Triton\ X\text{-}100}$ is the absorbance of Triton X-100 that was used as negative control.

Statistical analysis. The experimental results were expressed as Mean ± SD (standard deviation of three independent experiments). Minitab software version 16 (Statistical software, Minitab Inc. State College, PA, USA) was used to perform the analysis of variance (ANOVA), followed by the Tukey's test; p values less than 0.05 were considered significant.

Results and Discussion

The results of GC-MS analysis of the methanol, n-hexane, chloroform, and ethyl acetate extracts of *C. cordifolia* were expressed in percentages (%) and are shown in Table I. Various chemical components such as erucic acid, oleic acid, palmitic acid, p-cymene, m-xylene, durene, cetyl ethylene, 2-methyldecalin, 5-eicosane, bisphenol AF, vitamin E, luteolin, longifolenaldehyde, quercetin and many others were observed at varying degrees in the investigated extracts. Oleic acid and erucic acid were common in four extracts. Oleic acid was a major component of chloroform and ethyl acetate extracts and was also reported in other *Crambe* species (*Crambe orientalis* L., *Crambe tataria* Sebeok, *Crambe hispanica* subsp. *abyssinica* (Hochst. ex R.E.Fr.) Prina) [8, 11]. Oleic acid exhibits anticancer potential by induction of apoptosis in human carcinoma cells [6]. The extent (5.7% to 20.8%) of erucic acid in the crude methanol extract and different solvent fractions of *C. cordifolia* was lower than previously reported values (60% to 67%) for seeds of *C. abyssinica* [9].

Table I

Chemical composition (%) of methanol, n-hexane chloroform and ethyl acetate extracts of *C. cordifolia* roots

Retention time	Compounds	Methanol	n-Hexane	Chloroform	Ethyl acetate
2.53	Toluene	----	3.5 ± 0.01	0.02 ± 0.01	2.9 ± 0.01
3.34	Cyclohexane, ethyl	----	5.3 ± 0.06	0.92 ± 0.06	4.2 ± 0.13
3.52	N-Ethylpropanamide	1.4 ± 0.01	1.1 ± 0.03	3.5 ± 0.02	----
5.49	Methanesulfinyl chloride		----	4.2 ± 0.07	0.1 ± 0.04
6.26	Butyl pentyl carbonate	0.8 ± 0.02	2.6 ± 0.01	1.1 ± 0.04	1.2 ± 0.01
6.27	1,2,3-Trimethylbenzene	0.2 ± 0.01	----	2.6 ± 0.03	----
7.41	Oxalic acid, decyl propyl ester	1.9 ± 0.07	0.88 ± 0.02	3.2 ± 0.05	----
8.11	Octatriacontyltrifluoroacetate	----	4.1 ± 0.16	----	2.2 ± 0.01
8.33	Carbonic acid, butyl tridecyl ester	0.1 ± 0.01	----	4.5 ± 0.14	0.03 ± 0.07
8.65	Erucic acid	20.8 ± 0.01	8.7 ± 0.04	13.3 ± 0.24	5.8 ± 0.03
9.65	p-Cymene	----	20.9 ± 0.01	----	12.6 ± 0.14
10.1	2-Methyldecalin	----	----	1.2 ± 0.07	----

Retention time	Compounds	Methanol	n-Hexane	Chloroform	Ethyl acetate
10.3	m-Xylene	1.4 ± 0.21	----	2.7 ± 0.03	----
10.6	Octyl acetate	----	2.2 ± 0.05	----	1.6 ± 0.08
10.8	Pentyl cyclohexane	----	2.9 ± 0.26	----	----
11.4	Durene	----	0.5 ± 0.01	----	----
27.1	Cetyl ethylene	----	0.5 ± 0.02	0.3 ± 0.01	----
30.6	Palmitic acid	15.1 ± 0.43	14.3 ± 0.56	19.2 ± 0.24	----
31.1	5-Eicosene	----	2.7 ± 0.01	2.9 ± 0.02	4.4 ± 0.02
32.7	Bisphenol AF	2.1 ± 0.05	----	4.2 ± 0.03	----
34.2	Oleic acid	18.6 ± 0.07	17.2 ± 0.49	16.5 ± 0.17	12.2 ± 0.01
38.7	Tetradecamethyl-cyclopentasiloxane	1.45 ± 0.03	----	----	3.8 ± 0.03
38.7	A-Neogammacer-22(29)-en-3-one	0.65 ± 0.01	4.3 ± 0.14	----	3.1 ± 0.05
40.2	Longifolenaldehyde	2.75 ± 0.04	----	----	4.9 ± 0.01
40.6	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	0.63 ± 0.03	3.9 ± 0.08	----	3.7 ± 0.08
41.1	Tetradecamethylhexasiloxane	0.56 ± 0.02	----	----	----
42.1	(3beta)-ergost-5-en-3-ol	1.01 ± 0.01	----	----	5.6 ± 0.01
44.1	Vitamin E	7.3 ± 0.24	----	----	13.8 ± 0.46
44.4	Octadecamethyl cyclononasiloxane	0.33 ± 0.09	----	----	3.4 ± 0.05
80.2	Luteolin	11.2 ± 0.31	----	10.1 ± 0.08	----
94.4	Quercetin	9.6 ± 0.05	----	6.9 ± 0.03	----
Total		97.88	95.58	97.34	85.53

The chemical contribution of palmitic acid in methanol, n-hexane and chloroform extract was 15.1, 14.3 and 19.2%, respectively. Luteolin and quercetin were identified in considerable amount in the methanolic and chloroformic extracts. Onyilagha *et al*. reported the presence of leuteolin and quercetin in *Crambe hispanica* L. and other species of *Brassicaceae* [22]. An important component of ethyl acetate (12.6%) and hexane (20.9%) extracts was p-cymene. Kisko *et al*. showed that p-cymene had antibacterial efficacy against *Escherichia coli* [16]. Vitamin E plays significant role against cancer, inflammation and microbial infections [12].

We also observed vitamin E in methanol (7.3%) and ethyl acetate (13.8%) extracts of *C. cordifolia*. The presented data was also flawed by artifacted siloxane derivates caused by the adsorption of samples to surface of fused silica of column during chromatography. Reports on the chemical composition of *C. cordifolia* are scant in literature. To the best of our knowledge, our study is the first to explore the chemical composition of methanol, n-hexane, chloroform, and ethyl acetate extracts of *C. cordifolia* roots. It is interesting to note that each extract (methanol, hexane, chloroform and ethyl acetate) of roots of *C. cordifolia* had a good profile of bioactive components with pharmacological potential.

Table II

Total phenolic contents (TPC), total flavonoid contents (TFC) and haemolytic activity of the extracts of *C. cordifolia* roots and positive controls

C. cordifolia plant extracts and positive controls	Total phenolic contents (µg GAE/mg of dry plant material)[a]	Total flavonoid contents (µg QE/mg of dry plant material)[b]	Red blood cells lysis (%)
Methanol	210.3 ± 0.74[a]	75.5 ± 0.32[c]	1.99 ± 0.05[b]
Hexane	10.7 ± 1.27[e]	7.09 ± 0.01[b]	7.41 ± 0.31[c]
Chloroform	122.5 ± 0.08[b]	48.2 ± 0.26[e]	2.23 ± 0.16[b]
Ethyl acetate	35.1 ± 0.14[d]	18.8 ± 0.65[d]	9.55 ± 0.02[d]
Phosphate Buffer Saline	------	------	0.015 ± 0.01[e]
Triton X-100	------	------	99.99 ± 0.11[f]

(----) = not tested. All data are represented as Mean ± SD of triplicate determinations of each experiment. Mean with different superscript letters in the same column indicate significant ($p < 0.05$) difference among the tested solvent extracts and positive controls.
[a] Microgram gallic acid equivalent *per* milligram of plant extract; [b] Microgram quercetin equivalent *per* milligram of plant extract

The total phenolic and total flavonoid contents (TPC and TFC) of various extracts of *C. cordifolia* were expressed as µg gallic acid equivalent/mg of plant extract and µg quercetin equivalents/mg of plant extract, respectively. A significant difference ($p < 0.05$) was observed among total phenolic and total flavonoid contents with respect to extracting solvents (methanol, n-hexane chloroform and ethyl acetate). TPC and TFC extracted from roots of *C. cordifolia* ranged from 10.7 ± 1.27 (µg GAE/mg of plant extract) to 210.3 ± 0.74 (µg GAE/mg of plant extract) and from 7.09 ± 0.01 (µg QE/mg of plant extract) to 75.5 ± 0.32 (µg QE/mg of plant extract), respectively. The results (Table II) showed that the extraction rate of TPC with methanol and different solvent fractions was as follows: methanol (210.3 ± 0.74) >

chloroform (122.5 ± 0.08) > ethyl acetate (35.1 ± 0.14) > hexane (10.7 ± 1.27), while the hierarchy of solvents in the case of TFC was methanol (75.5 ± 0.32) > chloroform (48.2 ± 0.26) > ethyl acetate (18.8 ± 0.63) > hexane (7.09 ± 0.01). These findings indicate the richness of strongly polar extract (methanol) of *C. cordifolia* with polyphenolic (total phenolic and total flavonoid) compounds. Our results are in accordance with the study of Ashraf *et al*. in sense that they also examined the higher amount of total phenolic and total flavonoid contents in polar (methanol) extract, as compared to non-polar (hexane) one [4].

Ferric reducing antioxidant power assay was used to assess the reducing potential of crude extract (methanol) and different solvent fractions (n-hexane, chloroform and ethyl acetate) of *C. cordifolia*. A dose dependent (0.025 to 0.4 mg/mL) study was conducted and results are shown in Figure 1. An increase in ferric reducing antioxidant contents was observed with increasing the dose of crude extract and different solvent fractions.

Minimum reducing contents in five different extracts were observed at 0.025 mg/mL, while the maximum at 0.4 mg/mL. These results are in accordance with the study of Sindhu *et al*. [26], in which an increase in the reducing potential was observed with increasing the dose of a potent Indian medicinal plant (*Kyllinga nemoralis* (J. R. Forst. & G. Forst. Dandy ex Hutch. & Dalziel). The maximum ferric reducing antioxidant power was noticed in the methanolic extract followed by the chloroformic, ethyl acetate and hexane extracts, respectively. The appreciable ferric reducing anti-oxidant potency of the methanolic extract might be attributed to its high level of the total phenolic and total flavonoid compounds [24]. These bioactive compounds act as reductants by donating electrons in the reaction environment.

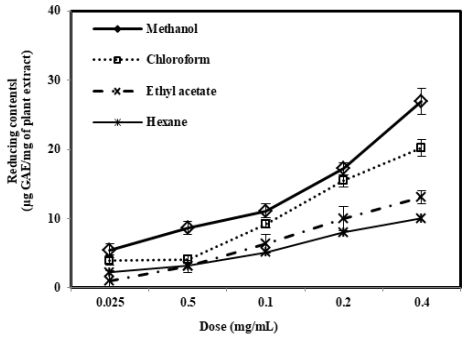

Figure 1.
The antioxidant activity of *C. cordifolia* plant extracts measured by ferric reducing antioxidant power (FRAP) assay

The different extracts (methanol, n-hexane, chloroform and ethyl acetate) of *C. cordifolia* roots were screened for their antimicrobial potencies against a panel of six microorganisms. The qualitative and quantitative anti-microbial potential was assessed by the measurement of the diameter of the inhibition zones and minimum inhibitory concentration (MIC) values. The results are shown in Table III and Table IV respectively.

Table III

Antimicrobial activity of *C. cordifolia* root extracts and positive controls measured by disc diffusion assay

Bacterial and fungal strains	*C. cordifolia* plant extracts				Positive controls	
	Methanol	n-Hexane	Chloroform	Ethyl acetate	*Rifampicin	**Terbinafine
	Diameter of inhibition zone (mm)					
Escherichia Coli	11.1 ± 0.1^{a}	18.1 ± 1.0^{b}	10.6 ± 0.9^{b}	----	21.6 ± 1.4^{e}	Nt
Bacillus subtillis	22.8 ± 2.7^{c}	----	19.4 ± 1.5^{bc}	12.6 ± 0.5^{a}	24.6 ± 0.5^{a}	Nt
Pasterulla multocida	14.3 ± 0.3^{df}	13.3 ± 0.9^{cd}	----	----	23.3 ± 1.7^{cd}	Nt
Staphylococcus aureus	17.8 ± 1.5^{e}	----^{f}	12.2 ± 0.4^{d}	----	28.1 ± 1.0^{b}	Nt
Aspergillus niger	13.5 ± 0.8^{a}	----	13.6 ± 0.1^{de}	15.3 ± 0.2^{cd}	Nt	25.6 ± 1.7^{ad}
Fusarium solani	16.6 ± 0.1^{d}	14.0 ± 0.2	7.27 ± 1.5^{a}	----	Nt	24.0 ± 0.8^{f}

Data is represented as Mean ± SD of triplicate determination of each extract against each microbial strain. Means with different superscript letter in the same column indicate significant difference ($p < 0.05$) among solvents tested.
(----) indicates no antimicrobial activity * Standard antibiotic for bacteria ** Standard antibiotic for fungi. Nt = Not tested

Table IV

Antimicrobial activity of *C. cordifolia* extracts and positive controls measured by minimum inhibitory concentration assay

Bacterial and fungal strains	*C. cordifolia* plant extracts				Positive controls	
	Methanol	n-Hexane	Chloroform	Ethyl acetate	*Rifampicin	**Terbinafine
	Minimum inhibitory concentration (µg/mL)					
Escherichia coli	0.86 ± 1.3^{c}	0.16 ± 0.7^{e}	1.24 ± 0.2^{c}	----	0.11 ± 0.0^{b}	Nt
Bacillus subtillis	0.14 ± 0.5^{f}	----	0.23 ± 0.4^{d}	0.86 ± 0.0^{a}	0.04 ± 0.0^{a}	Nt
Pasterulla multocida	0.43 ± 0.2^{b}	0.35 ± 0.1^{e}	----	----	0.09 ± 0.1^{a}	Nt
Staphylococcus aureus	0.16 ± 0.1^{f}	----	0.20 ± 0.5^{d}	----	0.01 ± 0.0^{a}	Nt
Aspergillus niger	0.35 ± 1.0^{a}	----	0.39 ± 0.2^{f}	0.43 ± 0.5^{e}	Nt	0.02 ± 0.5^{b}
Fusarium solani	0.21 ± 0.3^{b}	0.43 ± 0.1^{b}	2.51 ± 0.0^{a}	----	Nt	0.04 ± 0.1^{b}

Data is represented as Mean ± SD of triplicate determination of each extract against each microbial strain. Means with different superscript letter in the same column indicate significant difference ($p < 0.05$) among solvents tested.
(----) indicates no antimicrobial activity * Standard antibiotic for bacteria **Standard antibiotic for fungi. Nt = Not tested

Various extracts (hexanes, chloroform, ethylacetate and methanol) of *C. cordifolia* had significantly ($p < 0.05$) varying antimicrobial potential against the tested microbial strains. The difference in antimicrobial efficacy among the different extracts might be due to different chemical nature of compounds in these extracts. Overall, the methanolic extract of *C. cordifolia* showed maximum antimicrobial activity, indicated by larger inhibition zones diameters and smaller minimum inhibitory concentration (MIC) values that were comparable to the values of positive controls (as presented in Table III and Table IV). The potent antimicrobial activity of the methanolic extract might be due to higher level of polyphenolic (total phenolic and total flavonoid) compounds (Table I). Instead, a poor antimicrobial activity was recorded for n-hexane extract with the smallest inhibition zones (7.27 to 22.8 mm) and the largest MIC values (0.1 to 2.51 µg/mL). Many studies confirmed that polyphenolic compounds are directly linked with the antimicrobial activities [3, 10, 19, 25].

It was observed that the crude methanolic extract and different solvent fractions of *C. cordifolia* were more effective against *B. subtilis* than against *E. coli*. These results are consistent with a previous study [17] on a potent medicinal plant (*Ziziphus mauritiana* Lam.), where *B. subtilis* was reported as sensitive strain while *E. coli* was the resistant one. Chng *et al*. observed that outer membrane of Gram negative bacteria like *E. coli* is best owed with lipopolysaccharides which provide them protection by conferring a negative charge on the surface [9]. So, the *E. coli* is less susceptible to most of the examined extracts. In the case of fungi, the methanolic extract of *C. cordifolia* was found to be more effective against *A. niger* than against *F. solani*. These results are in agreement with a previous study [18] in which the methanolic extract of an Indian medicinal plant exhibited greater antifungal activity against *A. niger* as compared to *F. solani*.

The crude extract and different solvent fractions of *C. cordifolia* were examined for their haemolytic potency against human red blood cells (RBCs). The results are shown in Table II. The positive control (Triton X-100) significantly inhibited the viability of RBCs by 99.18%, whereas phosphate buffer saline, used as a negative control, exhibited no cell lysis. Among the tested extracts, the methanolic extract of *C. cordifolia* showed the poorest haemolytic potential as indicated by lower lysis (1.99%) of RBCs.

However, the ethyl acetate extract showed the maximum haemolytic activity (9.55%). The hierarchy of haemolytic potential of different extracts was as follows: ethyl acetate > n-hexane > chloroform > methanol. Overall, the poor haemolytic activity of different extracts of *C. cordifolia* revealed that they do not cause membrane disruption and are safe to be used as a source of herbal drugs for the human beings.

Conclusions

Our study for the first time reports the chemical composition, polyphenolic compounds, the reducing power, antimicrobial and haemolytic activity of different solvent fractions of *C. cordifolia*. The outcome of this study showed that the methanolic extract of *C. cordifolia* had the highest extent of total phenolic and total flavonoid compounds, potent ferric reducing antioxidant power and excellent antimicrobial activity. The crude extract and the solvent fractions were harmless for human erythrocyte membrane. The findings of this study would certainly help to ascertain the tendency of *C. cordifolia* root extracts as a potent source of natural antioxidant and antimicrobial agents.

Acknowledgement

We wish to thank to the Higher Education Commission (HEC) Pakistan (HEC scholarship for Ms. Sadia Noor) and University Malaysia Pahang (internal grant No. RDU 150109 and RDU 150349).

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