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Full Length Article

Antibacterial Potential of *Capparis spinosa* and *Capparis decidua* Extracts

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Abstract

Capparis spinosa and *C. decidua* are indigenous medicinal plants of Pakistan which are rich in antioxidant compounds. In present investigation, the aqueous menthanolic, ethanolic and acetone extracts of stem bark, shoot, fruit, flower and roots of both species were investigated for their antimicrobial potential in comparison with amoxicillin and ciprofloxacin (control). The effect of these extracts was evaluated on the growth of four bacteria i.e., *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pasteurella multocida* using disc diffusion and minimum inhibitory concentration (MIC) assays. After comparing all extracts, it was found that methanolic extracts of selected parts of both species significantly reduced the growth of all four bacteria although different extracts expressed varying efficacy in reducing bacterial growth. It was further noted that *S. aureus* growth was mainly inhibited by methanolic extracts of *C. decidua* flowers and roots (GIZ: 26.5 and 26.4 mm, respectively), *E. coli* population was effectively reduced by methanolic extracts of *C. decidua* roots and shoots (GIZ: 29.1 26.7 mm, respectively) while *B. subtilis* growth was inhibited maximally by methanolic extracts of *C. spinosa* stem bark and *C. decidua* fruits (GIZ: 26.8 and 26.8, respectively). Moreover, methanolic extract of *C. decidua* root markedly inhibited the growth of *P. multocida* (25.7 mm). On the basis of above mentioned findings, it can safely be concluded that *C. decidua* possess higher antimicrobial potential than *C. spinosa* and these can be used as natural antibacterial agent.

Keywords: Acetone; *Bacillus subtilis*; *Escherichia coli*; Ethanol; Methanol; *Pasteurella multocida*; *Staphylococcus aureus*

Introduction

The medicinal plants have been used traditionally in different civilization since ancient times due to their strong therapeutic effects on human beings. Recently, medicinal plants are acquiring good place in pharmaceutical industries owning higher nutritional quality and medicinal compounds which are capable for curing diseases (Shrishailappa *et al*., 2001). These are also used against microbial infections for treating ailments due to the presence of antimicrobial compounds (Iwu *et al*., 1999). In this modern era, the pharmacists and physicians mostly prescribe drugs discovered, isolated and manufactured synthetically which allow a fast recovery from diseases but, as their use increases, the side effects for the host and the resistance of infecting agents to these products also increase. Synthetic drugs have been preferred for over decades but now the use of the herbal medicines is being preferred to avoid blind dependency on synthetic ones. Recently, many plants have been explored and reported for their ability to inhibit the growth of microorganisms, which can replace the chemically manufactured drugs (Ilic *et al*., 2004; Mandalari *et al*., 2007). Consequently, plant based compounds like isoflavones, gamma thionin and homoisoflavinoids have been discovered and studied extensively (Hong *et al*., 2006; Franco *et al*., 2006; Mhaeswara *et al*., 2006). Additionally, sulfur rich plant extracts have been studied and their potential against bacterial pathogens has been reported (Iscan *et al*., 2002; Kalemba and Kunicka, 2003).

Many plant species have been reported for their medicinal uses around the world. According to a safe estimate, 6000 plant species have been reported in Pakistan (Shinwari *et al*., 2000). Many of these are traditionally used as therapy for curing and preventing different diseases. These plants belong to different families and genera including higher plants, trees, herbs, shrubs and grasses. *Capparis* species belonging to family Capparaceae are not exceptions for their medicinal attributes. This family consists of 250 species, out of which *C. spinosa* and *C. decidua* are native to Pakistan which have been reported for their traditional uses in folk medicine (Mabberley, 1997; Hamed *et al*., 2007). The aerial parts of these species have been reported as a potential source to restrain the bacterial growth. The extracts of fermented *C. spinosa* parts were reported to be effective to inhibit growth of different bacterial strains especially those which have acquired resistance to drugs like ciprofloxacin, vancomycin and teicoplanin (Perez *et al*., 2005; 2006a, b). Based on these findings, it is a dire need to explore the antimicrobial potential of different parts of *C. spinosa* and *C. decidua* as

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previously, no investigation has been carried out in Pakistan on these plants. So, the present study was designed aiming at exploring the antibacterial potential of aqueous methanol, aqueous ethanol and aqueous acetone extracts against Gram positive and negative bacteria.

Materials and Methods

Sample Collection

Five different plants of each species (*C. spinosa* and *C. decidua*) were selected and tagged from Cholistan desert, Bahawalpur region of Punjab. The samples of stem bark, shoots, fruits, flowers and roots of the both species were collected from the selected plants. The samples were further identified and authenticated by Dr. M. Hameed, Taxonomist, University of Agriculture Faisalabad, Pakistan. Average temperature (27°C), dew point (16°C) and relative humidity (47%) were recorded in sampling season (April).

Preparation of Extracts

The plant samples were ground to pass 2 mm sieve and the powder plant samples were separately extracted with three different solvents aqueous methanol (methanol: water, 80:20 v/v), aqueous ethanol (ethanol: water, 80:20 v/v) and aqueous acetone (acetone: water, 80:20 v/v). Dry powdered samples of each plant part (10 g) were mixed separately with 100 mL of each solvent for 6 h at room temperature in an orbital shaker (Pamico, Pak). The extract and residues were separated using Whatman's filter paper No. 1. The residues were extracted twice with the respective fresh solvents, and the obtained three extracts were pooled. The combined extracts were evaporated under reduced pressure at 45 ºC, using rotary evaporator (EYELA, N-N Series). The crude concentrated extracts were stored in refrigerator at -4ºC, until used for further studies.

Bacterial Strains

The pure cultures of Bacterial strains (*Staphylococcus aureus*: NCTC 6571, *Escherichia coli*: ATCC 8739, *Bacillus subtilis*: NCTC 10400 and *P. multocida*: wild type) were obtained from Department of Biochemistry, University of Agriculture Faisalabad, Pakistan and Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan which were further characterized from Institute of Microbiology, University of Agriculture Faisalabad, Pakistan. The bacteria were cultured overnight at 37ºC on nutrient agar (NA). Antibacterial activity of 80% aqueous methanolic, ethonolic and acetone extracts of selected parts of *C. spinosa* and *C. decidua* were individually evaluated against the above bacteria by following two methods.

a. *Disc diffusion method (NCCLS, 1997).* Bacterial suspension (100 µL) containing 10^8 colony-forming units (CFU)/mL was spread on petri plates (diameter × height: 150 × 25 mm) containing NA medium (50 mL media/plate). The wick paper discs (6 mm in diameter, 11 discs in each petri plate including two positive and one negative control) were separately saturated with 15 µL of each extract placed on the agar which had previously been inoculated with the selected test bacteria. Amoxycillin and ciprofloxacin were used as positive references for bacteria. Discs without samples were used as a negative control. Plates were kept at 4ºC for 1 h and incubated at 37ºC for 24 h. Antibacterial activity was assessed by measuring the diameter of the growth-inhibition zone (GIZ) in mm (including disc diameter of 6 mm) for the test organisms comparing to the controls.

b. *Resazurin microtitre-plate assay.* Modified resazurin microtitre-plate assay was also used for the measurement of MIC (minimum inhibitory concentration) of test bacteria against different extracts (Sarker *et al*., 2007). Briefly, 100 µL extract and standard drug (1 mg/mL in methanol) was pipetted into the first row of the 96 well plates. To all other wells 50 µL of nutrient broth was added. Two fold serial dilutions were performed using a multichannel pipette such that each well had 50 µL of the test material in serially descending concentrations. Then, 30 µL of 3.3× strength isosensitised broth and 10 µL of resazurin indicator solution (prepared by dissolving 270 mg tablet in 40 mL of sterile distilled water) were added in each well. Finally, 10 µL of bacterial suspension was added to each well to achieve a concentration of approx 5×10^5 CFU mL^{-1}. Each plate had a set of controls: a column with ciprofloxacin and second with amoxicillin as positive control, a column with all solutions with the exception of the test compound, a column with all solutions with the exception of the bacterial solution adding 10 µL of nutrient broth instead and a column with methanol as a negative control. The plates were prepared in triplicate. Plates were enfolded loosely with cling film and incubated at 37 °C for 24 h. The color change was then assessed visually. The growth was indicated by color changes from purple to pink or colorless. The lowest concentration at which colour change occurred was taken as the MIC value.

Statistical Analysis

Each parameter was analyzed individually in triplicate and data was reported as mean ± standard error. Data were analyzed by analysis of variance (ANOVA) using Minitab 2000 Version 13.2 statistical software (Minitab Inc. Pennysalvania, USA) at 5% significance level.

Results

The growth of all four bacteria was significantly affected by the application of plant extracts. It was found that aqueous methanolic extract of the stem bark of *C. spinosa* effectively inhibited the growth of all

Table 1: Antimicrobial activity (mm) of stem bark extracts of *C. spinosa* and *C. decidua* (assessed by disc diffusion assay)

Plant Species	Solvents	Bacteria			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pasteurella multocida*
C. spinosa	Methanol	15.7±0.5^{b}	17.9±0.7^{b}	26.8±0.7^{a}	24.7±0.4^{a}
	Ethanol	11.1±0.3^{b}	13.2±0.2^{b}	19.2±0.2^{a}	12.7±0.3^{b}
	Acetone	13.2±0.1^{bc}	18.6±0.7^{a}	13.7±0.4^{bc}	15.3±0.5^{b}
C. decidua	Methanol	20.4±0.8^{ab}	21.1±0.6^{ab}	23.4±0.9^{a}	19.7±0.7^{ab}
	Ethanol	14.2±0.6^{b}	14.3±0.5^{b}	17.1±0.6^{b}	21.2±0.9^{a}
	Acetone	13.7±0.5^{c}	16.9±0.4^{b}	12.9±0.5^{c}	19.0±0.4^{a}
Control	Amoxicillin	31.4±1.2	32.3±1.4	31.6±0.9	30.3±0.8
	Ciprofloxacin	29.6±1.1	30.9±1.2	27.8±1.3	27.9±1.3

Values (means ±SD) are average of three samples of each bacterium, analyzed individually in triplicate. Different Superscript letters within the mean rows indicate significant difference ($p < 0.05$) among the bacteria

Table 2: Minimum inhibitory concentration (µg mL^{-1}) of stem bark extracts of *C. spinosa* and *C. decidua*

Plant Species	Solvents	Bacteria			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pasteurella multocida*
C. spinosa	Methanol	145.2±5.1^{b}	184.6±4.8^{a}	123.2±5.2^{c}	126.7±8.4^{c}
	Ethanol	189.1±4.3^{b}	236.2±3.7^{a}	159.7±3.3^{c}	158.3±6.1^{c}
	Acetone	256.7±3.6^{b}	278.9±3.9^{a}	207.8±6.5^{c}	246.7±2.2^{b}
C. decidua	Methanol	236.7±4.5^{a}	212.3±4.5^{b}	128.9±3.1^{c}	199.0±6.2^{bc}
	Ethanol	241.2±7.9^{b}	268.9±5.3^{a}	179.1±3.9^{d}	213.2±7.7^{c}
	Acetone	303.4±6.3^{a}	310.1±8.1^{a}	233.4±8.6^{b}	296.7±7.5^{a}
Control	Amoxicillin	22.2±1.1^{ab}	13.7±0.6^{b}	10.8±0.5^{b}	29.5±1.4^{a}
	Ciprofloxacin	14.8±0.7^{a}	10.7±0.4^{ab}	9.2±0.4^{ab}	13.4±0.7^{a}

Values (means ±SD) are average of three samples of each bacterium, analyzed individually in triplicate. Different Superscript letters within the means row indicate significant difference ($p < 0.05$) among the bacteria

Table 3: Antimicrobial activity (mm) of shoot extracts of *C. spinosa* and *C. decidua* (assessed by disc diffusion assay)

Plant Species	Solvents	Bacteria			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pasteurella multocida*
C. spinosa	Methanol	21.0±0.2^{b}	21.9±0.2^{b}	24.6±0.5^{a}	23.6±0.7^{a}
	Ethanol	17.9±0.2^{b}	13.7±0.4^{c}	19.9±0.7^{a}	16.9±0.3^{b}
	Acetone	11.1±0.5^{b}	14.5±0.2^{a}	14.9±0.3^{a}	12.5±0.7^{b}
C. decidua	Methanol	19.8±0.3^{b}	26.7±0.7^{a}	12.4±0.4^{c}	20.7±0.1^{b}
	Ethanol	16.7±0.5^{b}	15.9±0.3^{b}	19.1±0.4^{a}	11.8±0.3^{b}
	Acetone	12.9±0.5^{c}	17.6±0.6^{a}	14.7±0.3^{b}	12.6±0.2^{c}
Control	Amoxicillin	31.5±1.2	30.6±1.0	30.6±1.2	29.3±0.8
	Ciprofloxacin	30.6±0.9	29.8±1.4	28.7±1.1	27.2±0.5

Values (means ±SD) are average of three samples of each bacterium, analyzed individually in triplicate. Different Superscript letters within the means row indicate significant difference ($p < 0.05$) among the bacteria

selected bacteria but its maximum inhibiting potential was recorded against *B. subtilis* with greater inhibition zones (26.8 mm) and lower MIC values (123.2 µg mL^{-1}) followed by *P. multocida* (GIZ, 24.7 mm; MIC, 158.3 µg mL^{-1}), while aqueous ethanol and acetone extracts of *C. spinosa* stem bark in that order were effective in inhibiting the corresponding population of *B. subtilis* and *E. coli* (GIZ, 19.2, 18.6 mm; MIC, 278.9, 159.7 µg mL^{-1}, respectively) as given in Table 1 and 2. In case of *C. decidua* stem bark, methanolic extract maximally inhibited *B. subtilis* (GIZ, 23.4 mm; MIC, 128.9 µg mL^{-1}), while its ethanol and acetone extracts showed considerable antibacterial activities with GIZ of 21.2, 19.0 mm and MIC values of 213.2, 296.7 µg mL^{-1} against *P. multocida* (Table 1 and 2).

Antibacterial activity of aqueous methanolic extract of *C. spinosa* shoot maximally controlled the population of *B. subtilis* (GIZ, 24.6 mm; MIC, 148.9 µg mL^{-1}) followed by *P. multocida* while its ethanol and acetone extracts effectively inhibited the growth of *B. subtilis* and *E. coli* with appreciable values of inhibition zones (GIZ) 13.7, 19.9 and 14.5, 14.9 mm minimum inhibitory concentrations (MIC) of 136.7, 198.9 and 251.9, 275.6 µg mL^{-1}, respectively (Table 3 and 4). The methanolic extract of *C. decidua* shoot maximally reduced the growth of *E. coli* followed by *P. multocida,* while showed less activity against *E. coli* and *S. aureus.* The antibacterial activity of aqueous ethanol and acetone extracts of shoot was good against the gram positive and gram negative bacteria in similar trend like *C. spinosa* shoot extracts (Table 3 and 4).

Aqueous methanol, ethanol and acetone extract of *C. spinosa* and *C. decidua* fruits exhibited varying antibacterial activity as shown by the inhibition zones (GIZ) and MIC values (Table 5 and 6). The best activity of methanolic

Table 5: Antimicrobial activity (mm) of fruit extracts of *C. spinosa* and *C. decidua* (assessed by disc diffusion assay)

Plant Species	Solvents	Bacteria			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pasteurella multocida*
C. spinosa	Methanol	17.7±0.5	20.9±0.1	23.9±0.4	24.9±0.6
	Ethanol	11.9±0.6	15.2±0.4	16.1±0.3	17.7±0.5
	Acetone	12.6±0.2	16.7±0.3	12.7±0.7	12.1±0.1
C. decidua	Methanol	21.4±0.3	24.1±0.7	26.8±0.2	21.7±0.1
	Ethanol	16.9±0.2	15.3±0.1	19.2±0.3	23.7±0.6
	Acetone	13.2±0.4	18.9±0.7	18.7±0.5	19.9±0.8
Control	Amoxicillin	30.1±0.9	32.2±1.6	34.1±0.9	29.9±1.4
	Ciprofloxacin	29.7±0.7	27.3±1.3	31.7±1.2	26.6±1.0

Values (means ±SD) are average of three samples of each bacterium, analyzed individually in triplicate. Different Superscript letters within the means row indicate significant difference ($p < 0.05$) among the bacteria

Table 6: Minimum inhibitory concentration (µg mL^{-1}) of fruit extracts of *C. spinosa* and *C. decidua*

Plant Species	Solvents	Bacteria			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pasteurella multocida*
C. spinosa	Methanol	110.9 ± 2.9^{b}	253.2 ± 8.4^{a}	126.7 ± 5.1^{b}	120.1 ± 2.5^{b}
	Ethanol	125.6 ± 3.5^{c}	266.7 ± 4.6^{a}	160.1 ± 2.2^{b}	176.7 ± 4.4^{b}
	Acetone	272.7 ± 4.7^{b}	306.9 ± 5.1^{a}	253.9 ± 6.2^{bc}	234.9 ± 8.2^{c}
C. decidua	Methanol	231.2 ± 7.9^{a}	158.3 ± 4.8^{c}	134.2 ± 5.7^{cd}	191.9 ± 4.3^{b}
	Ethanol	269.9 ± 8.3^{a}	188.9 ± 3.0^{c}	156.7 ± 2.9^{d}	233.7 ± 7.9^{b}
	Acetone	316.4 ± 9.8^{a}	260.1 ± 7.5^{c}	212.8 ± 6.8^{d}	285.7 ± 5.2^{b}
Control	Amoxicillin	21.2 ± 0.6^{b}	9.7 ± 0.5^{c}	12.4 ± 0.6^{c}	29.2 ± 0.9^{a}
	Ciprofloxacin	14.3 ± 0.4^{a}	8.9 ± 0.3^{ab}	6.8 ± 0.2^{b}	9.7 ± 0.3^{ab}

Values (means ±SD) are average of three samples of each bacterium, analyzed individually in triplicate. Different Superscript letters within the means row indicate significant difference ($p < 0.05$) among the bacteria

Table 7: Antimicrobial activity (mm) of flower extracts of *C. spinosa* and *C. decidua* (assessed by disc diffusion assay)

Plant Species	Solvents	Bacteria			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pasteurella multocida*
C. spinosa	Methanol	23.7 ± 0.5^{ab}	26.5 ± 0.7^{a}	17.6 ± 0.4^{bc}	20.7 ± 0.8^{b}
	Ethanol	14.8±0.2	18.2±0.9	19.7±0.1	15.9±0.3
	Acetone	12.1 ± 0.3^{b}	21.6 ± 0.7^{a}	11.2 ± 0.5^{b}	14.7 ± 0.2^{b}
C. decidua	Methanol	26.5 ± 0.4^{a}	23.9 ± 0.1^{b}	19.6 ± 0.3^{c}	22.3 ± 0.6^{b}
	Ethanol	19.1 ± 0.2^{a}	13.4 ± 0.9^{b}	11.3 ± 0.5^{b}	13.4 ± 0.3^{b}
	Acetone	21.3 ± 0.3^{a}	16.4 ± 0.5^{b}	13.2 ± 0.8^{c}	11.3 ± 0.2^{c}
Control	Amoxicillin	30.9±1.2	30.2±0.9	31.6±1.1	29.7±0.9
	Ciprofloxacin	28.5±1.1	29.3±1.1	30.3±0.9	27.9±0.7

Values (means ±SD) are average of three samples of each bacterium, analyzed individually in triplicate. Different Superscript letters within the means row indicate significant difference ($p < 0.05$) among the bacteria

Table 8: Minimum inhibitory concentration (µg mL^{-1}) of flower extracts of *C. spinosa* and *C. decidua*

Plant Species	Solvents	Bacteria			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pasteurella multocida*
C. spinosa	Methanol	122.1 ± 1.5^{c}	181.2 ± 3.1^{a}	111.2 ± 1.6^{cd}	146.7 ± 3.8^{b}
	Ethanol	147.8 ± 2.7^{bc}	215.6 ± 2.6^{a}	175.6 ± 3.7^{b}	158.9 ± 3.6^{bc}
	Acetone	266.7 ± 6.4^{a}	224.0 ± 5.3^{b}	196.7 ± 7.2^{c}	206.7 ± 7.1^{c}
C. decidua	Methanol	112.3 ± 3.7^{ab}	133.4 ± 3.6^{a}	96.3 ± 4.2^{b}	112.3 ± 3.9^{ab}
	Ethanol	143.1 ± 2.7^{b}	163.4 ± 2.6^{a}	138.2 ± 4.2^{b}	133.4 ± 1.9^{b}
	Acetone	204.5 ± 3.7^{d}	258.9 ± 2.6^{b}	295.6 ± 8.9^{a}	222.3 ± 2.7^{c}
Control	Amoxicillin	23.1 ± 0.6^{b}	11.2 ± 0.4^{c}	9.6 ± 0.3^{c}	27.2 ± 0.5^{a}
	Ciprofloxacin	17.4 ± 0.3^{a}	7.8 ± 0.2^{b}	7.4 ± 0.2^{b}	10.8 ± 0.4^{b}

Values (means ±SD) are average of three samples of each bacterium, analyzed individually in triplicate. Different Superscript letters within the means row indicate significant difference ($p < 0.05$) among the bacteria

extract of fruit was observed against *P. multocida* with large GIZ (24.9 mm) and small MIC values (120.1 µg mL^{-1}) values, while its acetone extract maximally reduced the growth of *E. coli* (GIZ, 16.7 mm; MIC, 3.6 µg mL^{-1}). Although the aqueous methanolic extracts of *C. decidua* fruits exhibited different trend against the selected bacteria, however it maximally reduced the population of *B. subtilis* and *E. coli* (GIZ, 26.8, 24.1 mm; MIC, 134.2, 158.3 µg mL^{-1}, respectively) while its ethanolic and acetone extracts effectively controlled the population of *P. multocida* with GIZ of 23.7, 19.9 mm and minimum inhibitory concentration 233.7, 285.7 µg mL^{-1} (Table 5 and 6).

Table 9: Antimicrobial activity (mm) of root extracts of *C. spinosa* and *C. decidua* (assessed by disc diffusion assay)

Plant Species	Solvents	Bacteria			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pasteurella multocida*
C. spinosa	Methanol	21.1±0.9	23.9±0.7	21.7±0.4	20.4±0.5
	Ethanol	14.9±0.4^{b}	19.2±0.1^{a}	16.1±0.6^{ab}	13.7±0.8^{b}
	Acetone	15.6±0.3	16.7±0.4	17.7±0.8	15.1±0.2
C. decidua	Methanol	26.4±0.2^{ab}	29.1±0.4^{a}	23.8±0.9^{b}	25.7±0.1^{ab}
	Ethanol	19.9±0.5^{ab}	22.3±0.3^{a}	18.2±0.6^{ab}	23.4±0.5^{a}
	Acetone	20.2±0.1^{a}	21.9±0.6^{a}	15.7±0.3^{b}	22.9±0.2^{a}
Control	Amoxicillin	32.1±1.6	34.2±1.7	32.1±1.3	29.7±1.2
	Ciprofloxacin	31.7±1.4	32.3±1.4	29.7±1.0	27.1±0.8

Table 10: Minimum inhibitory concentration (μg mL^{-1}) of root extracts of *C. spinosa* and *C. decidua*

Plant Species	Solvents	Bacteria			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pasteurella multocida*
C. spinosa	Methanol	156.7±1.4^{b}	142.6±6.3^{b}	157.8±4.2^{b}	207.9±8.1^{a}
	Ethanol	198.7±5.6^{b}	194.1±2.1^{b}	231.4±4.8^{a}	231.4±6.6^{a}
	Acetone	277.1±8.0^{a}	229.6±7.6^{b}	282.6±5.3^{a}	264.8±7.9^{a}
C. decidua	Methanol	182.3±5.3^{ab}	163.7±5.7^{b}	197.7±3.6^{a}	204.8±6.9^{a}
	Ethanol	189.3±4.6^{b}	179.6±4.1^{b}	232.3±4.8^{a}	232.7±7.5^{a}
	Acetone	263.4±5.5^{c}	221.7±6.2^{d}	318.1±7.9^{a}	286.4±8.1^{b}
Control	Amoxicillin	22.5±1.1^{b}	7.9±0.3^{c}	10.8±0.5^{c}	33.6±1.6^{a}
	Ciprofloxacin	15.7±0.7^{a}	8.0±0.4^{b}	5.6±0.2^{c}	11.8±0.5^{ab}

Values (means ±SD) are average of three samples of each bacterium, analyzed individually in triplicate. Different Superscript letters within the means row indicate significant difference ($p < 0.05$) among the bacteria

Screening of the antimicrobial activity of the aqueous methanol, ethanol and acetone extract of flowers of *C. spinosa* and *C. decidua* was also done against four pathogenic bacteria. The results of disc diffusion assay and measurement of inhibitory concentrations showed variation in the antibacterial activity (Tables 7 and 8). The methanol extract of *C. spinosa* flower showed stronger antibacterial activity against *E. coli* followed by *S. aureus*, *P. multocida* and *B. subtilis.* The values of GIZ offered for bacterial stains were 26.5, 23.7, 20.7 and 17.6 mm, respectively. Generally, the tested Gram positive bacteria were found to be more sensitive to flowers extract than Gram negative bacteria. Little differences were observed for the GIZ among these extracts, since all of them showed a strong activity for the strains assayed. However, more precise results on the antimicrobial properties were obtained through the determination of minimum inhibitory concentration that were found to be 181.2, 122.1, 146.7 and 111.2 μg mL^{-1} against *E. coli*, *P. multocida*, *S. aureus* and *B. subtilis*, respectively. Ethanolic extract of *C. spinosa* flower was effective against *B. subtilis* (GIZ, 19.7 mm; MIC, 175.6 μg mL^{-1}) followed by *E. coli* (GIZ, 18.2 mm, MIC, 215.6 μg mL^{-1}) while all three extracts of *C. decidua* flowers i.e., aqueous methanol, ethanol and acetone effectively reduced the population of *S. aureus* and *E. coli* (Table 7 and 8).

The antibacterial effectiveness of aqueous methanol, ethanol and acetone extract of *C. spinosa* and *C. decidua* roots was evaluated by the measurement of inhibition zones (GIZ) and minimum Inhibitory concentration (MIC) is in Table 9 and 10. The results from the disc diffusion assay indicated that aqueous methanol extract of *C. spinosa* root showed comparable antibacterial activity in selected strains of bacteria (*E. coli*, *S. aureus*, *Bacillus subtilis* and *P. multocida*) with GIZ 23.9, 21.7, 21.2 and 20.4 mm, respectively. The ethanol and acetone extracts of *C. spinosa* root exhibited best antibacterial activity against *E. coli* and *Bacillus subtilis* (GIZ, 19.2, 17.7mm; MIC, 142.6, 282.6 μg mL^{-1}, respectively) while methanolic extract of *C. decidua* roots showed best activity against the *E. coli* (GIZ, 29.1 mm; MIC, 142.6 μg mL^{-1}) and ethanolic and acetone extracts of *C. decidua* roots reduced the growth of *P. multocida* (GIZ, 23.4,22.9 mm; MIC, 232.7, 286.4 μg mL^{-1}, respectively) (Table 9 and 10).

Discussion

Resistance in bacteria against drugs is intensifying due to their extensive use in clinical medicines. No doubt, the scientists are continuously working on discovering and formulating new drugs to cure microbial infections but drug resistant bacterial strains are continuously opposing a challenge to pharmacists. It is urging for researchers to explore the natural products qualifying antibacterial potential in their extracts. Medicinal plants have been used in since ages but their antimicrobial potential as a source of drugs have not been explored extensively although these are used in folk medicine or indirectly in pharmaceutical drugs. The resistance in pathogenic bacteria against drugs and the traditional practices of using medicinal plants against infectious diseases is accelerating the research work related to explore the efficacy of plant extracts or plant derived phytochemical compounds (Chan *et al*., 2007; Pitchamuthu *et al*., 2012).

In present investigation, antibacterial potential of

selected parts of *C. spinosa* and *C. decidua* was evaluated against four Gram positive and negative bacteria. The growth of all four bacteria was significantly inhibited by the application of *Capparis* extracts in comparison with synthetic drugs i.e., amoxycillin and ciprofloxacin. Aqueous methanolic extracts were found to be more effective in comparison with ethanol and acetone extracts against all bacteria but extracts of different parts exhibited variable efficacy in limiting bacterial development. Previously, the inhibiting potential of aerial parts of *C. spinosa* plants has been reported against Gram-positive and negative bacteria (Mahasneh, 2002). In this study, *Capparis* stem bark aqueous methanolic extract effectively inhibited *Bacillus subtilis* and *P. multocida* growth, while other bacteria showed some resistance. The difference in bacterial response against solvents might be due to cell membrane permeability. Gram negative bacteria have an outer phospholipidic membrane, which makes the wall impermeable to antimicrobial chemical compound, while Gram positive bacteria have peptidoglycan layer, which is permeable to these substances (Sharma *et al*., 2010). It was further found that aqueous methanolic extract of *C. decidua* shoot has inhibiting potential against *Bacillus subtilis* and *Aspergillus niger* (Iqbal *et al*., 2006). Upadhyay *et al*. (2010) reported the antimicrobial potential of absolute and aqueous methanol extracts of *C. decidua* against *Klebsiella pneumoniae*, *E. coli*, *Micrococcus luteus*, *Streptococcus pneumoniae*, *S. aureus*, *Bacillus cereus* and *Lactobacilus acidophilus*. Those researchers reported 36 mm growth inhibition zone (GIZ) of *S. aureus* when induced with *C. decidua* shoot extract while in the present study 21.0 and 19.8 mm GIZ of *S. aureus* was recorded by aqueous methanolic extract of *C. decidua* and *C. spinosa* shoot extract, respectively. As, the fruits and flowers of *C. spinosa* and *C. decidua* are also rich in antioxidant compounds (Zia-ul-Haq *et al*., 2011), their extracts exhibited good antimicrobial potential in comparison with synthetic drugs i.e., amoxicillin and ciprofloxacin as found in the present investigation. Previously, *C. spinosa* flower extracts have been reported as 100 and 90% effective against Gram positive and negative bacteria, respectively (Ibrahim, 2012). Likewise, extracts obtained from fermented caper fruits have been found to contain effective antimicrobial agents against bacterial strains that had become resistant to drugs like ciprofloxacin, vancomycin and teicoplanin (Perez *et al*., 2005; 2006a, b).

The roots of *C. spinosa* and *C. decidua* are not exception to exhibit antibacterial activity. The root extracts of both species exhibited growth inhibiting effect on all studied bacteria (Table 9, 10). The potential of *C. spinosa* roots has also been studied on *Deinococcus radiophilus* (Boga *et al*., 2011). Aqueous methanolic extract of *C. decidua* roots showed larger GIZ for *S. aureus* and *E. coli,* while Mali *et al*. (2004) found that ethanolic extract of *C. decidua* roots is more effective against *S. aureus* and *E. coli* in comparison with other extracts. This difference might be attributed to extraction technique/solvent, germplasm, climatic factors and growing conditions. A few studies are available for antimicrobial potential of *C. spinosa* and *C. decidua* root extracts while the researchers have worked on a few other species' root extracts. The effectiveness of *C. grandiflora* root extract was studied against *S. aureus*, *Bacillus subtilis*, *Bacillus pumillus*, *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Candida albicans* and *A. niger* (Sini *et al*., 2011). Similarly, petroleum ether extract of *C. zeylanica* roots have inhibiting potential against *S. aureus*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Proteus vulgaris* (Rengapriya *et al*., 2012).

The efficacy of plant extracts against bacterial growth might be due to phytoconstituents like phenolics and flavonoids present in plant extracts. So, antibacterial potential of *Capparis* extracts might be due to higher phenolic and flavonoid compounds found in different parts of *Capparis* species (Zia-ul-Haq *et al*., 2011; Imran *et al*., 2014). Proestos *et al*. (2006) studied the correlation between antimicrobial activity of plant extracts and phenolic acids. The researchers studied the phenolic acids composition by employing RP-HPLC with UV detection and further GC-MS was used for phenolic acids characterization. It was found that plant extracts with higher phenolic and flavonoid compounds exhibit good antibacterial activity. *Capparis* extracts are also rich in antioxidant compounds, phenolics, flavonoids, rutin, tocopherols, carotenoids and vitamin C (Tlili *et al*., 2011a,b; Imran *et al*., 2014). The inhibited growth of *E. coli* and *S. aureus* can be correlated with flavonoid compounds in *C. decidua* extracts (Sharma and Kumar, 2009). Such studies show that the extracts rich in phenolic acids and flavonoids, exhibit better antimicrobial potential (Sivropoulou *et al*., 1995). Secondly, the difference in the antimicrobial potential of different solvents might be due to the difference in polarities and chemical characteristics of these solvents (Sultana *et al*., 2009).

Conclusion

Methanolic extracts of the selected parts of both species were more effective in inhibiting the growth of studied bacteria in comparison with other solvents. The results manifested that the bacterial population were maximally inhibited by *C. decidua* root extracts except *B. subtilis* while *S. aureus* was more sensitive to methanolic flower extract of *C. decidua*. Based on these findings, ex vivo confirmatory studies should be carried out for further explanation of the effectiveness of these species.

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