

Capparis Spinosa: A Potential Source Of Antioxidants And Antibacterial Activities Against Bacillus Cereus And Pseudomonas Aeruginosa

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Abstract

Antioxidant and antibacterial potential of ethanolic and acetone extracts of stem bark, shoot, fruit, flower and root of *Capparis spinosa* was studied in this study. Aqueous acetone was found as good solvent extracting phenolics and flavonoids. In case of parts, maximum phenolics and flavonoids were found in root and stem bark. While maximum antioxidant potential was exhibited by flower extracts. In case of solvents, aqueous ethanolic extracts showed maximum antioxidant potential in comparison with others. Seasonal variation was also recorded among *C. spinosa* parts collected in April and September. Moreover, all extracts effectively controlled the growth of selected bacteria while growth of *Bacillus cereus* and *Pseudomonas aeruginosa* were maximally inhibited by aqueous ethanol and aqueous acetone extracts. The present study concluded that *C. spinosa* is a useful source of antioxidant compounds possessing antibacterial potential.

Introduction

Medicinal plants are a reliable source of these natural antioxidants. Pakistan is rich in floral composition with >2000 medicinal plants, among which many are to be explored for their antioxidant potential. *C. spinosa*; belonging to the family Capparaceae, is native to the Cholistan desert of Pakistan. *C. spinosa* is a drought-tolerant plant that can easily be grown under harsh conditions of water scarcity and arid climatic conditions⁽¹⁾. In Pakistan and other Mediterranean countries, this perennial shrub blooms from May to October in the late summer and early winter. The flowers exhibit a loss of turgidity within a span of 12-16 hours, a process that ultimately leads to abscission, influenced by temperature conditions.

In addition, the flower nectar is secreted at night when the temperature is relatively low. Various scientists and traditional health practitioners have been using its parts especially stem bark, roots and flowers as preventive and curing medicine against cardiac, skin, biliousness and respiratory diseases⁽²⁾. The aerial parts of *C. spinosa* are reliable sources of nutrients and vitamins especially fruits (caper berries) are consumed by human beings as pickle and vegetable⁽³⁾. The present study was carried out to investigate the antioxidant potential of its various parts.

Medicinal plants are defined by their antioxidant compounds, which play a crucial role in scavenging reactive oxygen species (ROS) generated in living systems because of oxidative stress. Free radicals and reactive oxygen species can lead to chronic diseases such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts, and inflammation, primarily due to the oxidation of nucleic acids, proteins, and DNA. Antioxidant compounds scavenge peroxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO^{\cdot}) and lipid peroxyl (free radicals) and thus stop the oxidation process which causes chronic diseases. These antioxidant compounds initiate a neuroprotection mechanism, which contributes to scavenging free radicals and ROS⁽⁵⁾. Pharmacists and health practitioners express concerns regarding synthetic antioxidants such as tertiary butyl hydroquinone (TBHQ) and butylated hydroxyanisole (BHA), which could potentially lead to carcinogenic effects in the human body. Such reservations are motivating scientists to investigate potential antioxidant plants that could serve as alternatives to synthetic options⁽⁶⁾. This study, discovery, and extraction of active principles are receiving impetus as the knowledge about the plants is expanding. These antioxidant compounds are naturally found in whole grains, fruits, vegetables, spices, herbs and medicinal plants which had strong antioxidant activity and greater phenolic compounds⁽⁷⁾. This is why; the scientists are interested in exploring plant-derived antioxidants, especially phenolics, which have strong antioxidant potential.

The extraction of natural antioxidants is done through different solvents like methanol, ethanol, acetone and water. Among this water is considered as poor extraction solvent due to its weaker polarities because the extraction of antioxidants depends on the nature of solvents and their polarities. Ethanol and acetone are considered as good solvents for this purpose. Methanol has been noted as an effective solvent system; however, some have highlighted the effectiveness of acetone⁽⁸⁾. A few researchers have reported acetone as good solvents to extract such compounds and chemical constituents from stem bark, whole stem and roots while ethanol performs well in fruits and flowers⁽⁹⁾. Based on these research reports and traditional claims about the consumption of *C. spinosa*, the present investigation was aimed at exploring a better solvent for extracting antioxidant compounds from its selected parts (stem bark, shoot, fruit, flower and root) in different seasons.

Materials and Methods

Collection and identification of plant materials

The samples (stem bark, shoot, fruit, flower and root) of *C. spinosa* were taken in Cholistan desert of Bahawalpur, Pakistan (desert region, latitude 28-15 N; longitude 70-45 /E and altitude 89 m above mean sea level) in April and September. Plant samples were also identified and confirmed by a Taxonomist, Dr. Mansoor Hameed, Assistant Professor, Department of Botany, University of Agriculture Faisalabad, Pakistan.

Extraction by different solvent systems

The ground material of all samples was separately extracted with four solvents i.e., absolute ethanol, aqueous ethanol (80:20 v/v), absolute acetone and aqueous acetone (80:20 v/v). Dry sample (10 g of each) was mixed with 100 mL of solvent for 6 hours at room temperature in an orbital shaker (Pamico, Pak). The residues underwent extraction twice using the appropriate fresh solvents, and the resulting three extracts were combined. The combined extracts were devoid of solvents and concentrated to dryness under reduced pressure at 45 °C, utilizing a rotary evaporator (EYELA, N-N Series). The crude concentrated extracts were measured to determine the percentage yield and were subsequently stored in a refrigerator at -4 °C for future use.

Total Phenolic Contents (TPC)

Total phenolic contents were determined by Folin-Ciocalteu reagent method described by Hussain et al.⁽¹⁰⁾. The solution (250 µL) of each sample was taken in ninety-six well-plate and absorbance was noted at 755 nm using microplate reader (Bio Tek Instrument, Inc., VT, USA). The amount of TPC was calculated using gallic acid calibration curve.

Total Flavonoid Contents (TFC)

Total Flavonoid Contents were determined by using the protocol described by Hussain et al.⁽¹⁰⁾. Absorbance was read at 510 nm by spectrophotometer (Bio Tek Instrument, Inc., VT, USA). TFC were calculated using a calibration curve for catechin.

Spectrophotometric DPPH assay

The antioxidant activity of the extract was assessed by measuring their ability to scavenge 2,2'-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The assay was carried out spectrophotometrically as described by Hussain et al.⁽¹⁰⁾. Concentration of extract providing 50% scavenging (IC₅₀) was calculated from the graph-plotted scavenging percentage against extract concentration.

Percent inhibition of linoleic acid peroxidation

The antioxidant activity of the extract was determined in terms of measurement of % inhibition of linoleic acid peroxidation following the method described by Iqbal et al.⁽¹¹⁾.

Antibacterial activity

The pure cultures of Bacterial strains (*Bacillus cereus*: NCTC 7464 and *Pseudomonas aeruginosa*: NCTC 1662) were obtained from the Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan and Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan which were further characterized by the Department of Veterinary Microbiology, University of Agriculture Faisalabad, Pakistan. Antibacterial activity of plant extracts against selected bacterial strains was explored by using disc diffusion assay⁽¹²⁾.

Results and Discussion

Extract yield (%)

Data depicted in Table 1 shows the mean values of extraction yield in various parts of *C. spinosa* which demonstrate a significant difference in extract yields of selected parts of *C. spinosa* as affected by different solvent systems. It was found that the fruits exhibited extract yield ($21.2 \text{ g } 100\text{g}^{-1}$) while minimum yield was recorded in stem bark ($4.8 \text{ g } 100\text{g}^{-1}$) which was statistically at par with roots and shoot (6.5 and $7.0 \text{ g } 100\text{g}^{-1}$). Among solvents, aqueous ethanol gave maximum yield ($15.5 \text{ g } 100\text{g}^{-1}$) followed by aqueous acetone and absolute ethanol (12.1 and $11.7 \text{ g } 100\text{g}^{-1}$). A similar trend was observed in samples collected in September (Table 1). It has been previously reported in the literature that the extraction of antioxidant compounds from plants mainly depends on the polarities of solvent systems like methanol, ethanol and acetone because these are mostly present in outer tissues of plants in higher quantity than the inner ones which are soluble in different solvents depending upon the nature and polarities of solvents⁽¹³⁾. From these, ethanol has been extensively used for this purpose for different vegetables, fruits, and tree leaves^(14, 15).

Table 1: Extract yield (%) of different parts of *C. spinosa* in different solvent systems

Seasons	Solvents	Stem Bark	Shoot	Fruit	Flower	Root	Mean
April	Absolute Ethanol	$2.8 \pm 0.2^{\text{d}}$ _b	$6.0 \pm 0.7^{\text{c}}$ _b	$25.3 \pm 0.6^{\text{a}}$ _a	$18.9 \pm 0.5^{\text{b}}$	$5.5 \pm 0.03^{\text{c}}$	11.7 ± 0.4 AB
	Aqueous Ethanol	$10.0 \pm 0.3^{\text{c}}$ _a	$11.2 \pm 0.1^{\text{c}}$ _a	$28.5 \pm 0.6^{\text{a}}$ _a	$20.2 \pm 0.5^{\text{b}}$	$7.6 \pm 0.05^{\text{d}}$	15.5 ± 0.8 A
	Absolute Acetone	$1.4 \pm 0.2^{\text{c}}$ _c	$2.2 \pm 0.1^{\text{c}}$ _c	$12.7 \pm 0.5^{\text{b}}$ _c	$16.0 \pm 0.7^{\text{a}}$	$4.6 \pm 0.02^{\text{c}}$	7.4 ± 0.3 C
	Aqueous Acetone	$5.2 \pm 0.2^{\text{c}}$ _b	$8.6 \pm 0.7^{\text{b}}$ _{ab}	$18.4 \pm 0.4^{\text{a}}$ _b	$20.1 \pm 0.4^{\text{a}}$	$8.3 \pm 0.09^{\text{b}}$	12.1 ± 0.5 AB
	Mean	4.8 ± 0.1 B	7.0 ± 0.2 B	21.2 ± 0.8 A	18.8 ± 0.7 A	6.5 ± 0.2 B	
	September	Absolute Ethanol	$4.9 \pm 0.3^{\text{c}}$	$22.3 \pm 0.3^{\text{b}}$ _a	$44.6 \pm 0.4^{\text{a}}$ _b	$25.4 \pm 1.4^{\text{b}}$ _a	$6.7 \pm 0.4^{\text{c}}$ _b
	Aqueous Ethanol	$6.9 \pm 0.4^{\text{c}}$	$13.5 \pm 0.6^{\text{c}}$ _b	$63.7 \pm 0.5^{\text{a}}$ _a	$28.0 \pm 1.1^{\text{b}}$ _a	$9.4 \pm 0.5^{\text{c}}$ _{ab}	24.3 ± 1.7 A
	Absolute Acetone	$2.8 \pm 0.7^{\text{b}}$	$5.1 \pm 0.8^{\text{ab}}$ _c	$11.1 \pm 0.4^{\text{a}}$ _c	$8.1 \pm 0.12^{\text{a}}$ _b	$2.4 \pm 0.05^{\text{b}}$ _c	5.9 ± 0.3 C
	Aqueous Acetone	$3.7 \pm 0.5^{\text{c}}$	$7.3 \pm 0.9^{\text{b}}$ _c	$14.3 \pm 0.6^{\text{a}}$ _c	$13.7 \pm 0.23^{\text{a}}$ _b	$14.4 \pm 0.9^{\text{a}}$ _a	10.7 ± 0.4 B
	Mean	5.8 ± 0.7 C	12.1 ± 0.9 B	33.4 ± 1.9 A	15.6 ± 1.2 B	8.2 ± 0.8 C	

Values (means \pm SD) are average of three samples of each part, analyzed individually in triplicate ($p < 0.05$). Different Superscript letter within the mean's row indicates significant differences ($p < 0.05$) among the parts and subscript shows significant differences ($p < 0.05$) of means within the solvents.

Total phenolic contents

Total phenolic contents (TFC) were found significantly different in selected parts of *C. spinosa* affected under different solvent systems. Maximum (TFC) were recorded in stem bark and roots (395.3 and $371.5 \text{ mg GAE}/100\text{g}$ of DW, respectively) which were statistically similar with each other, while the minimum was found in flowers ($139.8 \text{ mg GAE}/100\text{g}$ of DW). In case of solvent systems, aqueous acetone ($445.8 \text{ mg GAE}/100\text{g}$ of DW) was more effective in extracting TFC in comparison with all others, followed by aqueous ethanol ($334.9 \text{ mg GAE}/100\text{g}$ of DW). The least quantity of TFC

was recorded in absolute acetone extracts i.e., 190.3 mg GAE/100g of DW. In September, more phenolic contents were found in *C. spinosa* samples in comparison with April. In September, maximum TPC were recorded in shoot, root and stem bark which were statistically at par with each other (Table 2). As evident from these findings that aqueous acetone was found as an effective solvent system for extracting TFC. Previously, methanol has been reported as an effective solvent for this purpose but acetone and ethanol are not exceptions⁽⁸⁾. Maximum TPC in aqueous of *Acacia nilotica* and *Moringa oleifera* leaves have also been reported by using ethanolic solvent extraction method⁽¹⁵⁾. TPC of *C. spinosa* found in the present study were found lower than the findings of another researcher⁽¹⁶⁾, this reduction might be due to different climatic condition, ecological zone and genetic variation as that study was conducted in Mediterranean region.

Table 2: Total phenolic contents (mg 100g⁻¹) of different parts of *C. spinosa* in different solvent systems

Seasons	Solvents	Stem Bark	Shoot	Fruit	Flower	Root	Mean
April	Absolute Ethanol	251.6±23.7 ^{bc}	285.3±14.5 ^b	218.4±18.7 ^b	98.6±19.3 ^d	328.4±18.9 ^a	236.5 ±12.5 C
	Aqueous Ethanol	381.9±34.2 ^b	449.7±11.6 ^a	315.7±16.5 ^a	195.8±15.5 ^d	331.4±16.1 ^c	334.9 ±15.7 B
	Absolute Acetone	385.3±13.5 ^a	117.3±32.6 ^c	112.5±14.8 ^c	46.0±12.3 ^d	290.3±12.5 ^b	190.3 ±9.3 D
	Aqueous Acetone	562.3±19.0 ^a	570.3±29.3 ^a	338.6±36.0 ^b	218.8±23.4 ^a	539.0±17.1 ^{ab}	445.8 ±11.8 A
	Mean	395.3±15.0	355.6±10.7	246.3±7.9	139.8±5.0	371.5±11.1	
		A	AB	B	C	A	
September	Absolute Ethanol	213.8±20.3 ^a	192.7±15.4 ^b	237.3±12.9 ^a	117.5±10.3 ^b	227.9±17.6 ^a	297.5 ±11.9A
	Aqueous Ethanol	461.2±29.4 ^a	398.2±31.2 ^b	443.6±23.7 ^a	323.8±23.8 ^a	473.1±29.4 ^a	311.6 ±9.3A
	Absolute Acetone	252.5±11.9 ^b	446.0±37.6 ^a	277.3±19.6 ^b	169.9±9.4 ^b	140.0±29.1 ^c	257.7 ±2.5AB
	Aqueous Acetone	366.0±23.7 ^b	332.7±24.5 ^b	136.8±17.8 ^d	157.5±6.9 ^d	511.9±32.3 ^a	290.8± 8.7A
	Mean	323.4±9.7	342.4±10.9	273.7±8.8	192.2±6.4	338.2±10.3	
		A	A	B	C	A	

Values (means ±SD) are the average of three samples of each part, analyzed individually in triplicate (p< 0.05). Different Superscript letter within the mean's row indicates significant differences (p< 0.05) among the parts and subscript shows significant differences (p< 0.05) of means within the solvents.

Total flavonoid contents

A significant difference was found among parts of *C. spinosa* and different solvent systems in expressing total flavonoid contents (TFC). The roots of *C. spinosa* exhibited maximum TFC (160.4 mg/ CE/100g of DW) while the least were found in flowers i.e., 107.7 mg/ CE/100g of DW. In case of solvent systems, aqueous ethanol and aqueous acetone were statistically at par with each other in offering TFC (147.6 and 144.2 mg/ CE/100g of DW, respectively). A likewise trend was observed in September samples but TFC were relatively higher than April (Table 3). The researchers have reported that the presence of TPC and TFC demonstrate higher antioxidant activity of the respective

plants or their parts⁽¹⁷⁾. Similar observations were recorded in the present investigation. The extracts that yielded maximum TPC and TFC, exhibited maximum antioxidant potential. As the roots of *C. spinosa* are rich in TPC and TFC, their effectiveness as antioxidants can be correlated with these compounds as has been reported for *C. spinosa* leaves.

Table 3: Total flavonoid contents (mg 100g⁻¹) of different parts of *C. spinosa* in different solvent systems

Seasons	Solvents	Stem Bark	Shoot	Fruit	Flower	Root	Mean	
April	Absolute Ethanol	118.2±11.6 ^{ab}	123.4±18.3 ^b	118.4±13.8 ^b	84.0±18.1 ^c	162.6±14.1 ^a	121.7 ±9.9 AB	
	Aqueous Ethanol	124.5±15.7 ^{ab}	161.7±11.8 ^a	152.3±17.5 ^a	132.5±10.6 ^b	167.0±20.4 ^a	147.6 ±8.4 A	
	Absolute Acetone	102.1±5.9 ^c	114.2±14.4 ^b	135.0±14.6 ^a	99.4±14.2 ^c	131.8±16.7 ^a	116.5 ±7.8 AB	
	Aqueous Acetone	145.8±7.8 ^a	131.4±16.3 ^{bc}	149.0±10.4 ^b	114.9±19.7 ^b	180.3±20.4 ^a	144.2 ±9.2 A	
	Mean	122.6±6.9 AB	132.7±8.2 AB	138.2±5.1 AB	107.7±3.23 B	160.4±8.0 A		
	September	Absolute Ethanol	130.8±11.7 ^b	175.3±5.9 ^a	120.9±11.9 ^b	73.1±19.3 ^c	152.1±23.2 ^{ab}	146.6± 4.3 B
		Aqueous Ethanol	175.3±8.6 ^a	181.0±11.2 ^a	176.9±14.6 ^a	129.1±18.0 ^b	188.5±11.4 ^a	198.5± 17.8 A
Absolute Acetone		191.0±7.4 ^b	174.3±9.4 ^b	97.6±13.7 ^{bc}	49.8±4.1 ^d	279.3±18.6 ^b	120.9 ±3.6 C	
Aqueous Acetone		214.8±15.4 ^b	151.4±6.8 ^c	109.2±16.3 ^{cd}	61.5±6.3 ^d	360.9±29.2 ^a	133.6 ±4.0 B	
Mean		178.0±5.7 B	170.5±5.6 B	126.2±4.3 BC	78.4±2.8 C	245.2±7.8 A		

Values (means ±SD) are the average of three samples of each part, analyzed individually in triplicate (p< 0.05). Different Superscript letter within the mean's row indicates significant differences (p< 0.05) among the parts and subscript shows significant differences (p< 0.05) of means within the solvents.

DPPH[•] scavenging activity

The antioxidant activity of the different parts of *C. spinosa* under different solvent system, as assessed by DPPH[•] radical scavenging assay as well as expressed in terms of 50% scavenging (IC50) is given in Table 4. Maximum DPPH[•] scavenging activity was shown by stem bark (89.4 µg mL⁻¹) followed by roots (69.4 µg mL⁻¹). Shoots of *C. spinosa* exhibited least scavenging activity in the present investigation (13.8 µg mL⁻¹). In case of solvent systems, aqueous ethanol expressed least IC50 value followed by aqueous acetone (25.3 and 51.6 µg mL⁻¹) which manifested higher DPPH[•] scavenging activity. In September, no significant difference was found among parts while aqueous ethanol and acetone solvents exhibited maximum DPPH[•] radical scavenging activity (Table 4). DPPH[•] are free radicals which accept an electron or hydrogen radical becoming a diamagnetic molecule. Polyphenols existing in plant extracts donate hydrogen atoms from their hydroxyl group to form a stable free DPPH[•] which inhibits further lipid oxidation⁽¹⁸⁾.

Table 4: DPPH[•] scavenging activity (IC 50) of different parts of *C. spinosa* in different solvent systems

Seasons	Solvents	Stem Bark	Shoot	Fruit	Flower	Root	Mean
April	Absolute	146.8	114.5	63.6	6.2	69.8	80.2
	Ethanol	±8.2 ^a _a	±6.1 ^b _a	±2.9 ^c _b	±1.7 ^d _b	±2.9 ^c _b	±4.7 A
	Aqueous	59.3	29.4	15.1	4.3	18.2	25.3
	Ethanol	±2.2 ^a _b	±1.5 ^b _{bc}	±1.4 ^c _c	±1.4 ^d _b	±1.5 ^c _{cd}	±1.6 D
	Absolute	137.4	55.3	80.6	39.4	39.3	70.4
	Acetone	±4.9 ^a _a	±3.6 ^c _b	±1.5 ^b _a	±2.9 ^d _a	±2.0 ^d _c	±3.9 B
	Aqueous	14.3	36.5	51.8	5.1	150.5	51.6
	Acetone	±1.5 ^d _c	±1.4 ^c _{bc}	±2.9 ^b _{bc}	±2.1 ^d _b	±4.1 ^a _a	±3.2 C
	Mean	89.4	58.9	52.8	13.8	69.4	
	±4.7 A	±3.7 C	±2.1 C	±1.4 D	±2.9 B		
September	Absolute	39.4	89.4	17.2	42.61	37.4	47.2
	Ethanol	±2.0 ^b _b	±3.0 ^a _a	±3.9 ^c _b	±3.2 ^b _a	±3.1 ^b _a	±1.8 B
	Aqueous	20.1	9.1	16.6	38.29	14.2	25.6
	Ethanol	±1.1 ^b _c	±1.3 ^{bc} _c	±3.22 ^b _b	±4.9 ^a _a	±1.1 ^b _{bc}	±1.0 C
	Absolute	96.2	21.88	88.5	46.24	21.1	60.8±
	Acetone	±4.3 ^a _a	±2.7 ^c _b	±4.2 ^a _a	±3.6 ^b _a	±2.4 ^c _b	1.8 A
	Aqueous	11.5	8.89	12.7	27.44	35.5	23.5±
	Acetone	±3.4 ^b _d	±2.1 ^b _c	±1.5 ^b _b	±1.0 ^a _b	±4.3 ^a _a	0.7 C
	Mean	41.8	32.3	33.7	38.6	27.1	
	±3.4	±2.9	±2.3	±2.8	±2.2		

Values (means ±SD) are average of three samples of each part, analyzed individually in triplicate (p< 0.05). Different Superscript letter within the mean's row indicates significant differences (p< 0.05) among the parts and subscript shows significant differences (p< 0.05) of means within the solvents.

Linoleic acid peroxidation inhibition

Further lipid peroxidation inhibition assay was conducted to clarify the antioxidant potential of *C. spinosa* extracts. Linoleic acid is a polyunsaturated fatty acid that, upon oxidation, produces peroxides, which then oxidize ferrous ions into ferric ions. The ferric ions subsequently form a complex with thiocyanate ions, and the intensity of this colored complex is measured at 500 nm. Table 5 manifested that stem bark and shoot extracts of *C. spinosa* exhibited maximum lipid peroxidation inhibition (72.5 and 67.1%) while the least was found in fruit extracts (48.0%). No significant difference was found among different solvent systems regarding lipid peroxidation inhibition. The inhibition power of *C. spinosa* extracts might be attributed to the total phenolics and total flavonoid contents, as Siddhuraju et al.⁽¹⁵⁾ reported that the reducing power of bioactive compounds is associated with antioxidant activity. Further, another researcher⁽¹⁶⁾ reported that *C. spinosa* extracts were supplied in 100 g/mL doses inhibited lipid peroxidation in 71.50% and an increase in ten times in these doses inhibited lipid peroxidation in 90% due to the contents of phenol compounds of *C. spinosa*.

Table 5: Inhibition of linoleic acid peroxidation (%) of different parts of *C. spinosa* in different solvent systems

Seasons	Solvents	Stem Bark	Shoot	Fruit	Flower	Root	Mean
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April	Absolute	73.7	64.4	38.4	43.4	52.5	54.5
	Ethanol	±3.5 ^a	±0.9 ^a _b	±3.5 ^b _b	±3.2 ^b _b	±3.2 ^{ab} _b	±4.7
	Aqueous	52.4	63.9	58.4	63.4	69.2	61.5
	Ethanol	±2.1 _b	±3.2 _b	±2.3 _a	±2.1 _a	±3.9 _a	±5.1
	Absolute	84.7	78.3	42.4	59.4	48.0	62.5
	Acetone	±2.8 ^a	±4.0 ^a	±2.9 ^b _b	±3.6 ^b _a	±4.7 ^b _b	±6.2
	Aqueous	79.3	61.9	52.7	53.7	75.9	64.7
	Acetone	±4.5 ^a	±1.6 ^b _b	±1.1 ^c _a	±2.8 ^c _a	±5.0 ^a	±6.1
	Mean	72.5	67.1	48.0	54.9	61.4	
	±1.9 A	±2.3 A	±1.8 B	±2.1 AB	±2.8 AB		
September	Absolute	74.9	75.6	69.3	61.4	64.8	69.2
	Ethanol	±4.0	±3.75 _a	±2.1 _a	±2.2 _a	±1.9 _b	±2.0 A
	Aqueous	84.7	46.3	23.3	17.4	83.6	51.0±
	Ethanol	±3.2 ^a	±2.8 ^b _b	±1.7 ^c _c	±0.6 ^c _c	±4.9 ^a	1.0 C
	Absolute	67.8	74.6	43.5	35.6	83.2	60.9±
	Acetone	±2.6 ^a	±2.7 ^a	±2.9 ^b _b	±2.0 ^b _b	±4.5 ^a	3.0 B
	Aqueous	70.9	83.4	77.9	68.9	58.7	71.9
	Acetone	±3.3 ^{ab}	±3.9 ^a	±3.2 ^a	±2.2 ^{ab} _a	±2.4 ^b _b	±2.8 A
	Mean	74.6	70.0	53.5	44.8	72.6	
	±2.6A	±2.4A	±1.8 B	±2.3 BC	±2.9 A		

Values (means ±SD) are average of three samples of each part, analyzed individually in triplicate ($p < 0.05$). Different Superscript letter within the mean's row indicates significant differences ($p < 0.05$) among the parts and subscript shows significant differences ($p < 0.05$) of means within the solvents.

Antibacterial activity of *C. spinosa* extracts

All *C. spinosa* extracts significantly inhibited the growth of selected bacteria. It was found that aqueous ethanol extracts were effective in controlling the growth of *Bacillus cereus* while aqueous acetone extracts limited the growth of *Pseudomonas aeruginosa*. Moreover, it was observed that *C. spinosa* parts, which were found rich in phenolics and flavonoids, exhibited better antibacterial activity in comparison with others. For example, root extracts of *C. spinosa* maximally inhibited the population of *Bacillus cereus*, while in the case of *Pseudomonas aeruginosa*, fruit and root extracts were more effective in comparison with other extracts (Table 6). Previously, the inhibiting potential of aerial parts of *C. spinosa* plants has been reported against Gram-positive and negative bacteria⁽¹⁹⁾. Likewise, *C. spinosa* flower extracts have been found effective against Gram-positive and negative bacteria. Caper fruit extracts have also been reported as effective antimicrobial sources against those bacteria which had become resistant to ciprofloxacin, vancomycin and teicoplanin⁽²⁰⁾.

Table 6: Antimicrobial activity (mm) of *C. spinosa* extracts (assessed by disc diffusion assay)

Solvents	Bacillus cereus					Pseudomonas aeruginosa				
	Stem Bark	Shoot	Fruit	Flower	Root	Stem Bark	Shoot	Fruit	Flower	Root
Absolute	14.1	11.2	16.2	16.9	21.7	11.8	10.7	12.3	10.7	9.3.9
Ethanol	±0.3 ^{bc} _b	±0.7 ^c _c	±0.8 ^b _c	±0.9 ^b _b	±1.8 ^a _b	±0.3 ^{bc} _c	±0.4 ^b _{bc}	±0.6 ^a _c	±0.2 ^b _{bc}	±0.4 ^b _d
Aqueous	19.4	20.2	24.7	23.7	27.1	14.1	13.3	17.6	13.4	15.9
Ethanol	±0.5 ^b _a	±0.6 ^b _a	±1.3 ^{ab} _a	±1.8 ^{ab} _a	±1.8 ^a _a	±0.4 ^b _b	±0.7 ^b _b	±0.7 ^a _b	±0.6 ^b _b	±0.8 ^b _b
Absolute	12.2	13.3	19.3	18.9	17.4	12.3	11.2	10.7	12.7	13.2

Acetone	±0.4 ^b _{bc}	±1.2 ^b _c	±1.0 ^a _{bc}	±0.9 ^a _b	±0.9 ^a _c	±0.5 _c	±0.5 _{bc}	±0.3 _c	±0.4 _b	±0.7 _c
Aqueous	18.8	17.4	21.9	11.7	22.7	16.2	17.6	19.4	17.2	19.7
Acetone	±0.9 ^b _a	±1.3 ^b _b	±1.4 ^a _b	±0.7 ^c _c	±1.3 ^a _b	±0.9 ^{ab} _a	±0.8 ^{ab} _a	±0.9 ^a _a	±1.1 ^{ab} _a	±1.0 ^a _a

Values (means ±SD) are the average of three samples of each part, analyzed individually in triplicate ($p < 0.05$). Different Superscript letter within the means row indicates significant differences ($p < 0.05$) among the parts and subscript shows significant differences ($p < 0.05$) of means within the solvents.

Conclusion

C. spinosa is a useful medicinal plant, being a reliable source of antioxidants and antibacterial potential. Root, shoot and fruits of this plant exhibited maximum antioxidant potential and antibacterial activities in the present investigation. Moreover, aqueous ethanol and aqueous acetone extracts performed better in extracting antioxidant compounds. Aqueous acetone was found to be effective in inhibiting the growth of *Bacillus cereus* while aqueous acetone performed well in controlling *Pseudomonas aeruginosa* growth.

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