



# Characterization of phenolics in different parts of selected *Capparis* species harvested in low and high rainfall season

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## Abstract

The stem bark, shoot, fruit, flower and root from *Capparis spinosa* and *Capparis decidua*, harvested in April and September (corresponding to low and high rainfall season, respectively), were investigated for variations in the contents of total phenols, flavonoids and individual phenolics. Aqueous methanol (80%) soluble extracts from different parts of the selected species, were evaluated colorimetrically for total phenolic contents (TPC), total flavonoid contents (TFC) and inhibition of linoleic acid peroxidation. Relatively, a higher extract yield (5.57–42.43%), TPC (157.3–348.6 GAE mg/100 g), TFC (229.2–584.9 CE mg/100 g) for both the species were recorded for September samples. Among the parts tested of both the species, fruits offered higher content of total phenolics (235.1–455.3 GAE mg/100 g) whereas flowers contained greater amount of flavonoids (96.7–269.9 CE mg/100 g). A notably variable content of phenolic compounds (0.24–94.22 mg/100 g) such as gallic acid, *p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic acid and sinapic acid were detected by RP-HPLC in different parts of the selected species; however, sinapic acid was not detected in the flowers of both the species. It can be concluded from the findings of the present study that season has significant effect on the phenolics profiling of *Capparis* plants and thus collection of different parts of the selected species in an appropriate season can be beneficial towards maximizing their functional food and nutraceutical benefits.

**Keywords** Capparaceae · Bioactive extraction · RP-HPLC · Phenolic acids · Total phenolics · Flavonoids

## Introduction

Medicinal plants are recognized for their wide array of high-value bioactives which can be extracted and employed as folk medicine as well as raw materials for the synthesis of novel drugs to control different diseases [1, 2]. Physiological benefits and biological/pharmaceutical activities of medicinal plants are mainly due to the presence of secondary metabolites such as alkaloids, terpenoids and polyphenols [1–3].

Among phytochemicals, phenolics are one of the most studied plant bioactivities with wide range of chemical structures and biochemical activities. These compounds, likewise other secondary metabolites, are naturally produced in plants to induce tolerance and resistance against insects or pest's attacks besides imparting coloring and astringent properties to plant foods [1, 4]. The distribution and profiling of phenolics in plant materials is subjective to various factors such as ecological and physiological conditions, development stage, germplasm, seasonal variability and cultivation techniques. Among these, seasonal factors are important that manipulate the nutritional composition of plants [5]. Therefore, the scientific knowledge of such variations during seasons and plant developmental stages is of high significance from nutritional, medicinal and agronomic perspectives. Now, due to revival of interest in the use of plants as a source of food and medicine, there is greater focus on investigating the plant phenolic compounds for their multiple biological activities such as antioxidant, anticancer, and anti-inflammatory and therapeutic potential as well as their abundance in our diet, and their credible role in preventing

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oxidative stress related degenerative diseases such as cancer and cardiovascular disorders [1, 6].

The use of wild plant as remedies for the treatment of different health disorders has been an ancient tradition in the Indo-Pak system of folk medicines [6–8]. One of the very important Genus “*Capparis*”, form family Capparaceae, is especially popular due to its several wild species of medicinal plants distributed across Indian Sub-continent, Mediterranean region, Atlantic Coasts to Black Sea Lands (Morocco) and Latin America [9]. Of the 250 species of genus *Capparis*, *C. spinosa* and *C. decidua* are widely distributed in deserts/dry lands of Afghanistan, India, Indonesia, Nepal, Pakistan, North Africa, South West Asia, Australia and South Europe up to an elevation of 1100 m [10] and are valued for their folk medicinal uses [11, 12]. The therapeutic effects and folk medicinal uses of *Capparis* species can be attributed to the occurrence of bioactive compounds such as phenolics, flavonoids and vitamins [13].

In Pakistan, there are huge reserves of economically and/or medicinally important flora with potential for bio-prospecting and isolation of natural bioactive compounds [6]. Especially, the indigenous wild flora due to high nutraceutical and medicinal potential can be explored as a valuable asset for the discovery and development of folk medicine and natural drugs [6, 14]. Among the indigenous wild medicinal plants, the species *C. spinosa* and *C. decidua*, locally known as “Karir”, are widely grown in deserts and arid to semi-arid areas of Pakistan. However, as such no earlier studies have been conducted on the evaluation and comparison of contents and profiling of phenolics in different parts of the selected *Capparis* species harvested in two different seasons from their natural habitats. The present study therefore was mainly designed to investigate whether or not the two harvesting seasons affect the phenolics composition

of different parts of widely distributed *C. spinosa* and *C. decidua* in desert areas of Bahawalpur, Pakistan.

## Materials and methods

### Collection of plant samples and pretreatment

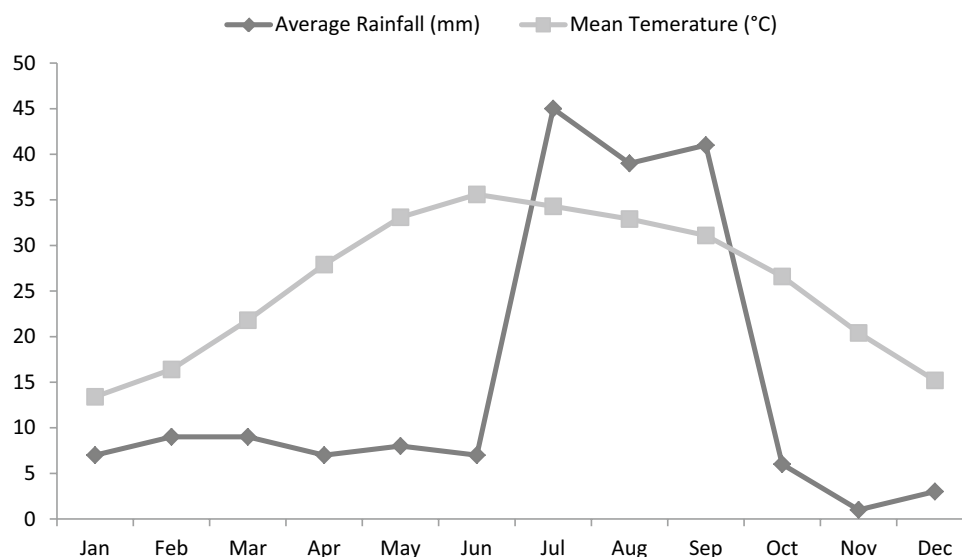
In studied area (Cholistan desert), 2 months i.e., April and September were selected for sampling based on low/high temperature and rainfall intensities. Rainfall and temperature data were obtained from Pakistan Council of Research in Water Resources (PCRWR), Bahawalpur, Pakistan (Fig. 1).

Different parts including stem bark, shoot, fruits, flowers and roots of *C. spinosa* and *C. decidua* were collected from Cholistan desert area 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 2013. The specimens were further identified and authenticated by Dr. Mansoor Hameed, Taxonomist, Department of Botany, University of Agriculture Faisalabad, Pakistan. The plant materials were washed with tap water to remove the dust and soil particles, and then were dried at room temperature in air blow at 25 °C. The dried material was ground to fine powder using commercial grinder (TSK-949, Wastpoint, France). The powder was passed through the sieve (mesh size 80) and used for extraction purposes.

### Preparation of extracts

In order to extract phenolic bioactive components from different parts of *C. spinosa* and *C. decidua*, the powder plant materials were extracted with aqueous methanol (methanol:water, 80:20 v/v). Briefly, a weighed quantity

**Fig. 1** Mean annual temperature and rainfall data of Cholistan desert, District Bahawalpur



(10 g) of dried sample was mixed with 100 mL of aqueous methanol and extracted for 6 h at room temperature in an orbital shaker (Pamico, Pak). The extract and residues were separated using filter paper Whatman No. 1. The residues were extracted twice with the fresh solvent, and the recovered three extracts pooled. The combined extracts were made free from solvent and concentrated to dryness under reduced pressure at 45 °C, using a rotary evaporator (EYELA, N-N Series). The crude concentrated extracts were weighted to calculate the % yield and stored in a refrigerator at -4 °C, until used for further analyses. The crude concentrated extracts produced from different parts of the selected species were subjected to different analyses.

### Total phenolic contents (TPC)

TPCs were estimated colorimetrically following Folin–Ciocalteu reagent method as described earlier [15]. Briefly, 50 mg of crude extract was taken in a test tube and mixed well with 0.5 mL of Folin–Ciocalteu reagent and 7.5 mL deionized water. The mixture was kept at room temperature for 10 min, and then 1.5 mL of 20% sodium carbonate (w/v) was added. The mixture was incubated in a water bath at 40 °C for 20 min and cooled in an ice bath. The solution (250 µL) of each sample was taken in 96 well-plate and absorbance was measured at 755 nm using microplate reader (Bio Tek Instrument, Inc., VT, USA). TPC were calculated using gallic acid calibration curve constructed by running a series of gallic acid standard solutions with concentrations ranging between 0 and 250 mg L<sup>-1</sup> (R<sup>2</sup>=0.9945) and reported as gallic acid equivalent (GAE).

### Total flavonoid contents (TFC)

TFCs were determined by using the protocol described by Hussain et al. [15]. Briefly, extract solution (250 µL) containing 25 µL sample extract was placed in 96 well-plate and mixed with 125 µL of distilled water, 7.5 µL of 5% NaNO<sub>2</sub>. After 5 min, 15 µL of 10% AlCl<sub>3</sub> was added. Further, 50 µL of 1M NaOH was added after 50 min and volume was made up to 250 µL with distilled water. Absorbance was read at 510 nm by spectrophotometer (Bio Tek Instrument, Inc., VT, USA). TFC were calculated using catechin calibration curve constructed by running a series of catechin standard solutions with concentrations ranging between 0 and 120 mg L<sup>-1</sup> (R<sup>2</sup>=0.9916) and reported as catechin equivalent (CE).

### Separation and quantification of individual phenolic acids by reverse phase high performance liquid chromatography (RP-HPLC)

The extracts from different parts (stem bark, shoot, fruit, flower and roots) of *C. spinosa* and *C. decidua* were

analyzed for phenolic acids (*p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic acid, gallic acid and sinapic acid) profiling by RP-HPLC.

### Extraction/hydrolysis

The plant phenolics are mostly found in bounded form and thus they were hydrolyzed prior to identification and quantification by HPLC [16]. In this treatment, 10 mL of 50% acidified aqueous methanol (1.2 M HCl) was mixed with 1 g of crude extract in a refluxing flask. Then mixture was refluxed at 80 °C for 2 h. After refluxing the extract was allowed to cool and volume was made up to 10 mL with methanol. The extract were filtered through 45 µm (Millipore) before injection into HPLC column.

### Chromatograph system and conditions used for phenolic acids analysis

The phenolic acids analysis was performed on an Agilent 1200-series HPLC system (Agilent Technology Germany) equipped with gradient model LC (G1312B) binary pump system, auto sample injection (G1367B), degasser (G1379), a (G513C) UV–VIS detector, and (G1316B) column oven. Separation of individual phenolic acids was done on hypersil Cold C18 column (250 × 4.6 mm, 5 µm particle size) (Thermo Fisher Scientific Inc., Massachusetts, United States). A non-linear gradient system consisting of solvent **A** (acetonitrile:methanol 70:30) and solvent **B** (water with 0.5% glacial acetic acid) at flow rate of 1 mL/min was used for elution purposes. The gradient programming used for the separation of phenolic acids included: 10–15% **A** from 0 to 5 min; 15–20% **A** from 5 to 18 min; 20–40% **A** from 18 to 40 min and maintained at 40% **A** from 40 to 45 min; 40–10% **A** from 45 to 50 min and kept at 10% **A** from 50 to 55 min. The detection of the targeted phenolic acids was made at 280 nm. The analytes were identified by matching the retention time and spiking samples with pure standards whereas quantification was based on an external standard calibration method.

### Statistical analysis

Three different samples for each part of the two selected species were taken and analyzed individually in triplicate. The values are reported as mean ± SD for triplicate determinations. Analysis of variance (two-way ANOVA) was applied to evaluate statistical differences of the means within the parts and harvest seasons.

## Results and discussion

### Extract yield

The plant phenolics are soluble in different polar solvents depending upon their chemical nature and polarities [17]. The aqueous forms of polar solvents such as methanol, ethanol, and acetone have been reported as suitable solvents for extracting antioxidant compounds and polyphenols from plant materials. Aqueous mixture of methanol has been most widely used for the extraction of phenolic antioxidants from different plant materials such as vegetables, fruits, and tree leaves [18–22].

In the present investigation, a higher extraction yield of phenolic antioxidant extract was recovered for September samples in comparison with April samples for both the selected species (Table 1). Regarding different parts of *C. spinosa*, maximum extraction yield was noted from fruits (33.23 g 100 g<sup>-1</sup>) which was statistically at par with flowers (31.03) followed by roots (24.37 g 100 g<sup>-1</sup>). In case of *C. decidua*, maximum extract yield was found in flowers and fruits (29.41 and 27.99 g 100 g<sup>-1</sup>) which were statistically at par with each other while the least yield was recorded in roots and stem bark (Table 1). In a previous

study, Baghiani et al. [23] reported maximum extract yield from roots of *Capparis* plants with chloroform. Such variation in extract yield might be due to choice of different polarity solvents employed for the extraction purposes. Moreover, the differences in the extract yield of antioxidant components might be attributed to the availability of phenolics and flavonoids in different plant tissues [24].

### Total flavonoid and phenolic contents

As shown by data in Table 2, the tested parts of the selected *Capparis* species contained appreciable contents of flavonoids. The roots of *C. spinosa* exhibited maximum amount of total flavonoids (314.0 mg 100 g<sup>-1</sup>) while fruits, flowers and shoot were statistically at par with each for their TFCs. Moreover, seasonal variability significantly affected the concentration of total flavonoids. In case of *C. decidua*, stem bark and roots exhibited maximum level of total flavonoids among others (Table 2).

The contents of TPCs determined in different parts of *C. spinosa* and *C. decidua* as given in Table 3, ranged from 229.2 to 535.8 mg 100 g<sup>-1</sup> and 262.1 to 584.9 mg 100 g<sup>-1</sup>, respectively. A considerable variation in phenolics concentration among different parts of both the selected species was noted. The highest contents of total phenolic were recorded

**Table 1** Seasonal variation in extract yield of different parts of *C. spinosa* and *C. decidua* in low and high rainfall season

Parts	<i>C. spinosa</i> Extract yield (%)			<i>C. decidua</i> Extract yield (%)		
	April	September	Mean	April	September	Mean
Stem bark	5.00 ± 0.35 <sup>e</sup>	5.57 ± 0.03 <sup>e</sup>	5.28 D	7.25 ± 0.17 <sup>de</sup>	10.87 ± 0.18 <sup>d</sup>	9.06 B
Shoot	12.00 ± 0.70 <sup>d</sup>	13.48 ± 0.25 <sup>d</sup>	12.74 C	10.67 ± 0.27 <sup>d</sup>	9.92 ± 0.04 <sup>d</sup>	10.29 B
Fruit	24.03 ± 0.68 <sup>bc</sup>	42.43 ± 0.60 <sup>a</sup>	33.23 A	25.20 ± 0.48 <sup>c</sup>	30.79 ± 0.35 <sup>b</sup>	27.99 A
Flower	29.50 ± 0.35 <sup>b</sup>	32.57 ± 0.36 <sup>b</sup>	31.03 A	24.05 ± 0.40 <sup>c</sup>	34.77 ± 0.67 <sup>a</sup>	29.41 A
Root	10.47 ± 0.18 <sup>d</sup>	38.28 ± 0.65 <sup>ab</sup>	24.37 B	7.03 ± 0.24 <sup>de</sup>	6.98 ± 0.17 <sup>de</sup>	6.99 BC

Values (means ± SD) are average of three samples of each part, analyzed individually in triplicate ( $p < 0.05$ ). Different small letters in superscript within the same column indicate significant differences ( $p < 0.05$ ) among the parts in each season. Different caps letter in means row indicate significant differences between two species on both the seasonal basis

**Table 2** Seasonal variation in total flavonoid contents of different parts of *C. spinosa* and *C. decidua* in low and high rainfall season

Parts	<i>C. spinosa</i> TFC (mg 100 g <sup>-1</sup> )			<i>C. decidua</i> TFC (mg 100 g <sup>-1</sup> )		
	April	September	Mean	April	September	Mean
Stem bark	139.2 ± 13.2 <sup>d</sup>	157.3 ± 12.8 <sup>d</sup>	148.2 C	208.1 ± 18.5 <sup>c</sup>	292.1 ± 25.8 <sup>a</sup>	250.1 A
Shoot	179.1 ± 15.2 <sup>d</sup>	250.8 ± 12.3 <sup>c</sup>	215.3 B	135.8 ± 7.9 <sup>de</sup>	173.1 ± 2.8 <sup>d</sup>	154.4 C
Fruit	189.4 ± 18.1 <sup>d</sup>	283.3 ± 23.4 <sup>b</sup>	236.3 B	144.4 ± 11.1 <sup>de</sup>	249.7 ± 18.5 <sup>b</sup>	197.0 B
Flower	231.7 ± 28.7 <sup>cd</sup>	235.6 ± 23.9 <sup>cd</sup>	233.6 B	96.7 ± 4.7 <sup>e</sup>	269.9 ± 18.5 <sup>ab</sup>	183.3 B
Root	279.4 ± 22.7 <sup>b</sup>	348.6 ± 28.0 <sup>a</sup>	314.0 A	277.0 ± 25.1 <sup>ab</sup>	202.0 ± 19.8 <sup>c</sup>	239.5 A

Values (means ± SD) are average of three samples of each part, analyzed individually in triplicate ( $p < 0.05$ ). Different small letters in superscript within the same column indicate significant differences ( $p < 0.05$ ) among the parts in each season. Different caps letter in means row indicate significant differences between two species on both the seasonal basis

**Table 3** Seasonal variation in total phenolic contents of different parts of *C. spinosa* and *C. decidua* in low and high rainfall season

Parts	<i>C. spinosa</i> TPC (mg 100 g <sup>-1</sup> )			<i>C. decidua</i> TPC (mg 100 g <sup>-1</sup> )		
	April	September	Mean	April	September	Mean
Stem bark	470.0 ± 21.8 <sup>b</sup>	408.4 ± 29.1 <sup>c</sup>	439.2 A	425.4 ± 50.1 <sup>cd</sup>	584.9 ± 37.2 <sup>a</sup>	505.1 AB
Shoot	344.9 ± 22.4 <sup>d</sup>	229.2 ± 28.9 <sup>e</sup>	287.0 C	304.0 ± 31.9 <sup>d</sup>	533.6 ± 21.9 <sup>b</sup>	418.8 B
Fruit	354.9 ± 29.3 <sup>d</sup>	535.8 ± 18.8 <sup>a</sup>	445.3 A	520.2 ± 18.2 <sup>b</sup>	575.1 ± 28.9 <sup>a</sup>	547.6 A
Flower	235.1 ± 29.3 <sup>e</sup>	416.0 ± 37.3 <sup>c</sup>	325.5 B	262.1 ± 31.2 <sup>e</sup>	455.3 ± 19.0 <sup>cd</sup>	358.7 C
Root	326.8 ± 32.3 <sup>c</sup>	531.9 ± 29.5 <sup>a</sup>	429.3 A	600.5 ± 14.8 <sup>a</sup>	487.5 ± 18.7 <sup>c</sup>	544.0 A

Values (means ± SD) are average of three samples of each part, analyzed individually in triplicate ( $p < 0.05$ ). Different small letters in superscript within the same column indicate significant differences ( $p < 0.05$ ) among the parts in each season. Different caps letter in means row indicate significant differences between two species on both the seasonal basis

in fruits of *C. spinosa* followed by stem bark and roots (5445.3, 439.2 and 429.3 g 100 g<sup>-1</sup>, respectively). Meanwhile, the fruits and roots of *C. decidua* ranked higher in phenolic contents (547.6 and 544.0 g 100 g<sup>-1</sup>, respectively) followed by stem bark (505.1 g 100 g<sup>-1</sup>). Moreover, harvesting season exhibited a significant effect on TPCs of different parts of both the species (Table 3).

Aqueous methanolic extracts from roots of both the species exhibited maximum total phenolic and flavonoid contents in comparison with other parts of the species. These findings are in line with Baghiani et al. [23] who reported the highest radical scavenging activities for the roots of *Capparis* species. Phenolics are an important group of plant bioactives which act as strong antioxidant and free radical scavengers [1]. A positive correlation has been reported among TPC, TFC and the antioxidant activities of different plant species [25, 26]. Given that caper leaves are rich in phenolic compounds, known as effective antioxidants, the effectiveness of caper leaves can also be attributed to the concentration of these compounds [27]. However, strong antioxidant properties of *C. spinosa* and *C. decidua* plant parts might be attributed to their high contents of polyphenols, tocopherols and carotenoids [28, 29]. Moreover, seasonal variation also affected the amount of phenolic and flavonoid (Tables 2, 3). Samples collected in September, offered higher amounts of

these compounds in comparison with April samples. In the present investigation, a higher intensity rainfall was recorded in August–September in comparison with other months as shown in Fig. 1. As, rainfall occurs often in the sampling areas in September, which improves the quality and quantity of these compounds; a significant correlation of rainfall with phenolic acids and flavonoid contents has been previously reported in several studies [30–32]. Matias et al. [33] reported somewhat similar trends as noted in our present study while investigating the chemical composition and biological activities of essential oil of *Cordia verbenacea* (Table 4).

### Quantification of selected phenolic acids

As per HPLC analysis five phenolic acids including gallic acid, *p*-coumaric acid, Caffeic acid, *p*-hydroxybenzoic acid and sinapic acid were identified and quantified in the stem bark, shoot, fruit, flower and root of *C. decidua* and *C. spinosa* (Figs. 2, 3, 4, 5). The quantitative data of the identified phenolic acids (mg 100 g<sup>-1</sup>) in different parts of *C. spinosa* and *C. decidua* are presented in Tables 5, 6, respectively.

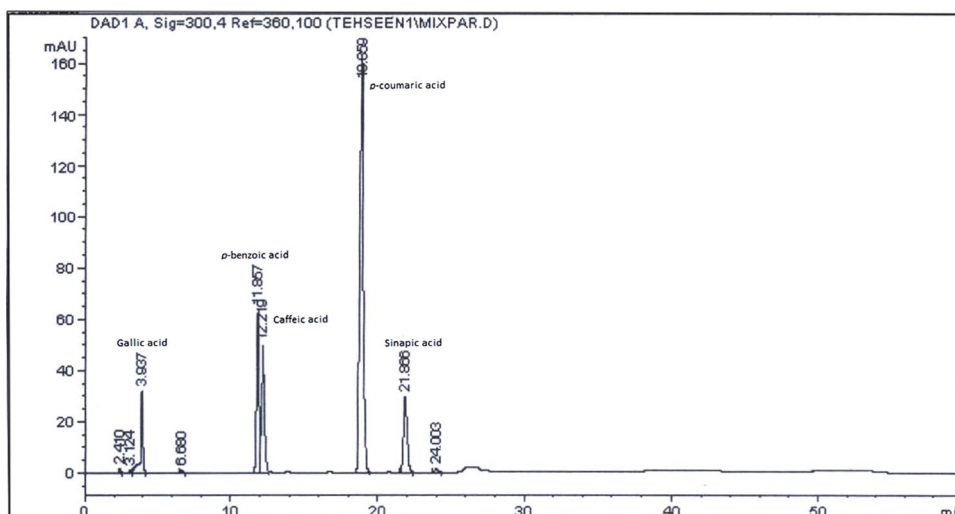
The stem bark of *C. spinosa* exhibited gallic acid as the major phenolic acid (14.24 mg 100 g<sup>-1</sup>) followed by *p*-coumaric and *p*-hydroxybenzoic acid (4.34 and 2.95 mg

**Table 4** Seasonal variation in inhibition of linoleic acid peroxidation of different parts of *C. spinosa* and *C. decidua* in low and high rainfall season

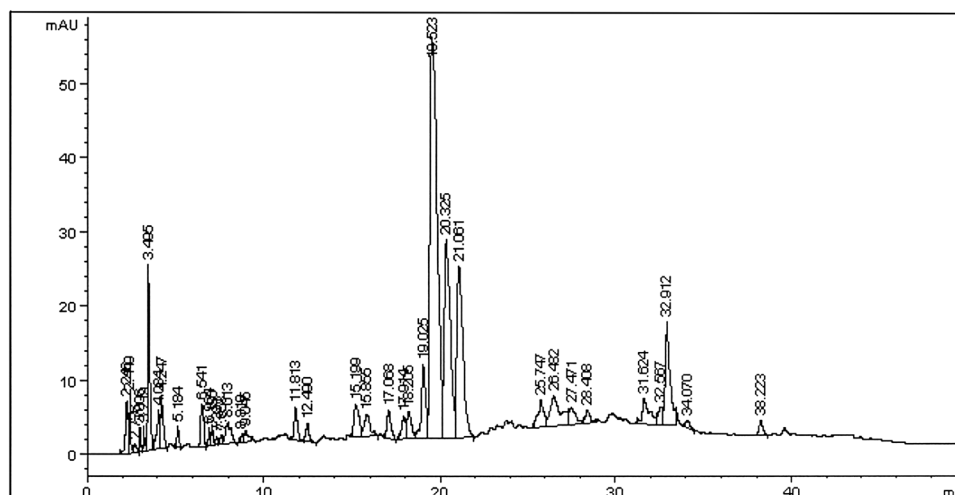
Parts	<i>C. spinosa</i> ILAP (%)			<i>C. decidua</i> ILAP (%)		
	April	September	Mean	April	September	Mean
Stem bark	83.1 ± 3.6 <sup>a</sup>	79.0 ± 2.2 <sup>a</sup>	81.0 A	44.2 ± 3.5 <sup>c</sup>	29.9 ± 0.9 <sup>d</sup>	37.0 C
Shoot	72.4 ± 2.1 <sup>ab</sup>	68.8 ± 3.1 <sup>b</sup>	70.6 B	54.0 ± 3.5 <sup>c</sup>	74.3 ± 3.3 <sup>ab</sup>	64.1 B
Fruit	81.2 ± 2.7 <sup>a</sup>	78.1 ± 3.2 <sup>a</sup>	79.6 A	92.6 ± 5.1 <sup>a</sup>	61.6 ± 1.9 <sup>b</sup>	77.1 A
Flower	69.3 ± 1.6 <sup>ab</sup>	66.2 ± 3.1 <sup>b</sup>	67.7 C	72.7 ± 3.2 <sup>ab</sup>	54.7 ± 2.2 <sup>b</sup>	63.7 B
Root	79.6 ± 2.3 <sup>a</sup>	84.6 ± 6.0 <sup>a</sup>	82.1 A	71.6 ± 4.6 <sup>ab</sup>	88.5 ± 5.8 <sup>a</sup>	80.0 A

Values (means ± SD) are average of three samples of each part, analyzed individually in triplicate ( $p < 0.05$ ). Different small letters in superscript within the same column indicate significant differences ( $p < 0.05$ ) among the parts analyzed in each season. Different caps letter in means row indicate significant differences between two species on both the seasonal basis

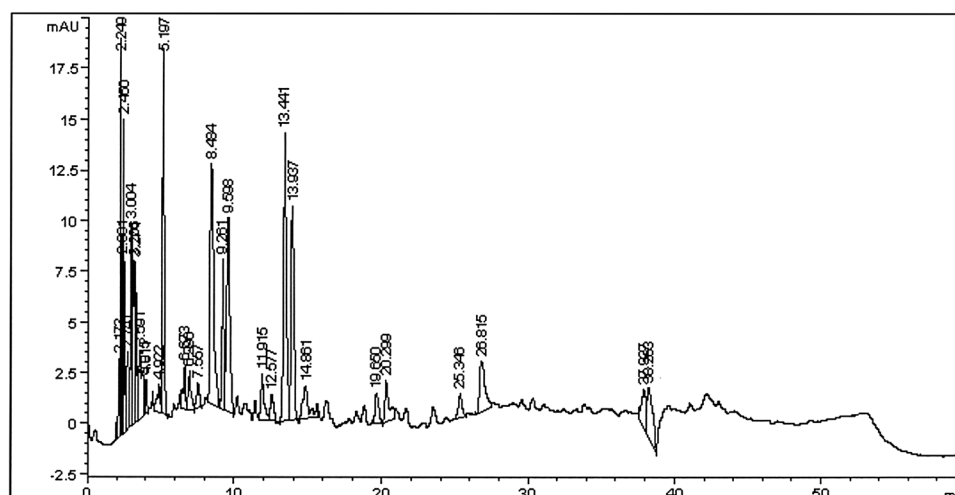
**Fig. 2** A typical RP-HPLC chromatogram of mixture of phenolics acid standards. Peak identification: 1: gallic acid (RT 3.96), 2: *p*-hydroxyl benzoic acid (RT 11.97), 3: caffeic acid (RT 12.44), 4: *p*-coumaric acid (RT 19.04), 5: sinapic acid (RT 22.13)



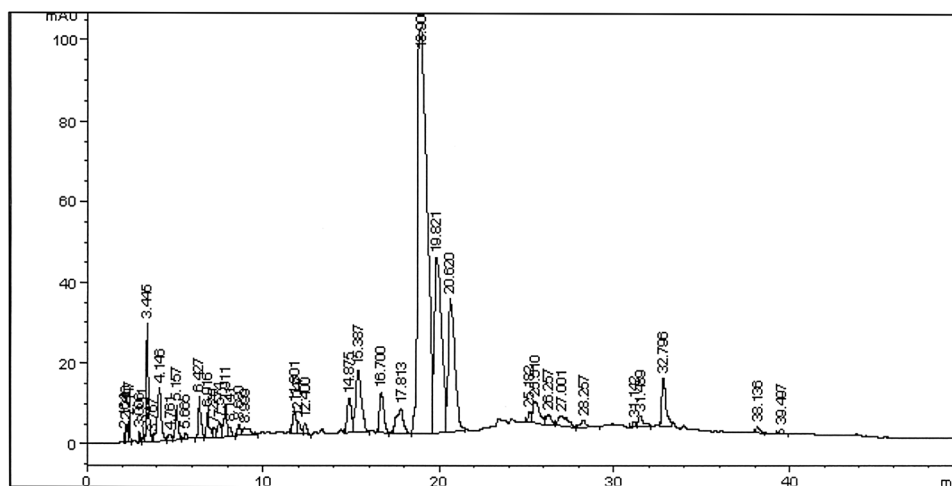
**Fig. 3** A typical RP-HPLC chromatogram showing separation of phenolic acids of *C. spinosa* stem bark. Peak identification: 1: gallic acid (RT 3.96), 2: *p*-hydroxyl benzoic acid (RT 11.97), 3: caffeic acid (RT 12.44), 4: *p*-coumaric acid (RT 19.04), 5: sinapic acid (RT 22.13)



**Fig. 4** A typical RP-HPLC chromatogram showing separation of phenolic acids of *C. spinosa* root. Peak identification: 1: gallic acid (RT 3.96), 2: *p*-hydroxyl benzoic acid (RT 11.97), 3: caffeic acid (RT 12.44), 4: *p*-coumaric acid (RT 19.04), 5: sinapic acid (RT 22.13)



**Fig. 5** A typical RP-HPLC chromatogram showing separation of phenolic acids of *C. decidua* shoot. Peak identification: 1: gallic acid (RT 3.96), 2: *p*-hydroxyl benzoic acid (RT 11.97), 3: caffeic acid (RT 12.44), 4: *p*-coumaric acid (RT 19.04), 5: sinapic acid (RT 22.13)



**Table 5** Quantification of selected phenolic acids (mg 100 g<sup>-1</sup>) in different parts of *C. spinosa* by RP-HPLC

Phenolic acids	Plant parts					
	Stem bark	Shoot	Fruit	Flower	Root	Total
Gallic acid	14.24 ± 0.71 <sup>a</sup>	1.24 ± 0.06 <sup>c</sup>	2.01 ± 0.10 <sup>c</sup>	8.63 ± 0.43 <sup>b</sup>	6.46 ± 0.32 <sup>b</sup>	32.58 <sup>b</sup>
<i>p</i> -Coumaric acid	4.34 ± 0.21 <sup>b</sup>	3.24 ± 0.16 <sup>b</sup>	52.71 ± 2.63 <sup>a</sup>	0.84 ± 0.04 <sup>c</sup>	1.08 ± 0.05 <sup>c</sup>	62.21 <sup>a</sup>
Caffeic acid	1.27 ± 0.06 <sup>b</sup>	1.75 ± 0.08 <sup>ab</sup>	1.58 ± 0.07 <sup>ab</sup>	0.78 ± 0.04 <sup>c</sup>	2.53 ± 0.12 <sup>a</sup>	7.91 <sup>c</sup>
<i>p</i> -Hydroxybenzoic acid	2.95 ± 0.14 <sup>bc</sup>	12.32 ± 0.61 <sup>a</sup>	4.56 ± 0.22 <sup>b</sup>	0.77 ± 0.03 <sup>c</sup>	0.95 ± 0.04 <sup>c</sup>	21.55 <sup>bc</sup>
Sinapic acid	14.69 ± 0.73 <sup>a</sup>	1.10 ± 0.05 <sup>b</sup>	14.68 ± 0.73 <sup>a</sup>	ND	0.54 ± 0.02 <sup>b</sup>	31.01 <sup>b</sup>

Values (means ± SD) are average of three samples of each part, analyzed individually in triplicate (*p* < 0.05). Different small letters in superscript within the same row indicate significant differences (*p* < 0.05) among the parts analyzed in each season

ND not detected

**Table 6** Quantification of selected phenolic acids (mg 100 g<sup>-1</sup>) in different parts of *C. decidua* by RP-HPLC

Phenolic acids	Plant parts					
	Stem bark	Shoot	Fruit	Flower	Root	Total
Gallic acid	17.34 ± 0.86 <sup>a</sup>	2.06 ± 0.10 <sup>d</sup>	10.13 ± 0.50 <sup>b</sup>	6.79 ± 0.33 <sup>c</sup>	8.45 ± 0.42 <sup>bc</sup>	44.77 <sup>b</sup>
<i>p</i> -Coumaric acid	94.22 ± 4.71 <sup>a</sup>	0.67 ± 0.03 <sup>c</sup>	7.18 ± 0.35 <sup>b</sup>	0.66 ± 0.03 <sup>c</sup>	1.79 ± 0.08 <sup>c</sup>	104.52 <sup>a</sup>
Caffeic acid	1.45 ± 0.07 <sup>c</sup>	2.46 ± 0.12 <sup>c</sup>	6.18 ± 0.30 <sup>b</sup>	11.22 ± 0.56 <sup>a</sup>	10.73 ± 0.53 <sup>a</sup>	32.04 <sup>b</sup>
<i>p</i> -Hydroxybenzoic acid	4.17 ± 0.20 <sup>b</sup>	1.35 ± 0.06 <sup>c</sup>	12.46 ± 0.62 <sup>a</sup>	0.89 ± 0.04 <sup>c</sup>	1.06 ± 0.05 <sup>c</sup>	19.93 <sup>c</sup>
Sinapic acid	22.51 ± 1.12 <sup>b</sup>	3.15 ± 0.15 <sup>c</sup>	79.39 ± 3.96 <sup>a</sup>	ND	0.24 ± 0.01 <sup>d</sup>	105.29 <sup>a</sup>

Values (means ± SD) are average of three samples of each part, analyzed individually in triplicate (*p* < 0.05). Different small letters in superscript within the same row indicate significant differences (*p* < 0.05) among the parts analyzed in each season

ND not detected

100 g<sup>-1</sup>, respectively) (Table 5). The stem bark of *C. decidua*, was rich in *p*-coumaric acid (94.22 mg 100 g<sup>-1</sup>) followed by sinapic acid and gallic acid (22.51 and 17.34 mg 100 g<sup>-1</sup>, respectively). While, caffeic acid was found in the least amount (1.45 mg 100 g<sup>-1</sup>) in stem bark (Table 6).

In shoots of *C. spinosa*, *p*-hydroxybenzoic acid was found as a major phenolic acid (12.32 mg 100 g<sup>-1</sup>) while *p*-coumaric acid, caffeic acid, gallic acid and sinapic acid

(3.24, 1.75, 1.24 and 1.10 mg 100 g<sup>-1</sup>, respectively) were statistically at par with each other (Table 5). In case of *C. decidua* shoots, the concentrations of sinapic acid, caffeic acid and gallic acid were statistically similar with each other and were ranked at higher level, 3.15, 2.46 and 2.06 mg 100 g<sup>-1</sup>, respectively followed by *p*-hydroxybenzoic acid and *p*-coumaric acid (1.35 and 0.67 mg 100 g<sup>-1</sup>, respectively) (Table 6).

The fruits of *C. spinosa* were rich in *p*-coumaric acid (52.71 mg 100 g<sup>-1</sup>) followed by sinapic acid (14.68 mg 100 g<sup>-1</sup>) while *p*-hydroxybenzoic acid, gallic acid and caffeic acid were statistically similar with each other (Table 5). On the other hand, in fruits of *C. decidua*, sinapic acid was recorded as a major phenolic acid (79.39 mg 100 g<sup>-1</sup>) followed by *p*-hydroxybenzoic acid > gallic acid > *p*-coumaric acid > caffeic acid (Table 6).

Sinapic acid was not found in the flowers of *C. spinosa* while gallic acid (8.63 mg 100 g<sup>-1</sup>) was detected as a major phenolic acid in flowers followed by *p*-coumaric acid > caffeic acid > *p*-hydroxybenzoic acid (Table 5). Sinapic acid was not detected in *C. decidua* flowers while caffeic acid contributed to maximum level followed by gallic acid (11.22 and 6.79 mg 100 g<sup>-1</sup>, respectively). *p*-hydroxybenzoic acid and *p*-coumaric acid were statistically at par with each other giving 0.89 and 0.66 mg 100 g<sup>-1</sup>, respectively (Table 6).

In roots of *C. spinosa*, gallic acid and caffeic acid were recorded as main phenolic acids (6.46 and 2.53 mg 100 g<sup>-1</sup>, respectively) while *p*-coumaric acid, *p*-hydroxybenzoic acid and sinapic acid with contribution 1.08, 0.95 and 0.54 mg 100 g<sup>-1</sup>, respectively were statistically at par with each other (Table 5). The roots of *C. decidua* exhibited caffeic acid and gallic acid (11.22 and 8.45 mg 100 g<sup>-1</sup>, respectively) as main components followed by *p*-coumaric acid and *p*-hydroxybenzoic acid (1.79 and 1.06 mg 100 g<sup>-1</sup>, respectively) while sinapic acid contributed as high as 0.24 mg 100 g<sup>-1</sup> (Table 6).

Phenolics are one of the most abundant secondary metabolites present in plants that mainly consist of flavonoids, tannins and phenolic acids [34]. The concentration of phenolics and flavonoids vary among plants depending upon the species, production technologies and environmental factors. The antioxidant potential of plants and their extracts is mainly attributed to the presence of phenolic compounds [1, 35].

In the present analysis, gallic acid was mainly found in stem bark of both the selected species. Gallic acid has been reported to possess anticancer activity [36, 37]. Moreover, its efficacy has also been reported against H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity [38]. In the present investigation, it was noted that *C. spinosa* shoots are good source of *p*-hydroxybenzoic acid. Benzoic acid exhibits antimicrobial property as against *Lactobacillus plantarum* and *Oenococcus oeni* [39, 40]. Moreover, it is an important constituent of vitamin B-complex occurring in wild plants that is being used in pharmaceutical industries [41]. The *p*-hydroxybenzoic acid was mainly detected in *C. spinosa* shoot in comparison with *C. decidua* shoot. While, *C. decidua* shoots were found to be a rich source of caffeic acid. So the shoots of *C. decidua* can be used as a natural antimicrobial agent for infectious disease prevention.

It has been reported that caffeic acid is an analogue of ferulic acid, one of the most well-known naturally

existing cinnamic acids that are helpful in immunoregulation diseases, asthma, and allergic reactions [42]. It has been reported that caffeic acid and its derivatives inhibit the growth of cells, hence these can be used against cancer cells but their activities are concentration and dose dependent. For example, hydroxycinnamic acid inhibits the microbial growth at higher concentration [43, 44]. Moreover, it is a strong ingredient in coffee, honey, grains and fruits with a potential antimicrobial and antioxidant properties [45, 46]. At concentrations of 500 and 1000 mg L<sup>-1</sup> of caffeic acid, the growth of *Lactobacillus collinoides* and *L. brevis* was maximally inhibited [47]. Aliyazicioglu et al. [48] reported sinapic acid and benzoic acid as the main phenolic acids in fruits of *C. spinosa*.

Further, caffeic acid was maximally found in shoot and fruit extracts of both the species. Imran et al. [49] did not report caffeic acid and coumaric acid in *C. decidua* roots while gallic acid was reported in trace amounts. Such variations in the qualitative and quantitative composition of phenolic acids of *Capparis* species can be linked to different climatic conditions, soil properties, production technology and extraction procedure employed [50, 51]. In conclusion, the concentration of phenolic acids varied significantly among different parts of both the species selected. This study manifested that *C. spinosa* and *C. decidua* are good sources of beneficial phenolic acids which are responsible for antioxidant activities. It is now well established that antioxidant activity of plant tissues can be predicted on account of quantity and chemical structure of phenolic acids and flavonoids. Moreover, antioxidant activities of phenolics and flavonoids are also related to the presence of two neighboring hydroxyl groups on the B-ring, the number of free hydroxyl groups, a C2–C3 double bond in the C-ring, or the presence of a 3-hydroxyl group [52].

## Conclusion

*Capparis spinosa* and *C. decidua* have been considered as a traditional source of food and folk medicine. As stated earlier that Pakistani germplasm of these species have not been studied earlier for their phenolics composition. In the present investigation, fruits, roots and flowers of both the selected *Capparis* species, native to Pakistan, were first time explored as a good source of total phenolics, total flavonoids and phenolic acids profiling. Phenolic acids are mainly responsible for antioxidant potential. The same is clear from the present investigation as *Capparis* fruits and flowers presented maximum amount of gallic acid, *p*-coumaric acid, caffeic acid and *p*-hydroxybenzoic acid. Based on these findings, it can be concluded that the fruits and flowers of *Capparis* species are relatively a good source for isolation of natural phenolic antioxidants and provided higher amounts of



phenolic acids i.e., gallic acid, *p*-coumaric acid, caffeic acid and *p*-hydroxybenzoic acid. On species basis, *C. decidua* displayed higher phenolic acids contents than *C. spinosa*. Moreover, the results also signify that the harvesting season is a considerable factor contributing towards the phenolic concentration in different parts of *C. spinosa* and *C. decidua*. Typically in this research, with few expectations, the samples, harvested in September (rainy month), of different parts of the selected species, exhibited superior phenolics profile. So, harvesting of *C. spinosa* and *C. decidua* in an appropriate season is recommended for getting maximum nutritional benefits from different parts of these species.

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