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HPLC-PDA-ESI-MS^n profiling of polyphenolics in different parts of *Capparis spinosa* and *Capparis decidua* as function of harvesting seasons

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Summary

HPLC-PDA-ESI-MS^n analysis of different parts such as stem bark, shoot, flower, fruit and root of *Capparis spinosa* (*C. spinosa)* and *Capparis decidua* (*C. decidua*), collected in rainy and dry seasons from the Cholistan desert of Pakistan, depicted the occurrence of a wide array of phenolics with quercetin, apigenin and kaempferol derivatives along with dicaffeoylquinic acid, caffeoylquinic acid and feruloylquinic acid as the main compounds. Kaempferol-3-glucoside (28.02-167.21 μg g^{-1}dw) was found to be the principal component in all tested parts of both species while dicaffeoylquinic acid was detected only in the flowers and roots. The roots exhibited maximum contents of flavonoids and hydroxycinnamic acid derivatives. The harvesting period significantly (p<0.05) affected the concentration of phenolics wherein the samples collected in rainy season offered greater levels of phenolics than their counterpart. The roots and fruits of both species were found to be rich sources of phenolics. The findings of this research suggest the harvesting of the selected wild *Capparis* species in rainy season to maximize their antioxidant and nutraceutical benefits.

Keywords: Capparaceae; Flavonoids; Hydroxycinamic acids; Polyphenols

Introduction

Plants are valued as a rich source of a wide array of secondary metabolites. Among secondary metabolites, phenolics are one of those bioactive compounds which are widely distributed in the plant kingdom (Muhammad et al., 2015; Sahib et al., 2013; Shahidi, 1997). It is widely accepted that nutraceutical and antioxidant attributes of plant foods are mainly associated with their phenolics (Shahidi, 1997). In this regard, consumption of selected vegetables and fruits as well as several other plant foods, is strongly linked with health benefits and/or reduced incidence of degenerative diseases such as aging, inflammation and certain cancers (Lodovici et al., 2001; Oomah, 2001; Robbins, 2003).

Capparis is one of the important genera with known 250 species distributed world wide. Of the *Capparis* species, *Capparis spinosa* and *Capparis decidua* are native to Pakistan (Gull et al., 2015a). These plants have been investigated for their anti-atherosclerosis, anti-hypertensive, anti-inflammatory, analgesic, anti-asthmatic, anti-hyperlipidemic, hepatoprotective, antibacterial, and antifungal activities (Chahlia, 2009; Duman et al., 2013; Hundiwale et al., 2005; Mali et al., 2005). Besides, stem bark, shoot, root, flower and fruits of both of these species have been reported as good sources of valuable nutrients such as minerals, crude fiber and protein (Gull et al., 2015b; Gull et al., 2015c; Gull et al., 2015d). Different parts of these species are also being employed in the traditional medicine systems for the treatment of several diseases (Gull et al., 2015a). For example, the root extract of *C. spinosa* has been reportedly used to prepare liver protecting drugs named Liv-52 and CM-52 which are used to cure hepatitis B and liver cirrhosis (Eddouks et al., 2005; Rajesh et al., 2009). The hepatoprotective efficacy of *C. spinosa* might be attributed to the potential antioxidant activities of its bioactives following the restoration of liver cell membrane and its permeability, while the exact mechanism of action needs to be explored. Moreover, *C. decidua* extracts have also been reported for hepatoprotective effects via decreasing the levels of the enzymes SGPT (serum glutamate pyruvate transaminase), SGOT (serum glutamic oxaloacetic transaminase), and ALP (alkaline phosphatase) (Jhajharia et al., 2010). Different parts of *Capparis* species are also used to cure/prevent diabetes, cardiac diseases, toothache and intermittent fever (Marwat et al., 2011; Özcan, 2005; Sharma and Kumar, 2008).

It has been reported in various studies that plant phenolics composition is affected by geographical zones, climatic conditions and seasonal variability (Nouman et al., 2016; Olson et al., 2016). Researchers are focusing on profiling of the phenolics in vascular plants but there are few studies available revealing the variation in these compounds under seasonal fluctuations (Sampaio et al., 2016) while no study is available explaining the variations in concentration of phenolic acids in different parts of *C. spinosa* and *C. decidua* as affected by harvesting seasons. The concentration and availability of phenolic bioactive compounds vary within plants of the same species and their parts. Variation in expression and profiling of these bioactive compounds also vary with respect to cultivar, geographical zones, climatic conditions and seasonal fluctuations (Cartea et al., 2008; Ciska et al., 2000; García-Salas et al., 2014; Ray et al., 2013). For example, variation in biological activities of different *Brassica* vegetables under seasonal fluctuations such as temperature and precipitation might affect the composition and concentration of bioactive compounds resulting in affecting antioxidant activities of *Brassica* vegetables (Aires et al., 2011). Likewise, variation in bioactive compounds, enzymatic antioxidants and antiradical activities of *Moringa oleifera* leaves as affected by tree age, climatic factors and soil condition has been reported (Nouman et al., 2016; Vázquez-León et al., 2017).

Keeping in view the possible variations among bioactive compounds of various plant species and genera, it can be hypothesized that different parts of *C. spinosa* and *C. decidua* can yield varying concentration and profile of bioactive compounds under seasonal effects. The present study was aimed to investigate whether or not the two

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different seasons such as rainy and dry affect the profile of phenolics in different parts of wild *C. spinosa* and *C. decidua* harvested in their natural habitat.

Materials and methods

Reagents

All LC-MS grade solvents were obtained from J.T. Baker (Phillipsburg, NJ). Formic acid was purchased from Panreac (Barcelona, Spain). The standards (-)-epigallocatechin, quercetin-3-*O*-glucoside, 5-*O*-caffeoylquinic acid were from Sigma Aldrich (Steinheim, Germany). Ultrapure water was produced using a Millipore water purification system.

Collection of Plant Samples and Pretreatment

As a representative of dry and rainy seasons, two months including April and September were selected for plant sampling based on low/high temperature and rainfall intensities, respectively. Rainfall and temperature data were obtained from Pakistan Council of Research in Water Resources (PCRWR), Bahawalpur, Pakistan. Fig. 1 depicts an average weather data for the sampling site while in particular sampling months (April and September), mean temperature (27.9 and 31.1 °C) and average rainfall (7 and 41 mm) was noted, respectively. Different parts including stem bark, shoot, fruits, flowers and roots of *C. spinosa* and *C. decidua* were collected from Cholistan desert area of Bahawalpur, Punjab, 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 samples of selected parts were harvested from ten different plants of each of the mentioned species and then these were pooled in three replications for further analyses. The specimen were further identified and authenticated by Dr. Mansoor Hameed, Taxonomist, Department of Botany, University of Agriculture Faisalabad, Pakistan. The collected samples were dried at room temperature for 24 hours followed by oven drying at 45 °C for further studies (Nouman et al., 2016).

Sample preparation and analysis

Samples of the selected parts including stem bark, shoot, flower, fruit and root of *C. Spinosa* and *C. decidua* were prepared for the analysis of phenolic compounds using HPLC-PDA-ESI-MS^n and HPLC-PAD following the protocol as described by our research group (Nouman et al., 2016). Briefly, samples were air-dried in an oven at 45 °C for 72 h and were ground to a fine powder and stored at -20 ºC for further analysis. Each sample (40 mg) was extracted with 1.5 mL of 70% methanol for 30 min at 70 ºC, vortexed every 5 min to improve extraction efficacy and then centrifuged (10000×*g* for 20 min at 4 ºC) (model Sigma 1-13, B Braun Biotech International, Osterode, Germany). Supernatants were collected and methanol was removed under reduced pressure using a rotary evaporator (EYELA, N-N Series, Rikakikai Co., Tokyo, Japan). The dried residue was reconstituted in ultrapure water (1 mL) and filtered through a 0.22 µm polypropylene membrane filter (ANOTOP 10 plus; Whatman, Maidstone, UK). Each sample (20 µL) was analyzed in a LC-PAD-ESI/MS^n (Thermo-Scientific, Loughborough, UK) and HPLC-PAD (Gilson Inc., Mettmenstetten, Switzerland) for phenolics identification/authentication and quantification, respectively. The method described by Nouman et al. (2016) was used for these analyses as mentioned below.

Analysis of phenolic compounds by HPLC-PDA-ESI-MS^n and HPLC-PDA

The phenolics profiling, using HPLC-PDA-ESI-MS^n chromatographic analysis, was conducted on a Luna C18 column (150 × 2.1 mm, 2.6 µm particle size; Thermo-Scientific, Loughborough, UK). The mobile phase was a mixture of distilled water/formic acid (99:1, *v*/*v*) (solvent A) and acetonitrile/formic acid (99:1, *v*/*v*) (solvent B). The flow rate was of 0.6 mL/min in a linear gradient following the scheme (t in min; %B): (0; 0%), (5; 20%), (30; 50%), (45, 100%), and (55; 0%). The chromatograms were recorded at 320 and 520 nm. The equipment consisted of a LC pump (SRVYR-LPUMP), an autosampler (SRVYR-AS), and a photodiode array detector (SRVYR-PDA5). The HPLC-PDA-ESI-MS^n analysis was performed in a mass detector in series, which was an ion trap spectrometer (model LCQ-Advantage-Max) equipped with an electrospray ionization interface controlled by Tune Plus 1.3 SR1 software (Fisher Scientific, Lisboa, Portugal) and operated in negative mode. The ionization conditions were adjusted at 250 ºC and 4.0 kV for capillary temperature and voltage, respectively. The nebulizer pressure and flow rate of nitrogen were 2.0 bar and 8.0 L/min, respectively. The full scan mass covered the range from *m*/*z* 100 up to *m*/*z* 1500. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 up to 2.0 V.

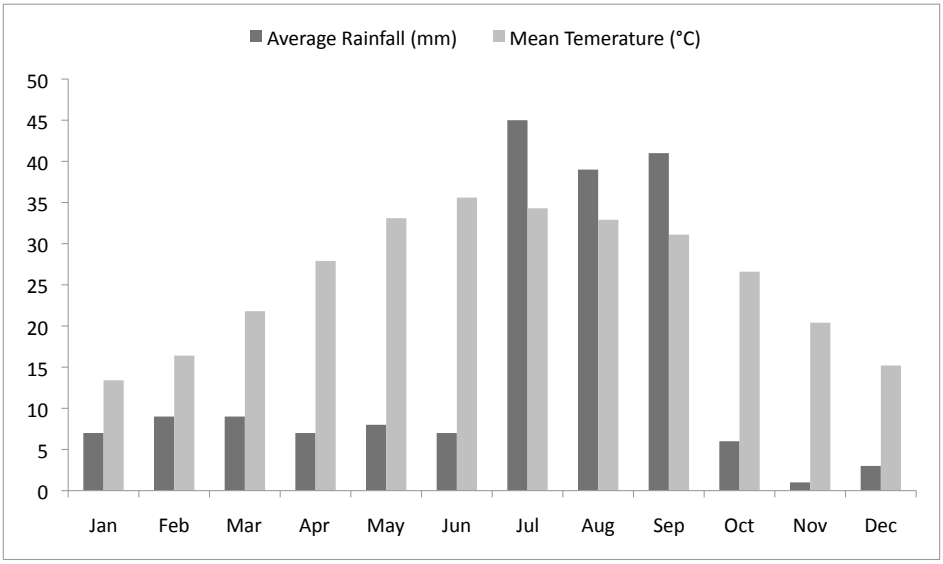

Fig. 1: Mean annual temperature and rainfall data of Cholistan desert, District Bahawalpur

Quantitative analysis was carried out by a HPLC-PDA system (Gilson Inc., Mettmenstetten, Switzerland) using the same chromatographic conditions as described for identification. The equipment consisted of a LC pump (Gilson, 305/306-PUMP), a dynamic mixer chamber (Gilson, 811C), an autosampler (Gilson, Autoinjector-234), and a photodiode array detector (PDA-Plus detector Finnigan Suriveyor, Thermo-Scientific). Cinnamic acids derivatives were quantified as 5-*O*-caffeoylquinic acid at 320 nm and flavonols as quercetin-3-*O*-glucoside at 360 nm.

Statistical Analysis

Data were processed using MSTAT-C Statistic Software Package (Michigan State University, Michigan, US). A multi factorial analysis of variance (ANOVA) and multiple range test (Tukey's test) were carried out to evaluate statistical differences. The level of significance was set at $p < 0.05$.

Results

In the present study, on the basis of retention time (RT), molecular masses, and fragmentation patterns, twelve flavonols and five hydroxycinnamic acids were detected and reported for the first time in the selected *Capparis* species from Pakistan. A significant ($p < 0.05$) variation in the qualitative and quantitative composition of flavonoids and hydroxycinnamic acids was recorded among parts of both species as well as function of two seasons. Overall, maximum concentration of total flavonoids was observed in *C. spinosa* in rainy season (554.53 µg g^{-1} dw) in comparison with dry months (488.07 µg g^{-1} dw) which were 11% lower (Tab. 1-5). A similar trend was recorded for hydroxycinnamic acid derivatives as well. Overall, in comparison with *C. spinose, C. decidua* offered 19.23 and 21.97% higher flavonoids and hydroxycinnamic acids, respectively (Tab. 1-5).

Stem bark of both species showed different compounds with varying concentration in both seasons. The stem bark of *C. spinosa* contained lower amount of kaempferol-3-glucoside (114.80 µg g^{-1} dw, on average) than that of *C. decidua* (144.78 µg g^{-1} dw, on average). The stem bark of *C. decidua* exhibited a reasonable level ofapigenin-7-C-glucoside (isorhoifolin) and quercetin-3-glucoside; however, these compounds were present only in traces in the stem bark of *C. spinosa*. *C. decidua* stem bark showed higher contents of the above mentioned phenolics in rainy season while *C. spinosa* stem bark presented more concentration in dry season (Tab. 1). Beside, 3-*p*-coumaroylquinic acid and feruloylquinic acid were also detected in *C. spinosa* stem bark while *C. decidua* stem bark showed the presence of only feruloylquinic acid (Tab. 1).

Shoot of *C. spinosa* and *C. decidua* showed lower concentration of these phenolics in comparison with other parts. Apigenin-8-C-glucoside (isovitexin) (3.15 µg g^{-1} dw, on average), quercetin-3-glucoside (5.23 µg g^{-1} dw, on average) and kaempferol-3-glucoside (45.93 µg g^{-1} dw, on average) were recorded in *C. spinosa* shoot while quercetin-3-rhamanoside was recorded in lower quantity. On the other hand, *C. decidua* shoot contained quercetin-3-glucoside (2.87 µg g^{-1} dw, on average), apigenin-7-C-glucoside (isorhoifolin) (5.48 µg g^{-1} dw, on average), kaempferol-3,7-diglucoside (4.34 µg g^{-1} dw, on average) and kaempferol-3-glucoside (51.71 µg g^{-1} dw, on average) in both seasons. In case of hydroxycinnamic acids, only feruloylquinic acid was recorded in both species with its concentration higher in *C. spinosa* shoot samples collected in rainy season than *C. decidua* shoot samples of both seasons (Tab. 2). The other flavonoid and hydroxycinnamic acids were detected in traces.

Fruits of both species were noted to be a good source of flavonoids and hydroxycinnamic acids compared to other parts with *C. decidua*

Tab. 1: Identification and quantification of phenolic acids and flavonoids (µg g^{-1} dw) in stem bark of *C. spinosa* and *Capparis decidua*

Compounds	*Capparis spinosa*		*Capparis decidua*	
	Dry season	Rainy season	Dry season	Rainy season
Flavonoids				
Quercetin-3,7-diglucoside	0.08±0.01 e	0.06±0.10 e	Traces	Traces
Quercetin-3,7-diglucoside (isomer)	Traces	Traces	Traces	Traces
Apigenin-8-C-glucoside (isovitexin)	Traces	Traces	Traces	Traces
Quercetin-3-glucoside	Traces	Traces	22±1.27 cd	13±0.43 d
Apigenin-7-C-glucoside (isorhoifolin)	Traces	Traces	39±0.94 c	44±1.41 c
Kaempferol-3,7-diglucoside	Traces	Traces	8±1.03 c	8±1.13 c
Apigenin-7-rutinoside	Traces	Traces	6±0.87-d	8±0.54 d
Quercetin-3-rhamanoside	0.46±0.09 e	0.42±0.07 e	0.34±0.02	0.39±0.05
Quercetin-3-sophoroside	0.24±0.05 e	0.12±0.02 e	Traces	0.49±0.08
Quercetin-3-acetyl-glucoside	Traces	Traces	0.11±0.03	Traces
Kaempferol-3-glucoside	122±3.59 a	107±2.98 ab	122±1.80 b	167±5.96 a
Kaempferol-7-glucoside	Traces	Traces	Traces	Traces
Total	123 A	108 B	197 B	242 A
Hydroxycinamic acids				
Dicaffeoylquinic acid	Traces	Traces	Traces	Traces
5-Caffeoylquinic acid	Traces	Traces	Traces	Traces
3-Caffeoylquinic acid	Traces	Traces	0.07±0.01	Traces
3-*p*-coumaroylquinic acid	31±1.11 c	52±1.73 b	Traces	Traces
Feruloylquinic acid	16±1.38 d	35±1.12 c	22±1.27 cd	35±1.18 c
Total	48 B	87 A	22 BC	35 B

Values (means ±SD) are average of three samples of each part, analyzed individually in triplicate ($p< 0.05$).

Tab. 2: Identification and quantification of phenolic acids and flavonoids (µg g^{-1} dw) in shoot of *C. spinosa* and *Capparis decidua*

Compounds	*Capparis spinosa*		*Capparis decidua*	
	Dry season	Rainy season	Dry season	Rainy season
Flavonoids				
Quercetin-3,7-diglucoside	Traces	Traces	Traces	Traces
Quercetin-3,7-diglucoside (isomer)	Traces	Traces	Traces	Traces
Apigenin-8-C-glucoside (isovitexin)	2.3±0.07 c	4.0±0.05 bc	Traces	Traces
Quercetin-3-glucoside	4.4±0.83 bc	6.0±0.19 b	2.4±0.22 d	3.4±0.32 d
Apigenin-7-C-glucoside (isorhoifolin)	Traces	Traces	7.75±1.23 c	3.22±0.71 d
Kaempferol-3,7-diglucoside	Traces	Traces	4.0±0.47 d	4.7±0.38 d
Apigenin-7-rutinoside	Traces	Traces	Traces	Traces
Quercetin-3-rhamanoside	0.73±0.11 d	0.29±0.08 d	Traces	Traces
Quercetin-3-sophoroside	Traces	Traces	Traces	Traces
Quercetin-3-acetyl-glucoside	Traces	Traces	Traces	Traces
Kaempferol-3-glucoside	43±0.57 a	48±1.89 a	42±1.40 b	61±2.08 a
Kaempferol-7-glucoside	Traces	Traces	Traces	Traces
Total	51 B	59 B	57 B	72 A
Hydroxycinamic acids				
Dicaffeoylquinic acid	Traces	Traces	Traces	Traces
5-Caffeoylquinic acid	Traces	Traces	Traces	Traces
3-Caffeoylquinic acid	Traces	Traces	Traces	Traces
3-*p*-coumaroylquinic acid	Traces	Traces	Traces	Traces
Feruloylquinic acid	7.5±0.96 b	17±0.99 b	2.4±0.22 d	3.9±0.47 d
Total	7.5 B	17 A	2.4 A	3.9 A

Values (means ±SD) are average of three samples of each part, analyzed individually in triplicate ($p< 0.05$).

Tab. 3: Identification and quantification of phenolic acids and flavonoids (µg g^{-1} dw) in fruit of *C. spinosa* and *Capparis decidua*

Compounds	*Capparis spinosa*		*Capparis decidua*	
	Dry season	Rainy season	Dry season	Rainy season
Flavonoids				
Quercetin-3,7-diglucoside	Traces	Traces	Traces	Traces
Quercetin-3,7-diglucoside (isomer)	Traces	Traces	Traces	Traces
Apigenin-8-C-glucoside (isovitexin)	17±1.03 c	20±0.1 c	2.6±0.42 e	2.9±0.31 e
Quercetin-3-glucoside	0.08±0.009	0.12±0.009	25±1.30 c	32±1.08 c
Apigenin-7-C-glucoside (isorhoifolin)	Traces	Traces	0.42±0.15 f	Traces
Kaempferol-3,7-diglucoside	Traces	Traces	0.21±0.02 f	1.2±0.14 ef
Apigenin-7-rutinoside	Traces	Traces	7.2±0.49 d	11±0.48 d
Quercetin-3-rhamanoside	0.47±0.08 e	0.89±0.12 e	0.41±0.08	1.11±0.18
Quercetin-3-sophoroside	0.12±0.15 e	0.78±0.23 e	Traces	Traces
Quercetin-3-acetyl-glucoside	0.11±0.05 e	0.43±0.09 e	0.19±0.009 f	0.97±0.10 f
Kaempferol-3-glucoside	123±1.66 b	147±1.34 a	116±1.54 b	131±5.38 a
Kaempferol-7-glucoside	Traces	Traces	Traces	Traces
Total	140 B	170 A	152 B	180 A
Hydroxycinamic acids				
Dicaffeoylquinic acid	Traces	Traces	Traces	Traces
5-Caffeoylquinic acid	Traces	Traces	Traces	0.49±0.08
3-Caffeoylquinic acid	Traces	Traces	0.09±0.012 f	1.1±0.17 ef
3-*p*-coumaroylquinic acid	4.1±0.59 de	5.0±0.47 de	2.6±0.42 e	5.0±0.97de
Feruloylquinic acid	7.6±0.85 d	8.2±0.37 d	24.9±1.30 c	29.0±1.03 c
Total	12 A	13 A	28 B	36 A

Values (means ±SD) are average of three samples of each part, analyzed individually in triplicate ($p< 0.05$).

Tab. 4: Identification and quantification of phenolic acids and flavonoids (μg g^{-1} dw) in flower of *C. spinosa* and *Capparis decidua*

Compounds	*Capparis spinosa*		*Capparis decidua*	
	Dry season	Rainy season	Dry season	Rainy season
Flavonoids				
Quercetin-3,7-diglucoside	Traces	Traces	1.18±0.56 b	1.16±0.32 b
Quercetin-3,7-diglucoside (isomer)	3.6±0.12 de	2.7±0.09	Traces	Traces
Apigenin-8-C-glucoside (isovitexin)	2.6±0.04 de	5.0±0.20 d	Traces	Traces
Quercetin-3-glucoside	2.8±0.40 de	3.5±0.13 de	1.8±0.09 b	2.2±0.11 b
Apigenin-7-C-glucoside (isorhoifolin)	Traces	Traces	2.2±0.31 b	2.1±0.27 b
Kaempferol-3,7-diglucoside	Traces	Traces	Traces	Traces
Apigenin-7-rutinoside	Traces	Traces	Traces	0.61±0.19
Quercetin-3-rhamanoside	0.82±0.05 e	1.3±0.13 e	0.9±0.03	1.4±0.09
Quercetin-3-sophoroside	Traces	Traces	Traces	Traces
Quercetin-3-acetyl-glucoside	Traces	Traces	Traces	Traces
Kaempferol-3-glucoside	41±0.78 b	52±1.77 a	40±1.07 ab	48±1.97 a
Kaempferol-7-glucoside	Traces	Traces	2.1±0.85	3.0±0.78
Total	51 B	65 A	48 B	59 A
Hydroxycinamic acids				
Dicaffeoylquinic acid	17±0.52 c	24±0.88 c	Traces	Traces
5-Caffeoylquinic acid	Traces	Traces	Traces	Traces
3-Caffeoylquinic acid	Traces	Traces	Traces	Traces
3-*p*-coumaroylquinic acid	Traces	Traces	Traces	Traces
Feruloylquinic acid	Traces	Traces	1.8±0.59 b	2.3±0.12 b
Total	17 B	24 A	1.8 A	2.3 A

Values (means ±SD) are average of three samples of each part, analyzed individually in triplicate ($p< 0.05$).

Tab. 5: Identification and quantification of phenolic acids and flavonoids (μg g^{-1} dw) in root of *C. spinosa* and *Capparis decidua*

Compounds	*Capparis spinosa*		*Capparis decidua*	
	Dry season	Rainy season	Dry season	Rainy season
Flavonoids				
Quercetin-3,7-diglucoside	0.10±0.01 e	0.32±0.01 e	Traces	Traces
Quercetin-3,7-diglucoside (isomer)	Traces	Traces	Traces	Traces
Apigenin-8-C-glucoside (isovitexin)	14.±1.5 cd	19.±0.65 c	18±1.25 f	19±0.62 f
Quercetin-3-glucoside	Traces	Traces	55±0.82 c	69±2.21 bc
Apigenin-7-C-glucoside (isorhoifolin)	Traces	Traces	48±0.45 d	59±2.15
Kaempferol-3,7-diglucoside	Traces	Traces	6.0±0.35 g	8.0±0.37 g
Apigenin-7-rutinoside	Traces	Traces	21±0.77 f	18±1.02
Quercetin-3-rhamanoside	0.48±0.08 e	0.97±0.09 e	51±1.20 d	69±2.07 bc
Quercetin-3-sophoroside	Traces	Traces	Traces	Traces
Quercetin-3-acetyl-glucoside	0.51±0.008 e	1.19±0.10 e	44±0.66 de	57±2.17 c
Kaempferol-3-glucoside	107±1.76 b	132±4.34 a	28±1.87 e	44±1.71 de
Kaempferol-7-glucoside	Traces	Traces	20±1.05	25±1.13 ef
Total	123 B	154 A	290 B	368 A
Hydroxycinamic acids				
Dicaffeoylquinic acid	Traces	Traces	29±1.29 e	34±1.19 e
5-Caffeoylquinic acid	Traces	Traces	3±0.16 g	4±0.19 g
3-Caffeoylquinic acid	Traces	Traces	78±1.03 b	97±3.21 a
3-*p*-coumaroylquinic acid	8±1.00 d	11±0.85 cd	18±1.25 f	30±1.13 e
Feruloylquinic acid	7.0±0.55 d	22±1.10 c	55±0.82 c	61±2.47 bc
Total	15 B	33 A	183 B	226 A

Values (means ±SD) are average of three samples of each part, analyzed individually in triplicate ($p< 0.05$).

fruits offering higher concentration of these compounds (Tab. 3). The fruits from *C. spinosa* exhibited maximum concentration of kaempferol-3-glucoside (134.80 μg g^{-1} dw, on average) followed by apigenin-8-C-glucoside (isovitexin) (18.62 μg g^{-1} dw, on average) while *C. decidua* fruit contained quercetin-3-glucoside (28.21 μg g^{-1} dw, on average) and kaempferol-3-glucoside (123.50 μg g^{-1} dw, on average). Moreover, 3-*p*-coumaroylquinic acid and feruloylquinic acid were recorded in fruits of both species in both seasons while 3-caffeoylquinic acid was recorded only in *C. decidua* fruit (Tab. 3).

Likewise, shoot and flowers of both species exhibited lower quantities of identified flavonoids and phenolic acids. Among flavonoids, only kaempferol-3-glucoside was detected in both species while the other compounds were present in traces. Dicaffeoylquinic acid (20.56 μg g^{-1} dw, on average) was quantified in flowers of *C. spinosa* while feruloylquinic acid was present in *C. decidua* flowers (Tab. 4).

It can be guessed from the data of Tab. 5 that roots of *C. decidua* contained higher amount of flavonoids and hydroxycinnamic acids than its counterpart. Meanwhile, the roots from *C. spinosa* were found to be a good source of kaempferol-3-glucoside (119.66 μg g^{-1} dw, on average) while the other flavonoids were found in traces. Regarding hydroxycinnamic acids, dicaffeoylquinic acid, 5-caffeoylquinic acid and 3-caffeoylquinic acid were identified in traces in *C. spinosa* roots while 3-*p*-coumaroylquinic and feruloylquinic acids were found in reasonable concentration. On the other hand, *C. decidua* roots exhibited maximum amount of 3-caffeoylquinic acid (87.58 μg g^{-1} dw, on average) followed by feruloylquinic acid (58.28 μg g^{-1} dw, on average), dicaffeoylquinic acid (31.40 μg g^{-1} dw, on average), 3-*p*-coumaroylquinic acid (23.69 μg g^{-1} dw, on average) and 5-caffeoylquinic acid (3.28 μg g^{-1} dw, on average) (Tab. 5).

Discussion

HPLC-PDA-ESI-MS^n analysis of aqueous methanolic extracts of different parts of *C. spinosa* and *C. decidua* demonstrated a variable composition of phenolic compounds in dry and rainy seasons. Among these compounds, flavonols were found as the main constituents as shown in Tab. 1-5. Beside these, hydroxycinnamic acids were also found in the tested parts of these species with varying concentrations. Previously, few studies have shown the presence of rutin, kaempferol, kaempferol rutinoside and quercetin rutinoside (Argentieri et al., 2012; Inocencio et al., 2000; Rodrigo et al., 1992) while the others were detected first time in the present study.

The fruits of *C. spinosa* presented highest amount of total flavonoids in rainy seasons (169.50 μg g^{-1} dw) followed by roots (153.61 μg g^{-1} dw) and fruits of dry season (140.35 μg g^{-1} dw). Total flavonoids, expressed in dry season by stem bark and roots of *C. spinosa,* were 27% lower than fruits (Tab. 1-5). Kaempferol-3-glucoside was noted as main flavonoid in *C. spinosa* fruits (134.80 μg g^{-1} dw, on average) followed by apigenin-7-C-glucoside (isorhoifolin) (Tab. 3). Apigenin and kaempferol have previously been reported in *C. spinosa* fruits collected from Xinjiang province of China (Yu et al., 2006; Zhou et al., 2010; Zhou et al., 2011) but these compounds were identified for the first time in capparis fruit samples collected from Pakistan. Beside this, a few other derivatives of kaempferol were reported in *C. spinosa* shoots which were confirmed in the present investigation (Siracusa et al., 2011). It was noted that *C. spinosa* shoot is a rich source of kaempferol derivative. Such variation in flavonoids distribution within plants of different origins may be linked to varying mode of extraction employed, and genetic and agro-climatic factors prevailing in different sites (Hashempour et al., 2010; Islam et al., 2003).

The flavonoid compounds detected in the present analysis of *Capparis* species were consisted of twelve compounds including quercetin-3,7-diglucoside, quercetin-3,7-diglucoside (isomer), apigenin-8-C-glucoside, quercetin-3-glucoside, apigenin-7-C-glucoside (isorhoifolin), kaempferol-3,7-diglucoside, apigenin-7-rutinoside, quercetin-3-rhamnoside, quercetin-3-O-sophoroside, quercetin-3-acetylglucoside, kaempferol-3-glucoside, and kaempferol-7-glucoside with their composition significantly differed among different parts of both species in either of the seasons. As stated earlier, fruits and roots of *C. spinosa* presented maximum content of flavonoids while a few such as apigenin-7-C-glucoside (isorhoifolin), kaempferol-3,7-diglucoside, apigenin-7-rutinoside and kaempferol-7-glucoside were detected only in traces amount in *C. spinosa*. In case on hydroxycinnamic acids, stem bark and fruits of *C. spinosa* exhibited maximum amount of these acids in either of the dry and rainy seasons, respectively while 5-caffeoylquinic acid and 3-caffeoylquinic acid were recorded in traces (Tab. 1-5). Kaempferol-3-glucoside was the most abundant constituent (437.0 and 486.9 μg g^{-1} dw) and represented 89.0 and 87.8% of flavonoids detected in in dry and rainy seasons, respectively. Apigenin-8-C-glucoside (isovitexin) was found as the second most abundant flavonoid in *C. spinosa* with contribution 36.09 and 48.49 μg g^{-1} dw in dry and rainy seasons, respectively. Apigenin-8-C-glucoside (isovitexin) was mainly present in the fruits representing 43.3% of total apigenin-8-C-glucoside (isovitexin) contents of *C. spinosa* while in stem bark, it was not detected. The least amounts of flavonoids were quantified in *C. spinosa* shoot.

Dicaffeoylquinic acid, 5-caffeoylquinic acid, 3-caffeoylquinic acid, 3-*p*-coumaroylquinic acid, and feruloylquinic acid were identified in *C. spinosa* (Tab. 1-5). As a whole, *C. spinosa* presented 99.10 and 173.80 μg g^{-1} dw of hydroxycinnamic acids in dry and rainy months, respectively (Tab. 1-5). Stem bark and roots exhibited maximum amount of these acids (47.85 and 33.06 μg g^{-1} dw, respectively) while the least count was recorded in shoots. Of the studied hydroxycinnamic acids, 3-*p*-Coumaroylquinic acid presented maximum amount in dry month (43.33 μg g^{-1} dw) while in rainy month feruloylquinic acid gave maximum quantity (81.44 μg g^{-1} dw). Both caffeoylquinic acid derivatives were found in traces while dicaffeoylquinic acid was as high as 17.38 and 23.74 μg g^{-1} dw (Tab. 1-5). The availability of these phenolic acids supports the traditional health benefits of capparis. The researchers reported that roots, fruits and flowers of these species are a good for curing infectious diseases (Iwu et al., 1999). Such functions of capparis tissues might be attributed to the phenolic acids (Sharma and Kumar, 2008). The researchers extracted kaempferol, apigenin and cinnamic acids from fruit extracts of *C. spinosa* and identified these as anti-inflammatory agents against induced rat paw edema (Zhou et al., 2010). So, the findings of the present investigation support that capparis tissues, especially roots can be used in pharmaceutical industries.

C. decidua presented more amounts of flavonoids and hydroxycinnamic acids. In dry and rainy months, *C. decidua* exhibited 65.5 and 52.9% more flavonoids than *C. spinosa*, respectively. These data also showed that *C. decidua* provided more flavonoids in rainy months rather than dry ones (920.38 and 734.49 μg g^{-1} dw, respectively) as was recorded in case of *C. spinosa*. Among different parts of *C. decidua*, roots ranked higher in flavonoids followed by stem bark and fruits. The least amount of flavonoids was recorded in shoots (64.42 μg g^{-1} dw, on average) which was 80.6% lower than roots and 70.7% lower than stem bark. Among all studied flavonoids, Quercetin-3,7-diglucoside (isomer) was found in traces while all others were also detected except quercetin-3-sophoroside and Quercetin-3,7-diglucoside (0.49 and 1.17 μg g^{-1} dw on average, respectively). Kaempferol-3-glucoside ranked highest among all detected flavonoids (400.10 μg g^{-1} dw, on average) followed by quercetin-3-glucoside and apigenin-7-C-glucoside (isorhoifolin). These three compounds represented 74.00% of total flavonoids. Interestingly, a few flavonoids like apigenin-7-rutinoside and quercetin-3-sophoroside were not detected in dry months but these were found in the samples (flowers and stem bark) harvested/plucked during rainy month. Previously, various compounds like apigenin, kaempferol, kaempferol-3-O-

rutinoside and quercetin-3-O-rutinoside have been isolated from *C. spinosa* fruits (YU et al., 2006; ZHOU et al., 2010; ZHOU et al., 2011). Beside these, *Capparis* leaves and shoots have also been reported as a good source of kaempferol and quercetin. Rutin, kaempferol, 3-O-rutinoside, and isorhamnetin 3-O-rutinoside have also been reported as major flavonoids in *C. spinosa* (SIRACUSA et al., 2011). Hydroxycinnamic acids such as dicaffeoylquinic acid, 5-caffeoylquinic acid, 3-caffeoylquinic acid, 3-*p*-coumaroylquinic acid, and feruloylquinic acid were also detected first time in *C. decidua*. These were found in all parts of *C. decidua* in appreciable amount with maximum levels recorded in roots of this species followed by fruits and stem bark. Roots represented 77.00 and 74.72% of total hydrocinnamic acids in dry and rainy months, respectively. Among all hydroxycinnamic acids, feruloylquinic acid ranked highest followed by 3-caffeoylquinic acid > 3-*p*-coumaroylquinic acid > dicaffeoylquinic acid > 5-caffeoylquinic acid.

The comparative profile of flavonoids and hydroxycinnamic acids of *C. spinosa* and *C. decidua* is first time investigated and reported in the present investigation. It can be noted that *C. decidua* is an important source of studied phenolics as it possesses 59.2 and 50.7% more flavonoids and hydroxycinnamic acids than *C. spinosa* on average, respectively. Both species exhibited higher contents of phenolics in rainy season than dry ones. The reasons for higher concentration of phenolic acids might be rainfall as researchers reported a positive correlation of rainfall with phenolics and flavonoid contents in several studies (SARRAZIN et al., 2015; SOUSA et al., 2010). YANG et al., (2006) studied 120 tropical and sub-tropical edible plants for their antioxidant potential and reported a higher antioxidant activity of these plants in hot wet season. Higher antioxidant activity is an indicator for the presence of higher phenolic acids (SIDDHURAJU and BECKER, 2003). The varying concentration of bioactive compounds such as rutin has been reported to be linked with varying daylight in different *Capparis* parts. For example, maximum rutin contents in flowers and leaf samples of *C. spinosa* were detected at morning and night times while floral buds of *C. spinosa* gave maximum rutin in the morning. Stem and fruits of this species provided maximum rutin contents in morning at 11:00 and afternoon at 16:00 h (BEHNAZ et al., 2013). In the present work, variations in the phenolics profile can be mainly linked to varying climatic factors such as temperature, humidity and rainfall fluctuation in both seasons.

Conclusion

In the present comprehensive study, we for the first time reported that how different parts of *C. spinosa* and *C. decidua* exhibit varying levels of polyphenolics in dry and rainy seasons. A notable variation in the profile of phenolics was recorded among selected species parts as function of both harvesting seasons. Different parts of both species exhibited impressive range of phenolics but the roots possessed maximum amount of these compounds which were mostly derivatives of quercetin such as kaempferol, apigenin, caffeoylquinic acid and feruloylquinic acid. These findings support the nutra-pharmaceutical potential of *C. spinosa* and *C. decidua* due to offering a wide array of polyphenols. Moreover, from the findings of this study, it may be concluded that the selected species exhibited more polyphenols in the rainy month (September) as compared to dry month (April) thus provoking harvesting of these species in an appropriate season to maximize their nutraceutical benefits.

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