Phenolic compounds and antioxidant activity of *Calligonum polygonoides* stem and buds

Muhammad Qasim Samejo[1], Shahabuddin Memon[2*], Khalid Mohammed Khan[3], Saba Muneer Rajput[2], Shabana Gul[2], Ghulam Zuhra Memon[1] and Qadeer Khan Panhwar[1]

[1]Dr. M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro, Pakistan
[2]National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan
[3]HEJ Research Institute of Chemistry, ICCBS, University of Karachi, Karachi, Pakistan

Abstract: In the present study we demonstrate the identification of phenolic compounds and the phenolic contents of the methanol extracts from stem and buds of *Calligonum polygonoides* with antioxidant activity. Eleven and nine phenolic compounds were identified and quantified from stem and buds, respectively by high-performance liquid chromatography (HPLC). *p*-Coumaric acid was predominant in stem and gallic acid in buds. In general, the samples with the highest phenolic contents had the highest antioxidant activities. Stem and buds sparked attention due to their high phenolic contents and antioxidant activities. The Results from present study reveal that the *C. polygonoides* could be considered as a promising source of antioxidant phytochemicals.

Keywords: *Calligonum polygonoides*, phenolic compounds, antioxidant activity, HPLC.

INTRODUCTION

Medicinal plants from ancient time are extensively used as substitute therapeutic tools for the treatment of various human diseases and ingredients of folk medicine (Sultana *et al.,* 2014; Kaur and Mondal, 2014). The significance of medicinal plants in various fields mainly originates due to the presence of active ingredients (biological active organic compounds) (Ahmad *et al.,* 2014; Ghani *et al.,* 2014; Khan *et al.,* 2014). Plants are rich in aromatic substances (sweet-smelling or fragrant) most of them are phenols or their oxygen substituted derivatives such as ascorbic acid, flavonoids, phenols, tocopherols, and carotenoids (Khan *et al.,* 2014). Phenolics ompounds (secondary metabolites) are an essential part of the human diet possessing one or more aromatic rings with one or more hydroxyl groups (-OH) directly to an aromatic hydrocarbon group. Crude extract of plants parts rich in phenolic compounds (Dai and Mumper, 2010). The main important of these compounds due to its antioxidant and potential health benefits.

Calligonum polygonoides Linn. is known for its medicinal properties. The flowers of *C. polygonoides* are useful against cough, asthma and cold. The juice of shoot is applied to the eyes as an antidote to scorpion sting, a roots decoction mixed with catechu is used as gargle for sore gum, and the latex is used for treating eczema, to cure bites of rabid dogs and to induce abortion. Methanol extract of the *C. polygonoides* showed strong toxicity in brine-shrimp lethality test. Phytochemical screening of *C. polygonoides* shows positive results for flavonoids, alkaloids, proteins, tannins, steroids, phenols, carbohydrates and terpenoids. The essential oil from buds and roots of *C. polygonoides* contain a complex mixture of terpenoids, hydrocarbons, phenolic compounds, acid derivatives and ketones. The literature survey revealed that the Calligonolides, tetracosan-4-olide, steroidal ester, *β*-sitosterol, *β*-sitosterol glucoside and ursolic acid isolated from *C. polygonoides* (Samejo *et al.,* 2013).

In our previous studies it was reported that *C. polygonoides* contains phytochemicals (flavonoids, alkaloids, proteins, tannins, steroids, phenols, carbohydrates and terpenoids) and its essential oil contains complex mixture of terpenoids, hydrocarbons and phenolic compounds (Samejo *et al.,* 2011; Samejo *et al.,* 2013; Samejo *et al.,* 2013; Samejo *et al.,* 2013). The present study demonstrates the phenolic compounds and the phenolic contents of the methanol extract from stem and buds of *C. polygonoides* and its antioxidant activity.

MATERIAL AND METHODS

Collection of plant material

The roots of *Calligonum polygonoides* were collected from Village Mehendri-Jo-Par (longitude: N 25˚ 34’ 2’’ and latitude: E 70˚ 11’ 20’’), District Umerkot in Sindh Province of Pakistan in August 2012. A voucher specimen (15173) of the plant was deposited in the herbarium of Institute of Plant Sciences, University of Sindh Jamshoro, Pakistan. The plant sample was identified by a Taxonomist of the same institution.

Chromatographic analysis of phenolic compounds

Phenolic compound extraction

6 g of dry powdered stem and buds of selected plant were extracted in methanol and water (3:2) by sonication at

*Corresponding author: e-mail: shahabuddinmemon@yahoo.com

room temperature (25°C) for 1 hour. The extracts of stem and buds were filtered separately through a normal paper filter. The filtrate was again filtered using disposable membrane filter (pores size 0.45µm). The filtrate was stored at 4°C.

HPLC conditions

Phenolic compounds were analysis by High Pressure Liquid Chromatography (Spectra System SCM 1000) with Diode-Array Detection (DAD) at National Centre of Excellence in Analytical Chemistry (NCEAC) laboratory. The Hypersil Gold C18 HPLC column (dimension: 250 mm × 4.6 mm; particle size 5µm) was used.

Solvent system A (water:formic acid 99.9: 0.1v/v), B (methanol) was used. The flow rate 0.8ml/min and injection volume 20µL were adjusted during analysis. The gradient elution was proceed such as, 20% of B (at 10 min), 40% of B (at 30 min), 60% of B (at 60 min), 80% of B (at 80 min), and 100% of B (at 90 min). Diode-Array Detection range 200 to 700 nm and detection windows 270, 320 and 254 nm were adjusted.

Characterization of each phenolic compound was done by two ways. (a) Retention time (b) UV-Vis spectra (the standard of phenolic compounds injected by *Memon in 2012* at NCEAC laboratory).

Radical scavenging activity (RSA) of stem and buds extracts

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) RSA was carry out by using scheme reported by *Memon in 2012* with some variation. 2mL ethanol extracts (stem and buds) mixed with 2mL of 0.1mmol DPPH methanol solution. The mixture was kept at room temperature for 30 min. After that the absorbance was measured at 517 nm (UV-Vis spectrophotometer: Perkin Elmer lambda 35). Each sample was measured three times then average of three values was calculated.

RSA in percentage (%) was calculated using formula

$$RSA\ (\%) = (A_{control}-A_{sample})/Acontrol\times 100\ \%$$

Where: $A_{control}$ is the absorbance of blank, A_{sample} is the absorbance of the sample

Total phenolics contents by Folin-Ciocalteu (FC) method

200µL of sample was mixed with 800µL FC reagent. The mixture was kept for 5min. Then, 2mL of Na_2CO_3 (saturated solution 75g/L) were added to the mixture. After incubation for 2h (at 25°C) absorbance was measured at 760nm. The result (Total phenolics contents) was expressed as milligrams (mg) of Gallic acid equivalent (GAE)/100g weight of stem and buds (Memon *et al.,* 2012).

RESULTS

A mixture of twenty two (ten phenolic acids, nine flavonoids and three catechins) different phenolic compound standards were injected into chromatographic column with a flow rate 0.8ml/min. The HPLC chromatogram phenolic compound standards are shown in table 1.

Table 1: Retention time and UV spectral characteristics of selected phenolic standards separated by HPLC analysis using diode-array detection

S.No	Compound	t_R (min)	λ (nm)
1.	Gallic acid	8.2	271, 227
2.	Catechin hydrate	14.3	234, 279
3.	Protocatechuic acid	15.8	259, 294, 222
4.	*p*-Hydroxybenzoic acid	15.8	255
5.	Chlorogenic acid	16.2	324, 241
6.	Epicatechin	16.4	234, 278
7.	Caffeic acid	17.4	306, 222
8.	Epicatechingallate	18.4	276, 233
9.	Vanillin	19.6	281, 308, 230
10.	*p*-Coumaric acid	22.2	309, 234
11.	Ferrullic acid	24.1	323, 240
12.	m-Coumaric acid	26.8	278, 233
13.	Rutin	29.3	256, 354
14.	*o*-Coumaric acid	32.9	277, 324, 233
15.	Myricetin	34.9	371, 253
16.	Morin	48.3	252, 353
17.	Diosmin	53.6	298
18.	Quercetin	53.7	257, 370
19.	Naringenin	55.5	288, 235
20.	Kaempferol	60.2	366, 265
21.	Chrysin	74.6	267, 314
22.	5-Hydroxyflavone	85.5	275, 239

Table 2: The content of some phenolic compounds in stem and buds extract.

S. No	Phenolic compounds	Content [mg/g]	
		Stem	Buds
1.	Gallic acid	0.32	0.65
2.	Catechin hydrate	0.72	0.34
3.	Protocatechuic acid	0.02	0.02
4.	Chlorogenic acid	0.32	0.01
5.	Caffeic acid	0.03	0.03
6.	Epicatechin	-	0.06
7.	Epicatechin gallate	0.53	-
8.	Rutin	1.57	0.12
9.	Vanillin	0.06	-
10.	*p*-Coumaric acid	2.77	-
11.	*o*-Coumaric acid	-	0.03
12.	Quercetin	0.04	-
13.	Naringenin	0.03	0.05

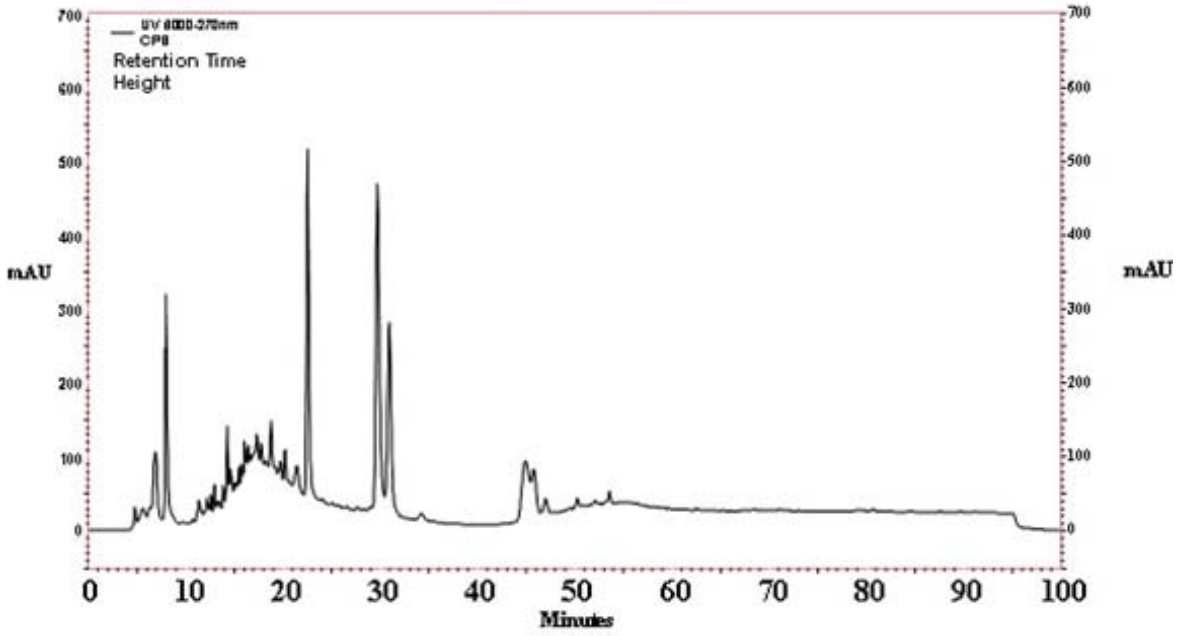

Fig. 1: HPLC chromatogram of *C. polygonoides* stem

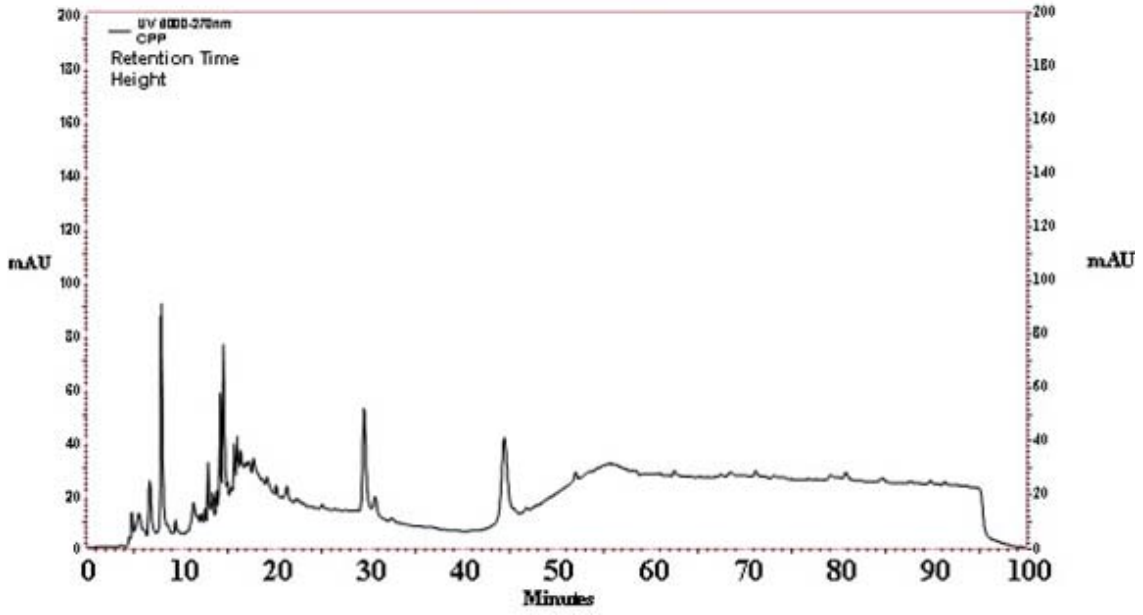

Fig. 2: HPLC chromatogram of *C. polygonoides* buds

The retention times (t_R) in minutes and UV absorbance (λ_{max}) of the phenolic compounds (as taken standard) are given in table 1. Phenolic compounds were identified on the basis of UV spectra and retention times (table 1).

The HPLC analysis of extracts of *C. polygonoides* shows the presence of eleven phenolic compounds in stem (shown in fig. 1) and nine buds (shown in fig. 2).

Using a HPLC method, such phenolic compounds as gallic acid, catechin hydrate, protocatechuic acid, chlorogenic acid, caffeic acid, epicatechin gallate, vanillin, *p*-coumaric acid, rutin, quercetin and naringenin were determined in stem extract whereas gallic acid, catechin hydrate, protocatechuic acid, chlorogenic acid, epicatechin, caffeic acid, rutin, *o*-coumaric acid and naringenin were determined in buds extract. The stem extract was found to be rich in *p*-coumaric acid whereas gallic acid in buds (shown in table 2).

DISCUSSION

Phenolic compounds are present in plants in various forms (a) as extractable with water (garlic acid esters) or

with methanol and aqueous acetone (flavonolglycosides, proanthocyanidins, flavonols), (b) as non-extractable forms due to their high molecular weight or covalently bonded with other plants constituents (Mueller-Harvey *et al.,* 1986).

The *C. polygonoides* buds and stem methanol extract were examined for its phenolic compounds, phenolic content and antioxidant activity. Due to poor extracting rate of other solvents (acetone or water) and possibility of extracting other compounds, methanol was used an extraction solvent. Precautions need to be taken when extracting phenolics because many phenolic compounds isomerize in sunlight (*trans-cis* conversions, react with oxygen in alkaline solution (quinone formation) and with methanol at room temperature and pH (Mueller-Harvey *et al.,* 1988).

Table 3: Total phenolic compounds as gallic acid eqiuvalents (GAE/100 g) determined by means of Folin–Ciocalteau reagent.

Analyzed Material	Total phenolics (mg/100 g) GAE Eq.	DPPH scavenging (%)
Stem	1911±0.2	70.11
Buds	1071±0.2	56.38

Total phenolic compounds and antioxidant activity were determined by FC reagent and RSA procedure respectively. FC method was chosen because it is simple, give a better estimate of total phenolic groups, reproducible essay and give a greater color response with phenols and a lesser response to non-phenolic compounds (MacDonald Wicks *et al.,* 2006). Spectrophotometric methods gives better estimate of total phenolic contents but these methods do not give quantitative amount of each compound in each class. DPPH method is simple, selective, quick and extensively used for the determination of antioxidant activity with good reproducibility.

CONCLUSION

Present study shows that total phenolic content of stem extract was high than buds extract, which suggests that stem of *C. polygonoides* may be a better source of phenolic compounds. Antioxidant activity of methanol extracts of stem and buds was measured by DPPH assay. This suggests that methanol extract from stem may provide better protective effect against free radical oxidative damage.

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REFERENCES

Ahmad R, Ahmad M, Mehjabeen and Jahan N (2014). Phytochemical screening and Anti-oxidant activity of the two plants *Ziziphus oxyphylla* Edgew and *Cedrela serrata* Royle. *Pak. J. Pharm. Sci.,* **27**(5 Special): 1477-1482.

Dai J and Mumper RJ (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules,* **15**: 7313-7352.

Ghani A, Ali Z, Islam T, Sanaullah S and Saeed S (2014). Nutrient evaluation and elemental analysis of four selected medicinal plants of soon valley Khushab, Punjab, Pakistan. *Pak. J. Pharm. Sci.,* **27**(3): 597-600.

Kaur S and Mondal P (2014). Study of total phenolic and flavonoid content, antioxidant activity and antimicrobial properties of medicinal plants. *J. Microbiol*., **1**(1): 00005.

Khan M, Rizwani GH, Shareef H, Cavar S and Zia-Ul-Haq M (2013). Assessment of total phenolic content and antioxidant potential of methanol extract of *peltophorum pterocarpum* (dc.) backer ex k. heyne. *Pak. J. Pharm. Sci.,* **26**(5): 967-972.

Khan ZA, Naqvi SA, Mukhtar A, Hussain Z, Shahzad SA, Mansha A, Ahmad M, Zahoor AF, Bukhari IH, Ashraf-Janjua MR, Mahmood N and Yar M (2014). Antioxidant and antibacterial activities of Hibiscus Rosa-sinensis Linn flower extracts. *Pak. J. Pharm. Sci.,* **27**(3): 469-474.

MacDonald-Wicks, LK, Wood LG and Garg ML (2006). Methodology for the determination of biological antioxidant capacity *in vitro*: A review. *J. Sci. Food Agric.,* **86**(13): 2046-2056.

Memon AA, Memon N, Luthria DL, Pittafi AA and Bhanger MI (2012). Phenolic Compounds and Seed Oil Composition *of Ziziphus mauritiana* L. Fruit. *Pol. J. Food Nutr. Sci.,* **62**: 15-21.

Mueller-Harvey AB, McAllan, MK, Theodorou and DE (1988). Beever. Phenolics in fibrous crop residues and plants and their effects on the digestion and utilisation of carbohydrates and proteins in ruminants. In Plant Breeding and the Nutritive Value of Crop Residues (ed. Reed, J. D., Capper, B. S., Neate, P. J. H.) *Proceedings of a workshop held at the International Livestock Centre for Africa, Addis Ababa, Ethiopia* (1987). pp.97-132.

Mueller-Harvey I, Hartley RD, Harris PJ and Curzon EH (1986). Linkage of p-coumaroyl and feruloyl groups to cell wall polysaccharides of barley straw. *Carbohydrate Research*, **148**: 71-85.

Samejo MQ, Memon S, Bhanger MI and Khan KM (2011). Preliminary Phytochemicals Screening of *Calligonum polygonoides* Linn. *J. Pharm. Res.,* **4**(12): 4402-4403.

Samejo MQ, Memon S, Bhanger MI, and Khan KM (2013). Chemical composition of essential oils of

Calligonum polygonoides. *Nat. Prod. Res*., **27**(7): 619-623.

Samejo MQ, Memon S, Bhanger MI and Khan KM (2013). Essential oil constituents of fruit and stem of *Calligonum polygonoides*. *Ind. Crops Prod.,* **45**: 293-295.

Samejo MQ, Memon S, Bhanger MI and Khan KM (2013). Isolation and Characterization of Steroids from *Calligonum polygonoides*. *J. Pharm. Res.,* **6**: 346-349.

Singleton VL and Rossi JAJr (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.,* **16**: 144158.

Sultana B, Anwar F, Mushtaq M, Aslam M, Sidra and Ijaz S (2014). *In vitro* antimutagenic, antioxidant activities and total phenolics of clove (*Syzygium aromaticum* L.) seed extracts. *Pak. J. Pharm. Sci.,* **27**(4): 893-899.