Original Article

Antioxidant Capacity and HPLC Determination of Phenolic in Different Organs of *Calligonum polygonoides* Subspecies *comosum*

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Abstract

Background: *Calligonum polygonoides* subsp. *comosum* is a perennial desert plant. Most of the previous chemical investigation of this plant was performed on the whole herb but there were no data about quantification of active constituent in different organs of *C. polygonoides*. **Materials and Methods:** *in vitro* antioxidant activity, total phenolic, and total flavonoid contents of the different organs were determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu, and aluminum chloride ($AlCl_3$) methods, respectively. Quantitative analysis of the phenolic compounds was determined in the different organs of the plant using high-performance liquid chromatography (HPLC). **Results:** Both bark and leaves showed the highest radical scavenging activity with the values of 450.30 and 398.10 µg/g ascorbic acid equivalent, respectively. The total phenolic content of the samples was in the range of 27.9–281.5 µg/g gallic acid equivalent and total flavonoid content of the samples was in the range of 53.9–257.4 µg/g rutin equivalent where the leaves and bark showed the highest contents. HPLC analysis showed that flavonol glycosides content was higher in all organs compared to the aglycones. Flowers and fruits were the richest organs in flavonols, whereas leaves, stems, and bark were the richest in taxifolin and catechin. **Conclusion:** Depending on the obtained results *C. polygonoides* is an excellent source of natural antioxidants.

Keywords: *Antioxidant, Calligonum polygonoides, HPLC analysis, total flavonoid, total phenolic*

Introduction

Poylgonaceae (smartweed) family comprises about 40 genera and 80 species distributed mainly in the temperate regions, only few are tropical. The plants of this family are mostly herbs; few are shrubs like *Calligonum* species known for high tolerance to xerophytic conditions. *Calligonum* species are distributed throughout Western Asia, Southern Europe, and North Africa. *Calligonum polygonoides* L. subsp. *comosum* L'Hér., from gonu, a knee joint that is referring to its leafless joint and *comosum* referring to long haired, is a tall woody perennial desert plant.[1,2] Its fruits have a single circular hairy carpel that is covered with brown–yellow hairs when ripe.[3] Traditional healers have been used the plant to treat various ailments. The stems and leaves are chewed for curing toothache and root decoction is used for gum sores.[4] Extracts prepared from this plant show diverse biological activities. This includes estrogenic,[5] antimicrobial,[6,7] cardioprotectiv,[8] lipoxygenase-inhibiting,[9] anti-ulcer, anti-inflammatory,[10] hypoglycemic,[11] cytotoxic and antioxidant activities.[12,13] Chemical constituents isolated from different organs of *C. polygonoides* were reported. Kaempferol-3-*O*-β-D-(6″-*n*-butyl glucuronide), quercetin 3-*O*-β-D-(6″-*n*-butyl glucuronide), kaempferol-3-*O*-β-D-(6″-methyl glucuronide), quercetin-3-*O*-β-D-(6″-methyl glucuronide), quercetin-3-*O*-β-D-glucuronide, kaempferol-3-*O*-β-D-glucuronide, quercitrin, astragalin, isoquercetin, taxifolin, (+)-catechin, dehydrodicatechin A, quercetin, and kaempferol were isolated from the hydroethanolic extract of the aerial parts of *C. polygonoides*.[14] Violaxanthin and neoxanthin were isolated from the herb.[15] Isoprunetin, genistein-6-*C*-glucoside, campesterol, stigmasterol, (3β,5α,24S)-stigmastan-3-ol, and stigmast-4-en-3-one were isolated from the methanol extract of the roots.[16,17] Essential oils extracted from the fruits, stem, buds, and roots of *C. polygonoides* were analyzed by gas chromatography, coupled with mass spectometry (GC-MS). The major chemical constituents extracted from the fruits of this plant by hydrodistillation are (Z,Z)-9,12-octadecadienoic acid (40.7%) and hexadecanoic acid (38.5%). Hexadecanoic acid (42.9%) and (Z,Z)-9,12-octadecadienoic acid (26.9%) were the major constituents from stem essential oil. Ethyl homovanillate was

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the main component in the buds essential oil (11.79%), and drimenol in the essential oil of the roots (29.42%).[18,19]

The objectives of this work were to quantify the compounds previously isolated from this plant species by means of high-performance liquid chromatography (HPLC) combined with diode-array detection and evaluate the antioxidant activity, total phenolic, and flavonoid contents.

Materials and Methods

General experimental procedures

Methanol, 2,2-diphenyl,1-picrylhydrazyl radical (DPPH), rutin, gallic acid, and ascorbic acid were purchased Sigma Chemical (St. Louis, Missouri) and Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). HPLC analysis was carried out on an HPLC (Agilent 1260 Infinity, Germany) instrument equipped with an Agilent 1260 Infinity preparative pump (G1361A), Agilent 1260 Diode array detector VL (G1315D), Agilent 1260 Infinity Thermo stated column compartment (G1361A), and Agilent 1260 Infinity preparative Auto sampler (G2260A). Separation and quantification were performed on a ZORBAX eclipse plus C8 analytical column (250 mm × 4.6 mm i.d., 5-µm particle size) (USA). Ultraviolet–visible (UV–visible) spectrophotometer: Shimadzu UV-1601 PC was used.

Plant material

Calligonum polygonoides subsp. *comosum* was collected from Western desert, Giza governorate, Egypt, during flowering stage. The plant was kindly authenticated by Dr. Abdelhalim Mohamed, Plant Taxonomy Department, Agricultural Research Institute, Egypt. A voucher specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University under the registration number BUPD-40. The different aerial parts were separated from freshly collected plant material, air-dried in shade at room temperature, finely powdered and stored in an airtight container till use.

Preparation of extract

Samples for measuring antioxidant activity, total phenolic, and total flavonoid contents were prepared as described by Bakar *et al* method with slight modification.[20] Briefly, 1 g powdered materials from the leaves, stems, bark, flowers, and fruits were separately extracted using aqueous MeOH (30 mL, 80%) for 2 h at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged for 20 min and the supernatant was transferred to a 100-mL volumetric flask. The procedure was repeated again, and respective supernatants were pooled. The final volume was adjusted to 100 mL and used for analysis.

Samples for HPLC analysis were prepared by extracting 5 g powdered materials from the different plant organs in a sonication bath for 20 min with 3 mL acidulated methanol at room temperature followed by centrifugation for 10 min at 3300 rpm.[21] The supernatant was decanted into a 10-mL volumetric flask. The procedure was repeated two times, and respective supernatants were combined. The final volume was adjusted to 10 mL then passed through a 0.45 µm nylon membrane filter (Sigma, USA). The first 1 mL was discarded and the remaining volume was collected in an HPLC sample vial.

Determination of antioxidant activity

Radical scavenging activity was estimated using DPPH method and ascorbic acid as positive control as described by Bakar *et al.*[20] Samples were prepared by mixing 300 µL of the extract or control (80% methanol) with 3.0 mL of 500 µM DPPH in absolute ethanol. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm. The free radical scavenging activity was calculated as follows:

$$\text{Scavenging effect } (\%) = \left[1 - (\text{As} / \text{Ac})\right] \times 100$$

where As is the absorbance of the sample and Ac is the absorbance of the control.

The result was expressed in equivalent µg ascorbic acid per 1 g of dried sample (µg AEAC/g).

Determination of total phenolic content

Total phenolic content was measured using Folin–Ciocalteu reagent and gallic acid as standard.[22] Briefly 300 µL of the extract and 2.25 mL of 10 fold diluted Folin–Ciocalteu reagent were mixed and allowed to stand at room temperature for 5 min. Then, 2.25 mL of 6% sodium carbonate solution was added and absorbance was measured at 725 nm. The result was expressed in µg equivalents of gallic acid per 1 g of dried sample (µg GAE/g).

Determination of total flavonoid content

Total flavonoid content was determined according to Amirul Alam *et al.*,[23] colorimetric method, using rutin as standard with slight modification. Samples were prepared by mixing 0.5 mL of the extract with 2.25 mL of distilled water then 0.15 mL of 5% $NaNO_2$ solution was added. After 6 min, 0.3 mL of a 10% aluminum chloride solution was added and allowed to stand for another 5 min followed by addition 1.0 mL of 1 M NaOH. The mixture was mixed well with vortex. The absorbance was measured immediately at 510 nm using spectrophotometer. The result was expressed as µg equivalents of rutin per 1 g dried sample (µg RE/g).

High-performance liquid chromatography analysis

Reversed-phase chromatography analyses were carried out with a ZORBAX eclipse plus C-8 column, 20 µL injection volume and gradient elution with water containing 0.03% formic acid (solvent A) and methanol containing 0.03% formic acid (solvent B); A/B 80/20–50/50; 20 min, 50/50–20/80; 5 min, 20/80–0/100; 5 min with a flow rate of 1ml/min. The UV absorption spectra were recorded at λ_{max} 365 nm.

Stock solutions of standards (gallic acid, quercetin, taxifolin, and catechin) were prepared in 100% methanol at five different concentration level ranging from 20 to 100 µg/mL for all the

compounds except catechin from 50 to 500 µg/mL). Also stock solutions of previously isolated compounds, quercitrin, astragalin, isoquercitrin, kaempferol-3-*O*-glucuronide, and quercetin-3-*O*-glucuronide,[14] were used. Samples and standards solutions as well as the mobile phase were filtered through 0.45 µm membrane filter (Millipore) and injected in triplicate. Identification of the compounds was verified by comparison of their retention's time and UV absorption spectrum with those of the standards. Standard samples purity was checked at retention times 26.21, 16.73, 7.51, and 4.10 for quercetin, taxifolin, catechin, and gallic acid, respectively. The mean values of AUPs (area under peaks) for each sample were plotted versus the concentration to obtain regression equation. Results of the flavanols were expressed as µg percentage w/w quercetin equivalent, whereas taxifolin, catechin, and gallic acid were calculated in µg percentage w/w of each equivalent

Statistical analysis

All the experiments were carried out in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software program, version 13.0 and Excel 2007. The values of $P < 0.05$ were considered statistically significant.

Results and Discussion

Radical scavenging activity was evaluated in different organs of *C. polygonoides*. Bark showed the highest activity followed by leaves, flowers, stems, and fruits with the values of 450.30, 398.10, 369.91, 148.11, and 2.95 µg AEAC/g, respectively. Significant difference was found between tested samples. Total phenolic content was determined using Folin–Ciocalteu reagent. There was a significant difference among the tested samples where phenolic content of the leaves and bark were the highest followed by stems, flowers and fruits, respectively. Total flavonoid content was determined using colorimetric method. The total flavonoid content in the different organs was relative to total phenolic in the order of leaves > bark > flowers > stems > fruits. Flavonoids are one of the most distributed groups of plant phenolic compounds. The phenolic compounds can scavenge the reactive oxygen species, chelate transition metals, and inhibit peroxidation so they are known to be responsible for the antioxidant activities of plants and this is in agreement with the obtained results.[24,25] These findings suggest that phenolic content could be used as an indicator of antioxidant properties. Data of the radical scavenging activity, total phenolic, and total flavonoids are shown in Table 1.

The phenolic compounds under investigation in this study were previously isolated from the hydroethanolic extract of the aerial parts of *C. polygonoides*. Their structures were determined based on spectroscopic methods including two-dimensional nuclear magnetic resonance spectroscopy (2D NMR).[14] The quantified compounds were kaempferol, quercetin, taxifolin, catechin, quercitrin, astragalin, isoquercitrin, kaempferol-3-*O*-glucuronoide, quercetin-3-*O*-glucuronoide, and gallic acid. In this study, the content of these compounds was determined in different organs using external standard method. Baseline separation of compounds under investigation was achieved using gradient elution. The concentration of isolated flavonols from the hydroethanolic extract was determined by applying the peak area in the regression equation. Retention time, correlation coefficient, and regression equations for phenolic standards were mentioned in Table 2.

Different organs of *C. polygonoides* showed significant difference in their relative content but leaf, stem, and bark showed little difference in-between. Flavonol glycosides content was higher in all organs compared to the aglycones [Table 3]. Flowers and fruits were the richest organs in flavonols (kaempferol, quercetin, quercitrin, astragalin, isoquercitrin, kaempferol-3-*O*-glucuronide, and quercetin-3-*O*-glucuronide) [Figure 1]. Leaves, stems, and bark were the richest organs in taxifolin and catechin.

Simple screening of the major phytochemical classes in the roots, stems, buds, flowers, and seeds of *C. polygonoides* was reported.[26] In addition, quantification of phenolic compounds in the stem and buds growing in Pakistan were reported.[27] p-Coumaric acid was predominant in stem and gallic acid in buds. Moreover, investigation of these phenolic compounds in the callus, shoot, and cell suspension cultures was reported.[28] Catechin and kaempferol-3-*O*-glucuronide were the major compounds in shoot culture. Isoquercitrin and catechin were prominent in cell suspension culture.

Table 1: Radical scavenging activity, total phenolic, and total flavonoid contents of different organs of *Calligonum polygonoides* L.

Plant organ	DPPH radical scavenging[A]	Total phenolic content[B]	Total flavonoid content[C]
Bark	$450.30^{a} \pm 0.08$	$280.60^{a} \pm 4.00$	$217.21^{a} \pm 7.31$
Fruits	$2.95^{b} \pm 0.00$	$27.88^{b} \pm 0.68$	$53.93^{b} \pm 5.51$
Flowers	$369.91^{c} \pm 0.05$	$74.71^{bc} \pm 1.92$	$101.91^{c} \pm 8.68$
Leaves	$398.10^{d} \pm 0.00$	$281.51^{a} \pm 7.99$	$257.41^{a} \pm 12.54$
Stem	$148.11^{e} \pm 0.03$	$95.01^{c} \pm 2.06$	$87.45^{bc} \pm 0.52$

Values are presented in mean ± SE ($n = 3$). Means with the different letters in each column are significantly different ($P < 0.05$) according to Tukey's multiple comparison test

[A] DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity was expressed as µg ascorbic acid equivalent antioxidant capacity in 1g plant dry weight

[B] Total phenolic was expressed as µg gallic acid equivalent in 1 g plant dry weight

[C] Total flavonoid was expressed as µg rutin equivalent in 1 g plant dry weight

The content of quercetin, kaempferol, and their glycosides varied between the leaves, flowers, and fruits. The conjugated form, as glucuronide and glucoside, showed higher contents than the corresponding aglycones for the two compounds. The highest content of quercetin, as glucouronide, was found in the fruits, whereas the content of kaempferol glucuronide was highest in the flowers. Quercetin-3-*O*-glucuronide content in the fruits was 40-fold the aglycone content. kaempferol-3-*O*-glucuronide content in the flowers was 115-fold the aglycone content. The leaves showed the lowest contents of all the flavonoids determined in this study. The obtained results showed that this plant is an excellent source of various natural antioxidants. *Calligonum polygonoides* can be distinguished from a closely related species based on this phenolic chemical composition and the previously studied chemical composition of the essential oils.[29]

Table 2: Retention time (*Rt*), regression equations, and correlation coefficient for phenolic standards determined by HPLC in *Calligonum polygonoides* L.

Compound	Retention time (min)	Correlation coefficient (r^2)	Regression equation
Quercetin	26.21	0.9933	$y = 25.24x–85.36$
Taxifolin	16.73	0.9982	$y = 34.07x–177.41$
Catechin	7.51	0.9992	$y = 13.11x–20.38$
Gallic acid	4.10	0.9975	$y = 80.85x–20.87$

y is the area under the peak (AUP)
x is the respective concentration expressed in µg/mL

Table 3: Quantification of different phenolic constituents isolated from different organs of *Calligonum polygonoides* L.

Compounds	Concentration (µg/g plant dry weight)				
	Leaves	Stem	Fruits	Flowers	Bark
Kaempferol [A]	$8.12^{ac} \pm 0.03$	$10.41^{b} \pm 0.68$	$9.64^{bc} \pm 0.05$	$21.28^{d} \pm 0.03$	$7.52^{a} \pm 0.00$
Quercetin	$14.80^{a} \pm 0.47$	$21.39^{b} \pm 0.72$	$85.91^{c} \pm 0.20$	$107.43^{d} \pm 0.27$	$10.13^{e} \pm 0.02$
Taxifolin	$55.05^{a} \pm 3.35$	$41.47^{a} \pm 7.03$	$15.59^{b} \pm 0.63$	$19.04^{b} \pm 0.21$	$57.93^{a} \pm 2.38$
Catechin	$734.36^{a} \pm 14.96$	$1702.63^{b} \pm 14.96$	$61.56^{c} \pm 10.39$	$192.11^{c} \pm 3.92$	$1606.23^{ab} \pm 23.39$
Gallic acid	$34.86^{a} \pm 1.50$	$63.49^{a} \pm 7.49$	$202.35^{b} \pm 0.77$	$399.93^{c} \pm 1.89$	$149.32^{d} \pm 12.12$
Quercitrin [A]	$14.90^{a} \pm 0.10$	$15.44^{a} \pm 0.08$	$132.42^{b} \pm 0.38$	$144.66^{c} \pm 1.23$	$15.31^{a} \pm 0.20$
Astragalin [A]	$7.52^{a} \pm 0.00$	$7.52^{a} \pm 0.00$	$53.64^{b} \pm 0.62$	$68.01^{c} \pm 0.82$	$7.52^{a} \pm 0.00$
Isoquercitrin [A]	$67.62^{a} \pm 1.76$	$93.60^{b} \pm 0.68$	$802.61^{c} \pm 2.14$	$780.84^{d} \pm 2.49$	$15.19^{e} \pm 0.98$
Kaempferol-3-*O*-glucuronide [A]	$811.56^{a} \pm 12.23$	$204.48^{b} \pm 2.21$	$1701.86^{c} \pm 13.74$	$2447.38^{d} \pm 3.39$	$13.28^{e} \pm 0.01$
Quercetin-3-*O*-glucuronide [A]	$1199.32^{a} \pm 18.26$	$210.25^{b} \pm 1.18$	$3484.91^{c} \pm 9.81$	$2690.96^{d} \pm 22.98$	$19.40^{e} \pm 1.21$

Values are expressed as the mean ± SD ($n = 3$) at 365 nm for flavonols and 275 nm for other compounds Means with the different letters in each row are significantly different ($P < 0.05$) according to Tukey's multiple comparison test
[A] Results are expressed in µg/g plant material dry weight as quercetin equivalent

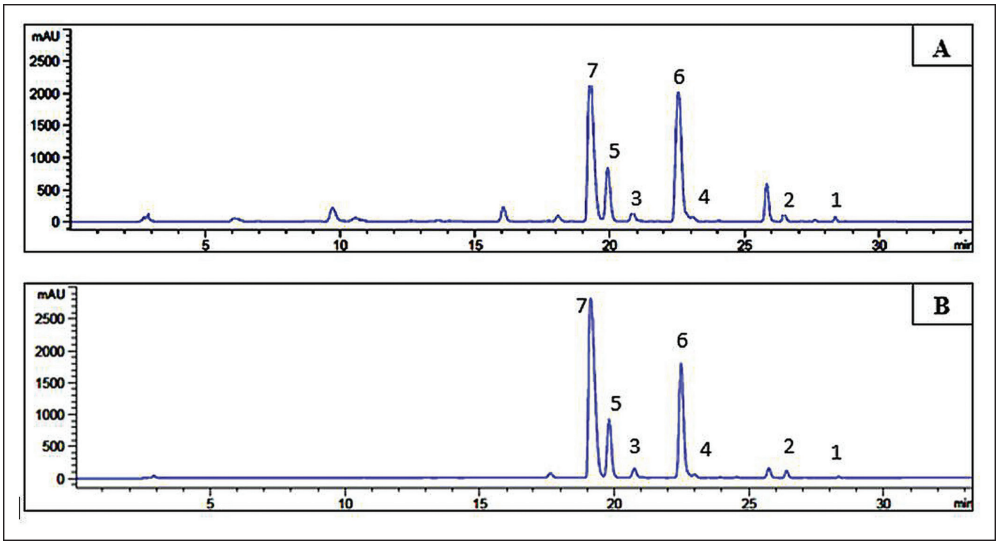

Figure 1: HPLC chromatogram (365 nm) of the flowers (A) and fruits (B) of *Calligonum polygonoides* L. 1: Kaempferol, 2: Quercetin, 3: Quercitrin, 4: Astragalin, 5: Isoquercitrin, 6: Kaempferol-3-O-glucuronide, 7: Quercetin -3-O-glucuronide

Conclusion

Investigation of antioxidant activity, total phenolic, and total flavonoid contents of different pant organs of *C. polygonoides* showed that bark and leaves were rich source in these phyto-constituents. Chromatographic analysis of the individual phenolic constituents in different organs of *C. polygonoides* showed that flowers and fruits were the richest organs in flavonols, whereas leaves, stems, and bark were the richest in taxifolin and catechin. Such studies are important in the use of this plant species as well as chemotaxonomic investigations.

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Conflicts of interest

There are no conflicts of interest.

References

1. Gouja H, Fernández AG, Garnatje T, Raies A, Neffati M. Genome size and phylogenetic relationships between the Tunisian species of the genus *Calligonum* (Polygonaceae). Turk J Botany 2014;38:13-21.
2. Soliman S, Mohammad MG, El-Keblawy AA, Omar H, Abouleish M, Madkour M, *et al*. Mechanical and phytochemical protection mechanisms of *Calligonum comosum* in arid deserts. PLOS ONE 2018;13:e0192576.
3. Umberto Q. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology. Boca Raton, FL: CRC Press; 2012.
4. Zouari S, Dhief A, Aschi-Smiti S. Chemical composition of essential oils of *Calligonum comosum* cultivated at the South-Eastern of Tunisia: A comparative study between flowering and fructification stages. J Essent Oil Bear PL 2012;15:320-7.
5. Ahmed HS, Moawad AS, Owis AI, AbouZid SF, Abdel-Rahman RF. Phytochemical screening and evaluation of biological activity of *Calligonum polygonoides* L. subsp. comosum. J Appl Pharm Sci 2015;5:022-6.
6. Al-Hammouri AA-N, Salman A-K, Ibbini J, Abusmier S, Sanogo S. Effect of biofumigation by *Calligonum polygonoides*, dry olive leaves, and ash of olive leaves on chilli pepper growth and recovery of *Rhizoctonia solani*. Acta Agric Sloven 2018;111:41-9.
7. Khan A, Khan RA, Ahmed M, Mushtaq N. In vitro antioxidant, antifungal and cytotoxic activity of methanolic extract of *Calligonum polygonoides*. Bangladesh J Pharmacol 2015;10:316-20.
8. Abushouk AI, Ismail A, Salem AMA, Afifi AM, Abdel-Daim MM. Cardioprotective mechanisms of phytochemicals against doxorubicin-induced cardiotoxicity. Biomed Pharmacother 2017;90:935-46.
9. Yawer MA, Ahmed E, Malik A, Ashraf M, Rasool MA, Afza N. New lipoxygenase-inhibiting constituents from *Calligonum polygonoides*. Chem Biodivers 2007;4:1578-85.
10. Shalabi M, Khilo K, Zakaria MM, Elsebaei MG, Abdo W, Awadin W. Anticancer activity of Aloe vera and *Calligonum comosum* extracts separetely on hepatocellular carcinoma cells. Asian Pac J Trop Biomed 2015;5:375-81.
11. Abdo W, Hirata A, Shukry M, Kamal T, Abdel-Sattar E, Mahrous E, *et al*. *Calligonum comosum* extract inhibits diethylnitrosamine-induced hepatocarcinogenesis in rats. Oncol Lett 2015;10:716-22.
12. Bannour M, Fellah B, Rocchetti G, Ashi-Smiti S, Lachenmeier DW, Lucini L, *et al*. Phenolic profiling and antioxidant capacity of *Calligonum azel* Maire, a Tunisian desert plant. Food Res Int 2017;101:148-54.
13. Cheruth AJ, Al Naqbi KM, El-Kaabi AAA, Odeh OW, Kandhan K, Maqsood S, *et al*. In vitro antioxidant activities and screening of phytochemicals from methanolic and ethyl acetate extracts of *Calligonum comosum* L'Her. Orient Pharm Exp Med 2016;16:209-15.
14. Ahmed H, Moawad A, Owis A, AbouZid S, Ahmed O. Flavonoids of *Calligonum polygonoides* and their cytotoxicity. Pharm Biol 2016;54:2119-26.
15. El–Sayyad S, Wagner H. A Phytochemical study of *Calligonum comosum* L. Henry. Planta Med 1978;33:262-4.
16. Toaima SM, Radwan MM, Asaad AM, El-sebakhy NA. Cytotoxic isoflavones from *Calligonum comosum* L'HÉR. Bulletin of Faculty of Pharmacy. Cairo University. 2007;45:171.
17. Samejo MQ, Memon S, Bhanger MI, Khan KM. Isolation and characterization of steroids from *Calligonum polygonoides*. J Pharm Res 2013;6:346-9.
18. Samejo MQ, Memon S, Bhanger MI, Khan KM. Chemical composition of essential oil from *Calligonum polygonoides* Linn. Nat Prod Res 2013;27:619-23.
19. Samejo MQ, Memon S, Bhanger MI, Khan KM. Essential oil constituents in fruit and stem of Calligonum polygonoides. Ind Crops Prod 2013;45:293-5.
20. Bakar MFA, Mohamed M, Rahmat A, Fry J. Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). Food Chem 2009;113:479-83.
21. Romani A, Pinelli P, Mulinacci N, Vincieri FF, Gravano E, Tattini M. HPLC analysis of flavonoids and secoiridoids in leaves of *Ligustrum vulgare* L. (Oleaceae). J Agric Food Chem 2000;48:4091-6.
22. Izzreen NMQ, Fadzelly MA. Phytochemicals and antioxidant properties of different parts of *Camellia sinensis* leaves from Sabah Tea Plantation in Sabah, Malaysia. Int Food Res J 2013;20:307-12.
23. Amirul Alam M, Juraimi AS, Rafii MY, Hamid AA, Aslani F, Alam MZ. Effects of salinity and salinity-induced augmented bioactive compounds in purslane (portulaca oleracea L.) For possible economical use. Food Chem 2015;169:439-47.
24. de Brum TF, Zadra M, Piana M, Boligon AA, Fröhlich JK, de Freitas RB, *et al*. HPLC analysis of phenolics compounds and antioxidant capacity of leaves of *Vitex megapotamica* (Sprengel) Moldenke. Molecules 2013;18:8342-57.
25. Chakraborty K, Joseph D, Praveen NK. Antioxidant activities and phenolic contents of three red seaweeds (division: rhodophyta) harvested from the gulf of mannar of peninsular India. J Food Sci Technol 2015;52:1924-35.
26. Samejo MQ, Memon S, Bhanger MI, Khan KM. Preliminary phytochemicals screening of *Calligonum polygonoides* Linn. J Pharm Res 2011;4:4402-3.
27. Samejo MQ, Memon S, Khan KM, Rajput SM, Gul S, Memon GZ, *et al*. Phenolic compounds and antioxidant activity of *Calligonum polygonoides* stem and Buds. Pak J Pharm Sci 2017;30:467-71.
28. Owis AI, Abdelwahab NS, Abul-Soad AA. Elicitation of phenolics from the micropropagated endangered medicinal plant *Calligonum polygonoides* L. (Polygonoaceae). Pharmacogn Mag 2016;12:465-70.
29. Dhief A, Zouari S, Abdellaoui R, Aschi-Smiti S, Neffati M. Comparative study of chemical composition of the essential oils from three *Calligonum* species growing-wild in Tunisian desert. J Essential Oil Bearing Plants 2011;14:11-22.