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Determination of total phenolic & flavonoids and antioxidant activity in *Calligonum polygonoides* L. from Thar Desert

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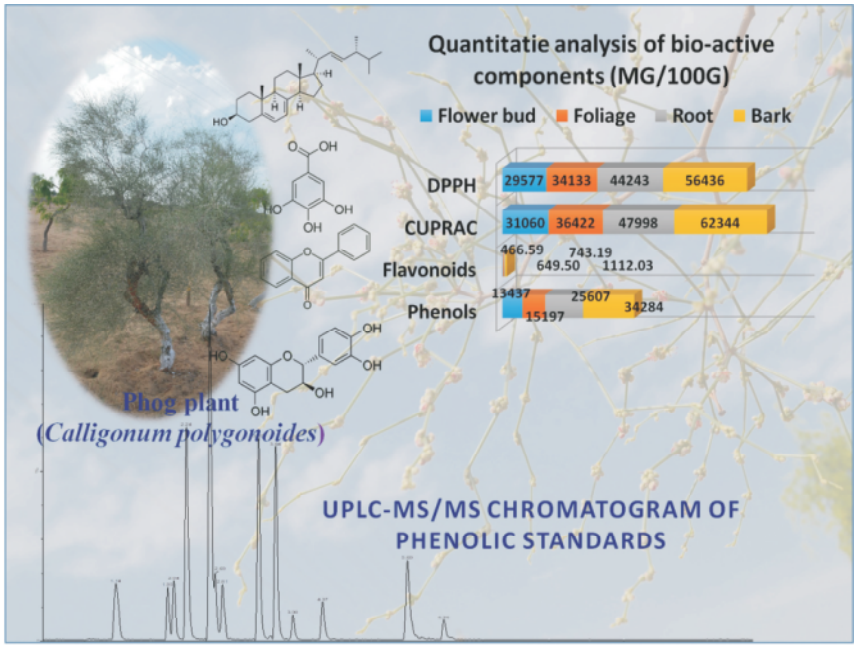
Abstract

Aim: The aim of the study was Identification and quantification of phenolic compounds, total flavonoides content and antioxidant potential of flower buds, foliages, bark and root of a perennial herb, *Calligonum polygonoides* (Phog) of Thar Desert.

Methodology: The methanolic extracts of different plant parts of *C. polygonoides* were subjected to LC-MS/MS (Waters Acquity UPLC-PDA, TQD) analysis for phenolic identification and spectrophotometric assay of total phenolic and flavonoids content and total antioxidant activity was estimated.

Results: A total of 15 phenolic compounds were identified and quantified, among which gallic acid content was abundant, followed by catechin. Besides these, the other major phenolic compounds detected in different plant parts were vanillic, chlorogenic acid, epicatechin, coumeric acid, catechol, vanillic acid, epicatechin and syringic acid. Extremely higher values were recorded for total phenolics, flavonoids and antioxidant capacity. The values for phenol, flavonoids and total antioxidant activity in bark extracts were higher than that of clove extract.

Interpretation: The identified phenolic compounds possess high antioxidant potential. These results provide scientific evidence for use of *C. polygonoides* as safe natural antioxidant compounds in nutraceutical and pharmaceutical industry.



Key words: *Calligonum polygonoides*, Nutraceuticals, Phenolics

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Introduction

Calligonum polygonoides L. is an endemic and threatened species reported from arid zones of India, especially 'Thar Desert'. It belongs to family Polygonaceae, and is popularly known as "*Phogala*" "*Phog*" and "*Phogaro*" by local community (Bhandari, 1978). *C. polygonoides* is a small high 3-4 feet perennial shrub with articulate, whitish and fragile branches, possessing winter shedding characteristics. It is highly drought and frost resistant in its native habitat of hot arid region of the 'Thar Desert' and is dominant biomass producer of this region (Khan, 1997). It grows on longitudinal transverse and parabolic dunes and considered as the major component of plant communities of *Psammophytic* scrub desert (Saxena and Singh, 1976). The western part of Rajasthan is hot arid region of India. In Rajasthan, *C. polygonoides* is widely distributed in Barmer, Bikaner, Churu, Jaipur, Jodhpur and Jaisalmer districts of Rajasthan (Kumar *et al*., 2015; Shetty, 1991; Sen, 1985). *C. polygonoides* is a multipurpose tree as all its components possess high economic values. The abortive flower buds and succulent fruits of *C. polygonoides* are important source as food for sustenance during frequently occurring famines (Goyal and Sharma, 2008; Kumar, 2005; Bhandari, 1990). The flower buds locally called "*Lasson*" is generally eaten by the local communities of the desert area along with butter milk (whey) or curd during summers for cooling, which detoxifying human body by neutralizing free radicals (Kumar, 2005; Singh and Pandey, 1998). The area of naturally occurring *C. polygonoides* plantation is reducing drastically due to its heavy demand in charcoal production industries, overgrazing and sand mining (Tadevosyan, 2001).

Among the secondary metabolites of plants, phenolic compounds comprise one of the most abundant groups of metabolites (Acosta-Estrada, 2014). They are widely distributed as secondary metabolites derived from phenylalanine or tyrosine amino acids. The phenolic compounds have been utilized as scavenging and inhibitory agents due to their pharmaceutical and medicinal properties (Saxena *et al*., 2020; Ayoub *et al*., 2016; Zivkovic, *et al*., 2009). However, the most important is their antioxidant property, which detoxify human body through neutralizing free radicals, and making efficient use of nutrients (Gomes *et al*., 2015). Recently, various studies have been carried out for identification and quantification of phenolic compounds and total antioxidant activities in different plants (Hem *et al*., 2015). To study the plant based phenolic compounds, the most common techniques are extraction with organic solvents like methanol, ethanol, acetones etc., in different concentration and combinations; while for quantification and identification of phenolic compounds, spectrophotometric and chromatographic techniques are mainly used.

Traditionally, *C. polygonoides* has been used as therapeutic agents against many diseases and disorders viz. the paste of *C. polygonoides* acts as an antidote against snake bite, heavy dose of opium and *Calotropis procera* has some medicinal properties for curing typhoid, asthma, cough and cold (Mohil, 2013; Katewa and Galav, 2005). Samejo *et al.* (2011) reported the presence of different secondary metabolites like phenolics, flavonoids, tannin, steroids and terpenoides in different parts of *phog* plant and higher scavenging activity against DPPH, ABTS and superoxide along with anti-fungal and cytotoxicity against *Aspergillus niger* and brine shrimp, respectively. Similarly there are some reports on identification of of some flavonoids compounds in *C. polygonoids* flower buds (Berwal *et al.*, 2021a; Gomes *et al*., 2015; Khan *et al*., 2015; Yawer *et al*., 2007). Inspite of these few studies, the reports on *C. polygonoids* scavenging properties are very scanty. Even two major international phenolic databases i) Phenol-Explorer database (Perez-Jimenez *et al*., 2010) and ii) USDA Database for the flavonoids content of selected foods (Bhagwat *et al*., 2014) don't have any report on *C. polygonoides*. Most of the the reports in literature regarding medicinal properties of *C. polygonoides* are based on rural wisdom and Indigenous Traditional Knowledge (ITKs) only. Therefore, the present investigation was planned to give scientific authenticity to the medicianal and nutraceutical properties of *C. polygonoides* through exploring the composition and concentrations of bio-active compounds like phenolics, flavonoids and total antioxidant activity present in it.

Materials and Methods

Plant material: Flower bud, foliage, bark and root of *C. polygonoides* were collected from the research farm of ICAR-Central Institute for Arid Horticulture, Bikaner (and subjected to shade drying in laboratory. Dried samples were ground and passed through 100 micron sieve for getting uniform samples. The sample powder was stored in air tight container at -20°C until further use. For comparison of phenolic content, flavanoids content and total antioxidant activity, clove was used as a standard. In our preliminarily study, we recorded very high amount of peholics and TAA in its different plant parts, than surveyed the literature and found that since clove is one of the highest phenolic containing plants food in literature, it has been used as positive standard for comparing our results (Perez-Jimenez *et al*., 2010).

Methanolic extract : Sample powder of different plant parts of *C. polygonoides* weighing 250 mg was homogenized with 20 ml of 70 % methanol using pestle and mortar. The solution was incubated at 70 ºC in water bath for 1 hr. and then subjected to centrifugation at 8000 rpm for 10 minutes at 4 ºC. The supernatant was collected. The residue was subjected to further extraction, twice, with 15 ml of 70% methanol and centrifuge. The supernatant was pooled and final volume was made up to 50 ml with 70 % methanol and stored at -20 ºC until further use.

Identification and quantification of phenolics using UPLC-

MS/MS: Methanolic extract was filtered and dried in vacuum concentrator without heating. The resulting residues were re-dissolved in 100 µl of absolute methanol. Phenolic compounds were analyzed through UPLC-MS/MS as described by Xia *et al.* (2011). Fifteen phenolic standards (gallic acid, epigallocatechin, catechin, catechol, chlorogenic acid, caffeic acid, vanillic acid, epicatechin, syringic acid, coumaric acid, ferulic acid, salicylic acid, quercetin, cinnamic acid and kaempferol) were used in this study. Each standard (500 µg ml^{-1} of each phenolic) was dissolved in 80 % methanol and separated individually. A mixture of standards was run and identified based on retention time and mass.

UPLC was performed on a Waters Acquity UPLC (Ultra Performance Liquid Chromatography) system (Waters, Milford, USA), equipped with a binary solvent delivery system with auto-sampler. The chromatographic separation was performed at 35 ºC on Acquity UPLC BEH C18 (1.7 µm, 2.1 x 100 mm) analytical column. The elution gradient consisted of mobile phase (A) methanol and mobile phase (B) 1 % acetic acid in water. The gradient program with the following proportions of solvent B was applied t (min), B %: (0, 95), (1, 80), (3, 65), (5, 50), (6, 25), (8, 25), (10, 50), (12, 85), (14, 95), (15, 95). The solvent flow rate was maintained at 0.4 ml min^{-1} and injection volume was set as 10 µm.

MS detection was performed using a Waters Acquity UPLC coupled to TQ mass spectrometer (Aquity) and MS system equipped with electro spray ionization (ESI) source operated in negative ion mode and a multiple reaction monitoring (MRM) scan mode. ESI ionization conditions were set at source temperature 150 ºC, negative ionization mode, source voltage " 3.2 kV. High purity nitrogen (> 99.999%) was used as curtain and auxiliary gas. The quantity of individual phenolics was calculated based on area and concentration of standards.

Total phenolics: The total phenolics in extracts were estimated following Folin-Ciocalteu method described by Medini *et al.* (2014). The total phenolics content of sample extracts was expressed as mg gallic acids equivalents (GAE) 100 g^{-1} d.wt. All samples were analyzed in triplicates.

Flavonoids: Total flavonoids content in extracts was determined by the aluminum chloride colorimetric assay (Medini *et al.,* 2014) with minor modifications. A volume of extracts (1 ml) was mixed with 0.3 ml each of 5 % $NaNO_2$ and 10 % $AlCl_3$ and 3.4 ml of 1 M NaOH. The mixtures were incubated for 15 min at room temperature and measured the OD at 510 nm against the reagent blank. The total flavonoids content was expressed as mg catechole equivalent (Ct. E) 100 g^{-1} d.wt. The complete flavonoids assay was carried out in three replications.

Total antioxidant activity by CUPRAC assay: The reducing capacity of the extracts was assayed by CUPRAC methods described by Apak *et al.* (2004) with some modifications. In this assay, 1 ml each of cupric chloride (10 mM), ethanolic neocuproin (75 mM) and ammonium acetate (1 M, pH 7.0) mixed simultaneously in a test tube containing 2 ml of distilled water followed by 100 µl methanolic extract. These mixtures were incubated in dark for 30 min at room temperature and the OD was read at 450 nm against the reagent blank. Ascorbic acid was used as positive reference standard and expressed in mg AAE 100 g^{-1}. The TAA as CUPRAC assays were replicated thrice to get mean values.

Total antioxidant activity by DPPH assay: The radical scavenging activity was assayed by DPPH method described by Re *et.al*. (1999) with some modifications. Accordingly, 100 µl methanolic extract was taken in test tubes and the volume was make up with 500 µl of distilled water followed addition of 0.006 % DPPH solution. The tubes were incubated under dark for 5 min and the OD was recorded at 517 nm. A blank set was also run simultaneously with distilled water. Ascorbic acid was used as positive reference standard and the results were expressed in mg AAE 100 g^{-1}. The TAA by DPPH assay was carried out in five replications.

Statistical analysis: The direction and magnitude of correlation between variables was performed using analysis of variance (ANOVA) and p values < 0.05 were regarded as significant. The assays were carried out in triplicate and the results were standardized and expressed as mean values. To assess the relationship between the activities of two different assays, Pearson's correlation coefficients (R) were calculated to determine their relationship.

Results and Discussion

Plant extracts have been extensively used to treat numerous diseases since centuries. The mode of action of these plant extracts is based on the presence of phenolic compounds (Sehitoglu *et al.*, 2015; Khoddami *et al.*, 2015). Phenolics are major phyto-chemical group widely occurring in plants having considerable physiological and morphological importance for plants with strong antioxidant potential. Several studies have been correlated with the antioxidant, anti-inflammatory, anti-cancer and antimicrobial activities of many plants species with their phenolic content (Kim *et al.*, 2016; Oueslati *et al.*, 2012). Thus, identification and quantification of phenolic compounds from different plant sources is becoming ever more important due to their potential application in pharmaceutical, nutraceutical and functional food industries.

In the UPLC-MS/MS chromatogram (Fig. 1), peak 1 exhibited a negative molecular ions at $[MS-H]^+$ at *m/z* of 168.91 and 124.82 for parent and daughter ions at RT of 1.18 min, respectively, which corresponded to gallic acid. Peak 2 had an *m/z* 305.06 and 124.82 at 1.99 RT, corresponding to epigallocatechin, peak 3 had an *m/z* 289.07and 108.84 at RT of

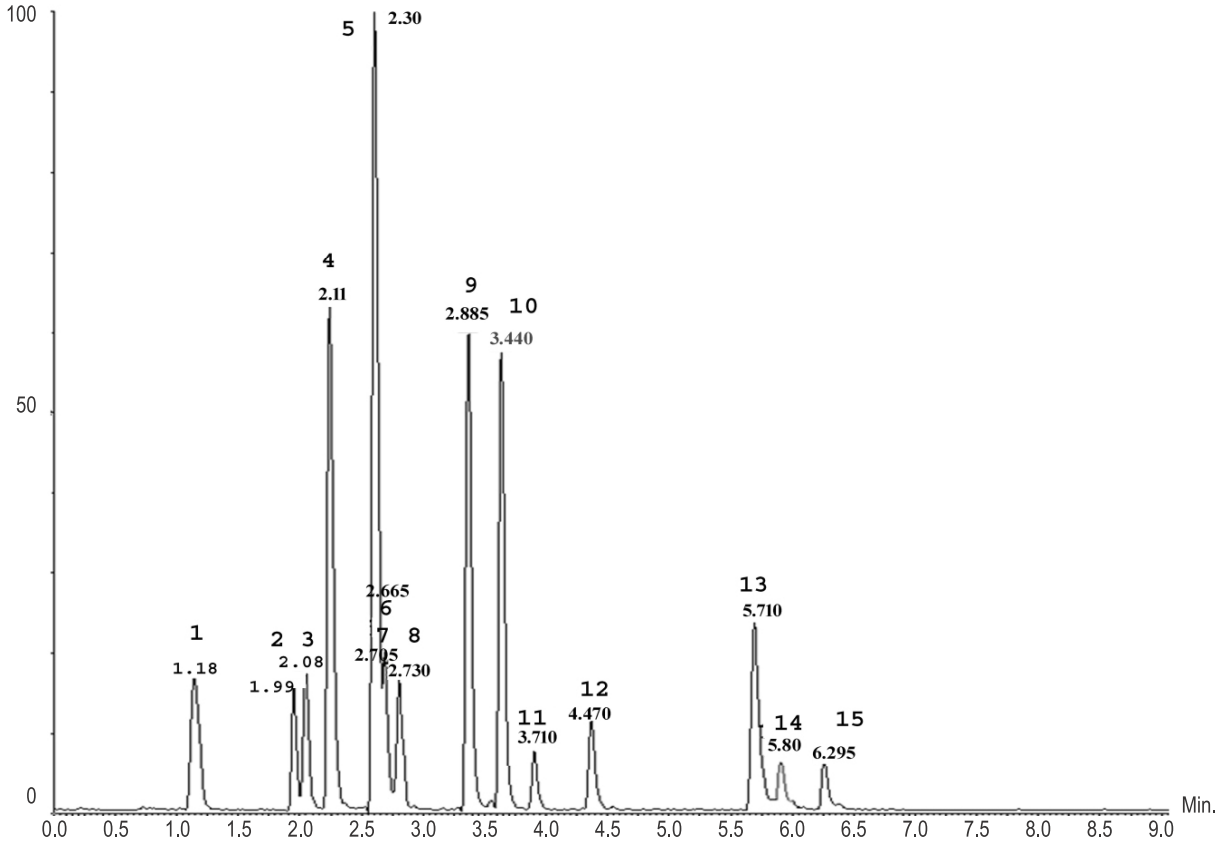

Fig. 1 : Standard chromatogram of mixture of 15 phenolic compounds (gallic acid, epigallocatechin, catechin, catechol, chlorogenic acid, caffeic acid, vanillic acid, epicatechin, syringic acid, coumaric acid, ferulic acid, salicylic acid, quercetin, cinnamic acid and kaempferol. Retention time (min.) of 1-15 compound is indicated.

2.08 min which indicated catechin. Peak 4 showed an *m/z* 108.89and 107.80 and were identified as catechol. Peak 5 had a negative molecular ion at an *m/z* of 353.12 and 190.11 at 2.30 RT and corresponding compound was identified as chlorogenic acid. Similarly, on the basis of negative molecular ions at different *m/z* of parental ion and daughter ion on different retention time, all fifteen peaks were identified for their corresponding phenolic compounds indicated in Table 1 according to their RT. In order to quantify the identified individual phenolic compounds, relationship was established between peak area and concentration through linear regression analysis of all the standards (Table 1). The correlation coefficient (r^2) of linearity curve between peak area and concentration was more than 0.999 for all the standards. The amount of each phenolic was expressed as mg 100 g^{-1} d. wt. basis. Interestingly, all the fifteen phenolic acids were detected in all plant parts of *C. polygonoides* in varying concentrations (Table 1). Among identified fifteen phenolic compounds, gallic acid was the main phenolic compound in all plant parts with more than 1181, 1163, 1097 and 1120 mg 100 g^{-1}, followed by catechin with 4.61, 65.09, 11.89 and 172.80 mg 100 g^{-1} in flower bud, foliage, bark and root extracts, respectively. The other phenolic acids in different plant parts showed different magnitudes. In flower buds, vanillic acid exhibited second highest content after gallic acid with 230.65 mg 100 g^{-1}, followed by catechin (4.91 mg 100 g^{-1}), chlorogenic acid (9.074 mg 100 g^{-1}), kaempferol (1.71 mg 100g^{-1}), coumaric acid (0.97 mg 100g^{-1}) and caffeic acid (0.79 mg 100 g^{-1}). The more abundant phenolic compounds found in foliages (after gallic acid) was epicatechin (182.10 mg 100 g^{-1}) followed by catechin (65.10 mg 100 g^{-1}), coumaric acid (11.73 mg 100 g^{-1}), catechol (5.92 mg 100 g^{-1}), epigallocatechin (4.40 mg 100 g^{-1}) and ferulic acid (1.91 mg 100 g^{-1}). In bark and root tissues, after gallic acid and catechin, epicatechin (2.42 and 4.94 mg 100 g^{-1}), syringic acid (1.42 and 2.26 mg 100 g^{-1}), vanillic acid (1.06 and 2.87 mg 100 g^{-1}) and epigallocatechin (0.98 and 3.16 mg 100 g^{-1}), respectively were the major phenolic acids present in *C. polygonoides*. The less abundant phenolics were salicylic acid, quercetin and cinnamic acid in all plant parts, while chlorogenic acid was observed in bark and root. Difference in composition and amount of phenolic content in different plant parts can be explained by the difference

Table 1 : UPLC-MS/MS parameters of selected phenolic compounds and content in flower bud, foliage, bark and roots of phog (mg $100g^{-1}$ d.wt.).

Phenolics	MRM transition (*m/z*)			MRM RT	Flower	Foliages	Bark	Root
	Parent ion	Daughter ion	Ionization mode	(min)				
Gallic acid	168.91	124.82	Neg	1.180	1181.81±10.13	1163.91±14.17	1097.14±12.06	1120.24±8.28
Epigallocatechin	305.06	124.82	Neg	1.990	0.21 ± 0.05	4.40 ± 0.08	0.98 ± 0.09	3.16 ± 0.18
Catechin	289.07	108.84	Neg	2.080	4.91 ± 0.17	65.09 ± 0.07	11.89 ± 0.71	172.80 ± 5.12
Catechol	108.89	107.80	Neg	2.110	0.17 ± 0.04	5.92 ± 0.03	0.17 ± 0.02	0.36 ± 0.05
Chlorogenic acid	353.12	190.11	Neg	2.300	9.07 ± 0.73	0.29 ± 0.02	0.05± 0.00	0.06 ± 0.00
Caffeic acid	178.95	134.83	Neg	2.665	0.79 ± 0.08	1.33 ± 0.14	0.085 ± 0.00	0.09 ± 0.00
Vanillic acid	166.95	107.81	Neg	2.705	230.65 ± 7.05	1.13 ± 0.13	1.06 ± 0.15	2.87 ± 0.37
Epicatechin	289.08	122.82	Neg	2.730	0.35 ± 0.01	182.09 ± 7.09	2.42 ± 0.03	4.94 ± 0.05
Syringic acid	197.00	122.80	Neg	2.885	0.27 ± 0.03	0.63 ± 0.09	1.423 ± 0.07	2.26 ± 0.03
Coumaric acid	162.94	118.84	Neg	3.440	0.97 ± 0.06	11.73 ± 0.35	0.09 ± 0.01	0.09 ± 0.00
Ferulic acid	192.97	133.85	Neg	3.710	0.35 ± 0.02	1.91 ± 0.01	0.46 ± 0.03	0.61 ± 0.01
Salicylic acid	137.92	93.82	Neg	4.470	0.34 ± 0.00	0.13± 0.01	0.08 ± 0.00	0.11 ± 0.00
Quercetin	301.03	150.81	Neg	5.710	0.38 ± 0.04	0.43± 0.04	0.21± 0.00	0.15 ± 0.02
Cinnamic acid	146.93	76.79	Neg	5.800	0.083± 0.00	0.35± 0.02	0.05 ± 0.01	0.28 ± 0.03
Kaempferol	285.03	92.81	Neg	6.295	1.71 ± 0.21	0.24± 0.01	0.162 ± 0.03	0.19 ± 0.01

*MRM-Multiple reaction monitoring; Parent ion (m/z): Molecular ions of the standard compounds (mass to charge ratio); Daughter ion-Molecular ions of the daughter ion of standard compounds (mass to charge ratio); RT: retention time; Neg- Negative-ion mode. Values are mean of replicates ±S.E.

in the tissue dependent bio-synthesis and accumulation of phytochemicals, physiological and morphological condition of the plant part like flower bud has a very short life span, foliages are photosynthetic tissues, bark is permanent hard tissue which has exposure to high temperature and light while roots are under underground tissues, and endogenous hormone levels (Chavan *et al.*, 2014). According to Amoo *et al.* (2012), the aerial plant parts are often considered appropriate for the bio-synthesis and accumulation of phytochemicals in abundance compared to the underground parts. Tuyen *et al.* (2017) reported variable composition and concentration of phenolic compounds in bark, flower, inner skin, kernel and leaf extracts of *Castanea crenata* Sieb. Similar findings were also reported from different plant parts viz. inner skin, flower, leaf and fruits of *Castanea sativa* Miller (Lee *et al.*, 2016; Barreira *et al.*, 2008).

The total phenolic contents of the extracts, expressed as gallic acid equivalents (GAE), varied from 13437 to 34284 mg GAE 100 g^{-1} in different plant parts *of C. polygonoides,* while clove possessed 25747 mg GAE 100 g^{-1} (Table 2). Bamdad *et al.* (2006) reported almost similar values for phenolic content (24391 mg GAE 100 g^{-1}) in clove. Among the different plant parts, bark contained the highest phenolic content (34284 mg GAE 100 g^{-1}) even higher than clove, the highest phenolic containing herbs reported till date (Perez-Jimenez *et al.*, 2010). The flower bud, foliage and roots of *C. polygonoides* contained 13437, 15197 and 25607 mg GAE 100 g^{-1} phenolics, respectively. Recently, Berwal *et al.*, (2021b) studied the seasonal changes in phenolic content of *C. polygonoides* foliages and reported as high as 8808 mg GAE 100 g^{-1} phenolic fresh weight basis. Phenolic content of root tissues of *C. polygonoides* was statistically at par with than that of clove ($p < 0.05$) whereas the phenolic content of flower bud and foliage was significantly lower than that of clove but was higher than that of Ceylan cinnamo (9700 mg GAE 100 g^{-1}), peppermint (8052 mg GAE 100 g^{-1}), cocoa powder (5624 mg GAE 100 g^{-1}) and star anise (5408 mg GAE 100 g^{-1}) etc., are highest phenolic containing plants reported after clove (Perez-Jimenez *et al.*, 2010).

The flavonoid content in different plant parts of *C. polygonoides* and clove, expressed as catechole equivalents (Ct.E) varied significantly ($p < 0.05$). Different plant parts of *C. polygonoides* namely flower bud, foliage, bark and root-contained 466.59, 649.50, 743.19 and 1112.19 mg Ct.E 100 g^{-1} flavonoids, respectively; while clove contained only 677.93 mg Ct.E 100 g^{-1}. When surveyed the USDA database for the flavonoids content of selected foods (Bhagwat *et al.*, 2014), it was revealed that only spices dried parsley (*Petroselinum crispum*) possesses higher flavonoids content with an average value of approximate 4850 mg 100^{-1} (varied from 2150 to 13851 mg 100 g^{-1}) than *C. polygonoides* (Huber *et al.*, 2009; Mattila *et al.*, 2000).

Like phenolic and flavonoid contents, TAA determined by both the systems varied significantly ($p < 0.05$) among the different plant parts with the magnitude of 31060 to 62344 mg AAE 100 g^{-1} (CUPRAC Assay) and 29577 to 56436 mg AAE 100 g^{-1} (DPPH radical scavenging activity) (Table 2). Bark tissue of *C. polygonoides* possessed significantly higher TAA than that of flower buds, foliages, roots as well as clove under CUPRAC and DPPH system. Whereas, flower bud, foliages and root tissues

Table 2 : Total phenolics, flavonoids and total antioxidant activity of different plant parts of *C. polygonoides* along with clove

Treatments	Total phenolics (mg GAE 100g^{-1} d.wt.)	Flavonoids (mg Ct.E 100g^{-1} d.wt.)	Total antioxidant activity (CUPRAC) mg AAE 100g^{-1} d.wt.)	Total antioxidant activity (DPPH) mg AAE 100g^{-1} d.wt.)
Flower bud	13437(115.92)*[a]	466.59 (45.39)[b]	31060 (176.24)[a]	29577 (172.13)[a]
Foliage	15197(123.28)[b]	649.50 (50.39)[c]	36422 (190.84)[b]	34133 (184.89)[b]
Root	25607(160.02)[c]	743.19 (59.35)[d]	47998 (219.09)[c]	44243 (210.68)[c]
Bark	34284 (185.15)[d]	1112.03 (73.15)[e]	62344 (249.69)[e]	56436 (237.86)[d]
Clove	25747(160.46)[c]	677.93 (36.78)[a]	57064 (238.88)[d]	33761 (183.85)[b]
SEm+	0.84	0.15	0.57	0.33
LSD (P < 0.05)	2.67	0.53	1.83	1.05
F calculated	1188.10	7476.96	2942.63	6411.66
Error degree of freedom	14	14	14	14

*Values in parenthesis are square root transformed. Value following different letter down the column are significantly different using Turkey's HSD test (p < 0.005). GAE- Gallic Acid Equivalent; Ct.E- Catechole Equivalent; AAE- Ascorbic Acid Equivalent and DW- Dry weight basis

Table 3 : Pearson's correlation matrix among total phenolic, flavonoids and antioxidant potential of *C. polygonoides*

	Total phenolics	Flavonoids	TAA (CUPRAC)	TAA (DPPH)
Total phenolics				
Flavonoids	0.984*			
TAA (CUPRAC)	0.994**	0.995**		
TAA (DPPH)	0.995**	0.994**	1.000**	1.000

**Significant at p < 0.01 (two-tailed); * Significant at p < 0.05 (two-tailed)

also exhibited high TAA but was significantly lower than that of clove in CUPRAC system. The values of TAA was recorded as 31060, 36422, 47998,62344 and 57064 mg AAE 100 g^{-1} in CUPRAC system and 29547, 34133, 44243, 56436 and 33761 mg AAE 100 g^{-1} under DPPH radical scavenging activity, respectively, in flower bud, foliage, roots, bark and clove. A significant positive correlation was observed among total phenolic, flavonoids and TAA in different plant parts of C. *polygonoids* along with clove with a magnitude >0.9 correlation coefficient (Table 3).

Most of the scientific reports on medicinal properties of *C. polygonoides* are based on rural wisdom and Indigenous Traditional Knowledge (ITKs). However, clinical or in-vitro study based reports on antioxidant, anti-fungal or cyto-toxic effect of C. polygonoides are very scanty. Khan et al. (2015) has reported its higher antioxidant activity on DPPH & ABTS scale along with anti-fungal acticity against Aspergillus niger and cyto-toxicity against brine shrimps. Few reports are available on phenolics and flavonoids content of *C. polygonoides* including a report on identification of some specific flavonoids by HPLC-EC (Gomes *et al.*, 2015). In this investigation we compared the phenolics, flavonoids and total antioxidant activity of *C. polygonoides* with clove as control, which ranked first in phenolic content and total antioxidant activity. The results of phenolic content of clove (highest phenolic containing plant food reported till date) in our study were completely resembling with previous reports (Perez-Jimenez *et al.*, 2010). The phenolic content of bark and root tissues of *C. polygonoides* is higher than that of some medicinal plants like red clover, Catharanthus roseus and Marrubium peregrinum, which posses abou 4688, 18280 and 8978 mg GAE 100 g^{-1} phenolics (Nisar *et al.*, 2017). The flavonoid content was higher in roots and bark while lower in flower bud and foliage than red clover (2661 mg 100 g^{-1}) (Esmaeili *et al.*, 2015). High values for bio-active compounds possessed by phog plant might be due to its resource poor habitat in The Thar Desert which includes high and low temperature, extremly resource poor soil, soil salinity and a prolonged drought period. A recent report of Lam *et al.* (2020) support this hypothese, they found that higher root zone temperature (28 ºC) drastically improved the accumulation of bio-active compounds in *Agastache rugosa*. Correlation analysis for phenolics, flavonoids and total antioxidant activity of different plant parts of *C. polygonoides* exhibited linear positive association among them (r = > 0.900) (Table 3). Erkan *et al.* (2016) also reported a close positive correlation between radical scavenging activity and total phenolic content of extract from various natural sources. like *Rosmarinus officinalis* L. and *Nigella sativa* L. extracts. Similarly, a strong positive association among phenolics, flavonoids and total antioxidant activity was reported in *Trifolium pratense* (red clover) by Esmaeili *et al.* (2015).

The results herein indicate that flower bud, foliage, bark and root extracts showed potential antioxidant activity along with one of the richest source of phenolic compounds. The beauty of

this plant is that it produces ample biomass and bioactive compounds under extreme resources poor conditions. There is indeed an urgent need to make available new plant-derived bioactive compounds; thus, these results scientifically authenticate the use of *C. polygonoides* as a source of natural effective and safe bio-active compounds for nutraceutical and pharmaceutical industry. Therefore, by the above mentioned high valued economic utilities of *C. polygonoides*, the regeneration and conservation of this plant is urgently required in 'Thar Desert'.

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Add-on Information

Author's contribution : M. K Berwal: Conceived, conceptualized, Investigations and writing of original draft; **S. M. Haldhar:** Sample preparations and investigations; **C. Ram:** statistical analysis and editing; **P.L. Saroj:** Reviewed and critically analyzed the manuscript.

Research content : The research content of manuscript is original and has not been published elsewhere.

Ethical approval : Not Applicable

Conflict of interest : The authors declare that there is no conflict of interest.

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