Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

GC-MS/MS-Based Phytochemical Screening of Therapeutic Potential of *Calligonum polygonoides* L. Flower Bud against Chronic Diseases

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Submitted: 02-Sep-2020 Revised: 08-Feb-2021 Accepted: 23-Mar-2021 Published: 10-Jun-2021

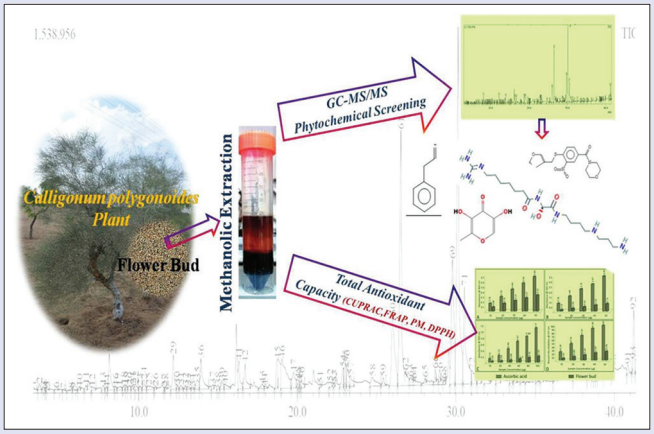
ABSTRACT

Background: *Calligonum polygonoides* is an endemic plant species, belongs to Polygonaceae family, native to the "Thar Desert" of India. It is highly tolerant to multiple stresses with dominant biomass and phytochemical producer under extreme niche. It has significant ethnopharmacological applications, but not yet scientifically validated. **Materials and Methods:** The methanolic extract of *C. polygonoides* flower bud was subjected to gas chromatography mass spectroscopy (GC-MS) analysis and antioxidant potential assay was done on different radical scavenging scales. The phytochemicals were identified based on retention time and matching their mass spectra to spectra in NIST 14 library. **Results:** The results revealed the presence of fatty acids, phenolics, terpenoides, flavanoids, alkaloids, tannins, steroids, ketones, esters, and amino acid derivatives, which comprises 93 compounds. Most of the detected compounds have been proved to possess important bio-activities such as anti-microbial, anti-inflammatory, anticancer, anti-diabetic, hepatoprotective, cardiovascular, antioxidant, and antimutagenic. Interestingly, some compounds such as furan-2,5-dimethyl, 2,3-dihydro-2,5-dihydroxy-6 -methyl-4H-pyran-4-one (DDMP), dehydromevalonic lactone, deoxyspergualin, 2-methoxy-4-vinylphenol, benzeneethanol-4-hydroxy-, quinic acid, lauric acid, linolenic acid, and squalene were detected which have proved pharmaceuticals applications against major diseases such as cancer, diabetics, cardiovascular, and some other chronic diseases. Furthermore, the methanolic extract also attributed very high level of antioxidant potential on cupric reducing antioxidant capacity, ferric reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazyl, and phosphomolybdenum assay scales. **Conclusion:** The identified phytochemicals with ample pharmaceutical application explore the worthiness of this endemic plant species. Along with pharmaceutical, it has an immense scope in nutraceutical and functional food industry. These medicinal importance advised for its conservation and artificial regeneration, to sustain the agro-ecological balance of Thar Desert of India.

Key words: Bioactive compounds, *Calligonum polygonoides,* GC-MS/MS, pharmaceutical applications, phog, phytochemical screening

SUMMARY

- In the present study, GC-MS/MS based phytochemical screening along with antioxidant potential on different scavenging scales like cupric-reducing antioxidant capacity, ferric-reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazyl, and phosphomolybdenum assay has been done in methanolic extract of *Calligonum polygonoides* flower bud. The presence of some distinct phytochemicals like furan-2,5-dimethyl, DDMP, dehydromevalonic lactone, deoxyspergualin, 2-methoxy-4-vinylphenol, benzeneethanol-4-hydroxy and some ω-3 fatty acids with well-established pharmaceuticals applications along with extremely higher total antioxidant activity on all scales revealed its medicinal properties against cancer, diabetics, cardiovascular, and other chronic diseases.



Abbreviations used: CUPRAC: Cupric reducing antioxidant capacity; FRAP: Ferric reducing antioxidant power assay; PM: Phosphomolybdenum assay; DPPH: 2,2-diphenyl-1-picrylhydrazyl assay; TAA: Total antioxidant activity; RT: Retention time.

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DOI: 10.4103/pm.pm_390_20

Access this article online
Website: www.phcog.com
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INTRODUCTION

Plant-based natural products either a solvent extracts or isolated pure compounds provide ample opportunities for discovery of new drugs because of the unparalleled accessibility and availability of phytochemical diversity. Therefore, plants play a significant role in prevention and cure of diseases and can also avoid and reduce the adverse effects of conventional treatments.[1] The medicinal and therapeutic properties of plants are due to the presence of phytochemicals such as phenolics, flavonoids, tannins, alkaloids, terpenoids, and steroids.[2,3] These compounds are produced in plants as secondary metabolites and have

Cite this article as: Berwal MK, Haldhar SM, Ram C, Gora JS, Singh D, Samadia DK. GC-MS/MS-based phytochemical screening of therapeutic potential of *Calligonum polygonoides* L. flower bud against chronic diseases. Phcog Mag 2021;17:S68-76.

been proved for their disease preventing and curing effects through their antioxidant, antibacterial, anti-inflammatory, antihypertensive, anti-aging, and anti-allergic activities. Antioxidant compounds are the gift of nature to scavenge free radicals through different ways, thus play an important role in protection of biologically important cellular components like DNA, proteins and membrane lipids, from ROS attacks leading to cell damage, which has been associated to aging, inflammation, atherosclerosis, ischemic injury, and finally to cancer.[4-6] Consequently, in the present scenario, plant-based phytochemicals/bioactive compounds are not only used as functional food ingredients but also in a large number of health promoting preparation, have become a potential topic for research and development.[7]

C. polygonoides (L.) is an endemic and threatened plant species belongs to the Polygonaceae family and known for its medicinal properties. *C. polygonoids* is highly tolerant to all type of abiotic stresses and emerged as predominant biomass producer in resource-limited environmental condition in its native habitat at "Thar Desert" of India.[8] It is popularly known as "*Phogala*" "*Phog*" and "*Phogaro*" by local community of "Thar Desert."[9] It grows on longitudinal transverse and parabolic dunes and considered as the major component of plant communities of *Psammophytic* scrub desert.[10] *C. polygonoides* possess high economic values as its all plant parts are being utilized in different purposes. The abortive flower buds and succulent fruits of *C. polygonoides* are the important source as food for sustaining during frequently occurring famines.[11-13] The flower buds locally called as "*Lasson*" is generally eaten by the local communities of the desert area along with of butter milk (whey) or curd during summers for cooling the body and to cure sun stroke.[12,14]

Conventionally, *C. polygonoides* has been used as therapeutic agents against many diseases and disorders, namely the paste of *C. polygonoides* acts as an antidote against snake bite, heavy dose of opium, and *Calotropis procera* and has some medicinal properties as for curing typhoid, asthma, cough, and cold.[15,16] Samejo*et al.*[17] reported the presence of different secondary metabolites in different parts of *Phog* plant, namely phenolics, flavonoids, tannin, steroids and terpenoides and showed its higher scavenging activity against DPPH, ABTS and superoxides along with anti-fungal and cytotoxicity against *Aspergillus niger* and brine shrimp, respectively. Similarly, some reports are also available to exploit its flower buds and identified few flavonoid compounds.[18-20] Recently, in our previous study, it has been reported that different plant parts such as flower bud, foliages, root, and bark possessed very high phenolic content (135, 151, 256, and 345 mg GAE/g DW, respectively) along with very high flavonoids and total antioxidant activity (TAA).[21] The literature survey revealed that some pytochemicals such as Calligonolides, tetracosan-4-olide, steroidal ester, b-sitosterol, b-sitosterolglucoside, and ursolic acid have been reported and isolated from *C. polygonoides* and also reported that its essential oil contains a complex mixture of hydrocarbons, terpenoids, phenolics, ketones, and acid derivatives.[22] The present investigation of GC-MS/MS analysis of endangered rare herb *C. polygonoides* has been taken up to carry out gas chromatography and mass spectra analysis of flower bud extract to decipher the major phytochemicals and its antioxidant capacity, responsible for its medicinal and therapeutic properties.

MATERIALS AND METHODS

Plant sample

Fresh flower buds of endemic herb *C. polygonoids* were collected during its flowering season i.e., 1st week of April, 2019 from plant grown at research farm of ICAR-Central Institute for Arid Horticulture, Bikaner (Rajasthan), India. The flower buds were air dried at room temperature for 4 days, powdered with milling machine and stored at −20°C till further use.

Chemicals and reagents

Ultrapure water (18.2 MΩ cm; Milli-Q Simplicity, Millipore, France) was used in all the assays employed. The chemicals and reagents used in present study with name, purity, grade, and make are as follows: Ammonium acetate (98%, GR, Merck India); Ascorbic acid (99%, GR, Himedia); Cuppric chloride (98%, GR, Merck India); DPPH (93.5%, GR, Merck India), $FeCl_3.6H_2O$ (99%, GR, Himedia); HCl (35%–38%, Merck India); Methanol (99.5%, HPLC Grade, Merck India); Methoxyamine (98%, GR, Merck India); Neocuproine (99.9%, GR, Merck India); Phosphomolebdate (99.9%, Sigma aldrich); Pyridine (99.9%, Sigma aldrich); TPTZ (99%, GR, Himedia).

Extraction of crude extracts

The dried and powdered sample of flower buds (200 mg) was homogenized in with prechilled mortar-pestle in 3 ml of precooled HPLC grade methanol (100%). The homogenate was shaken for 10 min at 70°C in a water-bath at 950 rpm and centrifuged for 10 min at 11,000 g. The supernatant was divided in two aliquots. One aliquot was used for TAA assays and second aliquot was collected in a Schott GL14 glass vial and 1.5 ml of prechilled chloroform and 3.0 ml of dH_2O (4°C) was added and vortex for 20 s. Thereafter, the mixture was centrifuged at 2200 g for 15 min. Both upper (polar) and lower (non-polar) phases were transferred into a separate test tube and evaporated to dryness in a nitrogen stream.

Total antioxidant activity

TAA of methanolic extract of *C. polygonoides* was determined on four different methods, namely cupric reducing antioxidant capacity (CUPRAC), ferric-reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and phosphomolybdenum (PM) assay with following the standard methods.

TAA was determined on CUPRAC assay in accordance with the methods described by Apak *et al.*[23] 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 test tubes containing 1.9 ml of distilled water. A volume of 100 µl methanolic extracts of *C. polygonoides* flower buds was added in each tube separately from serially diluted extracts with final concentrations of 100, 200, 300, 400, and 500 µg/ml. Simultaneously, ascorbic acid standard was also run with same concentrations, i.e., 10, 20, 30, 40, and 50 µg/ml. These mixtures were incubated for half an hourin dark and measured the absorbance (OD) at 450 nm against the reagent blank using ultraviolet-visible (UV-VIS) spectrophotometer (UV-2550, SHIMADZU). All assays were carried out in triplicate.

FRAP assay was carried out following the method described by Benzie and Strain[24] with some modifications. 100 µl methanolic extracts of *C. polygonoides* flower buds was added from serially diluted extracts with final concentrations of 100, 200, 300, 400, and 500 µg/ml separately in test tubes containing 2.9 ml of FRAP working reagent along with same concentrations of ascorbic acid as reference standard. The fresh FRAP working reagent wasprepared with mixing of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution in 10:1:1 ratio. The reaction mixture was allowed to react under dark for 30 min. The absorbance of coloured complex (ferrous tripyridyltriazine complex) was taken at 593 nm using UV-VIS spectrophotometer (UV-2550, SHIMADZU). All assays were carried out in triplicate.

The PM assay was performed according to the method described by Prieto *et al.*[25] Methanolic extract (100 µl) from serially diluted extracts

with final concentrations of 100, 200, 400, 600, 800, and 1000 µg/ml was added to each test tube individually containing 2.9 ml of distilled water and 1 ml of PM reagent. The tubes were incubated at 95°C for 90 min. After incubation, the tubes to room temperature and read the absorbance at 695 nm using UV-VIS spectrophotometer (UV-2550, SHIMADZU). Mean values from three independent replicates were calculated for each sample. Ascorbic acid in similar concentrations, i.e., 10, 20, 40, 60, 80, and 100 µg/ml was used as positive reference standard.

The DPPH scavenging assay was done according to the method reported by Berwal *et al.*[26] with some modifications. 100 µl methanolic extracts of *C. polygonoide* from serially diluted extracts with final concentrations of 100, 200, 300, 400, and 500 µg/ml was allowed to react separately with 2.9 ml of 0.006% methanolic DPPH for 10 min under dark condition. A control was also run simultaneously with 100 µl distilled water instead of extract. The absorbance was taken at 517 nm using UV-VIS spectrophotometer (UV-2550, SHIMADZU). Ascorbic acid was used as reference standard with similar concentrations of the sample. Whole assay was carried in five replicates and averaged. The percent inhibition was calculated by using the following equation:

$$\% \ Inhibition = \left(\frac{OD \ of \ Control - OD \ of \ Sample}{OD \ of \ Control} \right) \times 100$$

Derivatization of dried extract

The dried extract was derivatized by following the method described by Raval *et al.*[27] The dried extract was re-dissolved in 50 µl pyridine and sonicated for 10 min. Then, 100 µl methoxyamine hydrochloride (HCl) in pyridine (20 mg/ml) was added and vortexed for 30 s. The mixture was then sonicated again for 5 min and incubated for 90 min with constant agitation at 37°C. The trimethylsilylation step was performed with addition of 250 µl N-Methyl-N-(trimethylsilyl) trifluoroacetamide to the extract and vortexed for 30 s and the mixture was incubated for 1 h at 37°C for derivatization.

The GC–MS/MS analysis

The GC-MS/MS analysis of bioactive compounds from methanolic extract of the flower bud of *C. polygonoides* was performed using gas chromatography-mass spectrometer (GCMS-QP2010 Plus, SHIMADZU). For GC-MS analysis, 4 µl of derivatized extract was injected into a DB-17MS capillary column (30 m × 0.25 mm). The injection temperature was set to 280°C. After a solvent delay for 5 min, initial GC oven temperature was set at 65°C; after injection for 2 min, the GC oven temperature was raised to 290°C. The injection temperature was set at 280°C and ion source temperature to 230°C. Helium was used as the carrier gas with a stable flow rate of 1 ml/min. The measurement was performed with electron impact ionization (70 eV) in the full scan mode (*m/z* from 50 to 900) to a scan speed of 2000. Phytochemicals were putatively identified based on GC retention time on DB-17MS capillary column and matching their mass spectra to spectra in NIST 14 library. Preprocessing of total ion chromatograms such as baseline correction, alignment, peak picking and integration were performed using the ACD/Spec Manager v. 12.00 (Advanced Chemistry Development, Inc., ACD/Labs, Toronto, Canada). CSV comma delimited files were created for data analysis.

Statistical analysis

The statistical significance among the experiments was calculated using Student's *t*-test at $P < 0.05$ in Microsoft excel.

RESULTS

Phytochemical characterization of methanolic extract of *Calligonum polygonoides* by GC-MS/MS

The results pertaining to GC-MS/MS analysis of the methanolic extract of dried flower bud of *C. polygonoides* lead to the detection and identification of a number of compounds. The detected compounds were identified through matching their mass spectra to spectra in NIST 14 library. The details including RT (min), peak area (%), name, molecular formula, and molecular weight of various components present in the flower bud of *C. polygonoides* that were detected and identified through the GC-MS/MS [Table 1]. From the GC-MS/MS chromatogram [Figures 1 and 2], in the methanolic extracts of flower bud a total of 93 compounds were detected which includes fatty acids, phenolics, flavanoids, alkaloids, terpenoides, tannins terpenoids, steroids, ketones, amino acid derivaties, etc. Based on the literature, most of the constituents revealed by GC-MS/MS are biologically active compounds with proved nutraceutical and pharmaceutical applications. In identified phytochemicals, the major portions are fatty acids (>62%) followed by phenolic and flavonoids compounds (>13%), terpenoids (~5%), and alkanes (~2%) [Table 1]. Major portion of fatty acids in flower buds is essential fatty acids linoleic and linolenic acids and also contains 12 carbon long fatty acids called dodecanoic acid (lauric acid). It also contains 9, 12, 15-Octadecatrienoic acid an ω-3 fatty acid, which is not very common fatty acid in plant fats. Similarly, a number of other bioactive compounds were also detected and identified with established medicinal and therapeutic properties such as antioxidant, antimicrobial, anti-proliferative, anti-diabetic, hepatoprotective, and cardiovascular drugs. The major bioactive compounds, N-(2-Methylbutylidene) isobutylami (peak 3); Furan, 2,5-dimethyl-(peak 6); 1-Butanamine, 2-methyl-N-(2-methylbutylidene)-(peak 9); 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (peak 11); Butanoic acid, 4-hydroxy-(peak 14); Propanoic acid, 3-(trimethylsilyl)-(peak 18); Phenol, 2-methoxy-/o-guaiacol (peak 25); 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one, (peak 29); Benzoic acid trimethylsilyl ester (peak 31); 2-Pyrrolidinone (peak 30); 1,6-Octadien-3-ol, 3,7-dimethyl-, (. +/-.)-(peak 32); 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-(peak 33); 1,2-Benzenediol/catechol (peak 35); Benzofuran, 2,3-dihydro-/Coumaran (peak 36); Dehydromevaloniclactone (peak 37); deoxyspergualin (DSG) (peak 38); 2-Methoxy-4-vinylphenol (peak 41); 3-(4-Hydroxyphenyl) propionitrile (peak 42); 2H-Pyran-2-one, tetrahydro-4-hydroxy-4-methyl-(peak 43); Benzoic acid, 3-(1-methylethyl)-(peak 44); 1,2,3-Benzenetriol/pyrogallol (peak 45); Benzeneethanol, 4-hydroxy-(peak 50); 2,5-Pyrrolidinedione, 1-(2-methylene-3-butenyl)-(peak 51); 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-(peak 53); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (peak 57); (1R,3R,4R,5R)-(-)-Quinic acid (peak 60); Squalene (peak 85) etc., were detected in methanolic extracts of flower bud of *C. polygonoids* with established pharmaceutical applications.

Total antioxidant activity of methanolic extract of *Calligonum polygonoides*

The antioxidant potential of methanolic extract of *C. polygomoides* flower bud was also evaluated based on its reducing capacity by different methods such as CUPRAC, FRAP, DPPH and PM assay. The reducing power is directly reflected by absorbance in CUPRAC, FRAP and PM assays, while percent reduction in DPPH assay. Determinations of reducing power in amalgamation of different methods help in comprehend the real nature of the antioxidant present in it. Figure 3 portrays the total antioxidant capacity of flower bud methanolic

Table 1: Compounds detected in methanolic extract of *C. polygonoides* flower bud through gas chromatography mass spectroscopy/mass spectroscopy analysis

Peak number	RT (min)	Area (%)	Compounds	Molecular formula	Molecular weight
1	3.331	0.10	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	102
2	3.729	0.23	Butanoic acid, 2-msethyl- trimethylsilyl ester	$C_8H_{18}O_2Si$	174
3	3.993	0.12	N-(2-Methylbutylidene)isobutylami	$C_9H_{19}N$	141
4	4.132	0.06	4-Pyranone, 2,3-dihydro-	$C_5H_6O_2$	98
5	4.198	0.09	5-(2-Pyridylamino)-2-pyrrolidone	$C_9H_{11}N_3O$	177
6	4.781	0.08	Furan, 2,5-dimethyl-	C_6H_8O	96
7	5.249	0.04	DL-Glutamic acid	$C_5H_9NO_4$	147
8	5.748	0.08	Hexanoic acid	$C_6H_{12}O_2$	116
9	5.861	0.08	1-Butanamine, 2-methyl-N-(2-methylbutylidene)-	$C_{10}H_{21}N$	155
10	6.262	0.14	1H-Imidazole, 2,4-dimethyl-	$C_5H_8N_2$	96
11	6.673	0.24	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144
12	7.054	0.25	2-Hexenoic acid	$C_6H_{10}O_2$	114
13	7.746	0.23	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	110
14	7.981	0.13	Butanoic acid, 4-hydroxy-	$C_4H_8O_3$	104
15	8.141	1.86	Ethyl trans-2-trimethylsilyl-cyclopropane-1-carboxylate	$C_9H_{18}O_2Si$	186
16	8.535	0.18	Decanal dimethyl acetal	$C_{12}H_{26}O_2$	202
17	8.795	0.32	1,3-Cyclohexanedione	$C_6H_8O_2$	112
18	9.085	0.26	Propanoic acid, 3-(trimethylsilyl)- ethyl ester	$C_8H_{18}O_2Si$	174
19	9.192	0.21	4-Pyridinol	C_5H_5NO	95
20	9.419	0.27	2,4-Pentadienoic acid, 1-cyclopenten-3-on-1-yl ester	$C_{10}H_{10}O_3$	178
21	9.636	0.25	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	128
22	9.802	0.29	Benzeneacetaldehyde/Phenylacetaldehyde;	C_8H_8O	120
23	10.058	0.19	Pantolactone	$C_6H_{10}O_3$	130
24	10.321	0.54	Thymine/2,4-Dihydroxy-5-methylpyrimidine	$C_5H_6N_2O_2$	126
25	10.613	0.49	Phenol, 2-methoxy- (o-Guaiacol)	$C_7H_8O_2$	124
26	11.052	0.14	Tridemorph/Morpholine, 2,6-dimethyl-4-tridecyl-	$C_{19}H_{39}NO$	297
27	11.609	0.05	3-Hexanone, 2,2-dimethyl-	$C_8H_{16}O$	128
28	11.700	0.11	Methanone, [4-(2-furfurylthio)-3-nitrophenyl](morpholino)-	$C_{16}H_{16}N_2O_5S$	348
29	12.175	1.61	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144
30	12.412	0.18	2-Pyrrolidinone	C_4H_7NO	85
31	12.643	0.23	Benzoic acid trimethylsilyl ester	$C_{10}H_{14}O_2Si$	194
32	12.953	0.15	1,6-Octadien-3-ol, 3,7-dimethyl-, (.+/-.)-	$C_{10}H_{18}O$	154
33	13.104	0.17	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	$C_6H_6O_4$	142
34	13.373	0.10	Cyclopentanethiol, 1-methyl-	$C_6H_{12}S$	116
35	13.703	0.72	1,2-Benzenediol catechol	$C_6H_6O_2$	110
36	13.917	2.08	Benzofuran, 2,3-dihydro- Coumaran	C_8H_8O	120
37	14.657	0.23	Dehydromevalonic lactone	$C_6H_8O_2$	112
38	15.000	0.18	Deoxyspergualin	$C_{17}H_{37}N_7O_3$	387
39	15.255	0.49	2-Oxepanone, 7-methyl-	$C_7H_{12}O_2$	128
40	15.442	0.45	Cyclopentanecarboxylic acid, allyl ester	$C_9H_{14}O_2$	154
41	16.317	1.28	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150
42	16.700	1.53	3-(4-Hydroxyphenyl) propionitrile	C_9H_9NO	147
43	17.784	0.33	2H-Pyran-2-one, tetrahydro-4-hydroxy-4-methyl-	$C_6H_{10}O_3$	130
44	17.935	0.12	Benzoic acid, 3-(1-methylethyl)-	$C_{10}H_{12}O_2$	164
45	18.742	1.25	1,2,3-Benzenetriol :Pyrogallol	$C_6H_6O_3$	126
46	19.033	4.09	Dodecanoic acid	$C_{11}H_{22}O_2$	186
47	19.755	1.32	Benzaldehyde, 2-hydroxy-6-methyl-	$C_8H_8O_2$	136
48	20.036	0.31	3,10-Dioxa-6,7-dithia-2,11-disiladodecane, 2,2,11,11-tetramethyl-	$C_{10}H_{26}O_2S_2Si_2$	298
49	20.159	0.23	DL-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143
50	20.288	0.46	Benzeneethanol, 4-hydroxy-	$C_8H_{10}O_2$	138
51	21.444	0.13	2,5-Pyrrolidinedione, 1-(2-methylene-3-butenyl)-	$C_9H_{11}NO_2$	165
52	22.065	0.34	1,3-Diethylimidazolidine	$C_7H_{16}N_2$	128
53	22.337	0.13	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180
54	22.896	0.96	Tetradecanoic acid	$C_{14}H_{28}O_2$	228
55	23.028	1.14	Tetradecanoic acid, trimethylsilyl ester	$C_{17}H_{36}O_2Si$	300
56	23.132	1.21	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
57	23.354	0.76	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
58	24.745	1.19	Nonanoic acid	$C_9H_{18}O_2$	158
59	25.417	0.35	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
60	26.022	6.02	(1R,3R,4R,5R)-(-)-Quinic acid	$C_7H_{12}O_6$	192
61	26.533	17.51	Hexadecanoic acid (n-Palmitic acid)	$C_{16}H_{32}O_2$	256
62	26.826	0.05	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216
63	27.145	0.10	(1R,3R,4R,5R)-(-)-Quinic acid	$C_7H_{12}O_6$	192
64	27.345	0.03	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$	278

Contd...

Table 1: Contd...

Peak number	RT (min)	Area (%)	Compounds	Molecular formula	Molecular weight
65	28.064	0.18	Eicosanoic acid	$C_{20}H_{40}O_2$	312
66	28.698	0.36	9-Octadecenoic acid (Z)-	$C_{19}H_{36}O_2$	296
67	28.976	0.35	9,12-Octadecadienoic	$C_{19}H_{34}O_2$	294
68	29.243	0.04	1,2-Benzenedicarboxylic	$C_{20}H_{30}O_4$	334
69	29.792	8.46	Oleic acid	$C_{18}H_{34}O_2$	282
70	30.140	20.22	9,12-Octadecadienoic	$C_{19}H_{34}O_2$	294
71	30.542	6.49	9,12,15-Octadecatrienoic acid	$C_{19}H_{32}O_2$	292
72	31.024	1.27	Octadecane	$C_{18}H_{38}$	254
73	31.439	0.82	13-Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	254
74	31.843	0.55	Octadecanamide	$C_{18}H_{37}NO$	283
75	32.639	0.68	Eicosanoic	$C_{20}H_{40}O_2$	312
76	33.465	0.21	7-Hexadecenal,	$C_{16}H_{30}O$	238
77	3.781	0.18	Eicosane	$C_{20}H_{42}$	282
78	34.123	0.14	9-Hexacosene	$C_{26}H_{52}$	364
79	34.508	0.06	2-Decanone	$C_{10}H_{20}O$	156
80	35.102	0.17	Tetratriacontane	$C_{34}H_{70}$	478
81	35.400	0.22	Eicosanoic acid	$C_{20}H_{40}O_2$	312
82	36.319	0.27	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330
83	37.576	0.31	n-Docosane	$C_{22}H_{46}$	310
84	38.371	0.33	3,6-Nonadecadione	$C_{19}H_{36}O_2$	296
85	38.597	0.15	Squalene	$C_{30}H_{50}$	410
86	38.751	0.12	Cyclodocosane, ethyl-	$C_{24}H_{48}$	336
87	38.851	0.06	6-Pentadecanone	$C_{15}H_{30}O$	226
88	38.979	0.18	1-Triacontanol	$C_{30}H_{62}O$	438
89	39.357	0.20	2-Nonadecanol	$C_{19}H_{40}O$	284
90	39.929	0.41	Trichloroacetic acid hexadecyl ester	$C_{18}H_{33}Cl_3O_2$	386
91	0.852	0.40	3,6-Nonadecadione	$C_{19}H_{36}O_2$	296
92	41.221	2.30	Pentafluoropropionic	$C_{20}H_{35}F_5O_2$	402
93	41.341	0.37	9-Hexacosene	$C_{26}H_{52}$	364

RT: Retention time

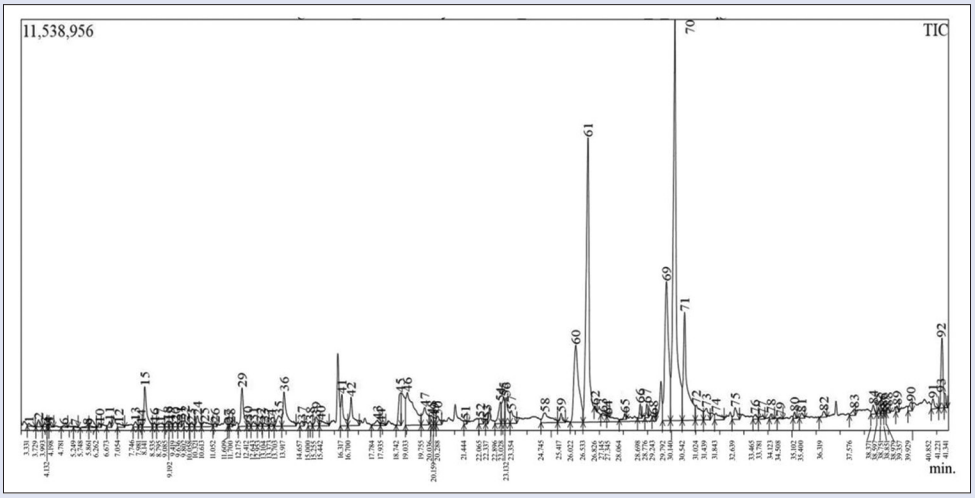

Figure 1: GC-MS chromatogram of methanolic extract of *Calligonum polygonoides* flower bud

extract along with positive reference ascorbic acid assayed with different methods, the reducing capacity is increasing with increased concentration of sample and reference standard. The TAA of methanolic extracts of *C. polygonoides* in amalgamation of different methods show about 16%–53% reducing power that of positive reference ascorbic acid at same level of concentrations. The TAA of methanolic extract exhibited 46%–50% reducing power under CUPRAC; 16%–22% under FRAP; 16%–19% under PM and 22%–26% under DPPH that of ascorbic acid, respectively, at the same level of concentrations. Among all methods, CUPRAC assay shows superiority over other methods in TAA.

DISCUSSION

The GC-MS/MS results for elucidation of the phytochemicals of this particular study are in accordance with the previous results of Mukhatar *et al.*[28] who have qualitatively reported the presence of these compounds in *C. polygonoides* plant extracts. Major portion of fatty acids in flower buds is essential fatty acids linoleic and linolenic acids and also contains 12 carbon long fatty acids called dodecanoic acid (lauric acid) precursor of mono laurines which is used in treatment of chronic fatigue syndrome and boost immune system.[29] A very novel compound DSG (peak 38) was also detected which is well known

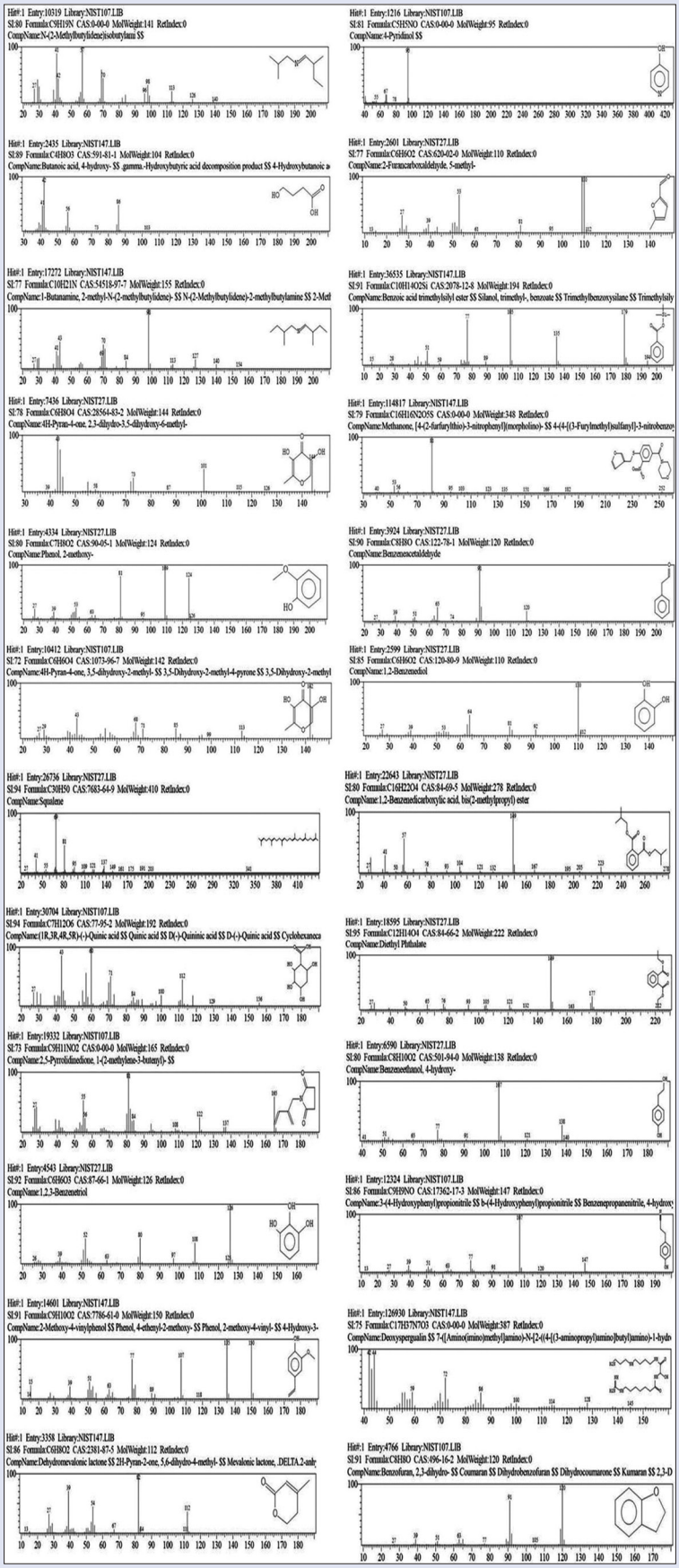

Figure 2: Mass spectrum showing of the methanol flower bud extract of *Calligonum polygonoides*

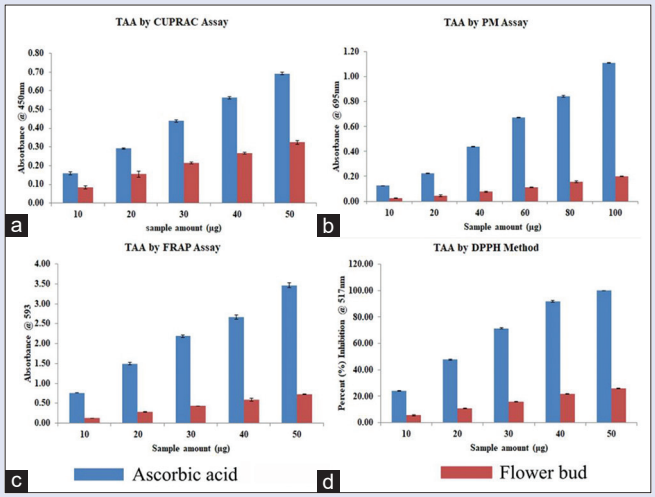

Figure 3: Total antioxidant potential of Ascorbic acid and methanolic extract of *Calligonum polygonoides* flower bud. (a) CUPRAC assay; (b) PM assay; (c) FRAP assay and (d) DPPH assay

established immunosuppressive drug with tumoricidal and antimalarial properties, has been implicated in the inhibition of a diverse array of cellular processes including polyamine synthesis and protein synthesis. It is also reported that DSG inhibit the cell growth through inactivation of eukaryotic translation initiation factor 5A (eIF5A).[30] Similarly, DSG has been extensively used against autoimmune disease like cancer, lupus nephritis, etc.[31-34]

Similarly, the other bioactive compounds detected and identified with established medicinal and therapeutic properties such as antioxidant, antimicrobial, anti-proliferative, anti-diabetic, hepatoprotective, cardiovascular drugs. Phutdhawong *et al.*[35] reported the anticancer and antibacterial activity of Furan, 2,5-dimethyl-(Peak 6) and its derivatives. They studied the cytotoxic effect against cancer cell lines HeLa, HepG2 and Vero and Gram (+) and Gram (-) bacteria and reported that its amine derivatives has potential bioactivity against the HeLa cell line (IC_{50} 62.37 µg/mL) and against the photogenic bacteria (MIC 250 µg/mL). Similarly, 2-Methoxy-4-vinylphenol (peak 41) also called 2M4VP is a naturally occurring phenolic compound used as flavoring agent, has also been reported as cancer preventive drug. Joeng and Jeong[36] reported that 2M4VP arrested the growth of BaP-treated NIH 3T3 cells through blocking the hyper-phosphorylation of Rb via expression regulation of cell cycle-related proteins. Al-Rubaye *et al.*[37] reported the anti-microbial activity of 1-Butanamine, 2-methyl-N-(2-methylbutylidene)-(peak 9), when studied the phytochemical screening of *Malva sylvestris* leaf extract. Accordingly, some common phenolic compounds like 2-methoxyphenol/o-Guaiacol (peak 25), Benzoic acid trimethylsilyl ester (peak 31), 1,2-Benzenediol/catechol (peak 35), Benzofuran, 2,3-dihydro-/Coumaran (peak 36), Benzoic acid, 3-(1-methylethyl)-(peak 44), 1,2,3-Benzenetriol/Pyrogallol (peak 45), Benzaldehyde, 2-hydroxy-6-methyl-(peak 47), quinic acid (peak 60) etc., has been proven to possess antioxidant and pharmacologic activities.[38] The compound detected at peak no 29, i.e., DDMP is reported as very strong antioxidant compound[39,40] and also reported as stimulus for autonomic nerve activities in rats.[41] Similarly, Cechovska *et al.*[42] reported that DDMP was the principle component responsible for the increasing the antioxidant capacity of prunes prepared at high temperature.

The compound detected in GC-MS/MS chromatogram at peak no 37, i.e., dehydromevalonic lactone also called as mevalonic acid, which exists in an equilibrium between its open (–)-(R)-1 and cyclic form (mevalonolactone) (–)-(R)-2).[43] The carboxylate anion of mevalonic acid, also called mevalonate, is the predominant form in biological environments and has of major pharmaceutical importance. The major pharmaceutical application of mevalonolactone are in production of cholestrol lowering drugs through inhibiting reductase activity.[44] It is also the biogenetic precursor of most steroids, terpenoids, isoprenoids, and carotenoids and therefore has been a synthetic target of substantial interest.[45] Similarly, Benzeneethanol, 4-hydroxy-a phenolic compound popularly known as tyrosol, as a strong antioxidant, an anti-arrhythmia and cardiovascular drug with protective effect. It has been reported that tyrosol exert antioxidant activity and ability to protect Low-Density Lipoprotein (LDL) particles from oxidantion through binding with these particles.[46-48] Some other phytochemicals detected in methanolic extracts of *C. polygonoides* flower bud with significant medicinal and therapeutic properties are Quinic acid has been reported as starting material for synthesis of many pharmaceuticals against influenza A and B strains called Tamiflu.[49] Squalene, a triterpene is the biochemical precursor for the synthesis of whole steroid family.[50] It is commonly used as immunologic adjuvant with many vaccines which stimulate the immune system and increase the response to the vaccine in human body. When squalene was added in influenza vaccines, it stimulated the immune response of human body through production of CD4 memory cells.[51,52] Squalene also exhibited antioxidant, chemopreventive, antitumor, and hypo-cholesterolemic activities.[53,54] In accordance with the above, the bioactivities of 9-Hexacosene (peak 93) has also been reported an analgesic and anti-inflammatory.[55]

The results of total antioxidant potential of *C. polygonoides* on different scavenging scales viz. CUPRAC, FRAP, DPPH and PM assay [Figure 3] are in accordance with the results reported in fresh leaves of *Kalanchoe pinnata*.[56] The exhibited very high antioxidant potential on different scale is in accordance with previous results and also supported the GC-MS/MS results on identification of novel phytochemicals with established bioactivity in terms of antioxidant, antimicrobial, anti-proliferative activities.[18-21] The superiority of antioxidant activity under CUPRAC assay is due to the involvement of both complexometric and redox reactions along with some specific features of CUPRAC reagent, namely more sensitive, selective and produce more stable colored chelate of Cu-(I)-Nc which is very less affected with air, light, solvent and pH.[25] The presence of significant level of antioxidants in the methanolic extracts of *C. polygonoides* flower buds are also a positive indicator of its vast pharmaceutical and nutraceutical applications.

CONCLUSION

These bio-activities of the phytochemicals present in *C. polygonoides* flower bud methanolic extract support the curative and therapeutic implications of the plant which has been reported in literature. The identified bioactive compounds with ample pharmaceutical application explore the worthiness of this endemic plant species. Along with pharmaceutical, it has an immense scope in nutraceutical and functional food industry. These pharmaceutical applications advised for its conservation and artificial regeneration, to sustain the agro-ecological balance of "Thar Desert" of India.

Acknowledgements

The authors are thankful to Director, ICAR-CIAH, Bikaner for providing all facilities for smooth conducting of this research and critically reviewing the manuscript. Authors are also thankful to the Indian Council of Agricultural Research, New Delhi, for all financial support as in-house project.

Financial support and sponsorship

Indian Council of Agricultural Research, New Delhi, India.

Conflicts of interest

There are no conflicts of interest.

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