Phytochemical Characterisation and Gas Chromatography-Mass Spectrometry Evaluation of Selected Medicinal Plant Species

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Background

- Plants had been used by mankind since antiquity as food, medicines and industrial raw material. Phytometabolite studies have attracted interest of R&D over many years.
- Plant polyphenolics such as flavonoids, tannins, etc. possess free radical-scavenging properties because of their favorable structural chemistry.
- However, the detailed phytochemical composition of certain medicinal plants remains underexplored.
- This study focuses on *Amaranthus viridis* L., *Chenopodium album* L., *Parthenium hysterophorus* L., and *Tridax procumbens* L.
- Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique used to determine and identify compounds present in these plant samples.
- GC-MS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active components.
- The present study comprehensively investigates antioxidant capacity, total phenolic and flavonoid content of successive extracts of leaves of these 4 plants at different concentrations using spectrophotometric assays.
- By using methanol, chloroform, and water extracts, the study aims to identify bioactive compounds supporting traditional medicinal applications.

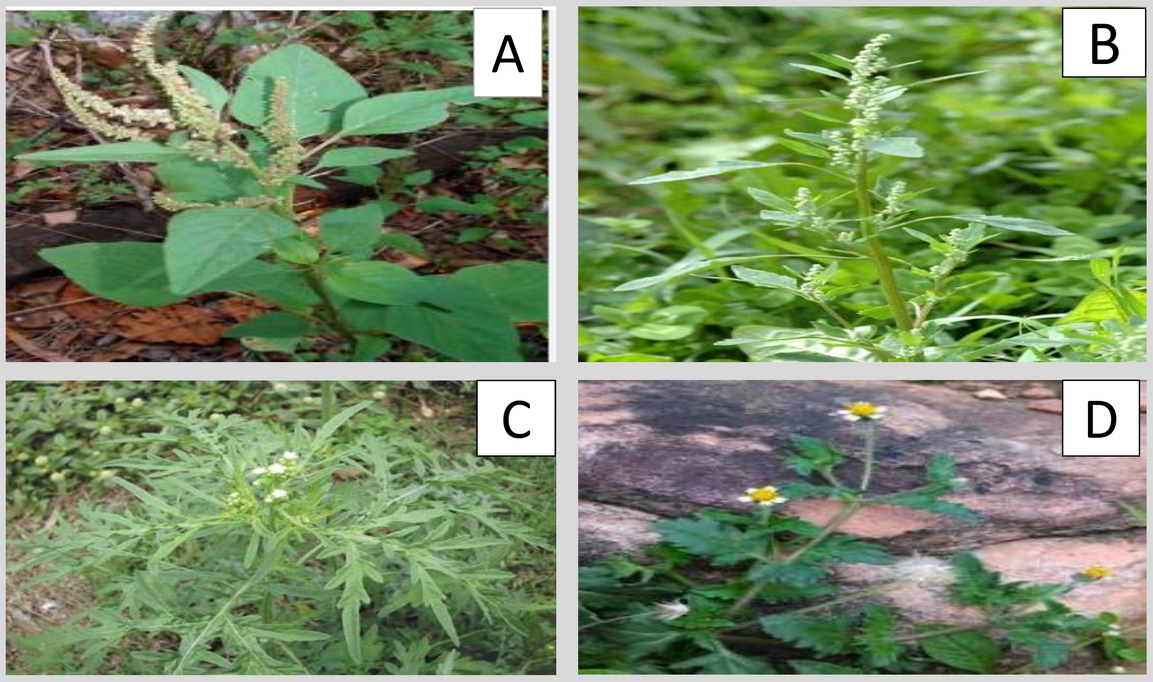

Fig.1 Pictures showing A. *Amaranthus viridis* B. *Chenopodium album* C. *Parthenium hysterophorus* D. *Tridax Procumbens*

Materials and methods

Chemicals and reagents

The analytical-grade chemicals and reagents utilised in this study were all provided by HiMedia, Sigma, and SRL.

Plant collection and authentication

The plants were collected from Banasthali Vidyapith Campus. After that, the voucher specimen was delivered to the herbarium of BURI (Banasthali University Rajasthan, India), and a voucher specimen number was generated for *Amaranthus viridis* L., *Chenopodium album* L., *Parthenium hysterophorus* L., and *Tridax procumbens* L.

Extract preparation

Plants are thoroughly washed with distilled water and dried at room temperature for 10-15 days. Then the dried plant made into powdered form. After that the extraction was done by Soxhlet method. The dried extracts were refrigerated between 2 and 8ºC for further analysis.

Quantitative analysis

Preparation of standard solution

Gallic acid and quercetin, both measured approximately 10 mg, were precisely weighed into dry, clean volumetric flasks. After dissolving them in methanol, the volume was raised to 10 ml and a solution concentration of 1 mg/ml was attained using the same solvent.

Analysis of Total Phenolic Content- This will be determined by Folin-ciocalteu method using UV-Visible spectrophotometer.

Analysis of Total Flavonoid Content- This will be determined by Aluminium chloride colorimetric method using UV- Visible spectrophotometer.

Antioxidant assay

The antioxidant assay will be carried out by a method called free radical scavenging assay using DPPH and NOSA assays.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was carried out in a combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer, fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m × 250 µm, film thickness 0.25 µm), interfaced with 5675C Inert MSD with Triple-Axis detector. Helium gas was used as carrier gas and was adjusted to column velocity flow of 1.0 ml/min.

Statistical analysis

Every determination was repeated in triplicate, and the mean±standard deviation was used to describe the results.

Results

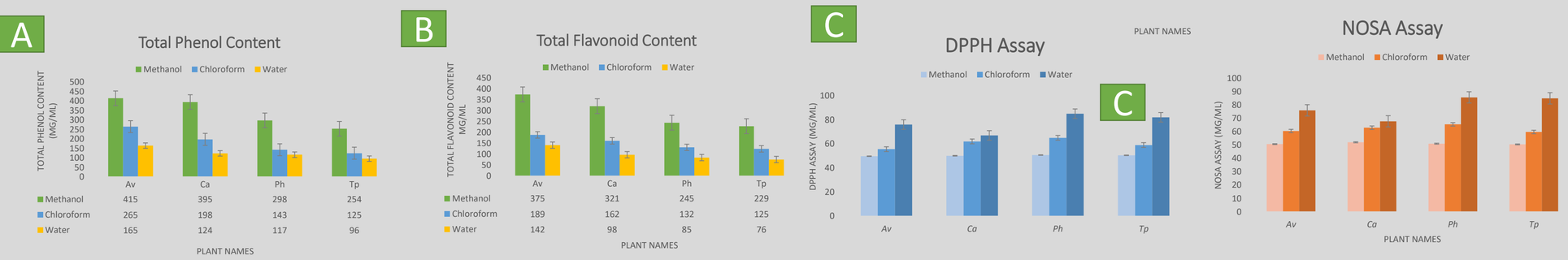

Fig.2 Quantitative and antioxidant analysis of 4 plants:
A. Total phenolic content B. Total Flavanoid content C. DPPH assay D. NOSA assay

- **Av = *Amaranthus viridis***
- **Ca = *Chenopodium album***
- **Ph = *Parthenium hysterophorus***
- **Tp = *Tridax procumbens***

A

S. No.	RT (min)	Peak Area (%)	Name of the compound	Molecular formula
1.	14.31	2.74	Phenol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$
2.	16.27	5.42	Diethyl Phthalate	$C_{12}H_{14}O_4$
3.	17.48	2.29	2-Aminophenol, 2TMS derivative	$C_{12}H_{23}NOSi_2$
4.	32.33	2.83	Silanol, trimethyl-, phosphite (3:1)	$C_9H_{27}O_3PSi_3$
5.	34.28	2.35	Tricyclo[4.2.1.0(2,5)]non-7-ene, 3,4-di(tris(trimethylsilyloxy)silyl)-	$C_{27}H_{64}O_6Si_8$
6.	36.32	2.62	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$
7.	39.19	3.83	Tetrasiloxane, 1,1,3,3,5,5,7,7-octamethyl-	$C_8H26O_3Si_4$
8.	40.35	3.14	7,7,9,9,11,11-Hexamethyl-3,6,8,10,12 ,15-hexaoxa-7,9,11-trisilaheptadecane	$C_{14}H_{36}O_6Si_3$
9.	41.89	3.89	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-h exadecamethyl-	$C_{16}H_{50}O_7Si_8$
10.	42.97	3.23	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl-	$C_{10}H_{32}O_4Si_5$
11.	43.55	2.68	3-Isopropoxy-1,1,1,5,5,5-hexamethyl- 3-(trimethylsiloxy)trisiloxane	$C_{12}H_{34}O_4Si_4$
12.	44.54	4.13	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$

B

S. No.	RT (min)	Peak Area (%)	Name of the compound	Molecular formula
1.	3.03	11.70	5-Benzyloxypyrimidine-2-carboxylic acid Thiodiglycol	$C_{12}H_{10}N_2O_3$ $C_4H_{10}O_2$
2.	3.40	15.52	Boron, trihydroxy (pyridine)-, (T-4)-	C_5H_8BN
3.	10.52	11.98	1-Chloromethyl-1-ethoxy-1-silacyclohexane	$C_8H_{17}ClOSi$
4.	13.53	6.29	2,4-Di-tert-butyl-phenol Phenol, 3,5-bis (1,1-dimethyl ethyl)-	$C_{14}H_{22}O$
5.	16.14	6.47	Octadecane, 3-ethyl-5-(2-ethylbutyl)- Heptacosane	$C_{26}H_{54}$ $C_{27}H_{56}$
6.	18.36	8.43	Stearic acid, 3-(octadecyloxy) propyl ester.	$C_{39}H_{78}O_3$
7.	18.76	10.87	1,2-Benzenedicarboxylic acid, Phthalic acid, butyl octyl ester, decyl isobutyl ester 6	$C_{20}H_{30}O_4$ $C_{22}H_{34}O_4$
8.	20.80	15.73	Methyl stearate	$C_{19}H_{38}O_2$
9.	23.54	6.18	Hexadecanoic acid, methyl ester Methyl glycocholate, 3TMS derivative	$C_{17}H_{34}O_2$ $C_{36}H_{69}NOSi_3$

D

Peak#	RT	Area %	Name of compound	Molecular formula
1	8.331	1.91	2-Nonadecanone 2, 4 dinitrophenyl hydrazine	$C_{25}H_{42}N_4O_4$
2	10.101	0.36	Ethyl iso-allocholate	$C_{26}H_{44}O_5$
3	10.898	0.30	4-(3-hydroxy-1-propenyl)-2-methoxy-phenol,	$C_{10}H_{12}O_3$
4	11.487	1.07	Hexadecanoic acid	$C_{16}H_{32}O_2$
5	12.869	2.09	1-β-D-Ribofuranosyl-3-[5-tetraazolyl]-1,2,4-triazole	$C_8H_{12}N_6O_5$
6	14.207	0.13	2,4-bis(1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$
7	14.954	0.31	Z,Z-4,16-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$
8	15.747	0.21	3-Pyridinol	C_5H_5NO
9	17.325	0.37	Hexadeca-9-en-1-ol	$C_{16}H_{32}O$
10	17.738	0.77	1-methyl-2-(3-methylpentyl)-cyclopropane	$C_{10}H_{20}$
11	18.252	1.21	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
12	21.025	0.24	9,12-octadecadienoic acid(Z,Z)	$C_{18}H_{32}O_2$
13	22.996	0.04	Beta-elemene	$C_{15}H_{24}$
14	23.2	0.21	Phytol	$C_{20}H_{40}O$
15	23.45	0.07	Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$

D

S. No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	13.158	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-	C14H22O	206	18.571
2.	16.904	PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(2-METHYLPROPYL)-	C11H18O2N2	210	6.789
3.	17.279	BUTANOIC ACID, PYRROLIDIDE	C8H15ON	141	3.799
4.	17.865	L-PROLINE, N-VALERYL-, HEXADECYL ESTER	C26H49O3N	423	3.403
5.	18.030	PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(2-METHYLPROPYL)-	C11H18O2N2	210	6.853
6.	18.140	L-(+)-ASCORBIC ACID 2,6-DIHEXADECANOATE	C38H68O8	652	33.988
7.	18.295	HEPTACOSYL HEPTAFLUOROBUTYRATE	C31H55O2F7	592	4.114
8.	19.975	OCTADECANOIC ACID	C18H36O2	284	9.943
9.	22.221	PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(PHENYLMETHYL)-	C14H16O2N2	244	5.751
10.	24.887	HENTRIACONTANE	C31H64	436	1.723
11.	25.588	HENTRIACONTANE	C31H64	436	1.911
12.	26.263	HENTRIACONTANE	C31H64	436	1.605

Fig. 3. Bioactive compounds found in methanolic extract of A. *Parthenium hysterophorus* B. *Tridax procumbens* C. *Amaranthus viridis* D. *Chenopodium album*

Discussion

- Methanolic extract of these plants have high phenolic and flavonoid content.
- Due to high phenolic and flavonoid content, these methanolic extracts also contain high antioxidant activity in comparison to chloroform and water extract.
- In GC-MS analysis, twelve compounds were found in the methanolic extract of *P.hysterophorus* and *C. album,* Nine in *T. procumbens* and Fifteen *in A. viriids.* These compounds were effectively matched and characterized.
- Overall, these compounds belong to alcohols, aldehydes, ketones, esters, terpenoids, and sesquiterpenoids.
- As a result of the presence of these important components, the methanol extracts of *Amaranthus viridis* could have an important therapeutic significance.

Conclusion

The current investigation aimed to identify several phytochemicals and GC-MS characteristics that may be useful for human and animal health. Our results demonstrated that various extracts of *Amaranthus viridis* contain considerable quantities of phytochemicals that can be potentially used for medicinal purposes. Additionally, we identified some major compounds that can be useful for *in vivo* and *in vitro* pharmacological screening. Also, methanolic extract was found to be more efficient in antibacterial treatment compared to the chloroform and water extract. Hence, our study paves the way for future in-depth investigations toward the discovery of efficient biomolecules that could be useful in human and animal health.