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GC-MS screening of bioactive constituents and antioxidant profiling in an invasive weed, *Malvastrum coromandelianum* (L.) Garcke

Shaiphali Saxena and PB Rao

Abstract
Phytoconstituents via. GC-MS (gas chromatography-mass spectrometry), antioxidant potential, total phenolic and flavonoid content along with enzymatic antioxidants were examined in the leaves of *Malvastrum coromandelianum* (L.) Garcke, an invasive alien weed species. Crude extraction of plant leaves in aqua methanol and acetone (20:80) was prepared. Antioxidant analysis of both extracts was performed by using 2,2'-diphenyl-1-picrylhydrazyl (DPPH), phosphomolybdenum (PM) assay, Fe^{2+} ion chelating activity (FCA), Fe^{3+} reducing antioxidant power (FRAP), total phenolic content (TPC) and total flavonoid content (TFC) accompanied with enzymatic assays (superoxide dismutase, catalase and peroxidase). Bioactive phytoconstituents were screened by GC-MS. Data analysis by ANOVA (using STPR 3) and significant range test by DMRT (using SPPS 16.0 version) were accomplished. Both aqua methanol (AM) and aqua acetone (AA) extracts were potent in all antioxidant assays in concentration dependent manner. However, the values were comparatively higher in AM than AA extracts except flavonoids, which was higher in AA than AM extract. GC-MS profiling of AM extract revealed 29 bioactive phytoconstituents categorized under phenols, fatty acids, flavonoids, vitamins and terpenes. The plant species may serve as a natural plant resource with immense antioxidative potential.

Keywords: Antioxidants, enzymatic-antioxidants, *GC-MS, Malvastrum coromandelianum, phytochemicals*

1. Introduction
Malvastrum coromandelianum (L.) Garcke, commonly called false mellow (Malvaceae), is an invasive alien weed distributed throughout India. Native distribution of this plant is in Tropical America. Ethnomedicinally, the plant possesses analgesic, anti-inflammatory, antimicrobial, antifungal, antidysentric, antihemorrhagic, hepatoprotective and antipyretic properties [1, 2, 3, 4, 5]. Further, its leaves are reported to possess malvastrone 1 (a sesquiterpene lactone), which is spectrally proved as 2-(pentyrolactone)-hendecane [6] with antidiarrhoeal property [7]. Presence of β-sitosterol (phytosterol), owning anthelmintic, analgesic, anti-inflammatory, hepatoprotective and antilipidemic properties, has been detected via HPTLC analysis [8]. These peculiarities of the species may be due to secondary metabolites, which aid immense antioxidants status.

Antioxidants are the compounds that suppress the havoc created by reactive oxygen species (ROS) by donating a hydrogen atom to them. Utilization of synthetic antioxidants like BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), etc. nowadays is being declined due to their carcinogenic outcomes [9]. This has been diverted the attention of researchers towards the use of natural and safe plant-based antioxidant resources. In the present investigation, an attempt is made aiming the bioactive phytochemical analysis and antioxidant profile in an invasive alien weed species, *M. coromandelianum*. Further, GC-MS analysis was taken as a pillar for strengthening the antioxidant status by correlating it to phenolic and flavonoid contents and to explore plant's phytochemistry as no clue of GC-MS has been traced on this plant species so far in literature.

2. Material and Methods
2.1 Collection and Authentication
Fresh stock of mature leaves of *M. coromandelianum* was collected from Pantnagar Campus (29⁰ 1′ 27.79″ N latitude and 79⁰ 29′ 22.47″ E longitude), Uttarakhand and authenticated by Dr. D.S. Rawat, Deptt. of Biological Sciences, College of Basic Sciences and Humanities,

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2.2 Solvent extraction

The fresh sample was washed with tap water (2-3 times) and finally with distilled water, shade dried, mechanically grounded and orbitally shaked (150 rpm) with aqua methanol and acetone (20:80) in ratio of 1:10 w/v for 10 days at 27 ^{0} C. The extracts were filtered through Whatmann no.1, evaporated in water bath (40 ^{0} C) and stored at 4 ^{0} C for further experimentation.

2.3 Qualitative phytochemical screening

The phytochemicals *viz.,* alkaloids, tannins, phenols, flavonoids, quinines, carbohydrate, protein, saponins, steroids, cardiac glycosides and terpenoids were examined [10, 11].

3. *In vitro* antioxidant evaluation

3.1 2,2'-diphenyl-1-picrylhydrazyl (DPPH•) scavenging assay

DPPH• scavenging activity (%) was evaluated by using Brand-Williams *et al.* [12] method with minor modifications. The reaction mixture containing 1 ml of BHT (standard) and plant extracts (20, 40, 60, 80 and 100 µg/ml conc) with 3 ml of DPPH solution (0.004%) was incubated for 1 hr. The absorbance (517 nm) of yellow product was measured and values were calculated by the following formula:

Scavenging activity (%) = $[1-(A_t/A_c)*100]$

Where, A_t and A_c are the absorbance of sample and control at 517 nm, respectively.

3.2 Phosphomolybdenum (PM) assay

Slightly modified phosphomolybdenum method of Prieto *et al.* [13], based on the molybdenum (VI) reduction into molybdenum (V), is considered for determining total antioxidant activity. The reaction of 1 ml of both plant extracts (200, 400, 600, 800 and 1000 µg/ml conc) with reagent (3 ml) prepared by mixing sulphuric acid (0.6 M), ammonium molybdate (4 mM) and sodium phosphate (28 mM) was performed and incubated for 90 min (95 ^{0} C) in water bath. At 695 nm, absorbance of resultant product was measured and values were expressed as µg ascorbic acid equivalent (AAE) per mg extract.

3.3 Ferrous ion (Fe^{2+}) Chelating Activity (FCA)

FCA activity was performed by the method of Hsu *et al.* [14] for measuring the ability of plant extracts to form ferrous ion-ferrozine complex. About 1 ml of both extracts (200, 400, 600, 800 and 1000 µg/ml conc) and Na_2EDTA (standard) were reacted with 2 mM $FeCl_2.4H_2O$ (0.1 ml) and 5 mM ferrozine (0.2 ml) and made 5 ml total volume with AM and AA solvents. After 10 min incubation, absorbance (562 nm) was read and FCA values were calculated by using following formula:

Chelating activity (%) = $[1-(A_t/A_c)*100]$

Where, A_t and A_c are the absorbance of sample and control at 562 nm, respectively.

3.4 Ferric Reducing Antioxidant Power (FRAP) assay

The Fe^{3+} reducing power of the plant extract was spectrophotometrically analyzed by using Benzie and Strain [15] method. Fresh FRAP reagent was prepared with 300 mM sodium buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM HCl) and 20 mM ferric chloride ($FeCl_3.6H_2O$) solution in 10:1:1 ratio, respectively. The reagent was incubated at 37 ^{0} C before using. Further, 1 ml of both plant extracts (200, 400, 600, 800 and 1000 µg/ml conc) was reacted with 3 ml of reagent for 30 min at 37 ^{0} C. The absorbance of blue coloured product (ferrous tripyridyltriazine complex) was read at 593 nm and the values were expressed as µg trolox equivalents (TE) per mg extract.

4. Quantitative phytochemical analysis

4.1 Total Phenolic Content (TPC)

Folin-Ciocalteu colorometric method proposed by Wolfe *et al.* [16] was used with minor modifications for TPC. Appropriately, 0.5 ml of both plant extracts (200 µg/ml conc) was reacted with Folin-Ciocalteu reagent (0.2 ml) for 5 min and neutralized with 7% Na_2CO_3 (saturated). The absorbance (at 765 nm) of blue product was measured after 1 hr incubation and values were expressed in µg gallic acid equivalents (GAE) per mg extract.

4.2 Total Flavonoid Content (TFC)

Method of Djeridane *et al.* [17] with minor modifications was applied for TFC. Both plant extracts (200 µg/ml conc) were reacted with 2% aluminium chloride (1:1 v/v). After 1 hr incubation, absorbance (at 420 nm) of yellow product was measured and the values were expressed as µg quercetin equivalents (QE) per mg extract.

5. Enzymatic antioxidant assays

5.1 Superoxide (SOD) activity assay

Giannopolitis and Ries [18] photochemical method was followed for assaying superoxide activity and value was expressed as units (U) per mg fresh weight (FW). Enzyme extraction of fresh mature leaves (1 g) in 4 ml of chilled extraction buffer solution (100 mM potassium phosphate buffer, pH = 7.0 and 0.1 mM EDTA) was performed and centrifuged (15000 rpm) for 15 min (4 ºC). The enzyme (100 µl) mixed with 3 ml of reaction mixture (50 mM phosphate buffer, pH=7.8; 0.1 µM EDTA; 13 mM methionine; 75 µM NBT and 2 µM riboflavin) was illuminated for 30 min against blank (non-illuminated identical mixture) along with control (illuminated reaction mixture without enzyme) and absorbance was measured at 560 nm. One unit of SOD required for 50% NBT photoreduction was calculated with following formula:

$Z = [(X-A)/X]*100$

Where, Z= photoinhibition% in sample; X= absorbance of control; A= absorbance of sample enzyme; and Z/50= total SOD unit.

5.2 Peroxidase (POD) assay

Peroxidase activity was assayed by the method proposed by Reddy *et al.* [19] and Kar and Mishra [20] with minor modifications. Fresh mature leaves (1 g) were homogenized with 125 µM potassium phosphate buffer (pH 6.8) in chilled mortar and pestle (1:8, w/v) and centrifuged (12000 rpm) for 20 min (4 ºC) for enzyme extraction. The enzyme extract (100 µl) was reacted with 1mM pyrogallol solution (3 ml) and then 1% H_2O_2 (0.5 ml) was added to record the absorbance change (470 nm) for 1 min (10 sec intervals) against blank (reaction mixture excluding H_2O_2). Reaction was then stopped by

adding 5% H_2SO_4 (1 ml) and value was expressed as nmol/mg protein.

5.3 Catalase (CAT) activity assay
Kar and Mishra [21] method with minor modifications was applied for assaying catalase assay and value was expressed as nmol/min/mg protein. Homogenate of fresh mature leaves (1 g) was prepared in ice-cooled pestle and mortar with 100 mM sodium phosphate buffer (6.8 pH) in 1:8 w/v ratio and centrifuged (12,000 rpm) for 20 min at 4^0 C for extracting enzyme. About 0.1 ml of enzyme was mixed with 2 ml of reaction mixture (200 mM potassium phosphate buffer, pH 7.0; 50 mM H_2O_2 and 0.1 mM EDTA) and absorbance (240 nm) was measured for 1 min at an interval of 10 sec.

6. Gas Chromatography-Mass Spectrometry (GC-MS) analysis
Bioactive component characterization of AM of *M. coromandelianum* (5 mg/ml) was performed by GC-MS. The syringe filtered supernatant of AM was examined via GCMS-QP2010 system equipped with a gas chromatograph interfaced to a mass spectrometer. Initial oven temperature was 80 ^{0} C for 3 min and then increased to 250 ^{0} C for 5 min at 10 ^{0} C/min rate. Finally, the temperature increased to 280 ^{0} C at a rate of 15 ^{0} C/min and kept isothermally constant for 23 min. The voltage of ionization was 70 eV. Flow rate of helium gas as a carrier was 1.21 ml-min^{-1}. Temperatures of interface and ion source were at 260 ^{0} C and 230 ^{0} C, respectively; while, injector temperature was set at 260 ^{0} C. Split mode ratio of injection was 10:0. Scan range of mass spectrum (m/z) was fixed at 40-650. Start and end time of mass spectra were 4 min and 50.24 min, respectively along with 3.50 min solvent cut time. National Institute of Standards Technology (NIST) library, possessing 62,000 plus patterns and mass spectral databases of Wiley (New York) were used for identification of unknown bioactive compounds.

7. Statistical analysis
The data (three replicates) was represented as mean±S.E. and analysis of variance was applied by using STPR3 software to investigate the antioxidant potential of plant extracts. The significant difference ($p < 0.05$) of all means was determined by Duncan's multiple range test (DMRT) of SPSS 16.0 version.

8. Results and Discussion
8.1 Phytochemicals and yield
The higher amount of different qualitative phytochemicals was obtained in AM than in AA extract along with the yield (%) (Table 1).

Table 1: Qualitative phytochemical analysis in AM and AA leaf extracts of *M. coromandelianum*.

Phytochemicals/ Yield (%)	Test	Extracts	
		AM	AA
Flavonoids	Sulfuric acid test	++	+
Carbohydrates	Molisch's test	++	+
Cardiac glycosides	Keller-Kiliani test	-	-
Proteins	Xanthoproteic test	+	+
Steroids	Liebermann-Burchard's test	+	+
Quinines	Hydrochloric acid test	-	-
Saponins	Froth test	-	-
Tannins	Lead acetate test	+	+
Terpenoids	Salkowski's test	++	-
Alkaloids	Mayer's test	+	+
Phenols	Ferric chloride test	++	+
Yield%		13.5% (w/w)	8.2% (w/w)

(+) Present and (-) Absent.

8.2 Antioxidant activity
8.2.1 DPPH• scavenging activity (%)
The DPPH• scavenging activity (%) of both AM and AA extracts increased in dose dependent manner (Fig. 1) with high significance level ($p < 0.05$). The DPPH scavenging effect (%) at 100 µg/ml was 86.53 ±0.25 in BHT > 80.55 ±1.04 in AM and > 76.43 ±0.67 in AA extracts. Malar *et al.* [22] reported the DPPH scavenging activity (%) in *Lepidium sativum* was 2.69±0.5 in stem < 10.21±0.7 in leaf < 11.63±0.3 in seed and < 12.19±0.2 in whole plant; Basma *et al.* [23] in *Euphorbia hirta* was 72.96±0.78 in leaves > 52.45±0.66 in flowers > 48.59±0.97 in roots and > 44.42±0.94 in stems; Devi and Kumar [24] in *M. coromandelianum* leaves was < 90, at 160 µg/ml conc.; and Qaisar *et al.* [25] in *Croton bonplandianum* plant extracts was 59.62 in methanol and < 39.37 in dichloromethane indicate that all these values are significantly lower than the present study. It may be due to the presence of polyphenols and better extraction in both AM and AA.

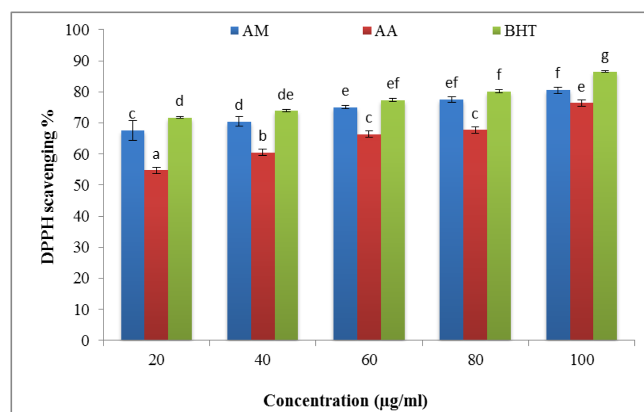

Fig 1: DPPH scavenging activity (%) in both AM and AA leaf extracts of *M. coromandelianum* and BHT at varying concentrations. Values with different lowercase letters indicate significant difference ($p< 0.05$).

8.2.2 Total Antioxidant Activity, Fe^{2+} ion Chelation and Fe^{3+} Reducing Power

The extracts in both AM and AA exhibited increased total antioxidant activity (µg AAE/mg extract) with increasing concentration and the difference was significant ($p < 0.05$). However, AM extract exhibited higher TAA (69.40±1.18 µg AAE/mg extract) than AA (59.89±0.64 µg AAE/mg extract) at 1000 µg/ml conc. (Fig. 2) indicating that the former one is more efficient to extract antioxidants than the later. The TAA values (mg GAE/g DW) reported by Chaouche *et al.* [26] in hydromethanolic extract of *Haloxylon articulatum* (128.79±7.3) and by Ibrahim *et al.* [27] in root extracts of *Rheum rhaponticum* (832.16±3.64 µg/ml in ethanol and 340.34±3.5 µg/ml in water) were maximum; while, remarkably lower TAA values (µg AAE/mg) were reported by Phatak and Hendre [28] in *Kalanchoe pinnata* (1.764±0.07 at 500 µg/ml conc.) than the present study values.

The extracts of both AM and AA exhibited increasing Fe^{2+} chelating activity (%) in dose dependent manner with significant difference ($p < 0.05$) (Fig. 2). The FCA (%) at 1000 µg/ml conc was comparatively higher in AM (55.39±0.61) than AA extract (52.02±0.49). The Fe^{2+} chelation value (%) reported by Mathew and Abraham [29] in methanolic leaf extract of *Cinnamomum verum* was >80 at 1000 µg/ml conc.; and by Farhan *et al.* [30] in *Euphorbia macroclada schyzoceras* aqueous leaf extract was 84.00±0.02 at 1 mg/ml conc., indicating higher chelation capacity than the present study. However, the present study values are significantly higher than the values reported by Mohan *et al.* [31] in methanol leaf extract of *Kalanchoe pinnata* (30% at 5 mg/ml conc.) and Jamuna *et al.* [32] in *Hypochaeris radicata* (38.69±0.23% at 5000 µg/ml conc.). Thus, the present values in the leaf extracts of *M. coromandelianum* exhibited a good amount of chelation.

The Fe^{3+} reducing power (µg TE/mg extract) in both AM and AA extracts increased in concentration dependent manner with significant difference ($p < 0.05$) (Fig. 2). The FRAP value (µg TE/mg extract) was 79.67±1.35 in AM and 67.52±0.95 in AA at 1000 µg/ml conc. More or less similar FRAP (%) values were reported by Sivakrishnan *et al.* [33] in *Albizia procera* ethanol extract (63.59±0.04 at 1000 µg/ml conc.). However, very low values were reported by Rajurkar and Hande [34] in methanol leaf extracts of *Vitex nigundo* (2.69±0.11 mg/g dw) > *Gymnema sylvestre* (1.00±0.07 mg/g dw) and > *Centella asiatica* (0.73±0.05 mg/g dw); and by Attanayake and Jayatilaka [35] in aqueous leaf extracts in *Pavetta indica* (1.86±0.67 µM), *Justicia adhatoda* (4.35±0.23 µM), *Azadirachta indica* (4.82±0.23 µM) and *Osbeckia aspera* (15.84±0.90 µM).

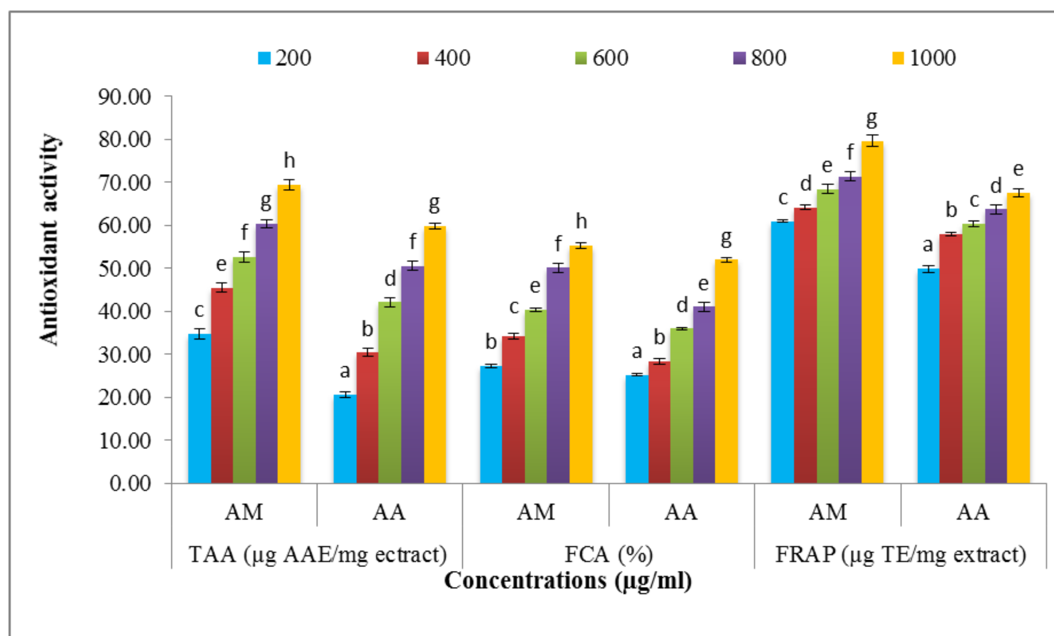

Fig 2: Total antioxidant activity (µg AAE/mg extract), Fe^{2+} chelating activity (%) and Fe^{3+} reducing power (µg TE/mg extract) in both AM and AA leaf extracts of *M. coromandelianum* at different concentrations. Values with different lowercase letters indicate significant difference ($p < 0.05$).

8.2.3 Total phenolic and flavonoid content

Phenols and flavonoids are most abundant secondary metabolites responsible for appreciable antioxidant activities. The total phenolic content (µg GAE/mg of extract) was higher in AM (88.45±1.72) than AA extract (67.50±1.58); whereas higher total flavonoid content (µg QE/mg of extract) was in AA (45.60±0.0015) than AM (40.68±0.0027) extracts at 200 µg/ml conc (Table 2) with significant difference at $p < 0.05$. The values of TPC (mg GAE/g) and TFC (mg QE/g) reported by Sanghai *et al.* [8] in *M. coromandelianum* leaf extracts at 100 µg/ml conc were 145.25±3.48 and 107.54±5.79 in methanol; and 41.78±7.86 and 29±2.36 in acetone, respectively, indicated that the lower TPC and TFC in acetone extracts as compared to present study values. However, TPC (mg GAE/g dw) and TFC (mg QE/g dw) at 200 µg/ml conc reported by Subedi *et al.* [36] in methanolic leaf extract of *Hibiscus rosa-sinensis* were 49.13±0.30 and 715.59±7.24, respectively indicating lower TPC and higher TFC than in present study.

Table 2: Total phenolic and flavonoid content in both AM and AA leaf extracts of *M. coromandelianum* (mean±S.E.).

Parameters	Extracts (200 µg/ml)	
	AM	AA
TPC (µg GAE/mg of extract)	88.45±1.72^{b}	67.50±1.58^{a}
TFC (µg QE/mg of extract)	40.68±0.0027^{a}	45.60±0.0015^{b}

Values with different lowercase letters indicate significant difference ($p < 0.05$).

8.2.4 Antioxidative enzyme activity assays

Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) assays were investigated to support enzymatic antioxidant potential concerning the medicinal value of *M. coromandelianum*. The present study values for SOD (units/mg FW), CAT (µmol/min/mg protein) and POD (nmol/mg protein) are 15.36±0.14, 21.58±2.91 and 14.10±1.42, respectively. When these values were compared to other studies, the SOD (U/ml) value was higher than *Rumex obtusifolius* (11.59±0.01600), while CAT (U/ml) and POD (U/ml) values (38.65±0.00026 and 195.24±0.0044, respectively) were low [37]. Nemade *et al.* [38] reported comparatively higher range of SOD (50.75±0.36 in *Vitex nigundo* to 207.70±3.48 U/mg protein in *Cynodon dactylon*) and lower POD (1.95±0.0008 in *Vitex nigundo* to 10.25±0.201 U/mg protein in *Acalypha indica*); while, both higher (24.11±0.61 in *Tridax procumbens* to 39.46±0.27 U/mg protein in *Pongamia glabra*) and lower range of CAT (1.38±0.04 in *Cynodon dactylon* and 17.36±0.39 U/mg protein in *Azadirachta indica*) values in the extracts of leaves.

8.2.5 GC-MS analysis

The GC-MS analysis of AM leaf extract of *M. coromandelianum* revealed that, a total of 29 bioactive compounds belonging to sterols, terpenes, phenols, vitamins, flavonoids, fatty acids, etc., and are presented with retention time, molecular weight, molecular formula and peak area (%) in Table 3 and chromatogram in Fig. 3. The amount of these bioactive phytoconstituents ranged from 0.22% (7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione) to 17.05% (guanosine). The major phytocomponents detected in GC-MS analysis are n-hexadecanoic acid (10.80%), 9,12,15-octadecadienoicacid, methyl ester, (Z,Z,Z)- (11.02%), guanosine (17.05%) and 9,12,15-octadecatrienoicacid, (Z,Z,Z)- (15.02%) along with the other tracer components like squalene, vitamin-E, phytol, diosgenin, neophytadiene, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, etc., which proved themselves as the antioxidative contributors along with other medicinal attributes. According to previous studies, out of 29 bioactive phytochemicals identified in the present study, about 20 phytoconstituents exhibited bioactive properties like antioxidant, ant-inflammatory, anticancerous, antimicrobial, hypoglycaemic, hepatoprotective, anticoronary, antiandrogenic, antiarthritic, etc. (Table 4). Some distinctive biological properties were also shown by the phytoconstituents like 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione (0.22%) treats hypokalemia, Conn's syndrome and hirsutism; 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (11.93%) acts as a feeding stimulant in Solanaceous members; 9,12-octadecadienoic acid (Z,Z)- (1.16%) is antihistaminic; diosgenin (1.25%) as antithrombotic; gamma.-Sitosterol (1.09%) as antiviral agent; while, octadecanoic acid (1.54%) emerged as antipsychotic agent.

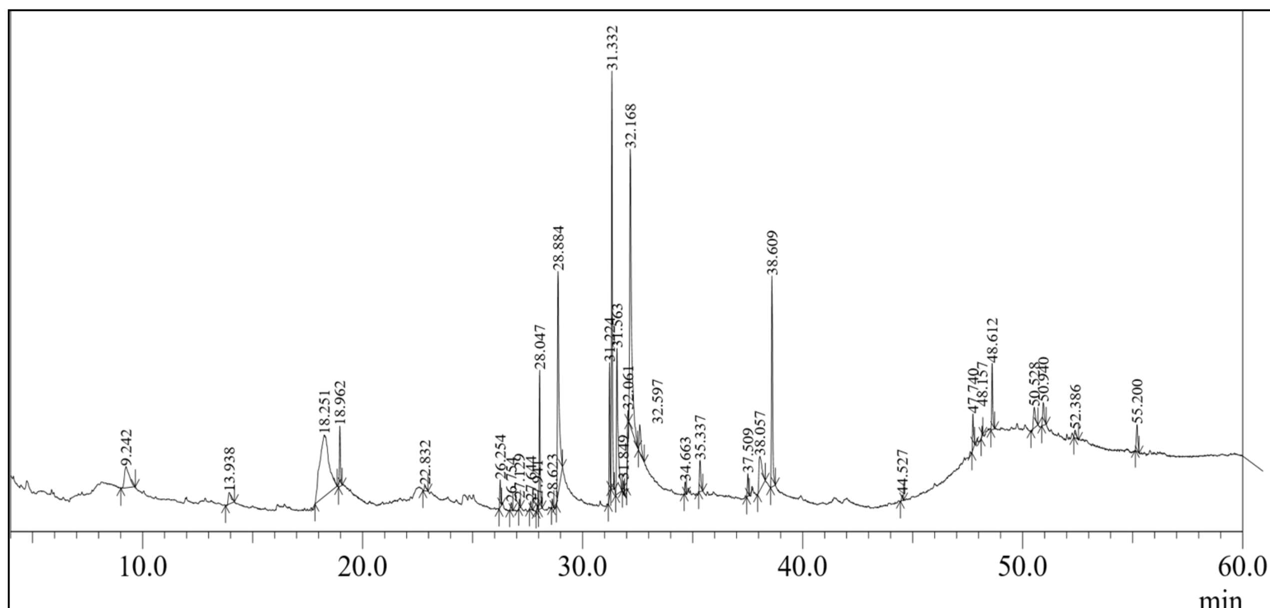

Fig 3: GC-MS chromatogram of *M. coromandelianum* in AM leaf extract.

Table 3: Bioactive phytochemical characterization of GC-MS analysis in AM leaf extract of *M. coromandelianum*.

S. No	Name of compound	RT (min)	Molecular weight	Molecular formula	Peak area (%)
1.	1,2-benzenedicarboxylic acid	38.609	166.132	$C_8H_6O_4$	6.93
2.	2-Methoxy-4-vinylphenol	13.938	150.177	$C_9H_{10}O_2$	0.98
3.	3-bromocholest-5-ene #	47.740	449.561	$C_{30}H_{50}Br$	1.10
4.	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester	37.509	213.316	$C_{12}H_{23}NO_2$	0.71
5.	4H-pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl-	9.242	144.1253	$C_6H_8O_4$	3.04
6.	5,8,11-Eicosatrienoic acid, methyl ester	35.337	320.5	$C_{21}H_{36}O_2$	1.29
7.	7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	27.644	276.376	$C_{17}H_{24}O_3$	0.22
8.	7.beta.-hydroxydiosgenin	52.386	430.629	$C_{27}H_{42}O_4$	0.43
9.	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	32.168	278.436	$C_{18}H_{30}O_2$	15.02
10.	9,12,15-octadecatrienoic acid, methyl ester,	31.332	292.46	$C_{19}H_{32}O_2$	11.93

	(Z,Z,Z)-				
11.	9,12-octadecadienoic acid (Z,Z)-	32.061	280.452	$C_{18}H_{32}O_2$	1.16
12.	9,12-octadecadienoic acid (Z,Z)-, methyl ester	31.224	294.472	$C_{19}H_{34}O_2$	3.42
13.	Carbonic acid, 2-dimethylaminoethyl neopentyl ester	34.663	203.279	$C_{10}H_{21}NO_3$	0.33
14.	Cholesteryl chloroformate	48.157	449.11	$C_{28}H_{45}ClO_2$	0.26
15.	Diosgenin	50.528	414.63	$C_{27}H_{42}O_3$	1.25
16.	d-Ribose, 2-deoxy-bis (thioheptyl)-dithioacetal	38.057	380.646	$C_{19}H_{40}O_3S_2$	3.18
17.	gamma.-Sitosterol	50.940	417.718	$C_{29}H_{50}O$	1.09
18.	Guanosine	18.251	283.244	$C_{10}H_{13}N_5O_5$	17.05
19.	Hexadecanoic acid, 15-methyl-, methyl ester	31.849	284.48	$C_{18}H_{36}O_2$	0.27
20.	Hexadecanoic acid, methyl ester	28.047	270.45	$C_{17}H_{34}O_2$	3.81
21.	Neophytadiene	26.254, 26.754, 27.129	278.524	$C_{20}H_{38}$	0.79, 0.26, 0.22
22.	n-hexadecanoic acid	28.884	256.43	$C_{16}H_{32}O_2$	10.80
23.	Octadecanoic acid	32.597	284.484	$C_{18}H_{36}O_2$	1.54
24.	Phenol, 2,4-Bis (1,1-dimethylethyl)-	18.962	278.511	$C_{17}H_{30}OSi$	1.98
25.	Phytol	31.563	296.531	$C_{20}H_{40}O$	6.92
26.	Pyridinium, 1-hexadecyl-, chloride, monohydrate	22.832	339.991	$C_{21}H_{38}CIN$	0.31
27.	Squalene	44.527	410.73	$C_{30}H_{50}$	0.16
28.	Tris (2,4-di-tert-butylphenyl) phosphate	55.200	662.936	$C_{42}H_{63}O_4P$	1.21
29.	Vitamin E	48.612	430.717	$C_{29}H_{50}O_2$	2.03

RT: Retention time.

Table 4: Bioactive properties of potent phytochemicals in AM leaf extract of *M. coromandelianum* analyzed via GC-MS.

Name of compound	Nature	Biological activity	Reference
2-Methoxy-4-vinylphenol	Phenolic compound	Antioxidant, antimicrobial, anti-inflammatory and flavouring agent.	[39, 40].
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Flavonoid	Antimicrobial, anti-proliferative, antioxidative and anti-inflammatory.	[41, 42].
7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	Steroid	Anti-androgen, hypotensive, diuretic, antihypokalemic, treats Conn's syndrome, cosmetic conditions like acne, hirsutism, baldness, androgenic alopecia and antimicrobial.	[43, 44].
9,12,15-octadecatrienoic acid, (Z,Z,Z)-	α-Linolenic acid (omega-3-fatty acid)	Antioxidative and reduces cardiovascular disease risks.	[45].
9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Methyl lineolate (polyunsaturated fatty acid)	Feeding stimulant of Solaneceous plant beetles and plant growth regulator.	[46].
9,12-octadecadienoic acid (Z,Z)-	Linoleic acid	Antioxidative, nematicide, hypocholesterolemic, antiarthritic, anti-androgenic, hepatoprotective, 5-α-reductase inhibitor, anticoronary, antiacne, antihistaminic, antieczemic and insectifuge.	[47, 48].
9,12-octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid ester	Antioxidative, hypocholestrolemic, androgenic, antihistaminic, insect repellent, anticoronary, nematicidal, antiacne, antieczemic, hepatoprotective, antiarthritic and 5-α-reductase inhibitor.	[47, 48].
Diosgenin	Steroidal saponin	Anticancerous, anti-inflammatory, hypocholesterolemic, antioxidant, neuroprotective, hypoglycaemic, hypolipidemic and antithrombotic.	[49].
gamma.-Sitosterol	Triterpenoid	Antiviral, antioxidative, antidiabetic, antimicrobial, anticancer, antidiarrhoeal, anti-inflammatory and anti-angeogenic.	[50].
Guanosine	Nucleoside	Antioxidant, neuroprotective.	[51].
Hexadecanoic acid, methyl ester	Methyl palmitate (saturated fatty acid)	Antifungal, antioxidative, hypocholesterorolenic, flavouring agent, antiandrogenic, nematicide, 5-α reductase inhibitor and antibacterial.	[52, 53, 44].
Neophytadiene	Terpenoid	Antifungal, antioxidant, antipyretic, anti-inflammatory, analgesic and antimicrobial.	[50].
n-hexadecanoic acid	Fatty acid (palmitic acid)	Anticancerous and antioxidative.	[54, 55].
Octadecanoic acid		Anti-inflammatory, anticancerous, hypocholeterolemic, nematicide, hepatoprotective, antieczemic, 5-α reductase inhibitor, anticoronary, antiarthritic, insectifuge, antipsychotic, lubricant, cosmetic, perfumery and antibacterial.	[43, 44].
Phenol, 2,4-Bis (1,1-dimethylethyl)-	Phenol	Antibacterial and antioxidative.	[44].
Phytol	Diterpene	Antioxidative, antimicrobial, hypocholesterolemic, anticancerous, anti-inflammatory, diuretic and immunostimulatory.	[41, 56].
Pyridinium, 1-hexadecyl-, chloride, monohydrate	Cationic quarternary ammonium compound.	Antimicrobial, plaque and gingivitis inhibitor.	[57].
Squalene	Triterpene	Antioxidative, antibacterial, pesticide, antitumor, anti-cancerous, anti-inflammatory, chemoprotective, stimulates immune system, antiaging,	[50, 41, 47].

		xenobiotic neutralizer, antiatherosclerotic, diuretic, analgesic, pesticide and hypoglycaemic.	
Tris (2,4-di-tert-butylphenyl) phosphate	Phenol	Antioxidative.	[58].
Vitamin E	Vitamin	Antiageing, analgesic, hypoglycaemic, antitumor, anti-inflammatory, antioxidative, anticancer and antileukemic.	[41, 47].

In the present investigation, screening of both AM and AA extracts of the plant species was accomplished for the antioxidant potential (in varying concentrations) with different *in vitro* assays (DPPH, phosphomolybdenum, FRAP and FCA) including TPC, TFC and enzymatic antioxidants (SOD, CAT and POD). Effective *in vitro* antioxidant potential in AM than AA extract may be the result of more polyphenols with high scavenging property against free radicals catalyzed by metals like copper, iron, etc. [59]. Different assays and chemical reagents used for antioxidant activity along with varied experimental conditions may bring differences in existing values when compared with previous literature. Even identical experimental and assay conditions may result variation in values as no common assay exists for quantitative and qualitative antioxidant evaluation. Further, inconsistency may arise due to complex phytocomposition in single antioxidant assay method. Presence of phenolics and flavonoids responsible for antioxidant property was further supported by GC-MS characterization of plant extract. Besides antioxidative, the bioactive compounds also displayed other medicinal aspects like anticancerous, antimicrobial, anti-inflammatory, antiatherosclerotic, antiandrogenic, hypocholesterolemic, etc., along with unique features (antihistaminic, antiviral, antithrombotic and antipsychotic).

9. Conclusions

The AM leaf extract of *M. coromandelianum* proved itself as a potent best in present antioxidant and can be used as a natural antioxidant resource in food and pharmacognostic fields. Several anticancerous, antibacterial, anti-inflammatory phytochemicals were identified through GC-MS that would help researchers for future studies. More focus is needed among explorers for utilizing invasive alien weeds species as a potent medicinal resource because of their endless availability in nature. This can be one of the better management programme of weed species in nature than spending money and energy on their eradication process.

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