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ORIGINAL ARTICLE

Phytochemical Profiling and Antioxidant Assessment of Indian *Cymbalaria Muralis* Extracts

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Abstract

Background/Aim: *Cymbalaria muralis* (ivy-leaved toadflax) is a small creeping plant native to Europe, South Africa and some regions of India. Indian *Cymbalaria muralis* is recognised as a plant of traditional importance, but scientific literature available for its pharmacological activities as well as its bioactive constituents is very limited, hence this plant draws more attention for research. Aim of this study was to access its phytochemical profile and potential antioxidative potential.
Methods: Phytochemical profiling of Indian *Cymbalaria muralis* methanolic (CMME) and aqueous extract (CMAE) was carried using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) techniques and LC-MS/MS techniques. In addition to determining total phenolic content (TPC) and total flavonoid content (TFC), antioxidant activity of both extracts was evaluated using DPPH radical scavenging method.
Results: The GC-MS spectrum of CMME and CMAE showed a presence of 52 compounds (86 peaks) and 8 compounds (11 peaks), respectively with majority of compounds corresponding to fatty acids or lipids. LC-MS/MS studies further confirmed the presence of some anticipated compounds, namely apigenin, luteolin, chrysoeriol 7-glucosides, 7-glucuronides, apigenin 7-O-glucoside, chrysoeriol 7-rutinosides, diosmin, catapol, linarin, glucosyringic, 8-epimuraloside and 8-epiloganic acid with discovery of 3 novel metabolites namely harpagide, aucubin and actinidine. The total phenolic content (TPC) was found to be 91.33 ± 0.67 mg/g (CMME) and 68.75 ± 0.65 mg/g (CMAE), total flavonoid content (TFC) in CMME and CMMA was 30.16 ± 0.15 mg/g and 10.04 ± 0.17 mg/g respectively. In DPPH radical scavenging activity, CMME was found as better antioxidant (IC50-40.82 g/mL) in comparison to CMAE (IC50-75.20 g/mL).
Conclusion: The Indian *Cymbalaria muralis* exhibited remarkable antioxidant activity and rich phytochemical profile.

Key words: Indian *Cymbalaria muralis*; Whole plant; *Cymbalaria muralis* extract, methanolic; *Cymbalaria muralis* extract, aqueous; Gas chromatography-mass spectrometry; Chromatography, liquid; Tandem mass spectrometry.

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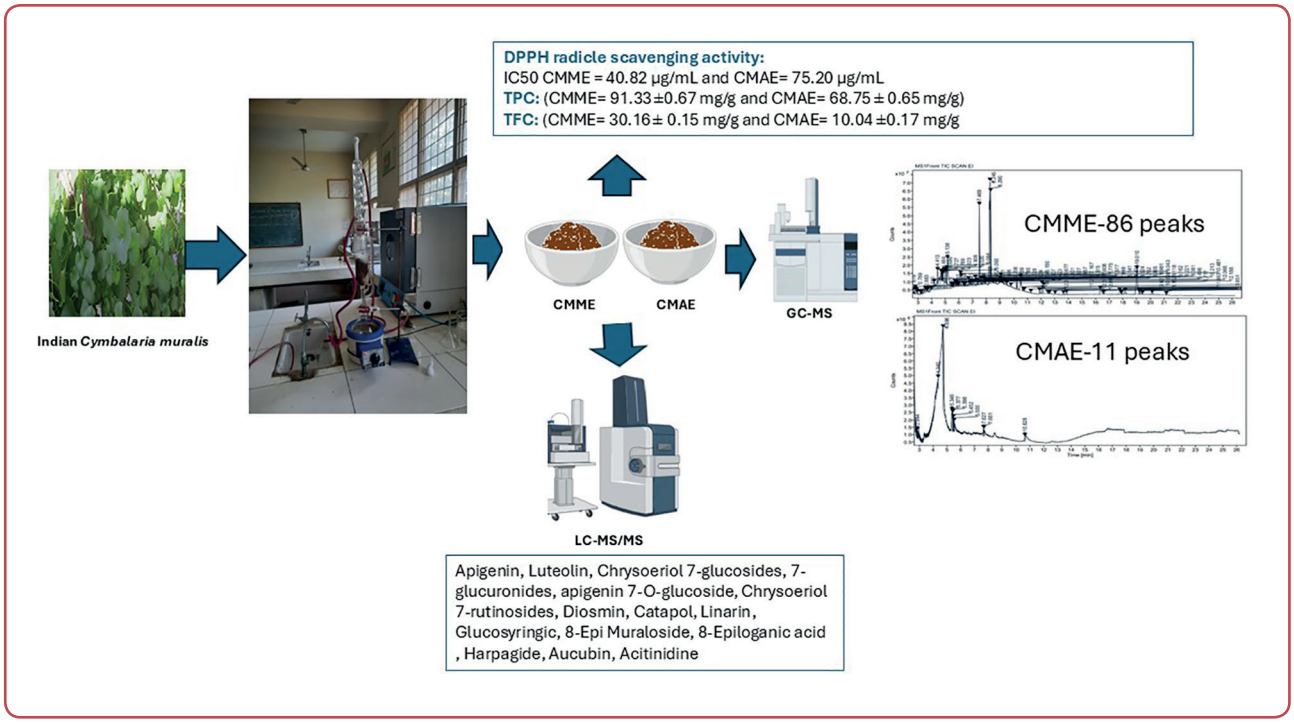

Graphical abstract

Introduction

Reactive oxygen species (ROS) and free radicals including peroxides, hydroxyl, peroxyl radicals are biologically produced inside the cells during various metabolic reactions such as breathing, inflammation, apoptosis, necrosis, etc.[1] Being highly reactive in nature, these free radicals pair their unpaired electrons within biological macromolecules including lipids, proteins and DNA causing oxidative damage.[2] This oxidative stress due to free radicals plays a major role in the prevalence and progression of many diseases such as atherosclerosis, diabetes, neurodegenerative and cardiovascular disorders.[3]

Antioxidants are the agents that counterbalance free radicals within cells providing protection against damage caused by them.[4, 5] Antioxidants function as reducing agents and eliminate free radical intermediates from chain events by oxidising themselves and thereby inhibiting oxidative stress.[6] Uric acid, glutathione and ubiquinol produced during normal metabolic processes in the body, vitamins like α-tocopherol, ascorbic acid or micronutrients (β-carotene) act as major sources of antioxidants. However, antioxidants like butylated hydroxytoluene (BHT), cannot be used by humans because of its adverse effects. Most plants and their derivatives, owing to their virtuous bioactivity and less toxicity are considered as a vital source of natural antioxidants with considerable efficacy to manage oxidative stress and alleviating various disease conditions (Figure 1).

Cymbalaria muralis (family Plantaginaceae) is a perennial herb which is distributed all over the world. This plant species was initially introduced in India for ornamental purposes but at present it is well acclimatised. In India this plant is mainly reported in Orissa and Mussoorie, particularly in rock crevices.[8, 9] Traditionally, this plant is widely used in various ailments like cicatriser for wounds, as diuretic and in diabetes.[10-13] Various phytoconstituents like iridoids including antirrhinoside; linarioside; antirrhide; linaride; 8- epiloganic acid; macfadienoside and muralioside and flavonoids *vis* apigenin; luteolin; chrysoeriol 7-glucosides and 7- glucuronides have been reported,[14-16] still this plant needs to be explored further.

Plant extracts screening is a novel methodology to discover therapeutically active compounds in various plant species.[17] Gas chromatography-mass spectrometry (GC-MS) and liquid chro-

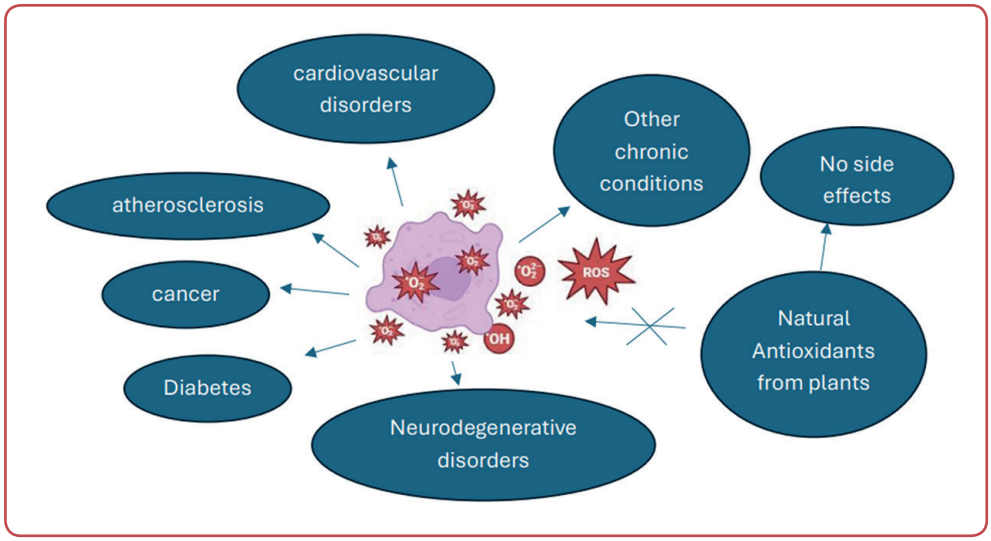

Figure 1: Role of natural antioxidants in alleviating numerous diseases caused by oxidative stress

matography–tandem mass spectrometry (LC-MS/MS) techniques have been widely accepted tools for comprehensive understanding and complete analysis of critical components in plants.[18] GC–MS analysis offers a universal mass spectral library for identifying molecules with relatively low molecular masses, featuring a broad dynamic range. It is considered one of the most effective techniques for identifying bioactive compounds, including long-chain hydrocarbons and various organic moieties such as alcohols, carboxylic acids, esters and alkaloids.[18, 19] LC–MS/MS is more capable of identifying and determining a wider range of compounds than GC–MS with minimal sample preparation.[20] It also encompasses a vast array of molecules primarily secondary metabolites like phenolics and terpenoids.

The Indian species of *Cymbalaria muralis* has not been extensively studied experimentally. To the best of authors' knowledge, this is the first study to investigate its phytochemical profile using both GC–MS and LC–MS/MS techniques, along with an evaluation of its antioxidant potential.

Methods

Collection and extract preparation

The plant was collected from wastelands of Mussoorie, Uttarakhand, India and authenticated by Central Ayurveda Research Institute (Authentication/SMPU/CARI/BNG/2022-23/663). The dried plant was grinded and successive extraction using soxhlation was used for preparation of extracts with methanolic extraction first at 40-50 °C followed by aqueous extraction at 80-90 °C. The percentage yield was found to be 29 % for the methanolic extract and 10 % for the aqueous extract. The prepared extracts were then used for preliminary phytochemical screening.

Phytochemical screening

Initially, a qualitative phytochemical screening was conducted on *Cymbalaria muralis* extracts as per the previous research reported using specified reagents to detect the occurrence of plant metabolites *vis* glycosides, carbohydrate, flavonoids, phytosterols, phenols, iridoids, tannins and alkaloids (Table 1).[21-23]

Antioxidant evaluation

DPPH radical scavenging activity

DPPH solution (0.1 mM) in methanol was prepared. The prepared extracts were diluted with methanol to get required concentrations between 12.50–150.00 µg/mL. 2.40 mL of DPPH solution was added to 1.60 mL of each diluted extract. The mixture was mixed properly and kept in dark at room temperature for about half an hour and their absorbance was observed spectrophotometrically at 517 nm using BHT as reference standard. The scavenging activity of both extracts and standard was calculated as:

$$\% \text{ DPPH radical scavenging avtivity} = \frac{A_{control} - A_{sample}}{A_{control}} * 100$$

IC_{50} was calculated from the graph plotted % inhibition versus concentration. The test was rep-

licated thrice for each concentration.[24] Based on IC_{50}, the antioxidant efficacy of the extracts can be determined, as per the classification of Phongpaichit et al, 2007, "IC_{50} greater than 250 indicates inactive nature of the compound"; "between 100 and 250-weakly active"; "between 50 and 100-moderately active"; "between 10-50-strongly active" and "less than 10-highly strongly active".[25]

Total phenols content (TPC)

TPC was determined using "Folin-Ciocalteu method". To 100 µL of extract (1.5 mg/mL) water was added (2 mL), 15 % w/v Na_2CO_3 (1 mL) and Folin-Ciocalteu reagent (2 mL). The mixture was incubated at 25 °C for 2 hours and then absorbance was measured at 765 nm. TPC was expressed as mg of gallic acid equivalents (GA)/g of extract.[26]

Total flavonoids content (TFC)

To 1 mL of extract (1.5 mg/mL), 5 % w/v $NaNO_2$ (0.3 mL) and distilled water (4 mL) was added; incubated for 5 minutes, followed by the addition of 10% w/v $AlCl_3$ (0.6 mL). Later, 1M NaOH (2 mL) and distilled water (2.1 mL) were also added. The absorbance was measured at 510 nm and TFC was then expressed as mg of quercetin equivalents (QE)/g of extract (Shah et al, 2021). Both TPC and TFC were determined in triplicate.[26]

GC-MS analysis

Agilent 7890B GC coupled with 5977B MSD using DB-5MS column was used for analysis. The oven temperature was kept initially at 150 °C and then increased to 250 °C. Helium was used as carrier gas at flow rate of 1 mL/min. The injection volume used was 1 µL. Ten mg of extracts were weighed and dissolved in 1 mL methanol. The mixture was vortexed for about 3 minutes followed by centrifugation at 10000 RPM for 6 minutes at 8 °C. The supernatant layer was transferred into Tarson tube. The solution was filtered through 0.22 µm and transferred to GC-MS vial. Compound identification from the GC–MS spectra was carried out using the Mainlib and Replib libraries (Table 2).

LC-MS/MS analysis

Agilent LC-1200 series MS-6400 series instrument with C_{18} column was used for LC-MS/MS analysis. A mixture of 5 mM ammonium formate in 0.1 % formic acid was used as mobile phase with injection volume kept at 5 µL. The temperature of instrument was maintained at 55 °C, gas temperature at 225 °C, gas flow at 6 L/min, nebuliser pressure at 50 psi, sheath gas temperature at 300 °C, sheath gas flow at 12 L/min, while capillary and nozzle voltage at 2000 V and 500 V respectively.

Five hundred mg of sample was weighed and extracted with 10 mL methanol for 24 hours in Soxhlet apparatus. Sample was dried at 40 °C using rotatory evaporator and volume was made up with 1 mL methanol. The mixture was centrifuged at 12000 RPM for 6 minutes and the supernatant layer was transferred into LC vial. This sample was injected into LC-MS/MS.

With LC-MS/MS, various flavonoids (apigenin, luteolin, chrysoeriol 7-glucosides, 7-glucuronides, apigenin 7-O-glucoside, chrysoeriol 7-rutinosides, diosmin, linarin) and iridoids (catapol, glucosyringic acid, 8-epimuraloside, 8-epiloganic acid, harpagide, aucubin) have been identified and quantified.

Results

On preliminary phytochemical screening, various secondary metabolites *vis* glycosides, carbohydrate, flavonoids, phytosterols, tannins and alkaloids were found present in methanolic and aqueous extracts of *Cymbalaria muralis* (Table 1).

DPPH radical scavenging activity

Although both extracts possessed pertinent antioxidant activity, *Cymbalaria muralis* methanolic (CMME) showed better scavenging activity as com-

Table 1: Phytochemical screening in methanolic (CMME) and aqueous (CMAE) extracts of Cymbalaria muralis

Extract	Glycosides	Phenols	Carbohydrates	Flavonoids	Phytosterols	Tannins	Alkaloids	Iridoids
CMME	+	+	+	+	+	+	+	+
CMAE	+	+	-	+	+	+	-	+

+ denotes presence and - denotes absence;

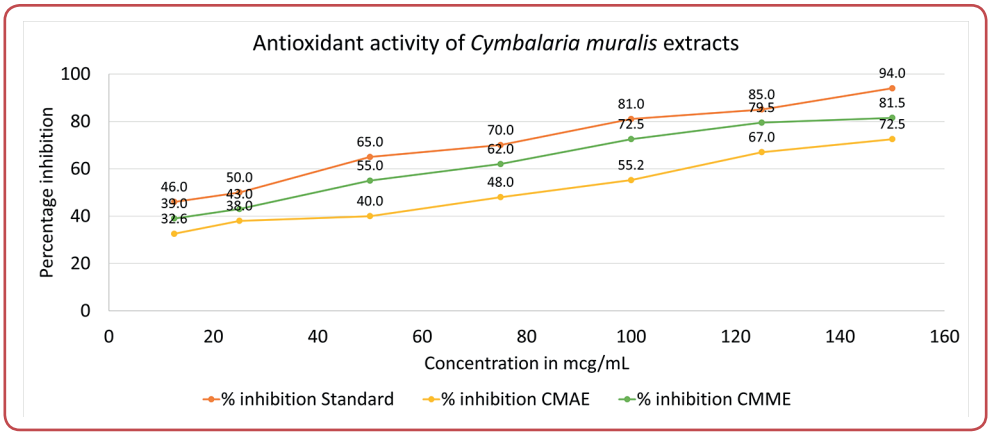

Figure 2: Antioxidant activity of Cymbalaria muralis extracts

pared to aqueous extract (CMAE). With increase in extract concentration, the antioxidant activity also increased. The methanolic extract showed the maximum scavenging activity (81.50 %) at concentration of 150.00 µg/mL (Figure 2). The IC_{50} of all the samples was also calculated by plotting "percentage inhibition against concentration". The IC_{50} was found to be 18.52 µg/mL (Standard), 40.82 µg/mL (CMME) and 75.20 µg/mL (CMAE). Based on the Phongpaichit classification,[25] CMME can be categorised as strongly active antioxidant and CMAE as moderately active antioxidant.

Total phenols content and total flavonoids content

TPC and TFC of *Cymbalaria muralis* extracts were determined in terms of gallic acid equivalent (standard curve equation: y = 0.009x -0.0233, R^2 = 0.997) and quercetin equivalent (standard curve equation: y = 0.0037x + 0.0296, R^2 = 0.9939) respectively. The TPC in CMME and CMAE were 91.33 ± 0.67 mg/g and 68.75 ± 0.65 mg/g respectively, whereas TFC was 30.16 ± 0.15 mg/g and 10.04 ± 0.17 mg/g, respectively.

GC-MS analysis

GC-MS spectrum of CMME demonstrated 52 compounds (86 peaks) with majority corresponding to fatty acids or lipids. Exceptionally, certain compounds produced multiple peaks at different retention times, despite being the same compound in both extracts. The maximum area was occupied by 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-/ methyl linolenate (18.29 %), followed by phytol (8.52 %), γ-sitosterol (7.64 %), hexadecanoic acid, methyl ester (6.96 %) (Figure 3, Table 2).

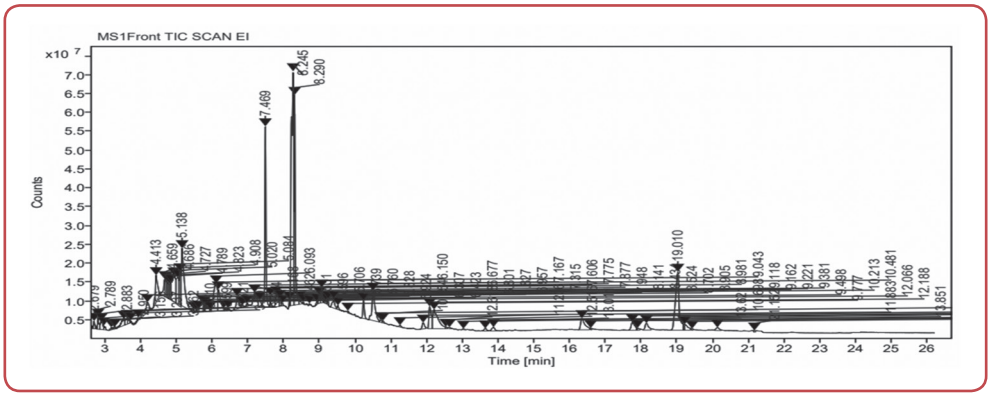

Figure 3: Gas chromatography-mass spectrometry (GC-MS) spectrum of Cymbalaria muralis methanolic extract (CMME)

Table 2: Gas chromatography-mass spectrometry (GC-MS) spectrum of Cymbalaria muralis methanolic extract (CMME)

N	Compounds	RT	Prob %	Area	Nature of compound	Activity
1	2- amino-6-hydroxy-4- methyl-4-hexenoic acid	2.68	8.94	0.32	Fatty acid	Immunosuppressive agent
2	Butyrolactone	2.79	28.87	0.59	Cyclic ester	Antidiabetic, antioxidant, anti-inflammatory
3	Trans-2,3-Epoxynonane	2.88	7.30	0.72	Epoxides	-
4	4-[N-(2- hydroxyethyl)-nitro]amino-1,2,4-Triazole	2.95	8.39	0.19	Heterocyclic	Antibacterial, antifungal, antiviral, anticonvulsant, anti-inflammatory
5	Alpha-l-rhamnopyranose	3.16	18.48	0.10	Monosaccharide	Tumor-killing agents, immunogenicity
6	Oxacyclododecan-2-one	3.24	7.19	0.08	Cyclic ketone	Antimicrobial
		3.26	11.29	0.07		
7	1,6-Anhydro-2,4-dideoxy-ß-D-ribo-hexopyranose	3.51	9.64	0.72	Sugar	-
8	Melezitose	3.60	12.20	0.44	Sugar	Present in honey, act as nutritive
9	2-Deoxy-D-galactose	3.62	21.68	0.26	Sugar	Kidney fibrosis
10	l-Gala-l-ido-octose	**3.70**	**10.32**	**0.08**	**Sugar**	**Insecticidal**
11	Desulphosinigrin	**3.91**	**12.30**	**0.14**	Glucosinolate derivative	Antidiabetic
12	d-Mannose	**4.17**	**21.03**	**1.38**	**Monosaccharide**	**UTIs**
13	6-methylene bicyclo[3.2.0]hept-3-en-2-one	**4.41**	**8.94**	**2.26**	**Cyclic ketone**	**Fungistatic**
14	1- acetyl-19,21-epoxy-15,16-dimethoxy -Aspidospermidin-17-ol	4.66	55.42	1.11	Alkaloid	Antidiabetic
		4.69	61.79	0.85		
		4.73	58.13	0.68		
15	Glycerin	4.79	26.96	0.44	Sugar	Wound healer
		4.82	32.38	0.74		
		4.91	32.09	3.39		
		5.02	50.85	0.44		
16	N- (phenylmethylene)-2-Propanamine	**5.08**	**24.94**	**1.77**	**Amines**	Anti-inflammatory, neuro-protective, anti-diabetic, anti-angiogenic
17	3,5-Dimethyl-5-[2- pyridyl]-1,3,4-thiadiazolidin-2-thione	**5.14**	**17.86**	**5.90**	**Heterocyclic**	**Antibacterial and antifungal**
18	Tetraacetyl-d-xylonic nitrile	5.46	41.62	0.15	Organic compound	Cholesterol modulating effect, antiviral and antioxidant
		5.53	26.99	0.11		
		5.92	17.34	0.08		
		7.95	26.73	0.16		
		6.96	52.60	0.21		
19	d-Lyxo-d-mannonononic-1,4-lactone	**5.71**	**14.31**	**0.87**	Lactone derivative of sugar	Insulin resistance
20	2-Myristynoyl pantetheine	5.83	23.45	0.27	Fatty acid derivative	Anti-inflammatory activity
		5.60	36.24	0.19		
		5.76	25.31	0.16		
		5.74	20.59	0.27		

21	Pyrazole[4,5-b] imidazole, 1-formyl-3-ethyl-6-ß-dribofuran	6.09	28.75	3.58	Heterocyclic	-
22	Decahydro-4,8,8,9- tetramethyl-1,4 -Methanoazulen-7-ol	6.15	9.66	2.10	Hydrogenated azulene derivative	-
23	10-Heptadecen-8-ynoic acid, methyl ester	6.31	38.08	0.15	Lipid-derived secondary metabolite	-
24	2-(7-heptadecynyloxy) tetrahydro-2H-pyran	6.42	15.44	0.10	Phenolic	Lipid-derived secondary metabolite
25	9-Octadecenoic acid, (2- phenyl-1,3 -dioxolan-4-yl) methyl ester	6.42 6.83 7.32 6.80	15.44 39.57 26.77 33.66	0.10 0.53 0.53 0.14	Fatty acid ester	Antimicrobial, anti-inflammatory
26	1,25-Dihydroxyvitamin D3, TMS derivative	7.17	21.81	1.64	-	Biological active form of Vitamin D
27	Hexadecanoic acid, methyl ester	7.47	74.01	6.96	Saturated fatty acids	Antioxidant, antimicrobial, antiarthritic, anticancer, anti-inflammatory, hepatoprotective, hypocholesterolaemic
28	2,6-Dimethyl-N-[3- (trimethylsilyl)-1,3 -hiazinam-2-ylidene]	7.61	12.77	0.32	Heterocyclic	-
29	Estra-1,3,5(10)-trien-17ß-ol	7.78 7.88	39.59 35.66	1.30 -	Steroidal lipid	Estrogenic
30	Cyclopropanedodecanoic acid, 2-octyl methyl ester	8.14 6.68	15.51 22.18	1.07 1.07	Fatty acid ester compound	-
31	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-/ Methyl linolenate	8.25	46.73	18.29	Polyunsaturated fatty acid	Hypocholesterolaemic, antiarthritic, hepatoprotective, anti-androgenic, antiacne
32	Phytol	8.29	57.35	8.52	Diterpene	Anxiolytic, cytotoxic, antioxidant, anti-inflammatory, immune-modulating, antimicrobial
33	Dasycarpidan-1- methanol, acetate	8.43 8.52 8.70	38.84 42.49 41.46	0.28 - 0.07	Alkaloid	Antibacterial, antifungal, anti-inflammatory and anticancer
34	3- octyl-cis-Oxiraneoctanoic acid	8.91 10.75 10.70 9.38	20.19 10.04 20.94 20.68	0.17 0.78 0.14 0.78	Fatty acid	-
35	Oleic acid	9.04	22.72	0.57	Lipids	Antifungal, antitumor, cardio-protective and improves insulin sensitivity
36	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentyl-cyclopropyl methyl] cyclopropyl] methyl]cyclopropyl] methyl]-, methyl ester	9.22	36.32	0.18	Ester	Anti-inflammatory, antibacterial, antifungal

37	Ethyl iso-allocholate	9.78	38.91	-	Steroidal alkaloid	Antioxidant and anti -inflammatory, antidiabetic
		11.24	52.87	0.11		
		9.16	27.32	0.13		
		9.50	24.95	0.20		
		9.12	18.39	0.14		
		13.01	35.16	0.07		
		8.98	12.58	0.42		
		19.20	12.31	0.42		
		17.86	56.24	0.07		
38	17-Pentatriacontene	10.21	9.43	-	Unsaturated hydrocarbons	Anti-inflammatory, anticancer, antiatherogenic and antiarthritic
		11.88	13.53	0.96		
39	Hexadecanoic acid, 2- hydroxy-1- (hydroxymethyl) ethyl ester	10.48	34.06	-	Lipids	Antioxidant, pesticide, flavour, nematicidal, hypocholestero-laemic
40	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1 -(hydroxymethyl)ethyl ester	12.07	46.05	1.93	Polyunsaturated fatty acid	Anti-inflammatory
41	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, ((Z,Z,Z)-/ 1-ß-linolenoylglycerol	12.19	33.30	3.58	Fatty acid esters	Anti-inflammatory and antibacterial properties
		12.52	22.33	0.09		
42	9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester	12.62	19.13	0.09	Fatty acid	Anti-obesity and antiviral
43	15,17,19,21- hexatriacontatetrayne	13.62	18.89	0.24	Polyenes	-
44	Vitamin E	16.32	27.59	1.68	Vitamin	Antioxidant
45	1,1'-dimethoxy-1,2,1',2'-tetrahydro-ψ,ψ-carotene/ .psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'- di-methoxy	16.57	14.39	-	Carotenoids	Antioxidant
		17.88	49.47	0.13		
		19.41	56.97	0.07		
46	Campesterol	17.74	33.75	0.98	Phytosterol	Cholesterol reduction, anti-inflammatory, antibacterial, breast cancer
47	Stigmasterol	18.13	65.23	0.81	Phytosterols	Antidiabetic, diabetic nephropathy
48	γ-Sitosterol	19.01	74.91	7.64	Plant sterol	Antioxidant, antidiabetic activity
59	β-Sitosterol	19.17	18.28	0.22	Plant sterol	Antioxidant, anti-inflammatory, lipid-lowering, Anti-diabetic
50	7,8-Epoxylanostan-11-ol, 3-acetoxy	20.11	19.78	0.38	Steroidal	Anti-inflammatory and antimicrobial properties
		13.85	12.10	0.38		
51	W-18	21.15	38.57	0.09	-	Weak peripheral benzodiazepine receptor

GC-MS spectrum of CMMA revealed 8 compounds with 11 peaks with major area covered by glycerine (48.33 %), followed by 4-(2,5-dihydro-3-methoxyphenyl) butylamine (8.02 %) and cyclopropane butanoic acid, 2-[[2-[[2-[(2-pentyl cyclopropyl) methyl] cyclopropyl] methyl] cyclo propyl] methyl]-methyl ester (4.08 %). Although many compounds were similar to that found in CMME, only few compounds namely, hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl

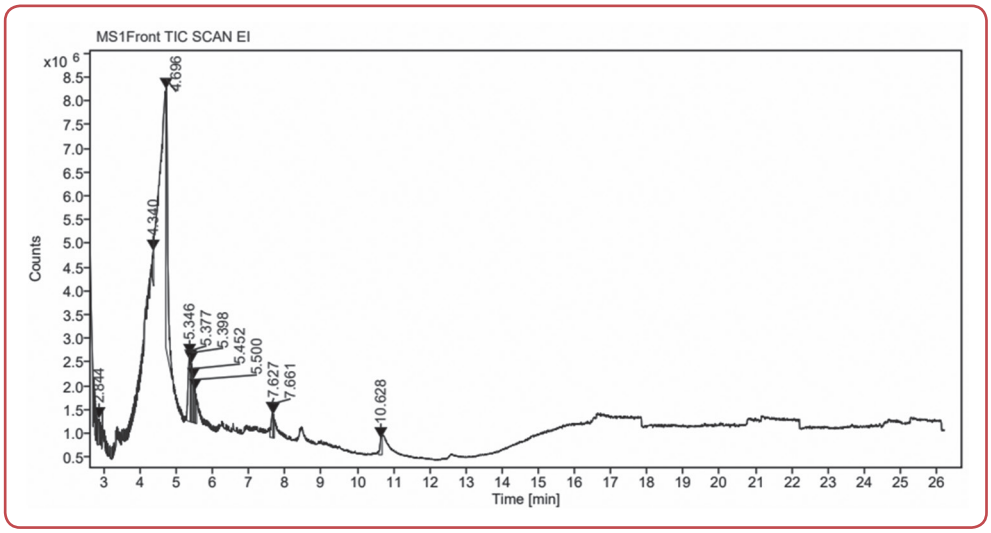

Figure 4: Gas chromatography-mass spectrometry (GC-MS) spectrum of Cymbalaria muralis aqueous extract (CMAE)

Table 3: Gas chromatography-mass spectrometry (GC-MS) spectrum obtained for Cymbalaria muralis aqueous extract (CMAE)

N	Compounds	RT	Prob %	Area	Nature of compound	Activity
1	9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester	4.70	89.71	48.33	Sugar	Wound healer
		4.34	84.01	3.81		
2	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcy-clopropyl) methyl] cyclopropyl] methyl]cyclopropyl] methyl]-,methyl ester	7.63	47.53	4.08	Fatty acid	Anti-inflammatory, antibacterial, antifungal
3	2- [[2-[[2-[(2- pentylcyclopropyl) cyclopropanebutanoic acid	7.66	55.65	2.56	Fatty acid	Antioxidant, hypocholesterolaemic
4	Hexadecanoic acid, 1- (hydroxymethyl)-1,2-ethanediyl ester	10.63	70.67	3.34	Fatty acid ester	Antioxidant, hypocholesterolaemia
5	1,2-Propanediol diformate	2.84	22.79	3.20	Ester	-
6	Tetraacetyl-d-xylonic nitrile	5.50	19.50	3.80	Organic compound	Antiviral and antioxidant
7	4-(2,5-Dihydro-3- methoxyphenyl)butylamine	5.40	21.00	8.02	Amine	Anti-inflammatory
		5.38	15.97	4.85		
		5.35	11.23	13.76		
8	3,5-Dimethyl-5-[2- pyridyl]-1,3,4- thiadiazolidin -2-thione	5.45	11.01	4.28	Heterocyclic	-

ester, 1,2-propanediol diformate and 4-(2,5-dihydro-3-methoxyphenyl) butylamine were additional in this extract (Figure 4, Table 3).

LC-MS/MS

Along with the previously reported secondary metabolites from different geographical regions of the same plant including apigenin, luteolin, chrysoeriol 7-glucosides, 7-glucuronides, apigenin 7-O-glucoside, chrysoeriol 7-rutinosides, diosmin, catapol, linarin, glucosyringic, 8-epi muraloside and 8-epiloganic acid, three novel metabolites like harpagide, aucubin and actinidine have also been discovered for the first time in this plant (Figure 5 and 6). In CMME, the apigenin 7-O-glucoside (flavonoid) was found abundantly with concentration of 70.41 ng/mL, followed by glucosyringic acid, an iridoid (60.78 ng/mL) whereas, in CMMA, diosmin (68.44 ng/mL) was found as a major component followed by glucosyringic acid (67.80 ng/mL) (Table 4).

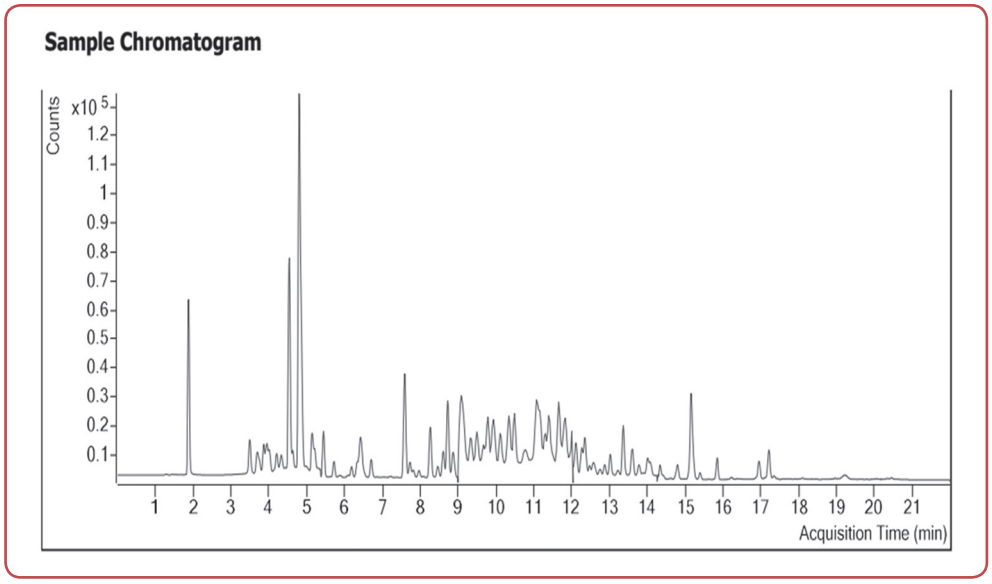

Figure 5: Liquid chromatography–tandem mass spectrometry (LC-MS/MS) spectrum of Cymbalaria muralis methanolic extract (CMME)

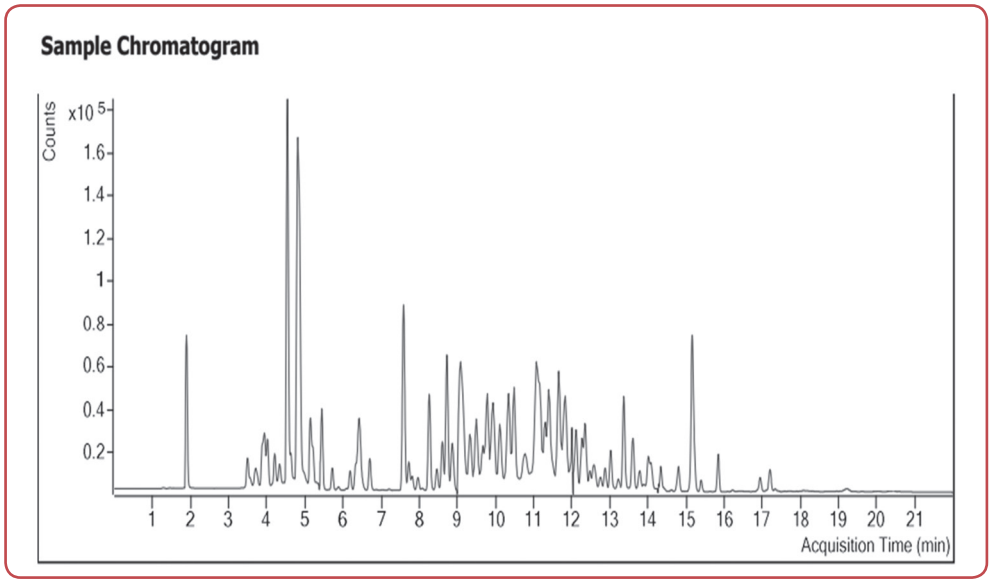

Figure 6: Liquid chromatography–tandem mass spectrometry (LC-MS/MS) spectrum of Cymbalaria muralis aqueous extract (CMAE)

Table 4: Retention time (RT) and concentration of all recognised iridoids and flavonoids

N	Compounds	Concentration in CMME (ng/mL)	RT CMME	Concentration in CMAE (ng/mL)	RT CMAE	Type of compound	Activity reported
1	Apigenin	54.64	3.88	65.34	3.92	Flavonoids	Antioxidant, anti-diabetic and anti inflammatory
2	Luteolin	10.46	4.55	8.12	4.55	Flavonoids	Antioxidant, antibacterial, anti-inflammatory and anti-cancer.
3	Chrysoeriol 7-glucosides	37.14	4.81	30.06	4.82	Flavonoids	Antioxidant, anti-inflammatory, anti-diabetic
4	7-glucuronides	28.19	5.15	24.65	5.15	Flavonoids	Antioxidant, anti-inflammatory

5	Apigenin 7-O -glucoside	70.41	5.45	62.50	5.45	Flavonoids	Antioxidant, anti-inflammatory
6	Chrysoeriol 7 -rutinosides	13.53	8.73	14.69	8.73	Flavonoids	Antioxidant, antimicrobial, anti-inflammatory
7	Diosmin	58.84	9.08	68.44	9.08	Flavonoids	Antioxidant, anti-inflammatory
8	Catapol	51.75	9.08	45.41	9.08	Iridoids	Antioxidant, anti-inflammatory anti-diabetic
9	Linarin	8.52	9.51	6.11	9.51	Flavonoids	Antioxidant, anti-inflammatory
10	Glucosyringic acid	60.78	9.80	67.80	9.80	Iridoids	Antioxidant, anti-inflammatory
11	8-Epi Muraloside	8.64	10.12	7.11	10.12	Iridoids	-
12	8-Epiloganic acid	41.38	10.37	48.99	10.37	Iridoids	Antioxidant, anti-inflammatory anti-diabetic
13	Harpagide	17.93	11.09	24.82	11.09	Iridoids	Antioxidant, anti-inflammatory
14	Aucubin	20.36	11.32	17.70	11.32	Iridoids	Antioxidant, anti-inflammatory
15	Actinidine	53.74	15.16	40.26	15.16	Enzyme	Antioxidant, α-amylase inhibitor

CMME: Cymbalaria muralis methanolic extract; CMAE: Cymbalaria muralis aqueous extract;

Discussion

Indian *Cymbalaria muralis* is recognised as a plant of traditional importance but scientific literature available for its pharmacological activities as well as its bioactive constituents is very limited. Till date, no study has been conducted on revealing its phytochemical profile and antioxidant potential. The initial results of the preliminary phytochemical screening were validated using GC-MS and LC-MS/MS analysis. Therefore, this study divulges all the phytochemicals present in the plant and opens prospects for future studies. The phytoconstituents present in the methanolic extract of this plant are namely γ-sitosterol, stigmasterol, ethyl iso-allocholate and β-sitosterol which are well known anti-diabetic constituents that support its traditional claim for the use in diabetes.

The phytochemical profiling was first done using GC-MS technique, after which, some expected or already reported metabolites (not detected by GC-MS) were confirmed through LC-MS/MS due to its higher specificity. The plant contains a significant number of flavonoids and iridoids as confirmed by LC-MS/MS analysis. The metabolites *vis* apigenin, luteolin, chrysoeriol-7-glucosides, 7-glucuronides, apigenin-7-O-glucoside, chrysoeriol-7-rutinosides, diosmin, catapol, linarin, glucosyringic, 8-epimuraloside, 8-epiloganic acid are previously reported in literature. [27] However, harpagide, aucubin, actinidine are reported for the first time in this plant.

A similar study has also been conducted by Cherie et al, [27] on Algerian *Cymbalaria muralis* for evaluation of its composition and biological activities of its aerial parts but the current study involved the whole plant extracts. The results of antioxidant potential, IC_{50}-40.82 µg/mL (CMME) and 75.20 µg/mL (CMAE), are concordant with the previous study. However, CMME (IC_{50}-40.82 µg/mL) possessed better antioxidant activity in comparison to Algerian *Cymbalaria muralis* (IC_{50}-63.05 µg/mL). The presence of secondary metabolites like carotenoids, iridoids, fatty acids, flavonoids, phenols and vitamins as described by GC-MS and LC-MS/MS analysis can be considered as a possible major reason behind their antioxidant efficacy.[28] Both extracts also show a prominent level of total phenolic and total flavonoid content, which also contributes to its strong antioxidant activity in the DPPH radical scavenging assay.

Conclusion

The GC-MS profile of the methanolic and aqueous extracts of Indian *Cymbalaria muralis* have shown presence of numerous secondary metabolites. GC-MS revealed that among all secondary metabolites, fatty acids or lipids were most abundant in both extracts, while targeted iridoids and flavonoids were quantified by LC-MS/MS. The Indian *Cymbalaria muralis* phytochemical profile is found similar to the available literature, however, three new iridoids namely harpagide, aucubin, actinidine are reported for the first time in this plant. The study concludes that Indian *Cymbalaria muralis* exhibited remarkable antioxidant activity and rich phytochemical profile. Most of the phytoconstituents present in the extracts are well known with documented pharmacological activities. Hence, Indian *Cymbalaria muralis* rich phytochemical profile can potentially be useful in combating various ailments.

Ethics

This study did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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