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In vitro Antioxidant Activity and Phytochemical Profiling of Methanolic Extract of *Lemna minor*

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The present study investigated the *in vitro* antioxidant potential of methanolic extracts of *Lemna minor* and characterised its phenolic and flavonoid constituents using liquid chromatography-mass spectrometry (LC-MS) and high-performance liquid chromatography (HPLC). Antioxidant activity was evaluated using phosphomolybdenum total antioxidant capacity (TAC), Folin-Ciocalteu total phenolic content (TPC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging assays, with L-ascorbic acid employed as the reference standard. The methanolic extract exhibited a total antioxidant capacity of 16.24 ± 6.00 mg AAE/g extract and a total phenolic content of 34.00 ± 5.43 mg GAE/g extract. The extract demonstrated strong free radical-scavenging activity, with EC_{50} values of 0.77 ± 0.04 mg/mL for the DPPH assay and 2.00 ± 0.08 mg/mL for the ABTS assay. LC-MS analysis identified several bioactive phenolic acids and flavonoids, including *p*-coumaric acid, ferulic acid, catechin and naringenin, which were subsequently confirmed by HPLC. The extract was characterised by low contents of antinutritional factors such as tannins and phytates. The presence of phenolic phytochemicals as well as the *in vitro* antioxidant activity indicate that *L. minor* may be considered a promising source of plant-based functional ingredients.

Keywords: *Lemna minor*, Duckweed, Methanolic extract, Antioxidant activity, LC-MS, Phytochemical profiling.

INTRODUCTION

Lemna minor L. (duckweed) is a free-floating perennial aquatic macrophyte of the family Lemnaceae, known for its rapid growth, short doubling time and high biomass productivity [1,2]. *L. minor* has a long history of use in folk medicine, with documented applications in the management of allergies, asthma, vitiligo, jaundice, glaucoma, rheumatism and gout. Beyond medicinal uses, duckweeds have been evaluated as protein-rich feed ingredients for livestock and as candidates for biofuel and functional-food applications [1,2]. The chemical composition of *L. minor* has been reported to include proteins, vegetable fibres, fats, polysaccharides, flavonoids, amino acids, aliphatic acids, phenolic acids, triterpene compounds, vitamins and micro- and macro-elements [3,4]. A survey by Vladimirova & Georgiyants [5] identified 32 biologically active compounds from the plant, including phytosterols (52.8 mg/kg), saturated hydrocarbons (23.1 mg/kg), fatty acids and their derivatives (11.1 mg/kg) and aldehydes and ketones (20.2 mg/kg).

The phytochemistry of *L. minor* has attracted considerable attention in past due of its diverse secondary metabolites. Petrova-Tacheva *et al*. [3] characterised the metabolite profile of *L. minor* by LC-MS/MS and identified twelve antioxidant compounds, with fraxetin as the predominant constituent (0.21 ± 0.02 mg/g in a 70% alcoholic extract). Other compounds too identified were phytol, dihydroactinidiolide, campesterol, loliolide, ascorbic acid, 2,3-dihydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, esculetin and esculin. Of particular interest are the phenolic acids and flavonoids reported in *L. minor*, namely caffeic, ferulic, p-coumaric, vanillic and chlorogenic acids, catechin and the low-molecular-weight antioxidant ascorbic acid, all of which have been associated with antioxidant activity in a variety of plant extracts. Catechin has been extensively studied for its ability to scavenge free radicals, inhibit lipid peroxidation and protect low-density lipoprotein (LDL) against oxidative damage in mammalian and cell-based systems [6,7]. Phenolic acids (*p*-coumaric, ferulic, caffeic, chlorogenic and vanillic acids) together with flavonoids such as catechin and naringenin are likewise recognised contributors to the antioxidant capacity of plant-derived materials [8,9].

Despite these findings, several aspects of the phytochemical and antioxidant profiles of L. minor remain unresolved. Petrova-Tacheva *et al*. [3] identified antioxidant constituents by LC-MS/MS, yet targeted HPLC quantification of the major phenolic acids (*p*-coumaric and ferulic acids) and flavonoids (catechin and naringenin) in a standardised methanolic oleoresin has not, to our knowledge, been described. Individual *in vitro* antioxidant assays have been reported for *L. minor* extracts, but a unified evaluation employing Folin-Ciocalteu total phenolics, phosphomolybdenum total antioxidant capacity, FRAP, DPPH and ABTS assays under the same analytical conditions with L-ascorbic acid as the reference standard is lacking.

Plant secondary metabolites, particularly phenolic acids and flavonoids, represent one of the largest classes of natural antioxidants due to their well-established redox properties [10,11]. With this background, the present study was designed to obtain a comprehensive phytochemical, antioxidant and antinutritional profile of *L. minor*. Phenolic acids and flavonoids were profiled by LC-MS/MS, followed by HPLC validation and quantification of the major constituents [9,12-14]. Antioxidant capacity was assessed using an integrated panel comprising Folin-Ciocalteu total phenolics, phosphomolybdenum total antioxidant capacity, FRAP, DPPH and ABTS assays, with L-ascorbic acid serving as the positive reference standard [6,8,15-17]. Condensed tannins (vanillin-HCl assay) and phytic acid/phytates were quantified in the same biomass to establish its anti-nutritional profile. The use of a single standardised methanolic extract for LC-MS profiling, HPLC quantification, integrated antioxidant assessment and anti-nutritional analysis provides a comprehensive characterisation of *L. minor* that has not been reported previously and forms the principal novelty of the present study.

EXPERIMENTAL

2,2-Azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri-(2-pyridyl)-5-triazine (TPTZ), ammonium molybdate, amylase, ascorbic acid, disodium hydrogen phosphate, FC reagent, ferric chloride, hexane, methanol, sodium bicarbonate, *trans*-*p*-coumaric acid, ferulic acid, naringenin were obtained from Sisco Research Laboratories (India). All the chemicals used in the present study were of analytical grade.

Collection of *L. minor*: Fresh *L. minor* was collected from Karangi Lake, Mysuru, India. A fresh live sample of the plant was deposited at the Survey of Medicinal Plants Unit, Central Ayurveda Research Institute (CARI), Bengaluru, India, for taxonomic authentication. Following authentication, a voucher specimen was deposited in the herbarium and assigned Voucher Specimen No. (RRCBI-mus428). The authenticated plant material was subsequently washed thoroughly with distilled water and to remove adhering debris and was further used for culturing. The cultured *L. minor* was then harvested, shade dried at room temperature, pulverised into a fine powder and stored in airtight containers until further analysis.

Plant cultivation and sample preparation: Following taxonomic authentication, *L. minor* was thoroughly washed with distilled water to remove adhering debris and cultured in modified 1× Hoagland nutrient medium prepared according to Hoagland & Arnon [18] with minor modifications to the nutrient composition. The composition of the modified medium is shown in Table-1. Cultures were maintained under controlled laboratory conditions until adequate biomass was obtained. The harvested biomass was washed with distilled water, shade-dried at room temperature, ground into a fine powder and stored in airtight containers until further analysis.

TABLE-1
COMPOSITION OF THE 1× HOAGLAND NUTRIENT MEDIUM USED FOR THE CULTIVATION OF *L. minor* UNDER LABORATORY CONDITIONS

Component	Concentration (mg/L)
Macronutrients	
Potassium nitrate	1000
Calcium nitrate	300
Magnesium sulphate	200
Monopotassium phosphate	250
Micronutrients	
Boric acid	1.00
Manganese sulphate	2.00
Sodium molybdate	0.12
Copper sulphate	0.08
Zinc sulphate	0.22
Ferric chloride	20.00
Ethylenediaminetetraacetic acid	50.00

Preparation of methanolic extract (LME): *L. minor* was cultivated in Hoagland's medium, oven-dried at 50 ºC and ground into a fine powder. The dried material was extracted with 95% methanol using a 1:10 (w/v) solvent-to-sample ratio for 24 h following the reported method [13]. The extraction mixture was agitated at 30 min intervals to improve solvent penetration and phytochemical recovery. Extractions were carried out in triplicate under identical conditions of solvent concentration, solvent-to-sample ratio, extraction time and processing to ensure reproducibility. The extract was filtered through Whatman No. 1 filter paper and the filtrate was concentrated under reduced pressure using a rotary evaporator. Residual solvent was removed by drying the concentrated extract at 50 ºC. The extraction yield was 17.7 ± 2.4% (mean ± SD, n = 3), with comparable yields obtained across the three independent extractions.

Antioxidant activity: All antioxidant assays were performed using two independently prepared methanolic extract batches. Each batch was analysed in duplicate under identical experimental conditions. Results from both batches were combined for statistical analysis and are expressed as mean ± standard deviation (SD). L-Ascorbic acid served as the positive reference standard for the Folin-Ciocalteu total phenolics, phosphomolybdenum total antioxidant capacity, DPPH and ABTS radical-scavenging and FRAP ferric-reducing power assays. Gallic acid was used as the calibration standard for the Folin-Ciocalteu total phenolic assay.

Total polyphenols: Folin-Ciocalteu method was employed for the quantification of the total phenol content in LME where the reagent is reduced to molybdenum-tungsten by the phenol in the extract which were read at 765 nm [15]. Gallic acid was utilised to compute the standard curve. The results were mean values ± standard deviations and expressed as milli-

grams of gallic acid equivalents (GAEs) per g of *Lemna minor* dry weight (DW).

Total antioxidant activity (TAC): Total antioxidant capacity (TAC) of the *L. minor* methanolic extract (LME) was determined by the phosphomolybdenum assay [6]. Under acidic conditions, Mo(VI) is reduced to Mo(V), forming a green phosphomolybdenum complex that was measured spectrophotometrically at 695 nm. L-Ascorbic acid served as both the calibration and positive reference standard. To minimise variation associated with extraction yield, methanolic extraction was performed independently on two batches of dried *L. minor* powder using the same extraction protocol. Each extract batch was analysed under identical experimental conditions in all antioxidant assays.

Radical scavenging activity: The free radical-scavenging activity of the LME was evaluated using the DPPH and ABTS assays. The DPPH assay is based on electron transfer, in which reduction of the purple DPPH radical by an antioxidant results in a decrease in absorbance measured at 514 nm. L-Ascorbic acid was used as the positive reference standard [17]. ABTS radical-scavenging activity was determined according to the method described by Xiao *et al*. [16]. The assay measures the ability of the extract to quench the ABTS radical cation, with L-ascorbic acid serving as the positive reference standard.

FRAP assay: FRAP assay was based on the principle of reducing ferric-tripyrdyltriazine to ferrous-tripyrdyl triazine at low pH by the sample antioxidant and read at 593 nm [16]. The calibration standards, working concentration ranges and coefficients of determination (R^2) were used for the antioxidant assays (Table-2).

Procedure for profiling of flavonoids and phenolic acids by LCMS: Individual phenolic acids and flavonoids were extracted for LCMS/MS analysis using 80% methanol following the reported methods [19,20] with minor modifications. A known quantity of powdered *L. minor* was homogenized in 80% methanol, centrifuged and the supernatant volume was adjusted to 20 mL. The extract was concentrated to near dryness under reduced pressure at 45 ºC, diluted with 5 mL of distilled water and finally extracted three times with petroleum ether. The aqueous phase was collected and extracted with ethyl acetate using a separating funnel. The ethyl acetate fraction was separated and evaporated to dryness under reduced pressure at room temperature. The residue was hydrolysed overnight with 4 mL of 2 N NaOH, acidified to pH 2 with 5 mL of 2 N HCl and re-extracted with 10 mL of ethyl acetate. The ethyl acetate fraction containing phenolic acids and flavonoids was evaporated to dryness under reduced pressure. The dried residue was dissolved in 1 mL of MS-grade methanol, filtered through a 0.2 µm nylon membrane filter and injected into the LC-MS/MS system for the analysis of phenolic acids and flavonoids.

LC and MS-MS conditions: Phenolic acids and flavonoids were separated on a BEH C18 analytical column (2.1 × 50 mm, 1.7 µm; Waters India Ltd.) protected by a Vanguard BEH C18 guard column (Waters, USA). Chromatographic separation was carried out using a gradient elution of aqueous and organic mobile phases at a flow rate of 0.1 mL/min. The column temperature was maintained at 25 ºC and a 6 µL sample was injected for each analysis. Eluted compounds were detected using a photodiode array (PDA) detector and the UPLC column effluent was introduced directly into a TQD-MS/MS system (Waters, USA) without flow splitting for the analysis of phenolic acids and flavonoids. Data acquisition and processing were performed using Waters MassLynx software.

Mobile phase: Solvent A: 0.1% formic acid in water and Solvent B: 0.2% formic acid in methanol.

HPLC analysis: Based on the preliminary LC-MS profiling, *p*-coumaric acid, ferulic acid and naringenin were selected as marker compounds for targeted HPLC quantification. These compounds represent the major phenolic subclasses identified in the extract and were quantified using authenticated reference standards to establish the phytochemical profile of the *L. minor* methanolic extract.

***trans-p*-Coumaric acid:** HPLC analysis was performed on a C18 reversed-phase column (Symmetry, 4.6 × 250 mm) under isocratic conditions using a mobile phase of water: methanol acetic acid (65:34:1, v/v/v) at a flow rate of 1.0 mL/min, following the method of Ramadoss *et al*. [14]. Standard *trans-p*-coumaric acid (0.4 mg/mL) and the methanolic extract (5 mg/mL) were prepared in the mobile phase and 20 µL of each solution was injected. Elution was monitored at 310 nm. Based on the LC-MS profiling results, *p*-coumaric acid, ferulic acid and naringenin were selected as marker compounds for targeted HPLC quantification. These analytes represent distinct phenolic subclasses identified in the extract and were quantified using authenticated reference standards to establish the phytochemical profile of the LME.

Analysis of ferulic acid: HPLC analysis of ferulic acid was performed on a C18 reversed-phase column (Symmetry, 4.6 × 250 mm) under isocratic conditions using methanol (48:52, v/v; pH 3.0) as the mobile phase at a flow rate of 1.0 mL/min, following the method of Nadal *et al*. [21].

Analysis of naringenin: HPLC analysis of naringenin was performed on a C18 reversed-phase column (Symmetry, 4.6 × 250 mm) under isocratic conditions using acetonitrile: 0.1 M ammonium acetate acid (30:69:1, v/v/v; pH 4.9) as the mobile phase at a flow rate of 1.0 mL/min. Standard naringenin (1 mg/mL) and the methanolic extract (5 mg/mL) were prepared in the mobile phase and 20 µL of each solution was

TABLE-2
CALIBRATION STANDARDS AND LINEARITY OF THE *in vitro* ANTIOXIDANT ASSAYS

Assay	Standard used	Calibration range (µg/mL)	Linearity (R^2)
Total polyphenol content (TPC)	Gallic acid	0–400	0.9991
Total antioxidant capacity (TAC)	Ascorbic acid	0–200	0.9956
DPPH radical scavenging assay	Ascorbic acid	0-200	0.9975
Ferric reducing antioxidant power (FRAP)	Ascorbic acid	0-200	0.9946
ABTS radical scavenging assay	Ascorbic acid	0-200	0.9974

injected. Elution was monitored at 292 nm. The concentration of each compound in the extract was calculated using the following equation:

$$\text{Amount of compound} = \frac{A}{A_1} \times \frac{B}{B_1} \times \frac{C}{C_1} \times \text{Mean weight}$$

where A = sample area; A_1 = standard area; B = standard amount; B_1 = standard dilution; C = dilution of sample; C_1 = sample amount.

Antinutrient activity

Determination of tannins: Condensed tannin content of the LME was determined by the vanillin-hydrochloric acid colorimetric method as described by Broadhurst & Jones [22]. Dried and powdered plant material (1.0 g) was extracted with 10 mL of 70% acetone by shaking for 30 min, followed by centrifugation. An aliquot of the supernatant (0.5 mL) was mixed with 3.0 mL of freshly prepared vanillin reagent (1%, w/v, in methanol) and 1.5 mL of conc. HCl. The reaction mixture was incubated at room temperature for 15 min and the absorbance was measured at 500 nm against a reagent blank. Tannic acid was used as the calibration standard and the results were expressed as milligrams of tannic acid equivalents per gram of dry sample (mg TAE/g).

Determination of phytates: Phytate content was determined using the Wade reagent method described by Latta & Eskin [23]. Dried and powdered plant material (1.0 g) was extracted with 10 mL of 0.2 M HCl by shaking for 1 h, followed by centrifugation to obtain a clear supernatant. An aliquot of the extract (200 µL) was mixed with 200 µL of Wade reagent containing 0.03% $FeCl_3$ and 0.3% sulphosalicylic acid. The reaction mixture was incubated at room temperature for 10-15 min and the absorbance was measured at 490 nm against a reagent blank. Sodium phytate was used as the calibration standard and the phytate content was expressed as milligrams per gram of dry sample (mg/g).

RESULTS AND DISCUSSION

Extraction yield and antioxidant: The methanolic extraction of *L. minor* (LME) yielded 17.7 ± 2.4% (mean ± SD), with comparable extraction efficiency across independent batches. This yield is consistent with previous reports describing methanol as an efficient solvent for recovering phenolic compounds from aquatic plants [13]. The extract obtained was subsequently used for phytochemical characterisation and antioxidant evaluation.

LC-MS profiling and HPLC quantification of phenolic acids and flavonoids: Preliminary LC-MS profiling identified *p*-coumaric acid, ferulic acid and naringenin as representative marker compounds for targeted HPLC quantification. LC-MS analysis detected a diverse range of phenolic acids and flavonoids in the methanolic extract (Fig. 1). Multiple reaction monitoring chromatograms of phenolic acid and flavonoid standards are shown in Tables 3 and 4. The predominance of these compounds is consistent with previous reports describing *L. minor* as a rich source of phenolic antioxidants. Data processing identified 15 phyto-chemical constituents, among which *p*-coumaric acid (390.0 µg/g) and ferulic acid (164.46 µg/g) were the predominant phenolic acids. Catechin (361.75 µg/g) and naringenin (228.18 µg/g) represented the major flavonoids detected (Table-5).

The relatively high catechin concentration measured in *L. minor* may account for a substantial proportion of the radical scavenging activity observed in the present investigation. Ferulic acid, *p*-coumaric acid and naringenin are well-recognised antioxidants that neutralise free radicals through hydrogen atom donation and electron transfer mechanisms while protecting cells against oxidative damage [24,25]. The combined presence of these phenolic compounds is likely to account for the strong antioxidant activity observed across the *in vitro* assays.

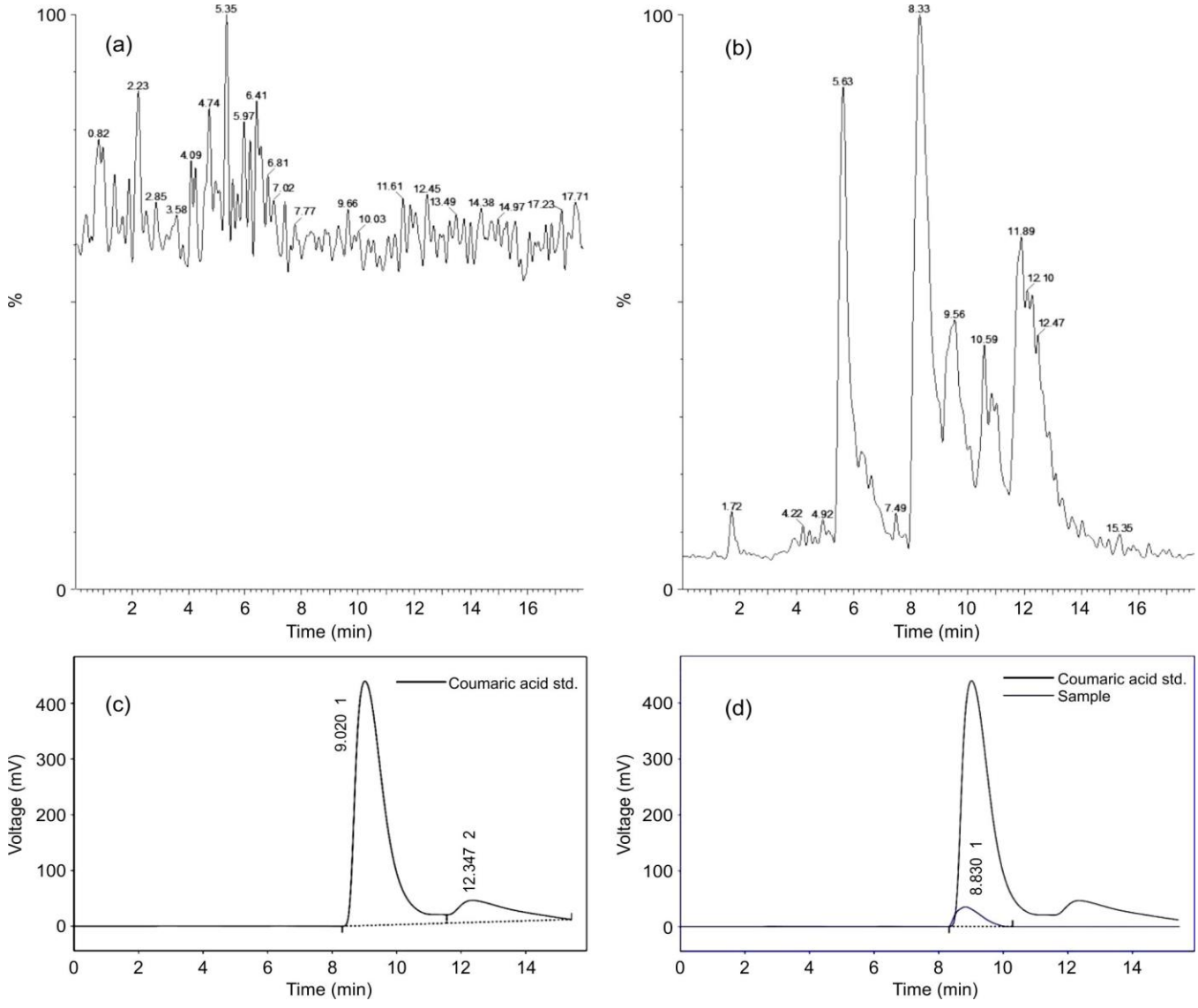
The phytochemical profile obtained by LC-MS served as the basis for targeted HPLC quantification of selected marker compounds. *p*-Coumaric acid, ferulic acid and naringenin were selected as they were among the predominant constituents detected by LC-MS, represent different classes of phenolic metabolites and possess well-documented antioxidant properties. Quantitative determination of these compounds using authenticated reference standards strengthened the reliability of the phytochemical characterisation.

TABLE-3
MRM OF FLAVONOIDS STANDARDS

Compounds	Retention time	Formula/ mass	Parent ion (*m/z*) $[M+H]^+$	Daughters	Conc. voltage (V)	Collision energy (eV)	Ion mode
Apigenin	7.07	270	268.97	107.04	46	30	ES-
Catechin	1.15	290	289.03	245.15	38	12	ES-
Hesperetin	6.62	302	300.97	286.15	42	16	ES-
Myricetin	5.21	318	317.03	151.06	42	28	ES-
Quercetin	6.19	302	301.03	151.12	36	20	ES-
Rutin	4.66	610	609.10	300.20	60	42	ES-
Umbelliferone	3.06	162	161.04	133.07	42	18	ES-
Luteolin	6.47	286	284.97	93.05	52	32	ES-
Kaemepherol	6.98	286	284.97	93.05	52	32	ES-
Naringenin	6.39	272	271.13	119.05	34	22	ES-
Epicatechin	1.67	290	289.05	109.08	36	22	ES-
Epigallocatechin	0.79	306	305.05	125.10	34	20	ES-
Fisetin	5.39	286	285.08	121.05	42	26	ES-
Galangin	8.59	270	269.08	168.53	52	28	ES-
Eriodictyol	5.66	288	287.09	135.12	32	20	ES-

TABLE-4
MRM OF PHENOLIC ACID STANDARDS

Compounds	Retention time	Formula/ mass	Parent ion (*m/z*) $[M+H]^+$	Daughters	Conc. voltage (V)	Collision energy (eV)	Ion mode
Caffeic acid	6.61	180	178.90	135.05	30	16	ES-
2,4-Dihydroxybenzoic acid	6.50	154	152.90	65.02	28	18	ES-
Chlorogenic acid	6.19	354	352.97	191.10	22	18	ES-
Ferulic acid	9.36	194	192.90	134.02	26	14	ES-
Gallic acid	2.10	170	168.90	125.03	28	12	ES-
Gentisic acid	19.7	154	152.90	108.98	24	12	ES-
o-Coumaric acid	8.39	164	162.90	119.06	22	12	ES-
p-Coumaric acid	8.34	164	162.90	119.05	24	14	ES-
p-Hydroxybenzoic acid	5.69	138	136.90	93.01	26	12	ES-
Protocatecuic acid	4.24	154	152.90	109.05	26	16	ES-
Salicylic acid	12.70	138	136.90	93.10	28	14	ES-
Syringic acid	6.99	198	196.97	182.07	26	10	ES-
t-Cinnamic acid	11.08	148	146.90	103.05	26	10	ES-
Vanillic acid	6.48	168	166.97	108.01	26	20	ES-
Benzoic acid	11.35	122	120.96	77.02	26	10	ES-
3-Hydroxy benzoic acid	6.76	138	137.02	93.80	28	10	ES-
Sinapic acid	9.66	224	223.11	149.06	32	14	ES-
Ellagic acid	7.67	302	301.09	144.00	70	38	ES-



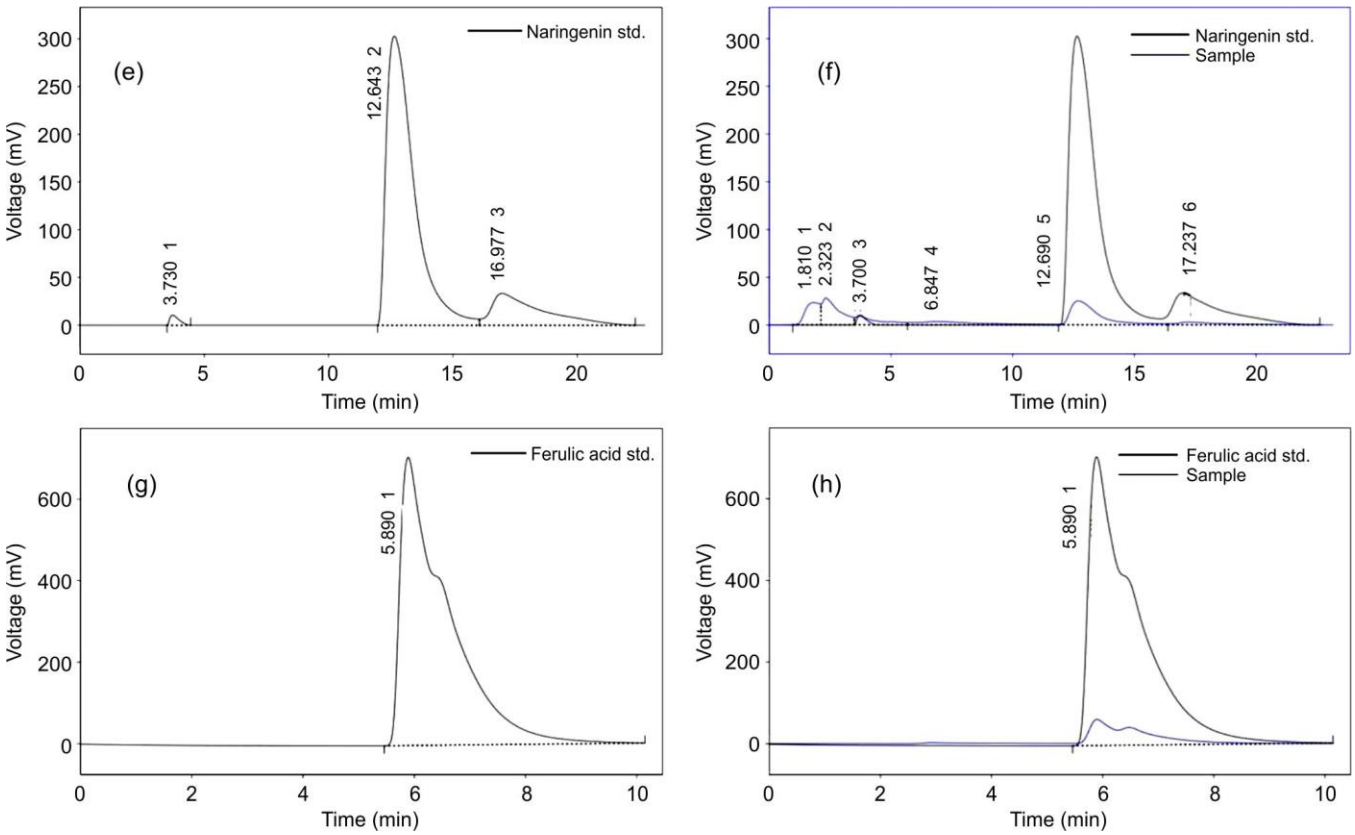


Fig. 1. LCMS chromatogram of (a) flavonoids, (b) phenolic acid profiling, (c) coumaric acid standard, (d) coumaric acid present in sample, (e) naringenin standard, (f) naringenin present in sample, (g) ferulic acid standard and (h) ferulic acid present in sample

TABLE-5
AMOUNT OF PHENOLIC ACIDS AND FLAVONOIDS PRESENT IN THE LME

Phenolic acids	Amount (μg/g)
p-Coumaric acid	390.0
o-Coumaric acid	3.37
Vanillic acid	4.51
Gallic acid	38.24
Caffeic acid	42.0
Ferulic acid	164.46
Flavonoids	**Amount (μg/g)**
Naringenin	228.18
Catechin	361.75
Epicatechin	63.61
Myricetin	112.0
Rutin	29.0
Epigallocatechin	37.07

HPLC analysis confirmed the presence of the selected marker compounds in the methanolic extract. The concentrations of *trans*-*p*-coumaric acid, ferulic acid and naringenin were 5 μg/mL, 7 μg/mL and 17 μg/mL, respectively. These results agreed with the LC-MS profiling, where *p*-coumaric acid (393 μg/g) and ferulic acid (166 μg/g) were the predominant phenolic acids, while catechin (362 μg/g) and naringenin (228 μg/g) represented the major flavonoids. The agreement between the two analytical techniques provides confidence in the identity and abundance of the principal antioxidant constituents present in the extract.

The phenolic acids identified in this study contribute significantly to antioxidant activity. *p*-Coumaric acid scavenges free radicals and interrupts oxidative chain reactions through hydrogen donation from its phenolic hydroxyl group [26], while ferulic acid exhibits similar activity, with its methoxy group enhancing resonance stabilisation of phenoxyl radicals and antioxidant efficiency [27]. Both compounds protect against lipid oxidation and oxidative damage, making them suitable markers of antioxidant-rich plant extracts. Naringenin, selected for HPLC quantification, is well known for its antioxidant and cytoprotective properties through scavenging reactive oxygen species, modulating cellular antioxidant defences and reducing oxidative stress. Its relatively high abundance in the methanolic extract supports the strong DPPH and ABTS radical-scavenging activities [28,29]. Although catechin was detected at a higher concentration by LC-MS, naringenin was selected for quantification due to its superior chromatographic suitability and the availability of validated analytical methods with authenticated reference standards.

Antioxidant activity: The integrated analytical strategy adopted in the present work provides a more complete assessment of the antioxidant potential of *L. minor* than either technique alone. LC-MS profiling identified abundant phenolic acids and flavonoids, while HPLC provided quantitative confirmation of representative marker compounds. The antioxidant properties of the methanolic extract are shown in Table-6. Total phenolic content was 34.0 ± 5.0 mg GAE/g

TABLE-6
In vitro ANTIOXIDANT ACTIVITY OF THE METHANOLIC EXTRACT OF *L. minor* (LME) DETERMINED BY DIFFERENT ASSAYS

Assay	Result
Total polyphenols	34.0 ± 5.0 (mgGAE/g of extract)
Total antioxidant capacity (TAC)	16.24 ± 6.0 (mg AAE/g of extract)
DPPH (α,α-diphenyl-β-picrylhydrazyl)	EC_{50}: 0.77 ± 0.04 mg/mL of extract
FRAP (Ferric reducing antioxidant power)	19 ± 0.8 (mg AAE/g of extract)
2,2′-Azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid)	EC_{50}: 2.0 ± 0.08 mg/mL of extract)

GAE (Gallic acid equivalent); AAE (Ascorbic acid equivalent); Data is presented as mean ± SD from four observations derived from two independently prepared extract batches analysed in duplicate.

extract, while total antioxidant capacity measured by the phosphomolybdenum assay was 16.24 ± 6.0 mg AAE/g extract. Ferric reducing antioxidant power (FRAP) reached 19.0 ± 0.8 mg AAE/g extract, reflecting the electron-donating ability of the extract. Radical-scavenging activity was equally pronounced, with EC_{50} values of 0.77 ± 0.04 mg/mL for the DPPH assay and 2.0 ± 0.08 mg/mL for the ABTS assay. Calibration curves prepared with gallic acid (0-400 µg/mL) for total phenolics and L-ascorbic acid (0-200 µg/mL) for TAC, FRAP, DPPH and ABTS exhibited excellent linearity, with R^2 values of 0.9991, 0.9956, 0.9975, 0.9946 and 0.9974, respectively. The relatively high phenolic content agrees with the antioxidant response observed across all five assays. The phenolic compounds readily donate hydrogen atoms or electrons to neutralise reactive oxygen species, while their hydroxyl groups stabilise free radicals through resonance. This mechanism explains the low EC_{50} values obtained in the DPPH and ABTS assays together with the high ferric-reducing capacity measured by FRAP. Similar relationships between phenolic concentration and antioxidant activity have been reported for numerous medicinal and edible plants [8].

The phenolic content measured in the present study exceeds values reported for several commonly consumed leafy vegetables. Gunathilake & Ranaweera [30] reported total phenolic contents of 4.60 ± 0.08 mg GAE/g dry weight for *Costus speciosus*, 1.56 ± 0.61 mg GAE/g dry weight for *Amaranthus caudatus* and 3.81 ± 0.16 mg GAE/g dry weight for spinach. The substantially higher phenolic concentration observed in *L. minor* supports its value as a rich source of natural antioxidants and reflects the abundance of phenolic metabolites detected during phytochemical analysis.

Antinutritional factors and nutritional significance: Antinutritional constituents are routinely assessed during the nutritional evaluation of plant-derived ingredients as excessive concentrations may reduce nutrient availability. Tannins can interact with proteins and digestive enzymes, lowering protein digestibility [31], whereas phytates chelate essential minerals such as iron, zinc, calcium and magnesium, reducing their intestinal absorption [32]. The concentrations measured in the present study are within the range reported for edible plant materials and aquatic plants, supporting the nutritional suitability of *L. minor*. The antinutritional profile of the LME was evaluated by determining condensed tannins and phytates. The tannin content was 5.01 ± 0.38 mg/g dry weight, while the phytate content was 9.31 ± 0.45 mg/g dry weight (mean ± SD, n = 3). These values indicate that the extract contains relatively low levels of antinutritional factors, when compared with many edible terrestrial plant materials. The values obtained in the present investigation are consistent with those observations and strengthen the nutritional profile of *L. minor* as a potential plant-based ingredient. Lower concentrations of tannins and phytates are particularly desirable since they improve protein utilisation and mineral bioavailability without diminishing the phytochemical richness of the plant [31,33].

Conclusions

The present study established the presence of biologically active phytochemicals in the methanolic extract of *Lemna minor* (LME) and demonstrated its significant *in vitro* antioxidant activity through total phenolic content, total antioxidant capacity, FRAP, DPPH and ABTS assays using L-ascorbic acid and gallic acid as reference standards. LC-MS profiling identified a diverse range of phenolic acids and flavonoids, while targeted HPLC quantification of *p*-coumaric acid, ferulic acid and naringenin provided quantitative confirmation of representative antioxidant constituents. The extract contained low levels of tannins and phytates, supporting the favourable nutritional profile of *L. minor*, as lower concentrations of these antinutritional factors are associated with improved protein digestibility and mineral bioavailability. The combined phytochemical, antioxidant and antinutritional findings indicate that *L. minor* is a valuable source of natural antioxidants with potential for use as a functional food ingredient and sustainable plant-based protein resource.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI.

All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

REFERENCES

1. Y. Xu, Q. Zhao, A.M. Stomp and J.J. Wang, *Plant Biol.*, **14**, 734 (2012); https://doi.org/10.1111/j.1438-8677.2011.00553.x
2. K.-J. Appenroth, K.S. Sree, V. Böhm, S. Hammann, W. Vetter, M. Leiterer and G. Jahreis, *Food Chem.*, **217**, 266 (2017); https://doi.org/10.1016/j.foodchem.2016.08.116
3. V. Petrova-Tacheva, V. Ivanov and A. Atanasov, *Trakia J. Sci.*, **18(Suppl. 1)**, 157 (2020); https://doi.org/10.15547/tjs.2020.s.01.029
4. P. Khvatkov, M. Chernobrovkina, A. Okuneva and S. Dolgov, *Plant Cell Tissue Organ Cult.*, **136**, 85 (2019); https://doi.org/10.1007/s11240-018-1494-6
5. I. Vladimirova and V. Georgiyants, *Pharm. Chem. J.*, **47**; 599 (2014); https://doi.org/10.1007/s11094-014-1016-8
6. M.N. Alam, N.J. Bristi and M. Rafiquzzaman, *Saudi Pharm. J.*, **21**, 143 (2013); https://doi.org/10.1016/j.jsps.2012.05.002
7. C. Yu, C. Sun, L. Yu, M. Zhu, H. Xu, J. Zhao, Y. Ma and G. Zhou, *PLoS One*, **9**, e115023 (2014); https://doi.org/10.1371/journal.pone.0115023
8. N. May, J. Shi, H. Clunas, J.L.S.A. Neri, C. Kelso, J. Morgan, Y. Yu, K. Charlton and K. Weston-Green, *Food Funct.*, **15**, 11537 (2024); https://doi.org/10.1039/d4fo02942a
9. C.G. Fraga, M. Galleano, S.V. Verstraeten and P.I. Oteiza, *Mol. Aspects Med.*, **31**, 435 (2010); https://doi.org/10.1016/j.mam.2010.09.006
10. B. Halliwell and J.M.C. Gutteridge, Free Radicals in Biology and Medicine, Oxford, U.K.: Oxford Univ. Press, edn 3 (1999).
11. N. Saeed, M.R. Khan and M. Shabbir, *BMC Complement. Altern. Med.*, **12**, 221 (2012); https://doi.org/10.1186/1472-6882-12-221
12. A. Plazonić, F. Bucar, Ž. Maleš, A. Mornar, B. Nigović and N. Kujundžić, *Molecules*, **14**, 2466 (2009); https://doi.org/10.3390/molecules14072466
13. Z. Leng, B. Zhong, H. Wu, Z. Liu, A. Rauf, S. Bawazeer and H.A.R. Suleria, *ACS Omega*, **7**, 4563 (2022); https://doi.org/10.1021/acsomega.1c06532
14. B. Mahdi-Pour, S.L. Jothy, L.Y. Latha, Y. Chen and S. Sasidharan, *Asian Pac. J. Trop. Biomed.*, **2**, 960 (2012); https://doi.org/10.1016/S2221-1691(13)60007-6
15. R. Karthikeyan, *Int. J. Anal. Chem.*, **2015**, 201386 (2015); https://doi.org/10.1155/2015/201386
16. T.J. Herald, P. Gadgil and M. Tilley, *J. Sci. Food Agric.*, **92**, 2326 (2012); https://doi.org/10.1002/jsfa.5633
17. F. Xiao, T. Xu, B. Lu and R. Liu, *Food Front.*, **1**, 60 (2020); https://doi.org/10.1002/fft2.10
18. K.J. Lee, Y.C. Oh, W.K. Cho and J.Y. Ma, *Evid. Based Complement. Alternat. Med.*, **2015**, 165457 (2015); https://doi.org/10.1155/2015/165457
19. D.R. Hoagland and D.I. Arnon, The Water-Culture Method for Growing Plants Without Soil, University of California, Berkeley, USA, California Agricultural Experiment Station Circular no. 347, pp. 1-32 (1950).
20. S. Weidner, R. Amarowicz, M. Karamać and E. Frączek, *Plant Physiol. Biochem.*, **38**, 595 (2000); https://doi.org/10.1016/S0981-9428(00)00774-9
21. H. Chen, Y. Zuo and Y. Deng, *J. Chromatogr. A*, **913**, 387 (2001); https://doi.org/10.1016/S0021-9673(00)01030-X
22. J.M. Nadal, M.G. Toledo, Y.M. Pupo, J. Padilha de Paula, P.V. Farago and S.M.W. Zanin, *J. Anal. Methods Chem.*, **2015**, 1 (2015); https://doi.org/10.1155/2015/286812
23. R.B. Broadhurst and W.T. Jones, *J. Sci. Food Agric.*, **29**, 788 (1978); https://doi.org/10.1002/jsfa.2740290908
24. M. Latta and M. Eskin, *J. Agric. Food Chem.*, **28**, 1313 (1980); https://doi.org/10.1021/jf60232a049
25. C.A. Rice-Evans, N.J. Miller and G. Paganga, *Free Rad. Biol. Med.*, **20**, 933 (1996); https://doi.org/10.1016/0891-5849(95)02227-9
26. M. Cavia-Saiz, M.D. Busto, M.C. Pilar-Izquierdo, N. Ortega, M. Perez-Mateos and P. Muñiz, *J. Sci. Food. Agric.*, **90**, 1238 (2010); https://doi.org/10.1002/jsfa.3959
27. R. Mishra, S. Gupta, P.K. Gaur, N. Lal, K. Lata and A. Yadav, *ChemistrySelect*, **11**, e73556 (2026); https://doi.org/10.1002/slct.73556
28. N. Kumar and V. Pruthi, *Biotechnol. Rep.*, **4**, 86 (2014); https://doi.org/10.1016/j.btre.2014.09.002
29. P. Badhe, V. Nanaware, A. Badhe, G.F. Wondmie, Y.A. Bin Jardan, and M. Bourhia, *Sci. Rep.*, **14**, 15314 (2024); https://doi.org/10.1038/s41598-024-63498-7
30. Y. Zhao, D. Fan, B. Ru, K.-W. Cheng, S. Hu, J. Zhang, E.T.S. Li and M. Wang, *Eur. J. Cancer*, **68**, 38 (2016); https://doi.org/10.1016/j.ejca.2016.09.001
31. K.D.P.P. Gunathilake K.K.D.S. Ranaweera and H.P.V. Rupasinghe, *Int. J. Food Sci.*, **2016**, 528 (2016); https://doi.org/10.1002/fsn3.832
32. F. Cosme, A. Aires, T. Pinto, I. Oliveira, A. Vilela and B. Gonçalves, *Molecules*, **30**, 800 (2025); https://doi.org/10.3390/molecules30040800
33. G. Ma, Y. Jin, J. Piao, F. Kok, G. Bonnema, and E. Jacobsen, *J. Agric. Food Chem.*, **53**, 10285 (2005); https://doi.org/10.1021/jf052051r
34. U. Schlemmer, W. Frølich, R.M. Prieto and F. Grases, *Mol. Nutr. Food Res.*, **53**, S330 (2009); https://doi.org/10.1002/mnfr.200900099