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Phytochemical profiling of little millet (*Panicum sumatrense* Roth.)

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The little millet (*Panicum sumatrense* Roth.) has great potential to develop as functional food and source of nutraceuticals to prevent metabolic disorders. The aim of the current study aim is to identify the bioactive compounds present in little millet (*Panicum sumatrense*). The bioactives were analysed using GC-MS and UHPLC-QTOF-MS. GC-MS analysis majorly showed the presence of hydrocarbons, fatty acids, and terpenes, while UHPLC-QTOF-MS analysis showed 22 secondary metabolites, including quercetin, palmitic acid, β-stigmasterol, luteolin, and kaempferol. ICP-MS detected 21 macro- and micro-elements, with potassium (K), magnesium (Mg), and molybdenum (Mo) as the major elements. FAME analysis revealed the presence of linoleic acid (42%), oleic acid (34.1%), and palmitic acid (15.7%)as the major fatty acids. Amino acid profiling indicated the presence of essential, non-essential, and non-proteinogenic amino acids. These findings suggest that *P. Sumatrense* could be a valuable natural source of bioactive metabolites and can be utilised to develop value-added functional foods.

Keywords: Amino acids, GC-MS, ICP-MS, Little millet, *Panicum sumatrense* Roth., Phytochemicals, UHPLC-QTOF-MS

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Introduction

Malnutrition and food security are major public health issues in India, affecting millions of people of all ages and socio-economic backgrounds. In light of the growing population, it becomes increasingly important to address these issues to maintain the food balance among everyone. India, being the highest producer of millets, holds great potential for addressing both malnutrition and food security issues through the use of millets[1].

Millets are small-seeded edible grass of the Poaceae family that can be found growing in marginal dry lands in tropical and subtropical regions of the world[2]. These millets help increase the genetic diversity of the food basket and food and nutritional security. Millets, like foxtail millet, pearl millet, sorghum, and finger millet, are a great option for those with celiac disease and diabetes because of their high nutritional content and low glycemic index[3]. Millets have been a staple food in traditional Indian diets for centuries, especially in rural areas. However, millet consumption has fallen drastically due to modernisation and the promotion of rice and wheat as the main grains.

Panicum sumatrense, commonly known as little millet or kutki, is an underutilised minor millet widely grown in countries like India, Africa, and China. It is a yearly crop that has resistant starch, phytates, phenolics, sterols, lignans, and gamma-aminobutyric acid as prominent phytochemicals[4]. It is regarded as a "cool food" because of its cooling effect on the body when consumed in summer[5]. The nutritional potential of little millet is entrenched with a good proportion of vitamins, minerals, and bioactive compounds. According to some previous studies, its high fibre content contributes to reducing fat deposits in the human body[6].

Severe chronic diseases in humans, such as cardiovascular disease, diabetes, cancer, cognitive dysfunction, and a variety of other normal activities, have been related to the oxidation of cellular molecules by reactive dietary antioxidants to protect against oxidative damage and maintain a healthy metabolic balance. Recently, plant bioactive compounds have gained noticeable attention from researchers for their numerous health benefits in reducing the risk of cancer, neurodegenerative, and cardiovascular diseases.

In view of the above, it is necessary to evaluate the phytochemicals and their bioactivity in little millet to utilise this millet as a food ingredient for the

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development of functional foods. Being nutritional and underutilised minor millet, our study was carried out with the objective of investigating the phytochemical and nutritional profile of little millet. The phytochemicals in little millet seeds were studied using GC-MS and UHPLC-QTOF-MS analysis, nutritional profile by proximate analysis, elemental analysis by ICP-MS, fatty acid profiling by FAME analysis, amino acid profiling, antimicrobial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive), and antioxidant activity by DPPH free radical scavenging method.

Materials and Methods

Plant material and reagents

Little millet (Lm) was procured from the Indian Council of Agricultural and Research (ICAR), Hyderabad, India. The little millet (collected in April 2022) was cleaned and sun-dried. All the reagents and chemicals were of analytical grade and purchased from Sigma-Aldrich.

Preparation of extract

Little millet was coarsely grounded for extraction. 50 g of the sample was extracted with n-hexane (69ºC), dichloromethane (DCM, 40ºC), and methanol (65ºC) in a Soxhlet apparatus. Exhaustive extraction was applied with each solvent for 10-12 hours[7] to ensure a complete extraction process. The extract was concentrated at 40ºC under reduced pressure using a rotary evaporator and was stored at 4ºC until further use.

Proximate analysis

To determine dietary fibre, protein, fat, and carbohydrate content in the selected little millet, proximate analysis was carried out according to the procedure of the Association of Official Analytical Chemists (AOAC)[8].

Phytochemical analysis

The phytochemical analysis of n-hexane, DCM, and methanol extract of little millet was done for the identification of alkaloids, flavonoids, tannins, phenols, glycosides, reducing sugars, anthocyanins, and coumarins, following the standard method[9].

Elemental composition by ICP-MS

About 0.25 g of little millet sample was taken in digestion vessel and 10 mL of nitric acid was added to it for digestion. After successful digestion, sample was transferred to volumetric flask and made upto 50 mL and then analysed by ICP-MS. The same procedure was used to analyse NIST standards. The blank sample solution was also prepared following the above procedure without adding the sample. The instrument calibration was assessed by analysing three certified samples from NIST[10].

Fatty acid profiling

Fatty acid methyl ester (FAME) analysis was done to evaluate the fatty acid content as the amount of methyl esters present in the sample using the method described by Ryan *et al.*, with slight modifications[11]. 0.15 g of the extracted oil sample was accurately weighed in a 5 mL glass vial. 200 µL of 2 N methanolic KOH solution was then added and vortexed for 2 min. The mixture was heated in a water bath shaker at 55ºC for 10 min. After the mixture was cooled, 1.0 mL of 5% HCl solution was added and then heated for 10 min in a water bath shaker at 70ºC for 10 min. Exactly 2 mL of petroleum ether was added, and the mixture was vortexed for 2 min. The upper layer was separated and analysed by GC-MS (Shimadzu, GC-2010).

GC-MS analysis

Different crude extracts of little millet were injected in GC-MS (Shimadzu GCMS-QP2010) to determine the volatile compounds. Samples were injected in GC-MS under the following conditions: Helium gas was used as carrier gas; split mode at 260ºC; column flow rate at 1.23 mL/min; mode of ionisation was electron ionisation (EI); ion source temperature was 220ºC, and the interface temperature was 270 ºC. The oven temperature was planned for 60ºC (2 min) to 280ºC (26 min), and the solvent delay time was 3.50 min. The chromatographic separation of the compounds was done using column-Rxi®-5Sil MS(30 m × 0.25 mmID × 0.25 µm, film thickness). NIST (National Institute of Standards and Technology, version 1.10 beta, Shimadzu) mass spectral database was used to identify the separated peaks[12].

UHPLC-QTOF-MS identification

UHPLC-QTOF-MS was used to identify secondary metabolites in the n-hexane, DCM, and methanol extracts of little millet, using a previously described method with some modifications[13]. The mass spectrometric analysis was conducted in positive

mode (ESI+). ESI source conditions were as follows: source temperature, 120ºC; desolvation gas flow, 950 L/h; cone gas flow, 50 L/h; capillary voltage, 3.22 keV. HPLC (Waters, SYNAPT-XS HDMS, UK) fitted with a controller, AD pump, degasser, AD auto sampler, AD column, coupled with a quadrupole time-of-flight mass spectrometer (QTOF-MS), was used for UHPLC and mass spectrometric analysis. Briefly, the extracts were mixed with 1% formic acid (10 mL) in water and kept for 10 min. Exactly 10 mL methanol and 10 mL acetonitrile were added and vortex for 1 min and centrifuged at 5000 rpm for 5 min. The supernatant was diluted with acidified water. Then, the extract was injected into the instrument for the analysis. The chromatographic separation of the samples was done by 100 mm × 2.1 mm column C18 (Waters, Acquity BEH 2.1). The injection volume was 5 µL. Secondary metabolites were eluted using a binary mobile phase at a flow rate of 0.2 mL/min, where solvent A was LC-MS grade water containing 1% formic acid and solvent B was 1% formic acid with acetonitrile. The data acquisition and processing were performed in ChemSpider software.

Amino acid analysis

The amino acid composition of little millet was measured using Automatic Amino Acid Analyzer L-8900 (Hitachi Co. Ltd., Tokyo, Japan) as per the method described by Chakrabarti *et al.*[14]. Dried and finely powdered sample was hydrolysed with 6 N HCl at 110ºC for 22 h. The hydrolysed sample was dried in Nitrogen Evaporator (PCi Analytic Private Limited, Maharashtra, India). 0.02 N HCl was added to the sample and then kept in the Autosampler. 20 µL sample injection was used. Tryptophan, cysteine, and methionine were destroyed by acid hydrolysis; these amino acids were treated with specific reagents. Cysteine and methionine were oxidised with performic acid and treated with 48% hydrobromic acid. The sample was hydrolysed with 4 N methane sulfonic acid and 3-(2-aminoethyl) indole for the estimation of tryptophan. The rest of the methods were the same for all the amino acids. The ninhydrin derivative of hydroxyproline and proline was observed at 440 nm, and all other amino acids were observed at 570 nm. For the quantification of contents of amino acids in the selected millet, the peak areas of the detected amino acids were compared with those of authentic standards provided with the equipment. Amino Acids Mixture Standard Solutions, Type B and Type AN-2 (Wako Pure Chemical Industries, Limited) were used. Standard solutions for tryptophan and glutamine (Sigma-Aldrich, USA) were prepared before analysis.

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of different extracts (n-hexane, DCM, and methanol) of little millet was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, following the method described by Banjara *et al*., with slight modifications[15]. Different concentrations (60, 80, 100, 120, 140, 160, 180, and 200 µL) of methanol extract of little millet were made, and 1 mL of each extracted sample was mixed with 3 mL of DPPH solution. The mixture was incubated for 30 min at room temperature, and the absorbance was measured at 517 nm using a UV spectrophotometer. The % of DPPH free radical scavenging activity was measured using the following formula:

$$\% \text{ Scavenging activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of sample}}{\text{Absorbance of Control}} \text{ x } 100$$

Antimicrobial activity

Disc-diffusion method

The antimicrobial activity of methanol extract of little millet was performed, following the method with slight modifications[16]. After pouring 20 mL of the nutrient media into sterilised Petri dishes, it was allowed to solidify. After that, 20 µL of each extract was tested against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) bacteria. The bacteria were grown in an incubator at 37°C and left for 24 h. After the incubation period, the zone of inhibition was measured.

Results and discussion

Proximate analysis

The nutritional values of the studied millet are presented in Table 1. The highest component in little

Table 1 — Nutritional composition analysis of little millet

Parameter	Little millet
Crude protein (g/100 g)	8.7
Dietary fibre (g/100 g)	10.3
Carbohydrate (g/100 g)	72.2
Fat (g/100 g)	4.5
Saturated fat (%)	1.0
MUFA (%)	1.6
PUFA (%)	1.9
Trans fat (%)	BLQ

BLQ = below detection limit

millet was carbohydrate (72.2 g/100 g). The dietary fibre content was found to be 10.3 g/100g. The increase in the carbohydrate content in these different classes of millets is probably due to the decrease in the dietary fibre content[17]. Also, the dietary fibre in food is an indication of the level of non-digestible carbohydrates. The protein content was 8.7 g/100g, which was relatively higher than Kodo and barnyard millet. The fat content was 4.5 g/100g. Generally, the fats are relatively minor constituents in millets and range from 0.5 to 5.5 g[18,19]. The results of the nutritional composition of little millet were in agreement with the previous report[20]. The previously reported data slightly differed from the present data, probably due to the difference in the cultivars of the millets.

Phytochemical analysis

All three different extracts (n-hexane, DCM, and methanol) of the selected millet were screened for different phytocomponents. The data revealed the presence of reducing sugars and alkaloids. The alkaloids were identified in n-hexane and DCM extract of the selected millet, while reducing sugar was identified in methanol extract only. Flavonoids, tannins, glycosides, anthocyanins, phenol, and coumarins were not identified in any of the extracts. All the phytoconstituents found in little millet are responsible for various biological activities.

ICP-MS analysis

Elemental analysis was carried out for the selected millet. A total of 21 macro-elements and micro-elements were present at different concentrations (Table 2). The major elements found were potassium (K), magnesium (Mg), and molybdenum (Mo). Among all the analysed elements, K was found to have the highest concentration. It is an essential mineral for the efficient functioning of tissues, the body's cells, and organs. Increased potassium intake in diets can help maintain healthy blood pressure levels. The concentration of heavy metals like cadmium (Cd), arsenic (As), and lead (Pb) were below the standard limit values set by FSSAI.

FAME analysis

The different fatty acid methyl esters found in little millet are shown in Table 3. Thirteen fatty acids were detected and quantified as percentages using GC-MS analysis. The predominant constituents were linoleic acid (42%), oleic acid (34.1%), and palmitic acid (15.7%), accompanied by a very small amount of others (0.07- 5.2%). Among all, linoleic acid was the predominant component and was present in the highest quantity in little millet. Linoleic acid (ω-6 polyunsaturated fatty acid, PUFA) is an essential fatty acid which has various biological effects on human body cells, such as flexibility, cell membrane, lower cholesterol levels, reduced cardiovascular disease, inflammation regulation, and immune system function. It is the most abundant PUFA in legumes and millet, accounting for about 90% of dietary ω-6 PUFA consumption, a higher intake of which is associated with a 15% lower risk of coronary heart disease incidence[21]. According to Okereke & Banigo, the nutritional value of some crops can be significantly improved by the presence of a single essential fatty acid, which can benefit human health[22].

Table 2 — ICP-MS analysis of little millet

S. No.	Minerals	Sample concentration (ppm)
1	Na	118.7
2	Mg	1838.3
3	Al	17.8
4	K	2303.5
5	^{43}Ca	49.7
6	^{44}Ca	84.6
7	Cr	0.1
8	Mn	22.3
9	Fe	1.2
10	Co	0.03
11	Ni	1.7
12	Cu	5.3
13	Zn	20.3
14	As	0.003
15	Se	0.1
16	Rb	6.9
17	Sr	2.1
18	Mo	540.9
19	Cd	0.01
20	Pb	0.1
21	U	0.05

Table 3 — Fatty acid composition of little millet

S. No.	Type of fatty acid	Percentage
1	Myristic acid	0.1
2	Palmitic acid	15.7
3	Palmitoleic acid	0.2
4	Heptadecanoic acid	0.1
5	Stearic acid	5.2
6	Oleic acid	34.1
7	Linoleic acid	42.0
8	Arachidic acid	0.8
9	Linolenic acid	1.1
10	Cis-11-Eicosenoic acid	0.2
11	Behenic acid	0.2
12	Methyl cis-5,8,11,14	0.1
13	Lignoceric acid	0.2

GC-MS analysis

The phytochemicals present in the n-hexane, DCM, and methanol extracts of little millet are summarised in Table 4. The data revealed that the extract encompassed a total of 87 compounds. Three major compounds identified were cis-9-hexadecenal (57.1%) and palmitic acid (34.0%). Palmitic acid, dodecane, stearic acid, tetracontane, stigmasterol, δ-tocopherol, and γ-tocopherol were also present in little millet. The data obtained was comparable to the previous study, with some additional compounds[23]. According to Liu *et al.*, aldehydes and benzene derivatives are the primary volatile compounds found in the Poaceae family[24].

Table 4 — Phytochemicals identified by GC-MS in the different solvent extracts of little millet

RT	Compound name	Percentage	RRI	MW	MF	n-hexane	DCM	Methanol
5.40	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	0.8	963	144	$C_6H_8O_4$	-	-	+
5.41	Decane	0.1	964	142	$C_{10}H_{22}$	+	-	-
5.84	Caproic acid	0.7	990	116	$C_6H_{12}O_2$	-	-	+
6.22	1-Methylpyrrolidone	0.2	1014	99	C_5H_9NO	+	-	-
6.41	Benzeneacetaldehyde	0.1	1026	120	C_8H_8O	-	-	+
6.57	Neohexanoic acid	0.5	1036	116	$C_6H_{12}O_2$	-	-	+
6.63	Dihydrodicyclopentadiene	0.04	1039	134	$C_{10}H_{14}$	+	-	-
7.07	Tetrahydrodicyclopentadiene	0.03	1066	136	$C_{10}H_{16}$	+	-	-
8.18	Pyranone	5.1	1136	144	$C_6H_8O_4$	-	-	+
8.58	Dodecane	0.2	1162	170	$C_{12}H_{26}$	+	+	-
9.67	Dihydrobenzofuran	0.4	1234	120	C_8H_8O	-	-	+
9.72	5-Hydroxymethylfurfural	4.4	1238	126	$C_6H_6O_3$	-	-	+
10.52	4-Hydroxy-3-methoxystyrene	1.9	1292	150	$C_9H_{10}O_2$	-	-	+
11.06	Docosane	0.02	1332	310	$C_{22}H_{46}$	+	-	-
11.15	Isosorbide	0.5	1338	146	$C_6H_{10}O_4$	-	-	+
11.39	2-Heptyl acetate	0.8	1356	158	$C_9H_{18}O_2$	-	-	+
11.42	Tetradecane	0.3	1358	198	$C_{14}H_{30}$	+	+	-
12.39	6-Demethoxyageratochromene	0.3	1431	190	$C_{12}H_{14}O_2$	-	-	+
12.51	1-Dodecanol	0.04	1440	186	$C_{12}H_{26}O$	+	-	+
12.82	Benzaldehydemethylimine	0.2	1464	119	C_8H_9N	-	-	+
13.05	Di(tert-butyldimethylsilyl)- mandelic acid	0.1	1481	380	$C_{20}H_{36}O_3Si_2$	+	+	-
13.92	Hexadecane	0.2	1552	226	$C_{16}H_{34}$	+	+	-
13.93	1,6-Anhydro-beta-D-glucopyranose	2.0	1553	162	$C_6H_{10}O_5$	-	-	+
15.49	(2E)-3-(1-Methylcyclopropyl)-2-propenoic acid	5.0	1686	126	$C_7H_{10}O_2$	-	-	+
15.77	Cyclohexanone, 2-methyl- semicarbazone	3.7	1710	169	$C_8H_{15}N_3O$	-	-	+
16.17	Octadecane	0.1	1747	254	$C_{18}H_{38}$	+	+	-
17.50	Palmitic acid, methyl ester	0.7	1872	270	$C_{17}H_{34}O_2$	+	+	+
17.83	(+)-Dihydrorecifeiolide	0.3	1904	198	$C_{12}H_{22}O_2$	-	-	+
18.19	Palmitic acid	34.0	1940	256	$C_{16}H_{32}O_2$	+	+	+
18.95	Allyl stearate	0.3	2018	324	$C_{21}H_{40}O_2$	-	-	+
19.12	Methyl linoleate	0.3	2036	294	$C_{19}H_{34}O_2$	-	+	+
19.18	Methyl 7-octadecenoate	0.1	2042	296	$C_{19}H_{36}O_2$	-	-	+
19.21	Methyl (9E)-9-octadecenoate	1.0	2045	296	$C_{19}H_{36}O_2$	+	+	-
19.44	Methyl stearate	0.2	2070	298	$C_{19}H_{38}O_2$	-	+	-
20.04	Cis-9-hexadecenal	57.1	2134	238	$C_{16}H_{30}O$	-	+	+
20.14	Stearic acid	2.3	2145	284	$C_{18}H_{36}O_2$	-	+	+
20.53	6-Nitro-cylohexadecane-1,3-dione	0.2	2188	297	$C_{16}H_{27}NO_4$	-	-	+
20.85	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.2	2224	213	$C_{12}H_{23}NO_2$	-	-	+
20.96	Octadecanedioic acid	0.2	2236	314	$C_{18}H_{34}O_4$	-	-	+
21.21	3,5,5-Trimethyl-5,6-dihydro-2H-pyran-2-one	0.1	2265	140	$C_8H_{12}O_2$	-	+	-

(*Contd.*)

Table 4 — Phytochemicals identified by GC-MS in the different solvent extracts of little millet (*Contd*.)

RT	Compound name	Percentage	RRI	MW	MF	n-hexane	DCM	Methanol
21.46	Leinoleic acid	0.3	2295	280	$C_{18}H_{32}O_2$	+	+	-
21.66	Hexadecanoic acid, hexyl ester	0.3	2318	340	$C_{22}H_{44}O_2$	+	+	-
21.93	(Z)-11-Eicosenic acid	0.1	2351	310	$C_{20}H_{38}O_2$	+	-	-
21.95	Triphenyl phosphate	0.3	2353	326	$C_{18}H_{15}O_4P$	+	+	+
22.26	2-Mono-Linolein	0.3	2390	354	$C_{12}H_{38}O_4$	-	+	-
22.29	Fumaric acid, 2-dimethylaminoethyl nonyl ester	0.2	2394	313	$C_{17}H_{31}NO_4$	-	-	+
22.44	Methyl 2-cyclohexyl-2-methylpentanoate	0.5	2412	212	$C_{13}H_{24}O_2$	-	+	-
22.50	Fumaric acid, 2-dimethylaminoethyl octadecyl ester	0.3	2419	439	$C_{26}H_{49}NO_4$	-	-	+
22.56	Trans-9-Octadecenoic acid, pentyl ester	0.4	2427	352	$C_{23}H_{44}O_2$	+	-	-
22.60	Palmitoyl chloride	2.1	2432	274	$C_{16}H_{31}ClO$	-	-	+
22.80	Heneicosane	0.1	2456	296	$C_{21}H_{44}$	+	-	-
23.02	Trans, trans-9,12-Octadecadienoic acid, propyl ester	0.1	2484	322	$C_{21}H_{38}O_2$	-	+	-
23.35	Hexyl stearate	0.1	2526	368	$C_{24}H_{48}O_2$	+	-	-
23.39	1,22-Dibromodocosane	0.3	2531	466	$C_{22}H_{44}Br_2$	+	+	+
23.42	Hexadecanoic acid, phenylmethyl ester	0.05	2534	346	$C_{23}H_{38}O_2$	+	-	-
23.79	3-Isopentylthiophene 1,1-dioxide	1.0	2582	186	$C_9H_{14}O_2S$	-	+	-
23.99	Oleoyl chloride	3.5	2609	300	$C_{18}H_{33}ClO$	+	-	+
24.17	Pentacosane	0.2	2632	352	$C_{25}H_{52}$	+	-	-
24.18	Glycerin 1-monostearate	1.0	2633	358	$C_{21}H_{42}O_4$	-	-	+
24.46	Widdrol	0.3	2668	222	$C_{15}H_{26}O$	-	-	+
24.68	Lanost-7-en-3-one	0.1	2695	426	$C_{30}H_{50}O$	+	-	+
24.72	Dodecahydro-3a,6,6,9a-tetramethylnaphtho[2,1-beta] furan	0.2	2700	236	$C_{16}H_{28}O$	-	+	-
24.74	Linoleic acid, phenylmethyl ester	0.4	2703	370	$C_{25}H_{38}O_2$	+	-	-
24.95	Dioctylsebacate	2.0	2726	426	$C_{26}H_{50}O_4$	-	+	-
24.99	Carbonic acid, eicosyl prop-1-en-2-yl ester	0.1	2730	382	$C_{24}H_{46}O_3$	+	-	-
25.14	Squalene	0.8	2746	410	$C_{30}H_{50}$	+	-	-
25.29	Betulin	0.1	2764	442	$C_{30}H_{50}O_2$	-	-	+
25.45	(2e)-5-Hydroxy-3,4,4-trimethyl-2-hexenoic acid	0.04	2781	172	$C_9H_{16}O_3$	-	-	+
25.56	3-Methyl-3-(palmitoylperoxy)butyl palmitate	0.1	2792	596	$C_{37}H_{72}O_5$	-	-	+
25.69	3-Bromocholest-5-ene	0.1	2806	448	$C_{27}H_{45}Br$	-	-	+
25.87	Tetratetracontane	0.2	2823	618	$C_{44}H_{90}$	+	+	-
25.95	Tetratriacontane	0.9	2830	478	$C_{34}H_{70}$	+	-	-
26.05	Cholestadiene	0.6	2840	368	$C_{27}H_{44}$	-	+	+
26.61	Delta-tocopherol	0.1	2893	402	$C_{27}H_{46}O_2$	+	+	-
27.02	Tetracontane	0.05	2925	562	$C_{40}H_{82}$	+	+	-
27.35	(22E)-Stigmasta-4,7,22-trien-3-ol	0.2	2951	410	$C_{29}H_{46}O$	-	+	-
27.92	Gamma-tocopherol	0.1	2994	416	$C_{28}H_{48}O_2$	+	-	-
28.16	Stigmasterol acetate	0.3	3010	454	$C_{31}H_{50}O_2$	-	+	-
28.46	Stigmast-5-en-3-ol, oleate	0.2	3029	678	$C_{47}H_{82}O_2$	-	+	-
29.07	Cholesterol	0.2	3068	386	$C_{27}H_{46}O$	-	+	-
29.12	Cholest-5-en-3-ol	0.1	3071	386	$C_{27}H_{46}O$	+	-	-
31.21	Triacontanal	0.1	3185	436	$C_{30}H_{60}O$	+	-	-
31.89	3.beta-methoxy-delta.14-serratene	7.2	3216	440	$C_{31}H_{52}O$	+	+	-
32.42	Methyl commate b	0.7	3238	470	$C_{31}H_{50}O_3$	+	+	-
32.80	Gamma-sitosterol	1.1	3254	414	$C_{29}H_{50}O$	+	+	-
34.83	3,5-Stigmastadien-7-one	0.2	3339	410	$C_{29}H_{46}O$	-	+	-
35.89	Lupenyl acetate	1.4	3383	468	$C_{32}H_{52}O_2$	-	+	-

RT = Retention time
MF = Molecular formula
RRI = Relative Retention Index

Palmitic acid and stearic acid are the saturated fatty acids consumed most frequently in the Western diet[25]. They aid in regulating glucose metabolism and developing insulin resistance in type 2 diabetes[26]. The phytosterols like stigmasterol, γ-sitosterol, and β-sitosterol are plant sterols known to be beneficial for human health. According to the research, phytosterols could be a complementary treatment for obesity and diabetes[27]. Squalene, a bio-functional lipid molecule, was detected in the n-hexane extract of little millet. It has been found to have a variety of bioactivities, including antioxidant, cardioprotective, anti-cancerous, membrane-stabilising effects, and anti-lipidemic. It can potentially enhance serum high-density lipoprotein cholesterol levels and reduce oxidative stress[28]. Based on the GC-MS results, the most common volatile compounds in little millet extracts were hydrocarbons, fatty acids, and terpenes, which is comparable to the results of other researchers[29].

UHPLC-QTOF-MS analysis

UHPLC-QTOF-MS is used to identify the secondary metabolites in the n-hexane, DCM, and methanol extracts of little millet, which are presented in Table 5. All the identified compounds were analysed based on mass-to-charge (m/z) values of mass spectrometry in positive ionisation mode. To identify the maximum metabolites in little millet, we analysed all the extracts using targeted and non-targeted metabolomics. In the targeted approach, the focus is on identifying known compounds and monitoring a predefined list of analytes with high sensitivity and precision. In contrast, the non-targeted approach detects and identifies a wide range of compounds in a sample without prior knowledge, providing a comprehensive chemical profile ideal for discovering new bioactive metabolites. In the non-targeted approach, a total of 22 metabolites were identified, which majorly contain phytosterols, phenolic compounds, terpenoids, and flavonoids. The

Table 5 — Phytochemicals identified using UHPLC-QTOF-MS in little millet

Peak No.	Tentative metabolites	RT (min)	MF	MW	$[M-H]^+$	Error (ppm)	Compound ID
			n-hexane				
1	Palmitic acid	2.02	$C_{16}H_{32}O_2$	256.2402	257.2477	1.0	CSID960
2	Stigmasterol	19.57	$C_{29}H_{48}O$	412.3705	413.3789	2.5	CSID4444352
3	1-Linoleoyl-glycero-3-phosphocholine	25.65	$C_{26}H_{50}NO_7P$	519.3324	520.3399	1.2	CSID9181014
			DCM				
4	Gibberellin A44 diacid	12.96	$C_{20}H_{26}O_6$	362.1740	363.1844	2.9	CSID24784737
5	β-Stigmasterol	28.02	$C_{29}H_{48}O$	412.3705	413.3785	2.3	CSID4444352
6	Ergostan-3-ol	29.12	$C_{28}H_{50}O$	402.3861	403.3941	4.9	CSID532070
			Methanol				
7	D-Verbascose	1.51	$C_{30}H_{52}O_{26}$	828.2753	829.2831	0.7	CSID390167
8	Quercetin	1.75	$C_{24}H_{42}O_{21}$	302.0427	303.0505	1.8	CSID4444051
9	Fagopyritol a3	7.28	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID145907
10	Kestotetraose	7.28	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID19128807
11	1,6-Kestotetraose	7.28	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID24785159
12	Lychnose	7.28	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID30785504
13	A-1,4-tetraglucose	7.28	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID3493964
14	Luteolin	7.28	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.7	CSID4444102
15	Kaempferol	7.28	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.7	CSID4444395
16	Scutellarein	7.28	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.7	CSID4445014
17	Orobol	14.23	$C_{15}H_{10}O_6$	286.236	287.0548	-0.7	CSID4445113
18	2'-Hydroxygenistein	14.22	$C_{15}H_{10}O_6$	286.236	287.0548	-0.7	CSID4445299
19	Nystose	14.22	$C_{15}H_{10}O_6$	666.2225	667.2303	1.0	CSID145907
20	Stachyose	16.39	$C_{19}H_{36}O_5$	666.2225	667.2303	1.0	CSID388624
21	α-Tocotrienol	19.62	$C_{29}H_{44}O_2$	424.3341		1.7	CSID4445512
22	Fagopyritol b3	7.28	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID30777084

CSID = ChemSpider ID

present study is one of the first reports to identify secondary metabolites through a non-targeted approach for little millet using UHPL-QTOF-MS. In the targeted approach, a total of 9 metabolites were detected in the positive ion mode, as shown in Table 6. We found that catechin-7-O-glucoside, catechin, epicatechin, quercetin, gallocatechin, gallic acid, caffeic acid, para-coumaric acid, and glycitein were significantly present in little millet. Several studies have reported that catechin and its derivatives have been examined for their possible health advantages, particularly its function in the treatment of obesity[30].

Flavonoids are essential components in cosmetics, nutraceuticals, and medicinal applications due to their anti-tumour, anti-inflammatory, and antioxidant activities[31]. Kaempferol, identified in the methanol extract of little millet, is known to reduce chronic diseases like liver injury, cancer, diabetes, and obesity[32].

Amino-acid profiling

The amino acid profile of little millet revealed some interesting results, as shown in Table 7. It contained essential, non-essential, and non-proteinogenic amino acids. All the essential amino acids, viz. arginine, leucine, histidine, lysine, methionine, tryptophan, phenylalanine, valine, threonine, and isoleucine, were found in significant amounts. In little millet, the essential amino acids (39.2%), non-essential amino acids (54.1%), and non-proteinogenic amino acids (7.0%) were in adequate quantity. Valine and leucine consisted 41.0% of the essential amino acids. The most common and major essential and non-essential amino acids were leucine and glutamic acid, respectively. These amino acids have distinct health advantages, e.g., leucine promotes protein biosynthesis, and glutamic acid plays a significant role in brain disorders such as schizophrenia, Parkinson's disease, and epilepsy[33]. Several non-proteinogenic amino acids were also present, out of which hydroxyproline was the major amino acid. These amino acids play a variety of functions in the human body, including potential health advantages.

β-Alanineis the precursor of pantothenic acid (vitamin B5), and it has been widely used as a precursor for many significant industrial chemicals in food, medicine, and environmental applications[34]. Taurine was found to exhibit numerous health benefits, including protection against ischemia-reperfusion of the heart, antiatherogenic, and antioxidant, and it is known to play a significant role in fat digestion and formation of bile salt[35]. β-Aminoisobutyric acid which is present in little millet,

Table 6 — Targeted Molecules identified by UHPLC-QTOF-MS in little millet

S. No.	Compound Name	Molecular weight	Little millet
1	Catechin-7-O-glucoside	452.1319	+
2	Catechin	290.0790	+
3	Epicatechin	290.0790	+
4	Quercetin	302.0427	+
5	Gallocatechin	2037.4728	-
6	Gallic acid	170.1210	+
7	Caffeic acid	180.0423	+
8	Para-coumaric acid	164.0473	+
9	Glycitein	284.2610	+

Table 7 — Amino acid composition of little millet

S. No.	Type of amino acid	Percentage
	Essential amino acids	
1	Arginine (Arg)	0.4 ± 0.006
2	Histidine (His)	0.1 ± 0.001
3	Isoleucine (Ile)	0.4 ± 0.002
4	Leucine (Lue)	0.9 ± 0.005
5	Lysine (Lys)	0.1 ± 0.002
6	Methionine (Met)	0.1 ± 0.004
7	Phenylalanine (Phe)	0.5 ± 0.003
8	Threonine (Thr)	0.4 ± 0.002
9	Tryptophan (Trp)	0.03 ± 0.002
10	Valine (Val)	0.5± 0.006
	No-essential amino acids	
11	Alanine (Ala)	0.7 ± 0.005
12	Aspartate (Asp)	0.5 ± 0.004
13	Cysteine (Cys)	0.05 ± 0.001
14	Glutamic Acid (Glu)	1.8 ± 0.009
15	Glycine (Gly)	0.2 ± 0.013
16	Proline (Pro)	0.7 ± 0.011
17	Serine (Ser)	0.5 ± 0.005
18	Tyrosine (Tyr)	0.2 ± 0.001
	Non- Proteinogenic amino acids	
19	Phosphoserine	0.02 ± 0.001
20	Taurine	0.04 ± 0.001
23	Cystathionine	0.03 ± 0.002
24	β –Alanine	0.005 ± 0.00
25	β –Amino isobutyric acid	0.003 ± 0.00
27	Ethanol amine	0.002 ± 0.00
29	Ornithine	0.001 ± 0.00
31	1-Methylhistidine	0.05 ± 0.001
32	Hydroxyproline	0.4± 0.002

ND = Not detected
BLQ = Below detection limit

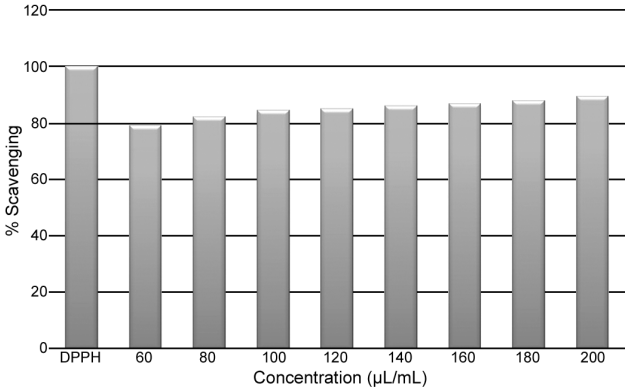

Fig. 1 — Antioxidant activity of methanol extract of little millet.

favourably affects lipid metabolism, decreases inflammatory reactions, and increases insulin sensitivity. According to some recent studies, it can protect from diet-induced obesity in animal models[36]. Thus, the results confirmed that little millet is nutritional as it is a rich source of both essential and non-essential amino acids. Moreover, the nutritional value of the little millet was enhanced by the presence of non-proteinogenic amino acids.

Antioxidant activity

DPPH radical scavenging activity

DPPH free radical scavenging activity is a widely used screening method for antioxidants in most plant products. It is a free-radical compound that has an absorbance in its oxidised form at 515-520 nm[37]. The data on the antioxidant activity of little millet at different concentrations is shown in Fig. 1. The results indicate that the highest antioxidant activity was observed at a sample concentration of 200 µL/mL.

Antimicrobial activity

Disc-diffusion method

The antimicrobial activity revealed that the methanolic extract of little millet possesses potent antimicrobial activity against Gram-negative bacteria (*Escherichia coli*), with a zone of inhibition of 16 mm, and Gram-positive bacteria (*Staphylococcus aureus)* with a zone of inhibition of 14 mm. From the results, it can be inferred that the methanolic extract of little millet could be a potent source of antimicrobial agents.

Conclusion

This study revealed that the underutilised little millet is not only nutritionally rich but also has many bioactive compounds that benefit human health. It is an excellent source of carbohydrates, dietary fibre, and protein. Our results revealed the existence of significant concentrations of fatty acids, terpenes, and hydrocarbons that may have various pharmaceutical and nutraceutical applications. UHPLC-QTOF-MS showed the presence of metabolites like catechin, epicatechin, quercetin, caffeic acid, para-coumaric acid, and gallic acid, which may be responsible for the activities such as anti-diabetic, anti-obesity and many more. In conclusion, our study indicates that this underutilised millet is a good source of bioactive metabolites and may be utilised to develop functional foods.

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Conflict of interest

The authors declare that there is no competing or conflict of interest.

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