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Profiling of *Naurangi* and *Kulthi Dal* used in traditional Indian system of cuisines and medicine

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The development of fortified foods and nutraceuticals based on legumes used in our traditional Indian system of cuisines & medicine has gained much appeal in recent times due to their exemplary biological activities. Specifically, the use of traditional and underutilized legumes holds much scope for exploration. This study demonstrates the biological profiling and phytochemical screening of *Naurangi dal* (rice beans) and *Kulthi dal* (horse gram/*Kulthi* beans) extracts. The bioactive compounds were identified using UHPLC-QTOF-MS and GC-MS. Fatty acid profiling, proximate, amino acid, and elemental analyses were carried out to evaluate the nutritional profile of the legumes. Using GC-MS, it was found that the legumes had high concentrations of terpenes, hydrocarbons, and fatty acids. Various secondary metabolites (quercetin, catechin-7-O-glucoside, epicatechin, and catechin) were found using UHPLC-QTOF-MS. The legumes demonstrated rich concentrations of essential, non-essential, and non-proteinogenic amino acids, as well as linoleic, oleic, and palmitic acid. Elemental analysis showed the presence of 21 elements with magnesium, potassium, and molybdenum being the most prevalent. In biological profiling, anti-microbial and anti-oxidant activities were performed on the selected legume extracts. The anti-oxidant activity of *Kulthi beans* extracts was greater than *rice beans* extracts. Additionally, methanolic extract of the legumes also showed promising anti-microbial activity. These results suggest that these underutilized legumes could be an excellent source of bioactive compounds, anti-microbial and anti-oxidantagents that could be utilized in food, pharmaceutical and cosmeticsindustries.

Keywords: Amino acids, Anti-microbial agent, ICP-MS, Phytochemicals, Secondary metabolites, UHPLC-QTOF-MS

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India is well-known for its agricultural diversity, with a vast array of legume species that have been grown for centuries. Legumes, classified under Fabaceae family, are considered as inexpensive and valuable sources of protein, and are ranked second most important food crop following cereals. Leguminous crops are not only rich in proteins, carbohydrates, minerals, vitamins, amino acids, and dietary fibre content, but they are also gluten-free and possess low fat and glycemic index.

Naurangi dal or rice beans (Family: Fabaceae), scientifically known as *Vigna umbellata*, are nutritionally rich grain legume which is cultivated primarily in hilly areas and utilized by traditional Indian system of medicine[1]. Owing to its immense nutritional benefits, it has been a staple food source in various cultures in form of stews, soups and curries[2]. This legume stands out for its dietary supremacy than other common traditional legumes in the *Vigna* family. Still, its potential to improve the well-being of humans is yet to be completely tapped. The beans are believed to have various health benefits, including aiding digestion and acting as a diuretic. It is a versatile crop traditionally utilized for its nutritional, agricultural, and cultural benefits across different regions. Besides, different parts of the rice bean plant are used in Chinese medicinal systems due to their nutraceutical potential[2]. According to recent research on rice bean, its protein hydrolysates may prevent breast and cervical cancer, while nutritional analysis of Himalayan accessions suggested it might improve food security[3,4]. While some Asian areas have a long history of its traditional use, it has yet to be adopted worldwide.

Similarly, *Kulthi* beans or horse gram (Fabaceae family), scientifically recognized as *Macrotyloma uniflorum*, are primarily grown in India, Africa, Australia, Malaysia, and Mauritius. It contains a substantial amount of proteins, amino acids, minerals, and vitamins. It also contains a plethora of natural

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compounds that have pharmaceutical/nutraceutical values[5]. It is used in traditional remedies for treating colds, coughs, and fever. In some cultures, a paste made from *Kulthi* beans is applied to joints to alleviate pain and inflammation[6]. *Sundal*, a traditional dish in South Indian cuisine, is made from stir-fried *Kulthi* beans and is often prepared during festivals and special occasions. It holds a significant place in Ayurvedic medicine, such as a decoction made from *Kulthi* beans, which is traditionally used to help dissolve kidney stones and facilitate their expulsion[7]. Recently, *Kulthi* beans flour extract was investigated for the green synthesis of silver nanoparticles that have anti-urolithiatic and anti-diabetic properties[8].

Legumes are rich in nutrients and phytochemicals such as flavonoids, alkaloids, and phenolic acids, which offer natural alternatives to synthetic compounds. These bioactives exhibit antioxidant, anti-inflammatory, antidiabetic, antihypertensive, antimicrobial, and anticancer properties, contributing to the prevention of chronic diseases[9,10]. While traditional legumes like chickpea, kidney bean, soybean, and cowpea are well-known, underutilized legumes are gaining attention for their high nutritional value, diverse bioactive profiles, and promising productivity.

The over dependency on a few staple crops, resulted in the loss of their traditional knowledge of cultivation and uses. *Kulthi* and rice beans, as underutilized legumes, have received limited attention regarding their nutritional composition and bioactive compounds, contributing to a lack of awareness among the population about their nutritional excellence.

Therefore, the present study explored the bioactive compounds of *Kulthi* and rice beans using UHPLC-QTOF-MS and GC-MS analysis, proximate analysis, fatty acid composition by fatty acid methyl ester (FAME) analysis, macro- and micro-elements analysis by ICP-MS, amino acid analysis, anti-oxidant property following DPPH assay, and anti-microbial property following disc-diffusion method against *Escherichia coli* (*E. coli*, Gram-negative) and *Staphylococcus aureus* (*S. aureus*, Gram-positive).

Materials and Methods

Plant materials collection and authentication

The selected rice beans were obtained from Himjoli Products in Delhi, India, while *Kulthi* beans were acquired from ICAR-National Bureau of Plant Genetic Resources (NBPGR), Delhi, India. Both beans were authenticated and identified by CSIR-NIScPR's Raw Materials Herbarium and Museum, Delhi (RHMD). All analytical-grade chemicals andreagentsused in the study were sourced from Sigma-Aldrich.

Preparation of extracts

About 50 g of selected sample was grounded and extracted with n-hexane (69ºC), dichloromethane (DCM, 40ºC), and methanol (65ºC) in a Soxhlet apparatus. To ensure that the extraction process was complete, each solvent was used exhaustively for 10-12 h. The extracts were concentrated at low temperature under reduced pressure using a rotary evaporator. All the concentrated extracts were stored at 4ºC until further use[11].

Proximate analysis

According to the procedure of the Association of Official Analytical Chemist (AOAC), the proximate analysis was carried out to identify protein, dietary fibre, carbohydrate and fat content in both beans[12].

Phytochemical analysis

Both legumes were identified qualitatively following the standard protocols. Several chemical tests were carried out for flavonoids (Shinoda's test),alkaloids (Dragendorff's test and Mayer's test), tannins (Ferric chloride test), phenols (Lead acetate test), glycosides (Killer killiani test),and steroids (terpenoids and Liebermann-Burchard test)[13].

FAME analysis

GC-MS (Shimadzu, GC-2010) was used to determine the fatty acid content in the beans[14]. The details of all the experimental methods are available as Supplementary (S).

Gas-Chromatography-Mass Spectrum analysis

The volatile compounds in the n-hexane, DCM, and methanol extracts of the legume samples were identified using GC-MS (Shimadzu GCMS-QP2010), following the previously described method[15].

UHPLC-QTOF-MS identification

The secondary metabolites in the n-hexane, DCM, and methanol extracts of the bean samples were analysed by UHPLC-QTOF-MS, following a previously described method with slight modifications[16].

Amino acid analysis

The estimation of amino acids in both samples were analysed using Automatic Amino Acid Analyzer

L-8900 (Hitachi Co. Ltd., Tokyo, Japan), following the protocol described by Chakrabarti *et al.*[17]

Elemental analysis

The macro-elements, micro-elements, and heavy metals in the selected beans samples were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), following previously described method[18].

Antioxidant activity

Different extracts of *Kulthi* and rice beans were evaluated for antioxidant activity using DPPH free radical scavenging assay, following the procedure explained by Banjara *et al.*[19], with slight modifications. Ascorbic acid served as the positive control standard. The % radical scavenging activity was measured using the following Eq. (1):

$$\% \text{ Scavenging activity} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of Control}}\right) \text{x } 100 \quad \ldots (1)$$

Anti-microbial activity

The anti-microbial activity of the methanolic extract of *Kulthi* and rice beans was performed, following the method with slight modifications[20].

Statistical analysis

All the data was obtained from three independent experiments in triplicates and were expressed as means ± standard deviation. The statistical analysis was done using Microsoft Excel and the error bar was inserted in the data points.

Results and Discussion

Proximate analysis

Supplementary Table S1 illustrates the nutritional analysis of the beans. Rice beans had the highest carbohydrate content (62.73 g/100 g), followed by *Kulthi* beans (62.22 g/100 g), while their dietary fiber content showed an opposite trend. Compared to *Kulthi* beans, rice beans had the highest dietary fibre concentration. The increase in the carbohydrate content is probably due to the decrease in dietary fibre[21]. The protein content of these beans ranged from 20.83 g - 21.26 g, which were relatively higher than maize and rice[22]. *Kulthi* beans exhibit the maximum protein content (21.26 g/100 g) as compared to rice beans (20.83 g/100 g). The highest fat content was found in *Kulthi* beans (2.10 g/100 g). Rice beans exhibited the lowest fat content (0.60 g/100 g). In comparison to other traditional legumes such as cowpea (4.8 g/100 g) and chickpea (5.2 g/100 g), both beans had a fat content that was relatively low[23]. The nutritional composition of the beans was found to be in agreement with the previous reports[1,24]. The slight difference between the present and previously reported data is probably due to the difference in the cultivars as well as place of cultivation of the beans.

Phytochemical analysis

The qualitative analysis of different extracts of the selected beans showed the presence of various phytochemicals, as presented in Supplementary Table S2. The phytochemical analysis revealed that both bean varieties contained reducing sugars, flavonoids, phenols, anthocyanin (identified in the methanolic extract) and alkaloids (found in the DCM and n-hexane extracts). However, tannins and saponins were not detected in any of the extracts examined. The phytochemicals present in beans are acknowledged to be biologically active compounds and are responsible for a range of activities, such as antioxidant, antifungal, antimicrobial, anticancer, and anti-inflammatory[25].

FAME analysis

Supplementary Table S1 illustrates the variations in the percentage of FAME among the selected bean varieties. GC-MS analysis identified and quantified twenty-three fatty acids. In *Kulthi* beans, the predominant fatty acid was linoleic acid (37.76%), followed by palmitic acid (20.64%), oleic acid (17.09%), and linolenic acid (11.40%), with smaller amounts (0.05-4.05%) of other fatty acids. Similarly, rice beans contained a high proportion of linoleic acid (37.73%) and palmitic acid (23.76%), followed by linolenic acid (20.72%) and minor quantities (0.05-7.25%) of other fatty acids. Among all detected components, linoleic acid was the most prevalent, appearing in the highest concentration in *Kulthi* beans (37.76%), closely followed by rice beans (37.73%).

Linoleic acid, a vital polyunsaturated fatty acid (PUFA), provides plentiful health benefits, such as lowering cholesterol levels, enhancing vascular elasticity, reducing the risk of cardiovascular disease, and supporting immune function. It is the most prevalent PUFA found in beans, contributing to approximately 90% of dietary ω-6 PUFA intake, with higher consumption linked to a 15% decrease in coronary heart disease incidence[23]. Similarly, oleic acid helps reduce the likelihood of heart disease and improves insulin sensitivity. Additionally, it exhibits

antioxidant and anti-inflammatory effects. Research by Okereke & Banigo suggests that the presence of a single essential fatty acid can significantly enhance the nutritional value of beans, making them a valuable component of a healthy diet[26].

GC-MS analysis

GC-MS analysis identified 76 volatile compounds in rice bean extracts and 74 in *Kulthi* bean extracts, as shown in Table 1 & Table 2. In rice beans extracts, the prominent phytochemicals were mome inositol (14.89%), hexadecanoic acid (14.02%), 9,12-octadecadienoic acid (10.64%), (7z)-7-tetradecenal (12.64%), 9,10-dibromopentacosane (30.26%), stigmasterol (10.87%), and β-amyrin (10.22%). In *Kulthi* beans extracts, mome inositol (52.87%), palmitic acid (15.27%), 9,12-linoleic acid (15.99%), and caprylic acid monoethanol amide (17.75%) were identified as the prominent phytochemicals. The findings aligned with earlier reports, which identified inositol as the primary component of *Kulthi* beans[27,28]. Significantly, both legume extracts shared several identical compounds, encompassing palmitic acid, dodecane, stearic acid, tetracontane, stigmasterol, δ-tocopherol, and γ-tocopherol. Palmitic and stearic acids represent prevalent saturated fats in Western dietary patterns, influencing blood sugar control mechanisms and associated with insulin resistance development in diabetes mellitus type 2[29]. Plant sterols such as stigmasterol, γ-sitosterol, and β-sitosterol demonstrate multiple health-promoting properties including tumor-fighting capabilities, oxidative stress protection, blood sugar management, cholesterol reduction, and inflammation control[30,31].

Table 1 — Quantitative identification of bioactive constituents in the different solvent extracts of *Kulthi* beans by GC-MS

Peak no.	RT	Compound name	% Area	MW	MF	n-Hexane	DCM	Methanol
1	5.441	Ethyl (trimethylsilyl)acetate	1.48	160	$C_7H_{16}OSi$	-	-	+
2	6.267	1-Methyl pyrrolidone	1.90	99	C_5H_9NO	+	-	-
3	6.338	N-α, n-omega-di-cbz-l-arginine	1.90	442	$C_{22}H_{26}N_4O_6$	+	-	-
4	6.631	Dihydrodicyclopentadiene	0.51	134	$C_{10}H_{14}$	+	-	-
5	7.021	Undecane	0.18	156	$C_{11}H_{24}$	+	-	-
6	7.066	2,3-Trimethylenenorbornane	0.39	136	$C_{10}H_{16}$	+	-	-
7	7.147	4,5-Dimethyl-1,3-oxazinane-2-thione	0.27	145	$C_6H_{11}NOS$	-	-	+
8	7.669	4,7-Methano-1h-indene, octahydro-2-methylene	0.54	148	$C_{11}H_{16}$	+	-	-
9	8.120	3-Methylundecane	0.14	170	$C_{12}H_{26}$	+	-	-
10	8.299	3-Hydroxy-2,3-dihydromaltol	1.44	144	$C_6H_8O_4$	-	-	+
11	8.577	Dodecane	1.43	170	$C_{12}H_{26}$	+	-	-
12	9.615	2,6,10-Trimethyldodecane	0.19	212	$C_{15}H_{32}$	+	-	-
13	9.964	5-Hydroxymethylfurfural	1.06	126	$C_6H_6O_3$	-	-	+
14	10.040	Tridecane	0.19	184	$C_{13}H_{28}$	+	-	-
15	10.595	2-Methoxy-4-vinylphenol	0.30	150	$C_9H_{10}O_2$	-	-	+
16	11.069	1,3-Dimethyl pyrogallate	0.28	154	$C_8H_{10}O_3$	-	-	+
17	11.413	Tetradecane	2.10	198	$C_{14}H_{30}$	+	-	-
18	11.430	Hydratropaldehyde	0.59	134	$C_9H_{10}O$	-	-	+
19	11.630	L- Glutamine	1.53	146	$C_5H_{10}N_2O_3$	-	-	+
20	13.106	1,3:2,5-Dimethylene-l-rhamnitol	1.11	190	$C_8H_{14}O_5$	-	-	+
21	13.774	3,5-Dimethoxyacetophenone	0.16	180	$C_{10}H_{12}O_3$	-	-	+
22	13.924	Pentadecane	1.43	212	$C_{15}H_{32}$	+	-	-
23	14.070	2-Ethylquinoline	1.35	157	$C_{11}H_{11}N$	-	-	+
24	14.289	N-Pentylcyclohexane	1.29	154	$C_{11}H_{22}$	-	-	+
25	16.176	Nonadecane	0.96	268	$C_{19}H_{40}$	+	-	-
26	16.442	Di(tert-butyldimethylsilyl)-mandelic acid	0.69	380	$C_{20}H_{36}O_3Si_2$	+	-	-
27	16.653	6,10,14-Trimethylpentade can-2-one	0.26	268	$C_{18}H_{36}O$	+	-	-
28	17.197	Mome inositol	52.87	194	$C_7H_{14}O_6$	-	-	+
29	17.502	Methyl hexadecanoate	0.91	270	$C_{17}H_{34}O_2$	+	+	+
30	18.074	Palmitic acid	15.27	256	$C_{16}H_{32}O_2$	+	+	+
31	19.144	Methyl linoleate	0.95	294	$C_{19}H_{34}O_2$	+	+	+
32	19.202	Lineoleoyl chloride	0.45	298	$C_{18}H_{31}ClO$	+	-	+
33	19.611	Lauric ethylolamide	7.67	243	$C_{14}H_{29}NO_2$	+	+	+
34	19.720	9,12-Linoleic acid	15.99	280	$C_{18}H_{32}O_2$	+	+	+
35	19.759	Z-9-Hexadecenal	3.78	238	$C_{16}H_{30}O$	-	+	+
36	19.924	9,10-Octadecenoic acid	0.41	282	$C_{18}H_{34}O_2$	-	+	-

… *Contd.*

Table 1 — Quantitative identification of bioactive constituents in the different solvent extracts of *Kulthi* beans by GC-MS (Contd.)

Peak no.	RT	Compound name	% Area	MW	MF	n-Hexane	DCM	Methanol
37	19.935	Stearic acid	0.68	284	$C_{18}H_{36}O_2$	+	-	+
38	20.179	N-Octadecyl ethanoate	0.32	312	$C_{20}H_{40}O_2$	+	-	-
39	20.507	Di(tert-butyldimethylsilyl)- Mandelic acid	1.42	380	$C_{20}H_{36}O_3Si_2$	+	-	-
40	20.547	3-Acetoxy- 7,8-Epoxylanostan-11-ol	0.07	502	$C_{32}H_{54}O_4$	-	-	+
41	20.859	2-Dimethylaminoethyl ester Octanoic acid	1.65	215	$C_{12}H_{25}NO_2$	-	-	+
42	20.978	3-Fluoro-4-(octyloxy)benzoic acid	0.63	268	$C_{15}H_{21}FO_3$	-	-	+
43	21.145	Caprylic acid monoethanol amide	17.75	187	$C_{10}H_{21}NO_2$	+	+	+
44	21.410	Lauric acid monoethanolamine	0.85	243	$C_{14}H_{29}NO_2$	+	+	-
45	21.795	Henicosane	0.21	296	$C_{21}H_{44}$	+	-	-
46	21.979	Triphenyl phosphoric acid ester	0.38	326	$C_{18}H_{15}O_4P$	+	+	+
47	22.274	2-(dimethylamino) ethyl 3-Cyclopentylpropanoate	2.19	213	$C_{12}H_{23}NO_2$	-	-	+
48	22.311	Fumaric acid, 2-dimethylaminoethyl nonyl ester	2.00	313	$C_{17}H_{31}NO_4$	-	-	+
49	22.416	Ethyl 3-oxooctadecanoate	0.66	326	$C_{20}H_{38}O_3$	-	+	-
50	22.422	Methyl 2-cyclohexyl-2-methylpentanoate	0.80	212	$C_{13}H_{24}O_2$	+	-	-
51	22.424	Cis-4-[(trimethylsilyl)oxy]-Cyclohexanol	0.33	188	$C_9H_{20}O_2Si$	-	-	+
52	22.601	Heneicosane	0.73	296	$C_{21}H_{44}$	+	-	-
53	22.627	2-monopalmitoylglycerol	0.29	330	$C_{19}H_{38}O_4$	-	-	+
54	23.377	2-Methyloctacosane	0.34	408	$C_{29}H_{60}$	+	-	-
55	23.763	1,1-Dichloro-2,2,3,3-tetramethylcyclopropane	0.90	166	$C_7H_{12}Cl_2$	+	+	-
56	23.780	3-Isopentylthiophene 1,1-dioxide	0.57	186	$C_9H_{14}O_2S$	-	-	+
57	23.900	Octocrilene	0.14	361	$C_{24}H_{27}NO_2$	+	-	-
58	24.059	5 -Hydroxy-3,6,12-tris[(trimethylsilyl)oxy] ergostan-25-yl acetate	5.31	724	$C_{39}H_{76}O_6Si_3$	-	-	+
59	24.372	Tetratetracontane	0.26	618	$C_{44}H_{90}$	+	-	-
60	24.637	Laurylethanolamide	0.28	243	$C_{14}H_{29}NO_2$	-	+	-
61	24.929	Carbonic acid, eicosyl prop-1-en-2-yl ester	0.79	382	$C_{24}H_{46}O_3$	+	-	-
62	24.942	Dioctyl sebacate	6.63	426	$C_{26}H_{50}O_4$	-	+	-
63	26.095	δ-3,5-cholestadiene	0.72	368	$C_{27}H_{44}$	+	+	+
64	26.566	8-Methyltocol	0.77	402	$C_{27}H_{46}O_2$	+	+	+
65	26.621	Stigmasterol acetate	0.64	454	$C_{31}H_{50}O_2$	+	+	+
66	27.859	O-Xylotocopherol	10.42	416	$C_{28}H_{48}O_2$	+	+	+
67	28.460	Stigmast-5-en-3-yl (9Z)-9-octadecenoate	2.59	678	$C_{47}H_{82}O_2$	-	+	+
68	28.333	Tetracontane	1.00	562	$C_{40}H_{82}$	+	-	-
69	28.458	Stigmasta-3,5-diene	1.15	396	$C_{29}H_{48}$	+	-	-
70	30.090	1-(Dimethyldodecylsilyloxy) butane	0.52	300	$C_{18}H_{40}OSi$	+	-	-
71	30.851	24-Epicampesterol	1.19	400	$C_{28}H_{48}O$	+	+	+
72	31.359	Stigmasterol	5.15	412	$C_{29}H_{48}O$	+	+	+
73	32.674	Stigmast-5-en-3-ol	6.19	414	$C_{29}H_{50}O$	+	+	+
74	33.602	Olean-12-en-3-ol	7.97	426	$C_{30}H_{50}O$	+	+	+
75	34.102	Stigmast-7-en-3-ol	2.03	414	$C_{29}H_{50}O$	+	+	-
76	34.776	Viminalol	2.34	426	$C_{30}H_{50}O$	+	+	-

UHPLC-QTOF-MS analysis

UHPLC-QTOF-MS is used to identify the secondary metabolites in the n-hexane, DCM, and methanol extracts of *Kulthi* and rice beans (Supplementary Table S3 & Supplementary Table S4). The mass-to-charge (m/z) values of all identified compounds were analyzed using positive ionization mode mass spectrometry. We used both non-targeted and targeted approaches to analyze all the extracts to identify the maximum metabolites in the beans.

Non-targeted metabolites

A comprehensive analysis identified 46 secondary metabolites in rice bean extracts and 13 in *Kulthi* bean extracts, predominantly flavonoids, phytosterols, and phenolic compounds, with oligosaccharides present in minor quantities. This study represents one of the first reports utilizing a non-targeted approach via UHPL-QTOF-MS for the characterization of secondary metabolites in these beans. Polyphenols are known for their antioxidant and anti-inflammatory properties, while flavonoids demonstrate anti-tumor, anti-inflammatory, and antioxidant activities, underscoring their significance in cosmetics, nutraceuticals, and medicinal applications[32]. Additionally, carboxylic acids such as para-coumaric acid, gypsogenic acid and vernolic acid were identified, which may facilitate microbial growth by acting as vitamin-like nutrients[33].

Table 2 — Quantitative identification of bioactive constituents in the different solvent extracts of Rice beans by GC-MS

Peak no.	RT	Compound name	% Area	MW	MF	n-Hexane	DCM	Methanol
1	5.404	Decane	0.52	142	$C_{10}H_{22}$	+	+	-
2	5.449	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	3.62	144	$C_6H_8O_4$	-	-	+
3	6.313	1-Methylpyrrolidin-2-one	0.26	99	C_5H_9NO	-	+	-
4	6.333	Benzyl alcohol	0.97	108	C_7H_8O	+	-	-
5	6.631	Tricyclo(5.2.1.0(2,6))dec-3-ene	0.33	134	$C_{10}H_{14}$	+	-	-
6	6.987	Glycerol	1.71	92	$C_3H_8O_3$	-	-	+
7	7.021	Undecane	0.12	156	$C_{11}H_{24}$	+	-	-
8	7.065	Trimethylenenorbornane	0.25	136	$C_{10}H_{16}$	+	-	-
9	7.425	Decamethylcyclopentasiloxane	0.13	370	$C_{10}H_{30}O_5Si_5$	+	-	-
10	7.507	Maltol	1.21	126	$C_6H_6O_3$	-	+	+
11	7.968	Ethyl 3-(2,6-dimehylmorpholino) propionate	0.06	215	$C_{11}H_{21}NO_3$	-	+	-
12	8.119	3-Methylundecane	0.09	170	$C_{12}H_{26}$	+	-	-
13	8.192	3-Hydroxy-2,3-dihydromaltol	6.49	144	$C_6H_8O_4$	-	-	+
14	8.570	Dodecane	0.17	170	$C_{12}H_{26}$	+	+	-
15	9.614	2,7,10-Trimethyldodecane	0.12	212	$C_{15}H_{32}$	+	-	-
16	9.731	5-Hydroxymethylfurfural	6.89	126	$C_6H_6O_3$	-	-	+
17	10.038	Tridecane	0.13	184	$C_{13}H_{28}$	+	-	-
18	11.009	2-Bromo dodecane	0.07	248	$C_{12}H_{25}Br$	+	-	-
19	11.400	Tetradecane	0.24	198	$C_{14}H_{30}$	-	+	-
20	11.414	Pentadecane	1.37	212	$C_{15}H_{32}$	+	-	-
21	12.719	2,5-Difluorobenzoic acid, 6-tetradecyl ester	0.11	354	$C_{12}H_{32}F_2O_2$	-	-	+
22	13.910	Hexadecane	0.19	226	$C_{16}H_{34}$	-	+	-
23	14.931	Methyl α-d-galactopyranoside	3.93	194	$C_7H_{14}O_6$	-	-	+
24	16.159	Mome inositol	14.89	194	$C_7H_{14}O_6$	-	-	+
25	16.159	Octadecane	0.65	254	$C_{18}H_{38}$	+	+	-
26	16.432	Isopropyl myristate	1.24	270	$C_{17}H_{34}O_2$	+	-	-
27	16.549	Neophytadiene	0.03	278	$C_{20}H_{38}$	-	+	-
28	16.637	Hexahydrofarnesyl acetone	0.26	268	$C_{18}H_{36}O$	+	+	-
29	17.485	Palmitic acid methyl ester	0.11	270	$C_{17}H_{34}O_2$	-	+	+
30	17.500	Methyl palmitate	0.41	270	$C_{17}H_{34}O_2$	+	-	-
31	18.063	Hexadecanoic acid	14.02	256	$C_{16}H_{32}O_2$	+	+	+
32	18.213	Heneicosane	0.16	296	$C_{21}H_{44}$	+	-	-
33	19.129	Linoleic acid, methyl ester	1.65	294	$C_{19}H_{34}O_2$	+	+	+
34	19.205	Linolenic acid, methyl ester	1.01	292	$C_{19}H_{32}O_2$	+	-	+
35	19.342	Phytol	0.08	296	$C_{20}H_{40}O$	-	+	-
36	19.609	Palmidrol	1.17	299	$C_{18}H_{37}NO_2$	-	+	+
37	19.709	9,12-Octadecadienoic acid	10.64	280	$C_{18}H_{32}O_2$	-	-	+
38	19.765	(7z)-7-Tetradecenal	12.65	210	$C_{14}H_{26}O$	-	-	+
39	19.866	9,10-Dibromopentacosane	30.26	508	$C_{25}H_{50}Br_2$	+	-	-
40	19.852	10,12-Hexadecadien-1-ol	15.83	238	$C_{16}H_{30}O$	-	+	-
41	19.931	Stearic acid	1.31	284	$C_{18}H_{36}O_2$	+	+	+
42	20.531	Tributyl acetylcitrate	0.68	402	$C_{20}H_{34}O_8$	+	-	-
43	20.854	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.57	213	$C_{12}H_{23}NO_2$	-	-	+
44	20.874	Octanoic acid, 2-dimethylaminoethyl ester	0.11	215	$C_{12}H_{25}NO_2$	-	+	-
45	20.973	Glycidyl palmitate	0.27	312	$C_{19}H_{36}O_3$	-	-	+
46	21.143	1-hydroxy-2,2,6,6-tetramethyl-3-(1-piperidinylmethyl)-4-piperidinone	3.41	268	$C_{15}H_{28}N_2O_2$	+	-	+
47	21.163	Caprylic acid monoethanol amide	1.91	187	$C_{10}H_{21}NO_2$	-	+	-
48	21.417	Laurylamidoethanol	0.34	243	$C_{14}H_{29}NO_2$	-	+	-
49	21.968	Triphenyl phosphate	0.20	326	$C_{18}H_{15}O_4P$	+	+	+
50	21.658	9-Octadecenamide	0.45	281	$C_{18}H_{35}NO$	+	-	-
51	22.265	N,6-Dimethyl-5-hepten-2-amine	0.08	141	$C_9H_{19}N$	-	+	-
52	22.270	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.56	213	$C_{12}H_{23}NO_2$	-	-	+
53	22.615	Palmitoyl chloride	0.27	274	$C_{16}H_{31}ClO$	-	+	+
54	22.911	1,2-Benzenedicarboxylic acid, bis(ethylhexyl) ester	0.17	390	$C_{24}H_{38}O_4$	-	-	+

… *Contd*.

Table 2 — Quantitative identification of bioactive constituents in the different solvent extracts of Rice beans by GC-MS (Contd.)

Peak no.	RT	Compound name	% Area	MW	MF	n-Hexane	DCM	Methanol
55	23.379	Tetratetracontane	0.28	618	$C_{44}H_{90}$	+	-	-
56	23.994	Trans, trans-9,12-Octadecadienoic acid, propyl ester	1.09	322	$C_{21}H_{38}O_2$	-	-	+
57	24.682	Benzyl linoleate	0.40	370	$C_{25}H_{38}O_2$	+	-	-
58	24.931	Carbonic acid, hexadecyl prop-1-en-2-yl ester	0.38	326	$C_{20}H_{38}O_3$	+	-	-
59	24.943	2-Ethylhexyl sebacate	4.99	426	$C_{26}H_{50}O_4$	-	+	-
60	26.055	Stigmasterol acetate	4.50	454	$C_{31}H_{50}O_2$	+	+	-
61	26.087	Cholesterilene	0.48	368	$C_{27}H_{44}$	+	-	+
62	26.551	Delta-tocopherol	7.62	402	$C_{27}H_{46}O_2$	+	+	+
63	26.679	β-Sitosterol monomethyl ether	0.43	428	$C_{30}H_{52}O$	-	+	-
64	27.315	(22E)-Stigmasta-4,7,22-trien-3-ol	0.29	410	$C_{29}H_{46}O$	+	+	-
65	27.832	γ-Tocopherol	3.97	416	$C_{28}H_{48}O_2$	+	+	+
66	28.445	Stigmast-5-en-3-yl 9-octadecenoate	1.65	678	$C_{47}H_{82}O_2$	-	-	+
67	28.338	Tetracontane	0.81	562	$C_{40}H_{82}$	+	-	-
68	28.747	2-Methyltetracosane	0.63	352	$C_{25}H_{52}$	+	-	-
69	30.830	24-Epicampesterol	0.65	400	$C_{28}H_{48}O$	+	+	-
70	31.326	Stigmasterol	10.87	412	$C_{29}H_{48}O$	+	+	+
71	32.787	γ-Sitosterol	8.48	414	$C_{29}H_{50}O$	+	+	-
72	33.138	Fucosterol	0.79	412	$C_{29}H_{48}O$	+	+	-
73	33.679	β-Amyrin	10.22	426	$C_{30}H_{50}O$	+	+	+
74	35.632	Tetracosamethyl-cyclododecasiloxane	0.24	888	$C_{24}H_{72}O_{12}Si_{12}$	-	+	-

Targeted metabolites

In the positive ion mode, nine secondary metabolites were identified in both beans (Supplementary Table S5). Gallic acid, gallocatechin, catechin-7-O-glucoside, glycitein, catechin, epicatechin, quercetin, para-coumaric acid and caffeic acid were identified in the beans. According to reported studies, catechin and its derivatives have been investigated for potential health benefits, especially in relation to their potential to treat obesity[34].

Amino-acid profiling

Amino acid profiling of both beans demonstrated significant nutritional value (Supplementary Table S1). Both rice beans and *Kulthi* beans exhibited a balanced distribution of amino acids, with rice beans containing 42.66% essential amino acids, 51.11% non-essential amino acids, and 6.22% non-proteinogenic amino acids. Similarly, *Kulthi* beans comprised 43.41% essential amino acids, 50.56% non-essential amino acids, and 6.01% non-proteinogenic amino acids. Notably, leucine and phenylalanine constituted a substantial proportion of the essential amino acid content-33.21% in rice beans and 36.95% in *Kulthi* beans. Leucine, an essential amino acid present in both legumes, plays a crucial role in several physiological functions, including protein synthesis, energy production, neurotransmitter formation, glucose metabolism, immune response, liver cell apoptosis, and regeneration[35,36]. On the other hand, glutamic acid, a non-essential amino acid, serves as a key excitatory neurotransmitter in the brain, influencing neural development by supporting differentiation, migration, and survival. It also contributes to synaptic integrity, neuroplasticity, cognitive processes, and memory retention[37,38].

The beans also contained appreciable quantities of non-proteinogenic amino acids, with hydroxyproline predominating in rice beans and 1-methylhistidine in *Kulthi* beans, consistent with previous findings reporting abundant non-proteinogenic amino acids in Fabaceae seeds[39]. β-Alanine, a precursor of pantothenic acid (vitamin B5), was identified in both beans, which has significance in food, pharmaceutical, and environmental applications. Current studies indicate that branched-chain essential amino acids, along with non-protein amino acids like taurine and ornithine, play beneficial roles in managing body weight, promoting muscle protein development, and maintaining blood sugar balance[40]. This evidence supports the exceptional nutritional value of these legumes, which serve as abundant sources of essential, non-essential, and non-protein amino acids-with the non-protein compounds further elevating their comprehensive nutritional benefits.

Elemental analysis

According to the elemental analysis of the selected beans, there were 21 elements (macro and micro-elements), as illustrated in Supplementary Table S1. Magnesium (Mg), molybdenum (Mo), and potassium (K) were the major elements, with potassium having

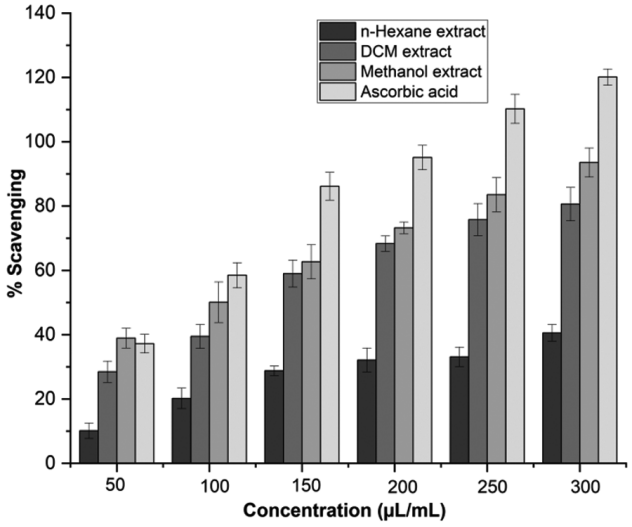

Fig. 1 — Anti-oxidant activity of different extracts of *Kulthi* beans

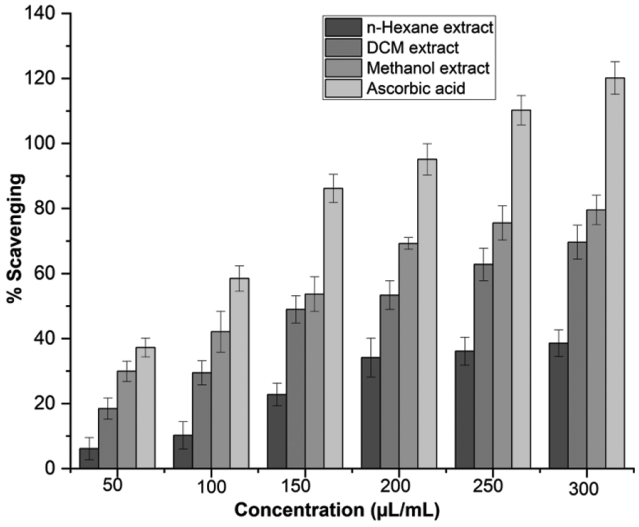

Fig. 2 — Anti-oxidant activity of different extracts of Rice beans

the highest concentration among all the analyzed elements. K is a vital mineral that supports the proper functioning of tissues, cells, and organs in the body. A higher dietary intake of K can aid in maintaining healthy blood pressure levels. Additionally, the concentrations of heavy metals such as arsenic (As), lead (Pb), cadmium (Cd) remained within the standard limit values established by FSSAI. The selected beans contain all the necessary elements to promote human health.

Anti-oxidant activity

The DPPH assay is a commonly used method for evaluating antioxidant properties in plant-based products. This free-radical compound exhibits absorbance in its oxidized state at 515-520 nm[41]. Ascorbic acid served as a positive control due to its well-documented ability to neutralize free radicals. The antioxidant activity of different rice bean and *Kulthi* bean extracts across various concentrations is presented in Figure 1 & Figure 2. The findings reveal that the methanolic extract of the selected beans demonstrated the highest antioxidant potential at a concentration of 300 µL/mL. Additionally, the methanolic extract of *Kulthi* beans exhibited slightly stronger antioxidant activity compared to rice beans.

Anti-microbial activity

The anti-microbial activity (following disc-diffusiion method) revealed that the methanolic extract of *Kulthi* beans possesses potent anti-microbial activity against Gram-negative bacteria (*Escherichia coli*), with a zone of inhibition of 17 mm, and Gram-positive bacteria (*Staphylococcus aureus*), with zone of inhibition of 15 mm. Methanolic extract of rice beans showed mild anti-microbial activity against gram-negative bacteria with a zone of inhibition of 14 mm, while no activity was observed in Gram-positive bacteria Figure 3. From the above results, it can be inferred that the methanolic extract of *Kulthi* beans is more potent source of anti-microbial agent than rice beans.

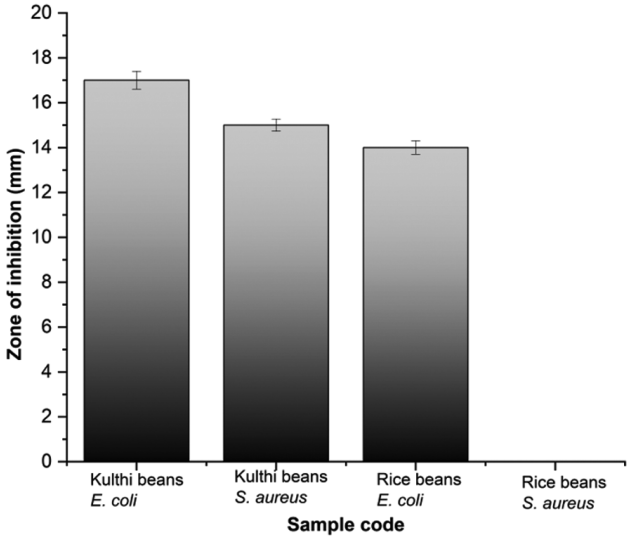

Fig. 3 — Anti-microbial activity of *Kulthi* and rice beans against *E. coli* and *S. aureus*

Conclusion

This paper explores the underutilized traditional legumes of India as potential nutraceuticals, focusing on their bioactive compounds and nutritional profiling. Based on the results of the current study, it can be

concluded that the two underutilized legumes, namely, *Kulthi* beans and rice beans (*Naurangidal*), are abundant in bioactive compounds. The nutritional analysis data revealed that legumes are an ample source of carbohydrates, dietary fibre, and protein. Different non-proteinogenic amino acids enhanced the nutritional values of the selected beans. The GC-MS and UHPLC-QTOF-MS data confirmed the existence of significant concentration of phytochemicals, which may be responsible for various biological activities. Moreover, the results of anti-oxidant and anti-microbial activity indicate that both the beans could be a potent natural anti-oxidant and anti-microbial agent. In conclusion, this work will enhance our knowledge of some underutilized legumes which are used in traditional Indian cuisines and ayurveda and provide valuable information for further research. These legumes could be used to isolate pure natural compounds, which could be utilized in food and pharmaceutical industries. This study may recommend further exploration of Indian legumes used in traditional system of Ayurveda and cuisines as a good source of nutraceutical and functional foods.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_24(7)(2025)698-707_SupplData.pdf

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Conflict of Interest

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

Author Contributions

RS: Writing- original draft, conceptualization, editing, data analysis; DK: Review, editing; RKG: Conceptualization, supervision, review & editing.

Ethical Approval

Not applicable.

Data Availability

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

References

1 Gong D, Zhang B, Yao Y, Wang S, Xiong T, *et al*., Assessment of the diuretic properties of rice bean accessions using a mouse model and identification of active polyphenolic compounds. *Nutrients*, 16 (11) (2024) 1603. doi: 10.3390/nu16111603.

2 Katoch R, *Ricebean: Exploiting the nutritional potential of an underutilized legume*, 1st ed, (Springer, Singapore), 2020. https://doi.org/10.1007/978-981-15-5293-9.

3 González-Cruz L, Morales-Cerda L, Teniente-Martínez G, Valadez-Vega C & Bernardino-Nicanor A, Isolation and hydrolysis of proteins from *Vigna umbellata* and the effect on their antioxidant and anticancer activities, *Separations*, 12 (3) (2025)55. https://doi.org/10.3390/separations12030055.

4 Katoch R, Sanadya S K, Pathania K & Chaudhary H K, Nutritional and nutraceutical potential of rice bean (*Vigna umbellata*) –a legume with hidden potential, *Front Nutr*, 10 (2023) 1126544. https://doi.org/10.3389/fnut.2023.1126544.

5 Chauhan A, Utility of horse gram: A narrative review, *Int J Food Nutr Sci*, 11 (8) (2022) 1742-1751.

6 Ingle K P, Al-Khayri J M, Chakraborty P, Narkhede G W & Suprasanna P, Bioactive compounds of horse gram (*Macrotyloma uniflorum Lam. [Verdc.]*), 1st ed, (Springer, Cham), 2020. http://dx.doi.org/10.1007/978-3-030-44578-2_36-1.

7 Gautam M, Katoch S & Chahota R K, Comprehensive nutritional profiling and activity directed identification of lead antioxidant, antilithiatic agent from *Macrotyloma uniflorum* (Lam.) Verdc, *Food Res Int*, 137 (2020) 109600. https://doi.org/10.1016/j.foodres.2020.109600.

8 Sudha S & Mary Saral A, Studies on phytochemical, mineral content, *in vitro* anti-urolithiatic and anti-diabetic activities of horse gram flour extracts and its biosynthesized Ag nanoparticles, *Heliyon*, 9 (6) (2023) e16572. https://doi.org/10.1016/j.heliyon.2023.e16572.

9 Hernández-Ruiz R G, Olivares-Ochoa X C, Salinas-Varela Y, Guajardo-Espinoza D, Roldán-Flores L G, *et al*, Phenolic compounds and anthocyanins in legumes and their impact on inflammation, oxidative stress, and metabolism: Comprehensive review, *Molecules*, 30 (1) (2025) 174. https://doi.org/10.3390/molecules30010174.

10 Priyadarshini R D, Beatrice D A & C Sivaraj S, Antioxidant potential, antidiabetic, and anti-inflammatory activities of white cowpea (*Vigna unguiculata* L.): An *in vitro* study, *Asian J Biol Life Sci*, 12 (3) (2024) 564-573. http://dx.doi.org/10.5530/ajbls.2023.12.74.

11 Sharma R, Kumar D & Gupta R K, Bioactive profiling of two varieties of Indian legumes: Adzuki and mung beans, *Int J Food Sci Technol*, 59 (9) (2024) 6218-6230. https://doi.org/10.1111/ijfs.17358.

12 Baur F J & Ensminger L G, The association of official analytical chemists (AOAC), *J Am Oil Chem Soc*, 54 (1977) 171-172.

13 Bankole A E, Adekunle A A, Sowemimo A A, Umebese C E, Abiodun O, *et al*, Phytochemical screening and *in vivo* antimalarial activity of extracts from three medicinal plants used in malaria treatment in Nigeria, *Parasitol Res*, 115 (1) (2016) 299-305. https://doi.org/10.1007/s00436-015-4747-x.

14 Ryan E, Galvin K, O'Connor T P, Maguire A R & O'Brien N M, Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes, *Plant Foods Hum Nutr*, 62 (3) (2007) 85-91. https://doi.org/10.1007/s11130-007-0046-8.

15 Beniwal T, Sharma R & Gupta R K, Phytochemical, antioxidant and antimicrobial activity of *Livistona chinensis* (Jacq.) R.Br. ex Mart. seeds, *Indian J Tradit Know*, 23 (2) (2024) 162-169. http://dx.doi.org/10.56042/ijtk.v23i2.3575.

16 Hong Y, Wang Z, Barrow C J, Dunshea F R & Suleria H A R, High-throughput screening and characterization of phenolic compounds in stone fruits waste by LC-ESI-QTOF-MS/MS and their potential antioxidant activities, *Antioxidants*, 10 (2) (2021) 234. https://doi.org/10.3390/antiox10020234.

17 Chakrabarti R, Clark W D, Sharma J G, Goswami R K, Shrivastav A K, *et al*, Mass production of *Lemna minor* and its amino acid and fatty acid profiles, *Front Chem*, 6 (2018) 479. https://doi.org/10.3389/fchem.2018.00479.

18 Ibrahim S, Aslam M & Musthafa M M, Trace elemental fingerprinting of selected herbs used in ayurveda using XRF and ICPMS, *J Pharmacogn Phytochem*, 8 (3) (2019) 3429-3433.

19 Banjara R, Sharma K R & Pokharel Y R, Phytochemical analysis, biological activities, and GC profiling of extracts of some medicinal plant growing in Nepal, *Indian J Nat Prod Res*, 13 (4) (2022) 505-515. https://doi.org/10.56042/ijnpr.v13i4.39187.

20 Rahman T U, Khan H, Liaqat W & Zeb M A, Phytochemical screening, green synthesis of gold nanoparticles, and antibacterial activity using seeds extract of *Ricinus communis* L, *Microsc Res Tech*, 85 (1) (2022) 202-208. https://doi.org/10.1002/jemt.23896.

21 García-Salcedo Á J, Fonthal G, Franco J F & Tirado-Mejía L, Analysis of compositional differences between commercial rice grains by the study of the photoluminescence response, *J Cereal Sci*, 111 (2023) 103681. http://dx.doi.org/10.1016/j.jcs.2023.103681.

22 Longvah T, Ananthan R, Bhaskarachary K & Venkaiah K, *Indian Food Compostion Tables*, (National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India), 2017.

23 Azmah U N, Makeri M U, Bagirei S Y & Shehu A B, Compositional characterization of starch , proteins and lipids of long bean, dwarf long bean, mung bean and French bean seed flours, *Meas Food*, 12 (2023) 100111. http://dx.doi.org/10.1016/j.meafoo.2023.100111.

24 Oguuniyi Q A, Ogbole O O, Akin-Ajani O D, Ajala T O, Liu Q, *et al*, Carbohydrate profiling of different accessions of three underutilized legumes: A potential source of valuable nutraceuticals and pharmaceuticals, *Starch/Staerke*, 2300184 (2023) 1-9.

25 Hailu T, Gupta R K & Rani A, Phytochemicals and antioxidant activity of *Sisymbrium irio* L. seeds, *Indian J Tradit Know*, 20 (1) (2021) 26-32. DOI: 10.56042/ijtk.v20i1.30046.

26 Obioma Okereke G & Banigo EOI, Proximate composition of some selected legumes and quality attributes of oils extracted from soybean, oil Bean and groundnut seeds, *Asian Food Sci J*, 20 (12) (2021) 49-60.

27 Senthilkumar R, Amutha S, Hemalatha G, Uma Maheshwari T & Mini M L, Identification of phytochemical profile of raw and cooked horse gram (*Macrotyloma uniflorum*) seeds by GC-MS/MS, *Pharma InnovJ*, 11 (11) (2022) 2365-2371.

28 Das S, Vasudeva N & Sharma S, Chemical composition of ethanol extract of *Macrotyloma uniflorum* (Lam.) Verdc. using GC-MS spectroscopy, *Org Med Chem Lett*, 4 (2014) 13. http://dx.doi.org/10.1186/s13588-014-0013-y.

29 Carta G, Murru E, Banni S & Manca C, Palmitic acid: Physiological role, metabolism and nutritional implications, *Front Physiol*, 8 (2017) 902. https://doi.org/10.3389/fphys.2017.00902.

30 E l Omari N, Bakrim S, Khalid A, Abdalla A N, Iesa M A M, *et al*., Unveiling the molecular mechanisms: dietary phytosterols as guardians against cardiovascular diseases, *Nat Prod Bioprospect*, 14 (2) (2024) 27.

31 Babu S & Jayaraman S, An update on β-sitosterol: A potential herbal nutraceutical for diabetic management, *Biomed Pharmacother*, 131 (2020) 110702. https://doi.org/10.1016/j.biopha.2020.110702.

32 Shabbir U, Tyagi A, Ham H J & Oh D-H, Comprehensive profiling of bioactive compounds in germinated black soybeans via UHPLC-ESI-QTOF-MS/MS and their anti-Alzheimer's activity, *PLoS One*, 17 (1) (2022) e0263274. https://doi.org/10.1371/journal.pone.0263274.

33 Dahibhate N L & Kumar K, Metabolite profiling of *Bruguiera cylindrica* reveals presence of potential bioactive compounds, *Peer J Anal Chem*, 4 (2022) e16. http://dx.doi.org/10.7717/peerj-achem.16.

34 Basu T, Selman A, Reddy A P & Reddy P H, Current status of obesity: Protective role of catechins, *Antioxidants*, 12 (2) (2023) 474. https://doi.org/10.3390/antiox12020474.

35 Grüngreiff K, Branched amino acids and zinc in the nutrition of liver cirrhosis, *J Clin Exp Hepatol*, 8 (4) (2018) 480-483. https://doi.org/10.1016/j.jceh.2018.04.004.

36 Holeček M, Branched-chain amino acids in health and disease: metabolism, alterations in blood plasma, and as supplements, *Nutr Metab*, 15 (1) (2018) 33. https://doi.org/10.1186/s12986-018-0271-1.

37 Garattini S, Glutamic acid, twenty years later, *J Nutr*, 130 (4S Suppl) (2000) 901s-909s. https://doi.org/10.1093/jn/130.4.901S.

38 Tapiero H, Mathé G, Couvreur P & Tew K D, II. Glutamine and glutamate, *Biomed Pharmacother*, 56 (9) (2002) 446-457. https://doi.org/10.1016/s0753-3322(02)00285-8.

39 da SilvaRodrigues-Corrêa K C & Fett-Neto A G, Abiotic stresses and non-protein amino acids in plants, *Crit Rev Plant Sci*, 38 (5-6) (2019) 411-430. https://doi.org/10.1080/07352689.2019.1707944.

40 Siddik M A B & Shin A C, Recent progress on branched-chain amino acids in obesity, diabetes, and beyond, *Endocrinol Metab*, 34 (3) (2019) 234-246. https://doi.org/10.3803/enm.2019.34.3.234.

41 Bandoniene D, Murkovic M, Pfannhauser W, Venskutonis R & Gruzdienė D, Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and on-line HPLC-DPPH methods, *Eur Food Res Technol*, 214 (2002) 143-147. http://dx.doi.org/10.1007/s00217-001-0430-9.