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

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Supplementary Data

Materials and Methods

Fatty acid methyl ester (FAME) analysis

GC-MS (Shimadzu, GC-2010) was used to evaluate the fatty acid content in the beans. A 0.25 g extracted oil sample was weighed accurately and mixed with 200 µL of 2 N methanolic KOH solution, and vortexed for 2 min. The mixture was heated in a water bath shaker for 10 min at 55ºC. After cooling the mixture, 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. 2 mL of petroleum ether was added to the mixture and it was vortexed for 2 min. The upper layer was separated and analysed by GC-MS.

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). The conditions for injecting the extracts into GC-MS were as follows: injection volume 1 µL, injector temperature at 260ºC, and helium was used as carrier gas at a column flow rate of 1.23 mL/min. The ion source temperature was 220ºC, and the interface temperature was 270ºC. The oven temperature was planned for 60ºC (2 min) to 280ºC (26 min), and the solvent delay time was 3.50 min. The ionization mode used was electron ionization. NIST (National Institute of Standards and Technology, version 1.10 beta, Shimadzu) mass spectral database was used to identify the separated peaks.

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.

The mass spectrometric analysis was conducted in positive mode (ESI+). The analysis was done under the following conditions: desolvation gas flow, 950 L/hour; source temperature, 120ºC; capillary voltage, 3.22 keV; cone gas flow, 50 L/hour. HPLC (Waters, SYNAPT-XS HDMS, UK) fitted with a controller, AD pump, degasser, AD auto sampler, and 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. 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 estimation of amino acids in the selected samples were analysed using Automatic Amino Acid Analyzer L-8900 (Hitachi Co. Ltd., Tokyo, Japan), following the method described by Chakrabarti *et al.* Dried and finely powdered samples were hydrolysed with 6 N HCl at 110ºC for 22 h. The hydrolysed samples were dried in

Nitrogen Evaporator (PCi Analytic Private Limited, Maharashtra, India). 0.02 N HCl was added to each sample and then kept in the Auto-sampler. 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 oxidized with performic acid and treated with 48% hydrobromic acid. Both the samples were 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 the contents of amino acids in the beans, the peak areas of the detected amino acids were compared with those of authentic standards provided with the equipment.

Elemental analysis

About 0.3 g of each sample was diluted to 10 mL nitric acid and then subjected to the analysis of macro-elements, micro-elements, and heavy metals using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The same procedure was used to analyse NIST standards. The instrument calibration was assessed by analysing three certified samples from NIST.

Antioxidant activity

The antioxidant activity of different extracts (n-hexane, DCM, and methanol) of *Kulthi*and rice beans were evaluated using DPPH free radical scavenging assay, following the procedure explained by Banjara *et al*., with slight modifications. Different concentrations of methanol extract of both the samples (50 µL, 100 µL, 150 µL, 200 µL, 250 µL, 300 µL) were made and 1 mL of each extract was mixed with 3 mL of DPPH solution. The prepared mixture was incubated for 30 min at room temperature, and the absorbance was measured at 517 nm using a UV spectrophotometer. Ascorbic acid was used as the positive control.

Anti-microbial activity

Disc-diffusion method

The anti-microbial activity of the methanolic extract of *Kulthi* and rice beans was performed, following the method with slight modifications. After pouring 20 mL of the nutrient media into sterilized petri dishes, it was allowed to solidify. After that, 20 µL of each extract was tested against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria. The bacteria were grown in an incubator at 37°C and then left for 24 h. After the incubation period, the zone of inhibition was measured.

Supplementary Table S1 — Nutritional composition analysis of *Kulthi* beans and rice beans on dry basis

Parameter	*Kulthi* beans	Rice beans
	Nutritional Profile	
Crude protein (g/100 g)	21.26±0.02	20.83±0.01
Dietary fibre (g/100 g)	9.51±0.01	12.46±0.02
Carbohydrate (g/100 g)	62.22±0.05	62.73±0.01
Fat (g/100 g)	2.10±0.01	0.60±0.04
Saturated fat (%)	0.67±0.0	0.20±0.01
MUFA (%)	0.37±0.03	BLQ
PUFA (%)	1.06±0.01	0.36±0.03
Trans fat (%)	BLQ	BLQ
	Mineral profile (ppm)	
Na	31.26±0.05	56.68±0.01
Mg	1886.09±0.01	3114.88±0.01
Al	18.21±0.00	8.17±0.02
K	13331.94±0.01	17198.02±0.03
^{43}Ca	371.40±0.02	599.51±0.09
^{44}Ca	606.41±0.03	1011.09±0.01
Cr	0.041±0.10	0.10±0.01
Mn	45.27±0.03	24.09±0.02
Fe	56.79±0.03	36.98±0.01
Co	0.37±0.20	0.08±0.00
Ni	5.01±0.01	0.88±0.02

… *Contd*.

Supplementary Table S1 — Nutritional composition analysis of *Kulthi* beans and rice beans on dry basis (Contd.)

Parameter	*Kulthi* beans	Rice beans
	Mineral profile (ppm)	
Cu	10.04±0.02	5.95±0.01
Zn	27.64±0.05	32.33±0.09
As	0.01±0.01	0.002±0.05
Se	0.09±0.01	0.16±0.04
Rb	37.50±0.04	15.06±0.01
Sr	11.96±0.09	5.21±0.02
Mo	2012.17±0.01	5772.41±0.02
Cd	0.09±0.00	0.09±0.05
Pb	0.02±0.01	0.01±0.00
U	0.01±0.03	0.02±0.01
	Fatty acid composition (Area %, g/100 g oil sample)	
Lauric acid	0.07±0.05	0.00
Myristic acid	0.49±0.03	0.34±0.01
Pentadecanoic acid	0.11±0.05	0.22±0.03
Palmitic acid	20.64±0.05	23.76±0.01
Palmitoleic acid	0.32±0.01	0.21±0.08
Heptadecanoic acid	0.15±0.01	0.34±0.02
Stearic acid	4.05±0.00	4.77±0.04
Elaidic acid	0.11±0.01	0.00
Oleic acid	17.09±0.01	7.25±0.01
Linoleic acid	37.76±0.04	37.73±0.09
Arachidic acid	1.19±0.00	0.96±0.06
γ- Linolenic acid	0.04±0.00	0.09±0.01
Linolenic acid	11.40±0.01	20.72±0.02
Cis-11-Eicosenoic acid	0.41±0.02	0.16±0.04
Heniecosanoic acid	0.22±0.06	0.15±0.09
Cis-8,11,14-Eicosatr	0.10±0.01	0.08±0.01
Behenic acid	2.98±0.07	1.30±0.03
Erucic acid	0.00	0.05±0.02
Cis-11,14,17-Eicosat	0.11±0.01	0.07±0.01
Methyl cis-5,8,11,14	0.63±0.01	0.49±0.05
Cis-5,8,11,14,17-eic	0.05±0.03	0.00±0.01
Lignoceric acid	1.81±0.01	0.95±0.00
Cis-4,7,10,13,16,19-	0.26±0.03	0.29±0.01
	Amino acid profile (g/100 g dry sample)	
	Essential Amino Acids (g/100 g dry sample)	
Arginine	0.948±0.015	0.997±0.013
Histidine	0.447±0.006	0.413±0.005
Isoleucine (Ile)	0.998±0.009	0.983±0.009
Leucine (Lue)	1.573±0.012	1.635±0.018
Lysine (Lys)	1.124±0.011	1.152±0.025
Methionine (Met)	0.115±0.003	0.077±0.001
Phenylalanine (Phe)	1.777±0.019	1.216±0.015
Threonine (Thr)	0.922±0.008	0.874±0.008
Tryptophan (Trp)	0.079±0.000	0.085±0.00
Valine (Val)	1.082±0.015	1.151±0.013
	Non- essential Amino acids (g/100 g dry sample)	
Alanine (Ala)	0.880±0.002	0.888±0.006
Aspartate (Asp)	1.831±0.019	2.540±0.032
Cysteine (Cys)	0.280±0.001	0.091±0.000
Glutamic Acid (Glu)	3.708±0.103	3.346±0.103
Glycine (Gly)	0.989±0.006	0.873±0.008
Proline (Pro)	1.091±0.016	1.06±0.005
Serine (Ser)	1.236±0.006	1.075±0.018
Tyrosine (Tyr)	0.544±0.002	0.409±0.009

… *Contd.*

Supplementary Table S1 — Nutritional composition analysis of *Kulthi* beans and rice beans on dry basis (Contd.)

Parameter	*Kulthi* beans	Rice beans
Non- Proteinogenic Amino acids (g/100 g dry sample)		
Phosphoserine	0.040±0.00	0.030±0.001
Taurine	0.044±0.00	0.037±0.002
Phospho ethanol amine	0.004±0.00	0.002±0.00
β-Alanine	0.058±0.00	0.064±0.00
Ethanol amine	0.001±0.00	ND
Ornithine	0.003±0.00	0.006±0.00
1-Methylhistidine	0.107±0.001	0.095±0.00
Hydroxyproline	0.999±0.005	1.018±0.016
Phosphoserine	0.040±0.00	0.030±0.001
Taurine	0.044±0.00	0.037±0.002

Data is expressed as mean value ± standard deviation
ND = Not detected
BLQ = Below detection limit

Supplementary Table S2 — Qualitative phytochemical analysis of *Kulthi* beans and rice beans

S. No.	Phytochemicals	*Kulthi* beans			Rice beans		
		n-hexane extract	DCM extract	Methanol extract	n-hexane extract	DCM extract	Methanol extract
1.	Tannins	-	-	-	-	-	-
2.	Flavonoids	-	-	+	-	-	+
3.	Glycosides	-	-	-	-	-	-
4.	Alkaloids	+	+	-	+	+	-
5.	Reducing Sugar	-	-	+	-	-	+
6.	Anthocyanins	-	-	+	-	-	+
7.	Coumarins	-	-	-	-	-	-
8.	Phenols	-	-	+	-	-	+

+ = presence of phytochemical
- = absence of phytochemical

Supplementary Table S3 — Identification of secondary metabolites using UHPLC-QTOF-MS in Kulthi beans

Peak No.	Tentative metabolites	RT (min)	MF	MM	$[M-H]^+$	Error (ppm)	Compound ID
			n-Hexane extract				
1.	3α,21-Dihydroxy-D-homo-5β-pregn-17a (20)-en-11-one	19.4659	$C_{22}H_{34}O_3$	346.2507	347.2587	1.6	CSID10128389
2.	1,4a-Dimethyl-8-methylenegibbane-1,10-dicarboxylate	21.1374	$C_{20}H_{26}O_4$	330.1842	331.1922	2.8	CSID24784772
3.	8-Hydroxy-2-(3-hydroxy-4,5-dimethoxybenzyl)-7-methoxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepinium	22.9102	$C_{21}H_{28}NO_5$	374.1961	375.2041	1.0	CSID24784686
4.	1-Naphthylacetylspermine	24.2378	$C_{22}H_{34}N_4O$	370.2732	371.2812	1.8	CSID114826
5.	1'-Hydroxy-γ-carotene glucoside	25.4244	$C_{46}H_{68}O_6$	716.5015	717.5095	3.1	CSID30792006
6.	Dehydrosqualene	26.2531	$C_{30}H_{48}$	408.3756	409.3836	4.9	CSID26332853
7.	1-Palmitoyl-2-lysophosphatidylcholine	28.3023	$C_{24}H_{50}NO_7P$	495.3324	496.3404	3.2	CSID405287
			DCM extract				
8.	α-Tocopherol	29.2322	$C_{29}H_{50}O_2$	430.3810	431.3890	4.1	CSID14265
9.	Stigmasterol	30.0348	$C_{29}H_{48}O$	412.3709	413.3787	2.3	CSID4444352
			Methanol extract				
10.	Nystose	2.1436	$C_{24}H_{42}O_{21}$	666.0902	667.0980	4.9	CSID145907
11.	D-(+)-catechin	6.1344	$C_{15}H_{14}O_6$	290.0790	291.0868	1.2	CSID8711
12.	Hexadecenal	23.1850	$C_{16}H_{30}O$	238.2035	239.2113	3.4	CSID4444172
13.	1,2-Linoleoylphosphatidylcholine	24.8049	$C_{44}H_{80}NO_8P$	781.5750	782.5830	4.6	CSID10716780

Supplementary Table S4 — Identification of secondary metabolites using UHPLC-QTOF-MS in rice beans

Peak no.	Tentative metabolites	RT (min)	MF	MM	$[M-H]^+$	Error (ppm)	Compound ID
			n-hexane				
1.	(+)-Secoisolariciresinol	13.60863	$C_{20}H_{26}O_6$	362.1729	363.1819	4.5	CSID28288853
2.	Palmitic acid	24.2378	$C_{16}H_{32}O_2$	256.2402	257.2477	0.72	CSID960
3.	1-Linoleoyl-glycero-3-phosphocholine	26.5970	$C_{26}H_{50}NO_7P$	519.3324	520.3399	0.14	CSID9181014
			DCM				
4.	1-Linoleoyl-glycero-3-phosphocholine	26.5970	$C_{26}H_{50}NO_7P$	519.3324	520.3399	0.14	CSID9181014
5.	Palmitic acid	24.2378	$C_{16}H_{32}O_2$	256.2402	257.2477	0.72	CSID960
			Methanol				
6.	6,7-(Methylenedioxy)coumarin	25.85572	$C_{10}H_6O_4$	190.0249	191.0327	-5.5	CSID2340777
7.	β-D-Fructofuranosyl-(2->1)- β-D-fructofuranosyl β-D-fructofuranosyl-(2->6)-alpha-D-glucopyranoside	2.015367	$C_{24}H_{42}O_{21}$	666.2221	667.2299	1.1	CSID10190528
8.	Nystose	2.015367	$C_{24}H_{42}O_{21}$	666.0902	667.0980	5.1	CSID145907
9.	Fagopyritol A3	2.015367	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID145907
10.	Kestotetraose	2.015367	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID19128807
11.	Lychnose	2.015367	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID30785504
12.	A-1,4-Tetraglucose	2.015367	$C_{24}H_{42}O_{21}$	666.2221	667.2299	1.1	CSID388711
13.	3F-α-D-Galactosylraffinose	2.015367	$C_{24}H_{42}O_{21}$	666.2221	667.2299	1.1	CSID389173
14.	Isolychnose	2.015367	$C_{24}H_{42}O_{21}$	666.2221	667.2299	1.1	CSID389173
15.	Mediose	2.015367	$C_{24}H_{42}O_{21}$	666.2221	667.2299	1.1	CSID847
16.	2-[(1H-Indol-3-ylacetyl) amino]-4-methylpentanoate	2.015367	$C_{24}H_{42}O_{21}$	287.1392	288.1470	-1.2	CSID24784918
17.	(+)-Leucopelargonidin	5.94455	$C_{15}H_{14}O_6$	290.0788	291.0866	1.0	CSID389080
18.	Luteoforol	5.94455	$C_{15}H_{14}O_6$	290.0788	291.0866	1.0	CSID389678
19.	(â˜)-Epicatechin	5.94455	$C_{15}H_{14}O_6$	290.0790	291.0868	1.0	CSID65230
20.	D-(+)-catechin	5.94455	$C_{15}H_{14}O_6$	290.0790	291.0868	1.0	CSID8711
21.	Cianidanol	9.200367	$C_{15}H_{14}O_6$	290.0785	291.0863	0.1	CSID1166
22.	P-Coumaroylquinic acid	9.423017	$C_{16}H_{18}O_8$	338.1007	339.1085	3.3	CSID4945466
23.	5,6-Dihydroxy-2-(4-hydroxyphenyl)-7-methoxy-2,3-dihydro-4H-chromen-4-one	9.423017	$C_{16}H_{14}O_6$	301.0797	303.0875	4.1	CSID58829719
24.	Cis-(-)-7,2'-dihydroxy-4',5'-methylenedioxyisoflavanol	9.423017	$C_{16}H_{14}O_6$	301.0797	303.0875	4.1	CSID58829833
25.	4-Methylumbelliferyl-β-D-glucoside	11.90338	$C_{16}H_{18}O_8$	302.0790	303.0868	1.7	CSID2015550
26.	2,6,7-Trihydroxy-3-(4-methoxyphenyl)-2,3-dihydro-4H-chromen-4-one	11.90338	$C_{16}H_{14}O_6$	302.0790	303.0868	1.8	CSID24785201
27.	Hesperitine	11.90338	$C_{16}H_{14}O_6$	302.0790	303.0868	1.8	CSID3467
28.	4'-Methoxy-2',3,7-trihydroxyisoflavanone	11.90338	$C_{16}H_{14}O_6$	302.0790	303.0868	1.8	CSID35015216
29.	Furcatin	12.0246	$C_{20}H_{28}O_{10}$	428.1656	429.1734	-4.8	CSID391122
30.	6,7,3',4'-Tetrahydroxyisoflavone	12.55728	$C_{15}H_{10}O_6$	286.0471	287.0549	-0.1	CSID4577544
31.	Indole-3-acetyl-glutamate-N-β-D-glucose	12.6785	$C_{21}H_{24}N_2O_{10}$	464.1453	465.1531	3.5	CSID24785300
32.	Homoeriodictyol	12.7659	$C_{16}H_{18}O_8$	302.0794	303.0872	3.0	CSID66296
33.	Solasodine	13.4198	$C_{27}H_{43}NO_2$	575.3821	576.3899	0.7	CSID58837434
34.	6-Hydroxy-2-(4-glucosyl-phenoxymethylene)-benzofuran-3-one	13.79748	$C_{21}H_{20}O_{10}$	432.1068	433.1146	4.0	CSID58837758
35.	Gypsogenic acid	14.00608	$C_{30}H_{46}O_5$	486.3346	487.3424	1.5	CSID10217372
36.	Calycosin	14.47115	$C_{16}H_{12}O_5$	284.0681	285.0759	0.7	CSID4444104
37.	Coreopsin	14.81502	$C_{21}H_{22}O_{10}$	434.1212	435.1290	0.9	CSID24784916
38.	8,3'-Dihydroxydaidzein	15.12507	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.4	CSID23255668
39.	Luteolin	15.12507	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.4	CSID4444102
40.	Scutellarein	15.77897	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.4	CSID4445014
41.	3-Hydroxy-1,2-propanediyl bis(2-propylpentanoate)	21.04988	$C_{19}H_{36}O_5$	344.2554	345.2632	-1.1	CSID21163206
42.	Phytoceramide	21.23872	$C_{18}H_{39}NO_3$	317.2921	318.2999	-0.9	CSID108921
43.	1-Hydroxy-1,2-ethanediyl dioctanoate	21.63617	$C_{18}H_{34}O_5$	330.2398	331.2476	-0.9	CSID58829678
44.	1-Linoleoyl-2-Hydroxy-sn-glycero-3-PC	21.70378	$C_{26}H_{50}NO_7P$	519.3322	520.3400	-0.3	CSID9181014
45.	Vernolic acid	21.98002	$C_{18}H_{32}O_3$	296.2348	297.2426	1.3	CSID4512106
46.	12(13)-Epoxy-9Z,15Z-octadecadienoic acid	22.84253	$C_{18}H_{30}O_3$	294.2195	295.2273	1.6	CSID17220744

Table S5 — Targeted Metabolites identified by UHPLC-QTOF-MS in the selected legumes

Peak No.	Compound Name	Molecular weight	*Kulthi* beans	Rice beans
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	+	+