Metabolite Profiling of Methanolic and Aqueous Extracts of Rice and Black Gram Pulse consumed in Indian Subcontinent by Untargeted LC-MS/MS

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Abstract

Rice (*Oryza sativa* L.) and black gram (*Vigna mungo* (L.) Hepper) are widely consumed dietary staples in India and represent important sources of nutrients and bioactive phytochemicals. The present study aimed to characterize and compare their phytochemical compositions using an untargeted liquid chromatography–tandem mass spectrometry (LC-MS/MS) metabolomics approach. Methanolic and aqueous extracts were prepared by maceration and subjected to qualitative analysis using a SCIEX QTRAP mass spectrometry system operated in enhanced mass scan–enhanced product ion (EMS–EPI) mode with information-dependent acquisition (IDA) under positive and negative electrospray ionization conditions. The acquired mass spectral data were processed using SCIEX OS software for tentative compound identification and characterization. The analysis revealed 62 and 91 putatively identified compounds in rice and black gram, respectively, representing diverse chemical classes, including phenolic compounds, flavonoids, organic acids, and other bioactive metabolites. Rice exhibited spectral features assigned to genipin, and citric acid, jasmonic acid, and nigerose. In contrast, black gram exhibited a chemically diverse profile comprising flavonoids such as kaempferol, vitexin, robinin, luteolin, and puerarin, together with citric acid. The complementary positive and negative ionization modes facilitated the detection of chemically diverse metabolites, providing broader qualitative coverage of the investigated food matrices. In addition to phytochemical characterization, the analysis revealed tentative matches to pharmaceutical-related compounds, indicating the potential application of untargeted metabolomics for preliminary contaminant screening. These findings demonstrate the utility of LC-MS/MS-based metabolomics for comparative chemical profiling of commonly consumed food grains and provide a foundation for future

applications in food authentication, quality assessment, and safety monitoring. Confirmation of putative contaminants using reference standards and targeted quantitative methods is required before assessing dietary exposure and associated health risks.

Keywords: Grains, Metabolomics, Rice, Black gram pulse, Phytochemical analysis, LC-MS.

1. Introduction

Cereal grains constitute an essential component of the regular Indian diet and serve as staple foods across diverse cultural and geographical regions of the country. Similarly, pulses are an integral part of traditional diets and contribute substantially to dietary protein intake. Among cereals, rice (*Oryza sativa* L.) is widely consumed across India, whereas black gram or urad dal (*Vigna mungo* (L.) Hepper) holds considerable culinary and nutritional importance as a major ingredient in several traditional foods throughout the Indian subcontinent. Cereals and pulses possess complementary amino acid profiles, thereby improving the overall protein quality of meals and making their combined consumption nutritionally significant. The Indian Council of Medical Research–National Institute of Nutrition (ICMR–NIN) Dietary Guidelines for Indians (2024) also emphasize the importance of consuming cereals and millets in combination with pulses and vegetables to achieve a nutritionally balanced diet[1].

In southern, eastern, and northeastern India, rice serves as a major source of dietary energy, while pulses provide complementary sources of protein and other essential nutrients. National dietary surveys have indicated considerable regional variation in cereal consumption, with rice predominating in several parts of India[2]. Cereals contribute significantly to dietary energy intake, particularly among rural and economically disadvantaged populations, while also providing various naturally occurring bioactive constituents[3]. Reports from NITI Aayog based on the National Sample Survey Office (NSSO) 2011–12 household consumption data indicated an average per capita rice consumption of approximately 5.85 kg per month, highlighting its substantial contribution to the Indian diet[4].

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Beyond their macronutrient composition, rice and black gram contain a diverse range of phytochemicals that contribute to their nutritional and functional properties. Rice contains several bioactive constituents, including phenolic acids, flavonoids, anthocyanins, tocopherols, γ-oryzanol, phytosterols, and phytic acid[5]. The distribution of phenolic compounds, including *p*-coumaric, gallic, ferulic, and vanillic acids, varies considerably among the bran, germ, and endosperm fractions of rice[6]. Similarly, black gram possesses a chemically diverse phytochemical profile in addition to its protein and carbohydrate content. Its reported phytochemical constituents include phenolic compounds, flavonoids, steroids, saponins, carotenoids, alkaloids, and fatty acids[7]. The concentrations and chemical forms of these constituents may vary depending on genotype, geographical origin, cultivar, grain fraction, and processing methods such as soaking, cooking, and fermentation. Moreover, a considerable proportion of grain-associated phytochemicals may occur in bound or insoluble forms, indicating that analysis of freely extractable compounds alone may not adequately represent their complete phytochemical composition[8].

In addition to phytochemicals, cereals and pulses contain essential mineral elements that play important roles in human nutrition and various physiological processes. Singh and Garg (2006) investigated the elemental composition of Indian cereals, vegetables, and spices using instrumental neutron activation analysis (INAA) and reported the occurrence of several essential trace elements, including iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn). The study highlighted the importance of evaluating trace-element composition when assessing the nutritional contribution of commonly consumed Indian foods[9]. Certain phytochemical constituents, particularly phytic acid and phenolic compounds, can interact with mineral elements through chelation or complex formation, potentially influencing mineral solubility and bioavailability. Such interactions are nutritionally relevant because the formation of phytate–mineral complexes may reduce the intestinal availability of essential elements, particularly Fe and Zn [10]. Therefore, the presence of both phytochemicals and mineral elements within cereal and pulse matrices warrants consideration when evaluating their nutritional and functional characteristics.

Pereira-Caro et al. identified diverse secondary metabolites, including anthocyanins, flavones, flavonols, flavan-3-ols, carotenoids, and γ-oryzanols while investigating the phytochemical profiles of black, red, brown, and white rice using HPLC-PDA-MS. The

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study, highlighted the utility of chromatographic separation coupled with mass spectrometry for comprehensive rice metabolite profiling among rice varieties [11.]

LC–MS-based research on rice and black gram has progressed from targeted constituent analysis to broader metabolomic characterization. In black gram (*Vigna mungo*), Lee et al. (1999) investigated saponins using LC/MS, while Singh et al. (2024) employed LC–MS/MS to characterize purified 3-O-methyl-D-glucose from the seed coat, demonstrating applications focused on specific constituents.[12] More recently, Ghallab et al. (2025) applied UPLC–MS/MS profiling to compare red, green and black mung beans, extending chemical characterization across related legumes.[13] In rice (*Oryza sativa*), Li et al. (2022) used untargeted LC–MS metabolomics to differentiate geographical origins, illustrating its potential for food authentication.[14] Singh et al., purified 3-O-methyl-D-glucose from the seed coat of *Vigna mungo* (L.) Hepper.[15] Although these studies establish the analytical value of mass spectrometry for both food matrices, their objectives differ from the comparative characterization undertaken here. The present study applies a common QTRAP LC–MS/MS workflow to aqueous and methanolic extracts of rice and black gram, combining positive- and negative-ion acquisition with information-dependent EMS–EPI analysis to examine their phytochemical diversity and generate candidate annotations relevant to subsequent food-quality and contaminant investigations.

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a sensitive and selective analytical technique for the detection and characterization of diverse phytochemical constituents in complex food matrices. LC-MS/MS-based profiling of commonly consumed cereals and pulses can provide valuable information regarding their chemical diversity and facilitate the identification of potentially bioactive metabolites.[16] In the present study, qualitative LC-MS/MS analysis was undertaken to investigate and compare the phytochemical profiles of two widely consumed Indian dietary staples, rice (*Oryza sativa* L.) and black gram (*Vigna mungo* (L.) Hepper). The study specifically aimed to characterize the detectable phytochemical constituents of these grains and examine similarities and differences in their chemical profiles. The resulting analytical profiles provide a basis for comparing the phytochemical composition of these two important food grains and identifying constituents of potential nutritional and functional relevance. The findings also provide a foundation for future investigations into the bioavailability of grain-associated phytochemicals and their possible interactions with essential mineral elements.

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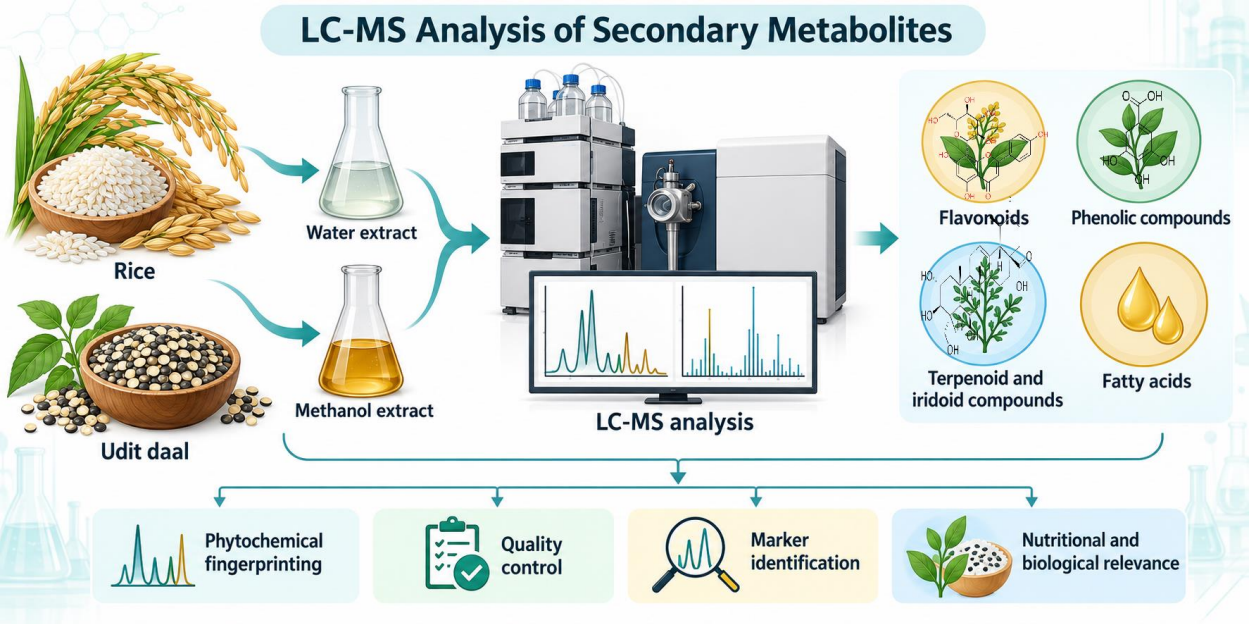


Fig 1: LC-MS Analysis of Rice and Black Gram Pulses and Phytochemicals

2. Experimental

2.1 Reagents and solvents

Analytical-grade methanol purchased from Spectrochem was used for extraction. LC-MS grade acetonitrile (JT Baker UN1648, CAS No-75-05-8), formic acid (JT Baker, UN-1779, CAS No. 64-18-6) and methanol (JT Baker UN1230, Cas No- 67-56-1) were used in LC-MS analysis.

2.2 Sample preparation and extraction

5 g of rice grains and black gram were individually macerated at room temperature for 48 hours in 50 mL of methanol and Milli-Q water, separately. After maceration was completed, it was filtered and concentrated using a rotary evaporator.[17] The dried samples were individually dissolved in 1 mL LC-MS grade methanol. Aliquot 100 µL of each was taken and made up to 1 mL using LC-MS grade methanol, which was injected into the system for analysis.

2.3 LC-MS/MS method

Phytochemical analysis of the rice and black gram pulse extracts was done using the Sciex LC-MS/MSQtrap 4500 system. The system is equipped with Shimadzu Nexera SIL-40C × 3 autosampler, Shimadzu Nexera SPD-M40 Photodiode Array (PDA) detector, Shimadzu Nexera CTO-40C column oven, Shimadzu SLC40 system controller, Shimadzu DGU-405 Degassing unit, and Shimadzu binary LC-40D × 3. Sciex Analyst and Sciex OS software

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were used for the data acquisition and processing of the results respectively. The mobile phase was composed of a mixture of 0.1 % (v) formic acid with Milli-Q water (solvent A) and acetonitrile with 0.1 % formic acid (solvent B), with modifications in gradient ratio[11]. The Kinetex 1.7 µm C18 100 Å LC Column 150 × 2.1 mm column was used for separation in gradient mobile phase in the following ratio: 10 % solvent B holding for 2 min, linear increase to 75 % B within 4 min, 90 % B till 10 min, maintained till 12 minutes, and finally, it changed to 10 % B till 16 minutes. The flow rate was maintained at 0.4 mL/min with an injection volume set at 10 µL. Phytochemicals were detected at UV absorbance of 254 nm, 382 nm, and 456 nm which was selected in PDA.

The method was developed in both positive and negative ionization modes using Electrospray Ionization (ESI) involving Information-Dependent Acquisition (IDA) to identify the maximum number of phytochemicals in the extracts. For positive ionization mode, source gas parameters were CAD 3.0, CUR 35, GS1 55, GS2 55, IS 5500, and TEM 500 and for negative ionization mode they were CAD − 3.0, CUR 35, GS1 55, GS2 55, IS − 4500, and TEM 500. The method was created by optimizing the compound parameters such as DP, EP, CS, and CES to obtain the best results. The full EMS-Scan for mass range of 100 to 1000 Da was done at DP 100 and CE 35 and ER resolution for top three intense peaks was carried out. For both ionization modes, 100 DP, 10 CE, 10 EP, and 15 CES were selected for EMS scan and to obtain the maximum phytochemicals, EPI with collision energy + 35 CE/-35 CE was used.

2.4 Analysis

IDA aided identification of phytochemicals based on their fragmentation was done with the help of the NIST library using SCIEX OS software.

3.0 Results and Discussion

We investigated the methanolic and water extracts of rice and black gram pulses, obtained by maceration. The method developed for LC-MS analysis in negative and positive polarity in EMS to EPI helped in the development of a metabolomic profile using the Information-dependent acquisition (IDA) method for untargeted analysis.

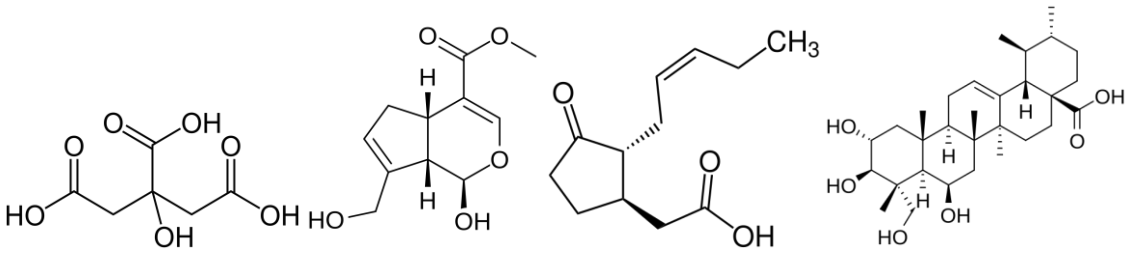
3.1 Metabolites identified in Rice-Water extract

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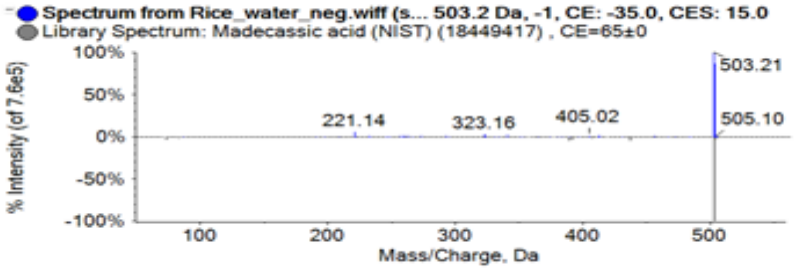
The water extract of rice in positive polarity showed the presence of 2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine, Gln-His, 1,2-Di-(9Z,12Z,15Z-octadecatrienoyl)-sn-glycero-3-

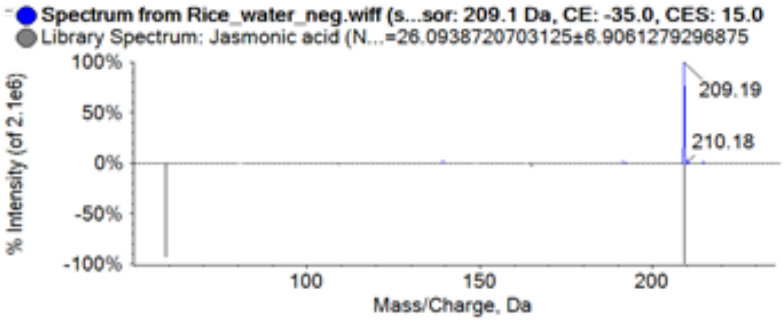
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phosphocholine, 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine, Salicylic acid ethyl ester, Nigerose, Citrinin, Glycerol tricaprylate, Genipin, and Citric acid.



Citric acid **Genipin** **Jasmonic acid** **Madecassic acid**





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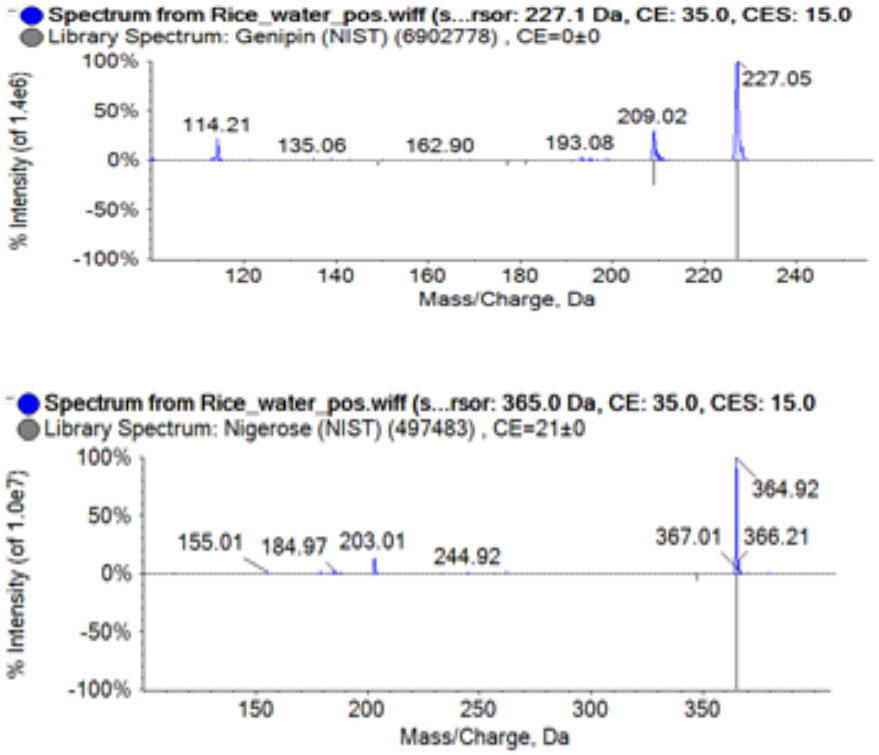


Fig 2: Phytochemicals structure and mass spectra identified in Rice water Extract in negative and positive polarity

The water extract of rice in negative polarity showed the presence of Madecassic acid, 1-Palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine, Citric acid, cis-5,8,11-Eicosatrienoic acid, Asp-Glu, .gamma.-Linolenic acid, 3-Hydroxyoctadecanoic acid, 3,4'-Dimethoxy-5,7,3'-trihydroxyflavone, 3-Hydroxydodecanoic acid, Jasmonic acid, 2-Hydroxytrimipramine, 3-Hydroxymyristic acid, Genipin, 3,5-Di-tert-butyl-4-hydroxybenzoic acid Trisaccharide, and 12(13)-Epoxy-9Z-octadecenoic acid. Dipeptides Asp-Glu and Gln-His are also detected. **Table-1** represents the list of compounds identified in the water extract of Rice. LC-MS spectra of Madecassic acid, Jasmonic acid, Genipin, and Nigerose are displayed in **Figure 2.**

Table-1: Rice water extract metabolites identified using LC-MS/QTRAP

Tentative library annotation	**InChIKey**	Mode	Retention Time (min)	Adduct / Charge	Precursor Mass (m/z)	Library Score
Salicylic acid ethyl	GYCKQBWUSAC	Pos	10.17	$[M+H]^+$	167.063	89.9

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ester	YIF-UHFFFAOYSA-N					
Nigerose	YGEHCIVVZVBCLE-NIKVEEOSSA-N	Pos	0.96	$[M+H]^+$	364.963	83.3
Citrinin	CQIUKKVOEOPUDV-IYSWYEEDSA-N	Pos	10.66	$[M+H]^+$	251.06	82.9
Genipin	AZKVWQKMDGGDSV-BCMRRPTOSA-N	Pos	5.75	$[M+H]^+$	227.03	76.2
2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine	WTJKGGKOPKCXLL-VYOBOKEXSA-N	Pos	14.56	$[M+H]^+$	760.589	100
1,2-Di-(9Z,12Z,15Z-octadecatrienoyl)-sn-glycero-3-phosphocholine	XXKFQTJOJZELMD-JICBSJGISA-N	Pos	12.37	$[M+H]^+$	778.244	92.8
1-Palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine	IIZPXYDJLKNOIY-JXPKJXOSSA-N	Neg	14.1	$[M-H]^-$	826.557	97.7
Citric acid	KRKNYBCHXYNGOX-UHFFFAOYSA-N	Neg	1.34	$[M-H]^-$	191.135	95.6
Madecassic acid	PRAUVHZJPXOEIF-AOLYGAPISA-N	Neg	1.04	$[M-H]^-$	503.254	100
cis-5,8,11-Eicosatrienoic acid		Neg	11.48	$[M-H]^-$	305.28	92.7
.gamma.-Linolenic acid	VZCCETWTMQHEPK-	Neg	11.32	$[M-H]^-$	277.292	89.2

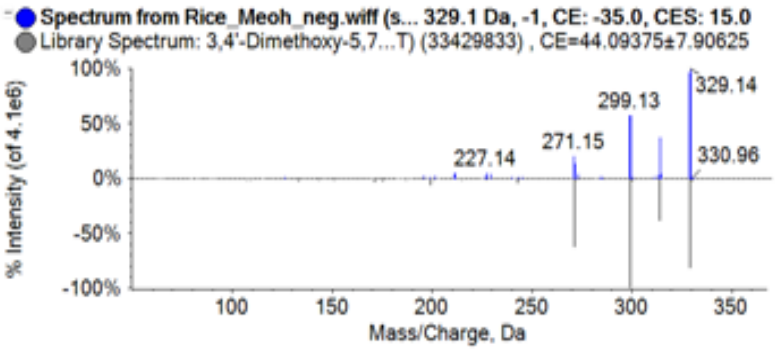
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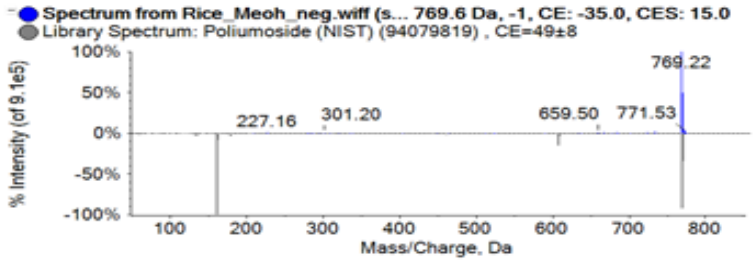
	QNEBEIHSSA-N					
3-Hydroxyoctadecanoic acid	POMQYTSPMKEQNB-UHFFFAOYSA-N	Neg	11.75	$[M-H]^-$	299.386	86.7
3,4'-Dimethoxy-5,7,3'-trihydroxyflavone		Neg	8.48	$[M-H]^-$	329.312	82.3
3-Hydroxydodecanoic acid	MUCMKTPAZLSKTL-UHFFFAOYSA-N	Neg	8.2	$[M-H]^-$	215.28	78.2
Jasmonic acid	ZNJFBWYDHIGLCU-HWKXXFMVSA-N	Neg	7.23	$[M-H]^-$	209.217	77.2
2-Hydroxytrimipramine	FQJSSUOYVSEYPF-UHFFFAOYSA-N	Neg	9.61	$[M-H]^-$	309.342	75.8
3-Hydroxymyristic acid	ATRNZOYKSNPPBF-UHFFFAOYSA-N	Neg	9.1	$[M-H]^-$	243.306	73.7
Blood Group B Trisaccharide	OZIOWHWTZWIMCZ-MPUPURDASA-N	Neg	6.73	$[M-H]^-$	487.422	72.7
12(13)-Epoxy-9Z-octadecenoic acid	CCPPLLJZDQAOHD-FLIBITNWSA-N	Neg	11.62	$[M-H]^-$	295.408	71.5
Asp-Glu	CKAJHWFHHFSCDT-WHFBIAKZSA-N	Neg	6.97	$[M-H]^-$	261.31	89.6
Gln-His	JZOYFBPIEHCDFV-YUMQZZPRSA-N	Pos	13.94	$[M+H]^+$	284.134	95.5

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3.2 Metabolites identified in Rice-Methanolic extract

The methanolic extract of rice in positive polarity showed presence of, Loganic acid, Arachidonoylthiophosphorylcholine, 1-Oleoyl-sn-glycero-3-phosphocholine, 1-Palmitoyl-sn-glycero-3-phosphocholine, 1,2-Benzenedicarboxylic acid, dinonyl ester, 1-Hexadecanoyl-2-octadecadienoyl-sn-glycero-3-phosphocholine, Solstitialin A, -dioleoyl-sn-glycero-3-phosphatidylcholine, Salicylic acid ethyl ester, Hexamethylcyclotrisiloxane, Citrinin, Bis(4-methoxyphenyl)methanol, Nigerose and Vincamone (**Table-2**).





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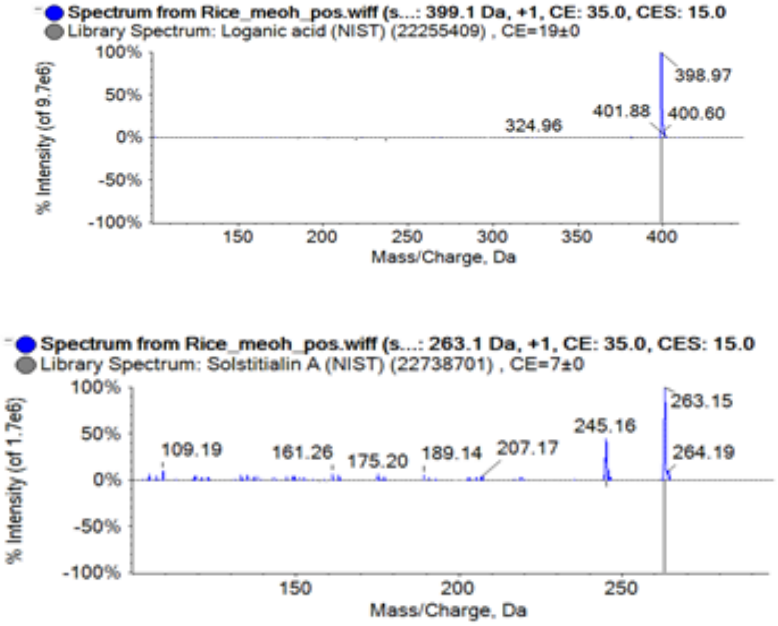


Fig 3: Phytoconstituents LC-MS spectra identified in Rice Methanolic extract

The methanolic extract of rice in negative polarity showed presence of 3,4'-Dimethoxy-5,7,3'-trihydroxyflavone, 3,5-Di-tert-butyl-4-hydroxybenzoic acid, 1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine, 1-Oleoyl-sn-glycero- 3-phosphoethanolamine, 2-Linoleoyl-1-palmitoyl-sn-glycero-3-phosphoethanolamine, 1-Palmitoyl-2-hydroxy-sn-glycero-3-phospho-(1'-rac-glycerol), Heneicosapentaenoic acid, 16-Hydroxyhexadecanoic acid, B-Pentasaccharide, (Z)-6-Octadecenoic acid, DL-.beta.-Hydroxypalmitic acid, N-Oleoylglycine, .gamma.-Linolenic acid, 9(10)-Epoxy-12Z-octadecenoic acid, cis-5,8,11-Eicosatrienoic acid, Jasmonic acid, Pinolenic acid, and Poliumoside. The LC-MS spectra of a few phytoconstituents, such as 3,4'-Dimethoxy-5,7,3'-trihydroxyflavone, Solstitialin A, Loganic acid, and Poliumoside are shown in **Figure 3**.

All the compounds, their retention times, polarities, precursor ions, and library scores matched with the NIST library are listed in **Table-2**.

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Table-2: Rice methanolic extract metabolites identified in using LC-MS/QTRAP

Tentative library annotation	InChIKey	Mode	RT (min)	Adduct / Charge	Pre Mass (Da)	Lib Score
Loganic acid	JNNGEAWILNVFFD-CDJYTOATSA-N	Pos	7.91	$[M+H]^-$	399.07	100
1,2-Benzenedicarboxylic acid, dinonyl ester	DROMNWUQASBTFM-UHFFFAOYSA-N	Pos	15.29	$[M+H]^-$	419.22	96.6
Solstitialin A	LWOZDCFSMDBOSA-AQEIEYEASA-N	Pos	13.04	$[M+H]^+$	263.05	94.4
Salicylic acid ethyl ester	GYCKQBWUSACYIF-UHFFFAOYSA-N	Pos	10.16	$[M+H]^+$	167.06	87.8
Citrinin	CQIUKKVOEOPUDV-IYSWYEEDSA-N	Pos	10.64	$[M+H]^+$	251.03	84.8
Nigerose	YGEHCIVVZVBCLE-NIKVEEOSSA-N	Pos	0.92	$[M+H]^+$	364.96	77.5
Vincamone	WYJAPUKIYAZSEM-MOPGFXCFSA-N	Pos	8.88	$[M+H]^+$	295.06	73.7
3,4'-Dimethoxy-5,7,3'-trihydroxyflavone	ZSPZNFOLWQEVQJ-UHFFFAOYSA-N	Neg	8.45	$[M-H]^-$	329.28	99.6
1-Hexadecanoyl-2-octadecadienoyl-sn-glycero-3-phosphocholine	ONCLVQFEAFTXMN-DHUJRADRSA-N	Pos	14.42	$[M+H]^+$	758.52	95.8
1-Oleoyl-sn-glycero-3-phosphocholine	YAMUFBLWGFFICM-PTGWMXDISA-N	Pos	11.01	$[M+H]^+$	522.36	97.4
1-Palmitoyl-sn-glycero-3-	ASWBNKHCZGQVJV-HSZRJFAPSA-N	Pos	10.76	$[M+H]^+$	496.28	96.8

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phosphocholine						
1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	YVYMBNSKXOXSKW-HXUWFJFHSA-N	Neg	10.7	$[M-H]^-$	452.31	99
1-Oleoyl-sn-glycero-3-phosphoethanolamine	PYVRVRFVLRNJLY-MZMPXXGTSA-N	Neg	10.95	$[M-H]^-$	478.39	98.8
2-Linoleoyl-1-palmitoyl-sn-glycero-3-phosphoethanolamine	HBZNVZIRJWODIB-XZBBILGWSA-N	Neg	12.1	$[M-H]^-$	714.57	98.4
1-Palmitoyl-2-hydroxy-sn-glycero-3-phospho-(1'-rac-glycerol)	FJEBZINYFPJJAW-IYZPEQFLSA-M	Neg	11.63	$[M-H]^-$	483.33	95.3
16-Hydroxyhexadecanoic acid	UGAGPNKCDRTDHP-UHFFFAOYSA-N	Neg	12.39	$[M-H]^-$	271.35	92.4
(Z)-6-Octadecenoic acid	CNVZJPUDSLNTQU-SEYXRHQNSA-N	Neg	13.75	$[M-H]^-$	281.38	90.5
DL-.beta.-Hydroxypalmitic acid	CBWALJHXHCJYTE-UHFFFAOYSA-N	Neg	10.71	$[M-H]^-$	271.33	81.8
N-Oleoylglycine	HPFXACZRFJDURI-KTKRTIGZSA-N	Neg	6.36	$[M-H]^-$	338.13	81.5
9(10)-Epoxy-12Z-octadecenoic acid	FBUKMFOXMZRGRB-XKJZPFPASA-N	Neg	10.93	$[M-H]^-$	295.33	81.2
cis-5,8,11-	CIYBEUWQVCQGPW-	Neg	11.41	$[M-H]^-$	305.28	80.5

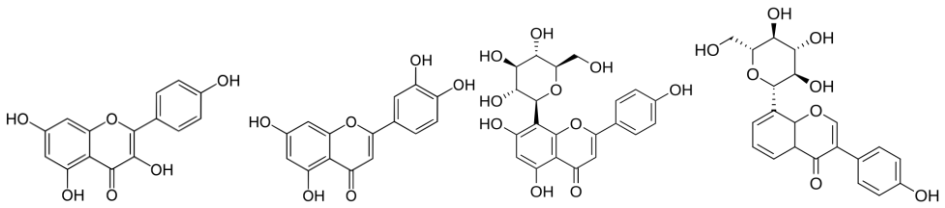
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Eicosatrienoic acid	LJURNQLPSA-N					
3-Hydroxydodecanoic acid	MUCMKTPAZLSKTL-UHFFFAOYSA-N	Neg	8.21	$[M-H]^-$	215.28	78.2
Jasmonic acid	ZNJFBWYDHIGLCU-HWKXXFMVSA-N	Neg	7.23	$[M-H]^-$	209.21	77.2
Pinolenic acid	HXQHFNIKBKZGRP-URPRIDOGSA-N	Neg	12.43	$[M-H]^-$	277.35	75.1
Salicin	NGFMICBWJRZIBI-UJPOAAIJSA-N	Neg	10.89	$[M-H]^-$	571.32	72.2
Poliumoside	YMWRMAOPKNYHMZ-LLVTZOIGSA-N	Neg	9.33	$[M-H]^-$	769.45	71.8
Asp-Glu	CKAJHWFHHFSCDT-WHFBIAKZSA-N	Neg	7	$[M-H]^-$	261.31	86.7
Ser-Cys	FFOKMZOAVHEWET-IMJSIDKUSA-N	Neg	9.53	$[M-H]^-$	207.24	99.2

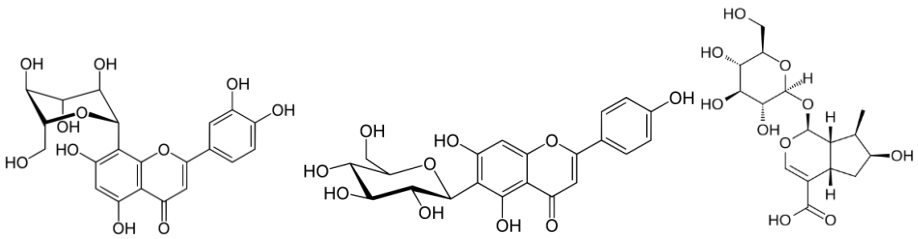
3.3 Metabolites identified in Black Gram Pulses Water Extract

The water extract of black gram showed the presence of 3,5-Di-tert-butyl-4-hydroxybenzoic acid, cis-7,10,13,16-Docosatetraenoic acid, Kaempferol, Ser-Cys, Vitexin, DELTA.2-cis-Eicosenoic acid, Lactitol, Citric acid, 16-Hydroxyhexadecanoic acid, Gly-Pro-Ala, 4-undecyl- , Heptadecanoic acid, Robinin, 9S-Hydroperoxy-10E,12Z-octadecadienoic acid, Dodeca-2(E),4(E)-dienoic acid, 5,6-Dehydroarachidonic acid, 3-Hydroxy-4'-methoxy-6-methylflavone, (Z)-6-Octadecenoic acid, 9S,11R-Dihydroxy-15-oxoprostanoic acid, 10,12-Tricosadiynoic acid, Luteolin, DL-.beta.-Hydroxypalmitic acid, cis-5,8,11-Eicosatrienoic acid, Puerarin, cis-4,7,10,13,16,19-Docosahexaenoic acid, 19,20-Dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid, Pinolenic acid, and 13-cis-Retinoic acid, in negative polarity.

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Kaempferol **Luteolin** **Vitexin** **Puerarin**



Orientin **Isovitexin** **Loganic acid**

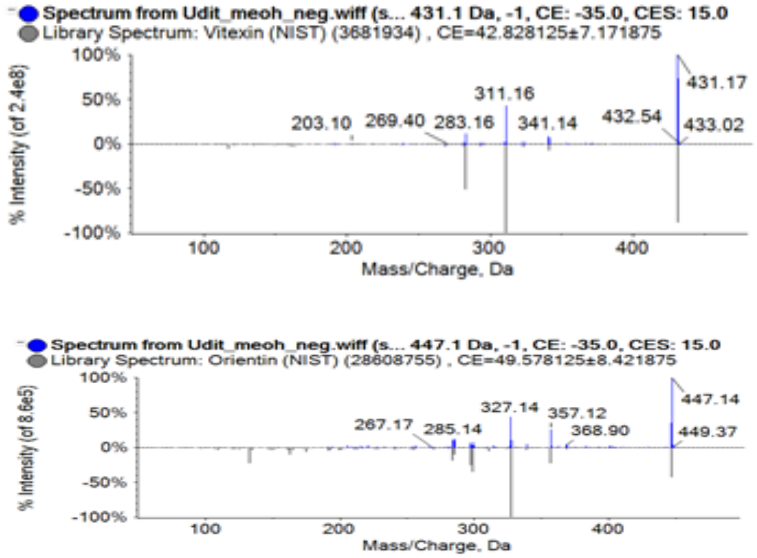


Fig 4: Structure of few phytomarkers and LC-MS spectra of Vitexin and Orientin identified in the methanolic extract of Black gram Pulse in negative polarity.

The water extract of Black Gram pulse in positive polarity showed the presence of Blood Group H Disaccharide, Geniposidic acid, Loganic acid, Isovitexin, Isoorientin, Vitexin-2"-O-rhamnoside, 3-Deoxy-D-glycero-D-galacto-2-nonulosonic acid, Salicylic acid ethyl ester, Vitexin, isopropyl ester, and Protoporphyrin IX.

.

Table-3: Black gram water extract metabolites identified using LC-MS/QTRAP

Tentative library annotation	InChIKey	Mode	RT (min)	Adduct / Charge	Pre Mass (Da)	Lib Score
Disaccharide	DLRVVLDZNNYCBX-ONPLHCRPSA-N	Pos	15.03	$[M+H]^+$	675.3	100
Geniposidic acid	ZJDOESGVOWAULF-OGJQONSISA-N	Pos	14.76	$[M+H]^+$	397.25	100
Loganic acid	JNNGEAWILNVFFD-CDJYTOATSA-N	Pos	7.91	$[M+H]^+$	399.12	100
Isovitexin	MYXNWGACZJSMBT-VJXVFPJBSA-N	Pos	6.71	$[M+H]^+$	432.96	99.3
Luteolin	IQPNAANSBPBGFQ-UHFFFAOYSA-N	Neg	6.97	$[M-H]^-$	285.22	82.7
Vitexin	SGEWCQFRYRRZDC-VPRICQMDSA-N	Pos	6.35	$[M+H]^-$	433.06	88.4
Isoorientin	ODBRNZZJSYPIDI-VJXVFPJBSA-N	Pos	6.49	$[M+H]^+$	448.97	97.9
Vitexin-2"-O-rhamnoside	LYGPBZVKGHHTIE-HUBYJIGHSA-N	Pos	6.69	$[M+H]^+$	579.11	95.9
3-Deoxy-D-glycero-D-galacto-2-nonulosonic acid	FQHUAUMYHAJTDH-GRCPKETISA-N	Pos	12.95	$[M+H]^+$	313.16	92.5

.

Salicylic acid ethyl ester	GYCKQBWUSACYIF-UHFFFAOYSA-N	Pos	10.15	$[M+H]^+$	167.06	89.5
Protoporphyrin IX	KSFOVUSSGSKXFI-VJCKZMALSA-N	Pos	15.02	$[M+H]^+$	563.28	79.6
3,5-Di-tert-butyl-4-hydroxybenzoic acid	YEXOWHQZWLCHHD-UHFFFAOYSA-N	Neg	10.09	$[M-H]^-$	249.34	100
Kaempferol	IYRMWMYZSQPJKC-UHFFFAOYSA-N	Neg	8.39	$[M-H]^-$	285.16	100
Vitexin	SGEWCQFRYRRZDC-VPRICQMDSA-N	Neg	6.75	$[M-H]^-$	431.22	100
Puerarin	HKEAFJYKMMKDOR-VPRICQMDSA-N	Neg	7.07	$[M-H]^-$	415.16	77.4
Robinin	PEFASEPMJYRQBW-HKWQTAEVSA-N	Neg	6.5	$[M-H]^-$	739.25	94.6
Lactitol	VQHSOMBJVWLPSR-JVCRWLNRSA-N	Neg	7.46	$[M-H]^-$	343.30	98.5
Citric acid	KRKNYBCHXYNGOX-UHFFFAOYSA-N	Neg	1.54	$[M-H]^-$	191.13	97.5
cis-7,10,13,16-Docosatetraenoic acid	TWSWSIQAPQLDBP-DOFZRALJSA-N	Neg	8.25	$[M-H]^-$	331.17	100
.DELTA.2-cis-Eicosenoic acid	FIKTURVKRGQNQD-	Neg	9.25	$[M-H]^-$	309.33	99.1

.

	HNENSFHCSA-N					
16-Hydroxyhexadecanoic acid	UGAGPNKCDRTDHP-UHFFFAOYSA-N	Neg	12.4	$[M-H]^-$	271.36	96.7
Heptadecanoic acid	KEMQGTRYUADPNZ-UHFFFAOYSA-N	Neg	10.86	$[M-H]^-$	269.33	94.9
9S-Hydroperoxy-10E,12Z-octadecadienoic acid	JGUNZIWGNMQSBM-UINYOVNOSA-N	Neg	12.82	$[M+Na-2H]^-$	293.33	94
Dodeca-2(E),4(E)-dienoic acid	BBRMJCAPNGJKEM-AQASXUMVSA-N	Neg	0.9	$[M-H]^-$	195.18	94
5,6-Dehydroarachidonic acid	GIOQWSLKUVKKAO-QNEBEIHSSA-N	Neg	6.94	$[M-H]^-$	301.12	93.8
3-Hydroxy-4'-methoxy-6-methylflavone	XBEINZKGCYZUQJ-UHFFFAOYSA-N	Neg	6.42	$[M-H]^-$	281.26	86.4
(Z)-6-Octadecenoic acid	CNVZJPUDSLNTQU-SEYXRHQNSA-N	Neg	5.61	$[M-H]^-$	281.24	86.2
9S,11R-Dihydroxy-15-oxoprostanoic acid	FVPKMMQYALWZHV-AKHDSKFASA-N	Neg	9.35	$[M-H]^-$	355.20	84.6
10,12-Tricosadiynoic acid	DIEDVCMBPCRJFQ-UHFFFAOYSA-N	Neg	7.57	$[M-H]^-$	345.24	84.3
DL-.beta.-Hydroxypalmitic acid	CBWALJHXHCJYTE-UHFFFAOYSA-N	Neg	7.06	$[M-H]^-$	271.33	81.8
cis-5,8,11-	UNSRRHDPHVZA	Neg	11.46	$[M-H]^-$	305.28	79.9

.

Eicosatrienoic acid	HH-YOILPLPUSA-N					
cis-4,7,10,13,16,19-Docosahexaenoic acid	MBMBGCFOFBJSGT-KUBAVDMBSA-N	Neg	8.21	$[M-H]^-$	327.29	76.8
19,20-Dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid	FFXKPSNQCPNORO-MBYQGORINA-N	Neg	6.94	$[M-H]^-$	361.36	75.2
Pinolenic acid	HXQHFNIKBKZGRP-URPRIDOGSA-N	Neg	10.65	$[M-H]^-$	277.26	75.1
13-cis-Retinoic acid	SHGAZHPCJJPHSC-XFYACQKRSA-N	Neg	3.49	$[M-H]^-$	299.16	74.6
Ser-Cys	FFOKMZOAVHEWET-IMJSIDKUSA-N	Neg	9.55	$[M-H]^-$	207.24	100
Gly-Pro-Ala	GGLIDLCEPDHEJO-BQBZGAKWSA-N	Neg	8.36	$[M-H]^-$	242.31	96.1

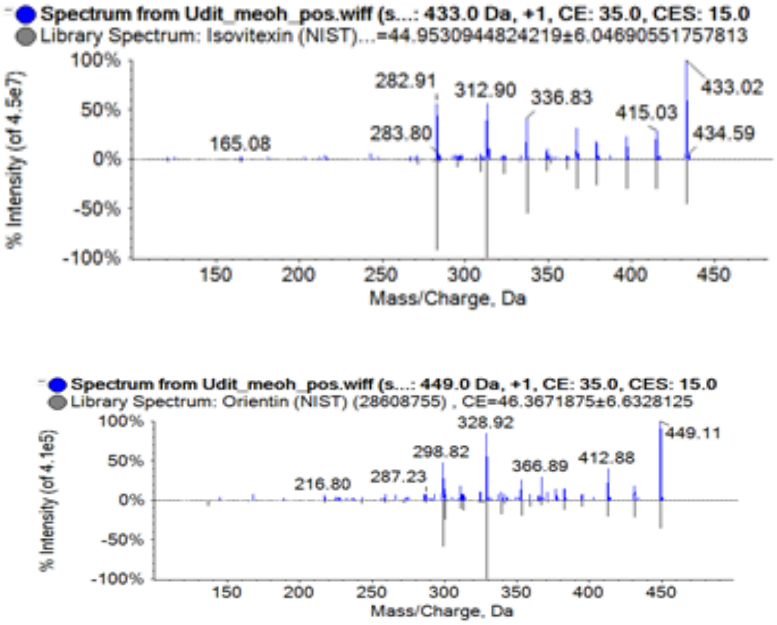
3.4 Metabolites identified in Black gram -Methanolic Extract

The methanolic extract of black gram pulse in negative polarity showed the presence of (Z)-6-Octadecenoic acid, .beta.-Carboline-1-propionic acid, DELTA.2-cis-Eicosenoic acid, 1,4-D-Xylobiose, 11-(Dansylamino)undecanoic acid,12(13)-Epoxy-9Z-octadecenoic acid, 16-Hydroxyhexadecanoic acid,1-Oleoyl-2-hydroxy-sn-glycero-3-phospho-(1'-rac-glycerol), 2-Linoleoyl-1-palmitoyl-sn-glycero-3-phosphoethanolamine, 3-Hydroxyoctadecanoic acid, , 7-Hydroxy-4-methylcoumarin-3-acetic acid, Asp-Ala, cis-4,7,10,13,16,19-Docosahexaenoic acid, cis-5,8,11-Eicosatrienoic acid, Citric acid, Gly-Pro-Ala, Heptadecanoic acid, Hyperoside, Isosteviol, Jasmonic acid, Orientin, Pygenic acid C, Robinin, Salicin, and

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Vitexin. **Fig 4** displayed the LC-MS spectra of Vitexin and Orientin detected in the methanolic extract of Black gram pulse.

The methanolic extract of black gram in positive polarity showed presence of (6aR)-5S-Hydroxy-4S-((R,E)-3aS-hydroxyoct-1-en-1-yl)hexahydro-2H-cyclopentabfuran-2-one, Arachidonoylthiophosphorylcholine,, 1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine, 3- Pinoresinol diglucoside, Isovitexin, Orientin, 1-Palmitoyl-sn-glycero-3-phosphocholine, 1-Hexadecanoyl-2-octadecadienoyl-sn-glycero-3-phosphocholine, Salicylic acid ethyl ester, Loganic acid, 1,2-Benzenedicarboxylic acid, dinonyl ester, Hexamethylcyclotrisiloxane, 3-Hydroxyphenylacetic acid, Resveratrol 3-O-D-glucuronide, (+)-Pinoresinol, Nigerose, and Loganin.



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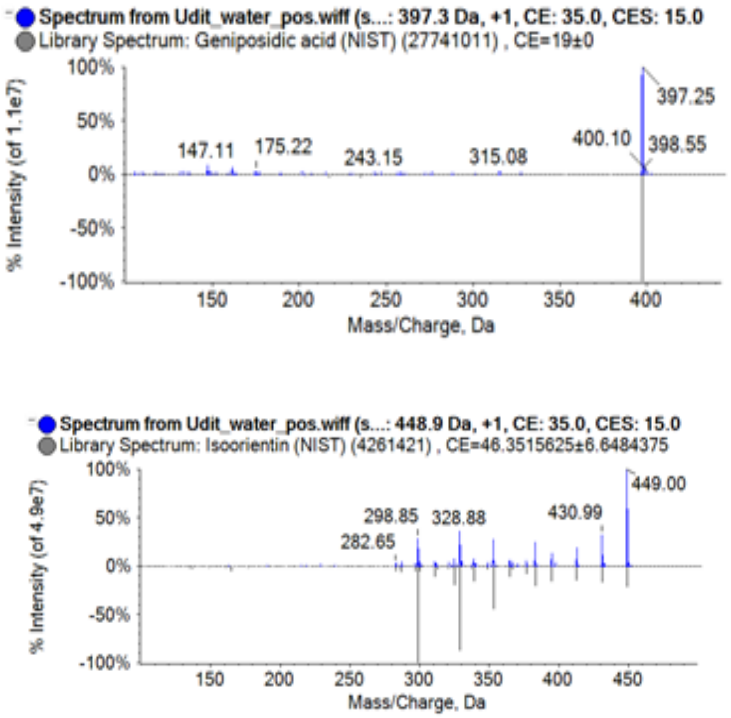


Fig 5: LC-MS spectra of Isovitexin geniposidic acid, Orientin, and Isoorientin identified in the methanolic extract of Black gram Pulse.

All the phytoconstituents identified in black gram pulse in water and methanolic extracts are mentioned in **Table-3** and **Table-4,** respectively.

Table-4: Black gram methanolic extract metabolites identified using LC-MS/QTRAP

Tentative library annotation	InChIKey	Mode	RT (min)	Adduct / Charge	Pre Mass (Da)	Lib Score
Pinoresinol diglucoside	ZJSJQWDXAYNLNS-FUPWJLLWSA-N	Pos	0.93	$[M+H]^+$	705.23	97.8
Isovitexin	MYXNWGACZJSMBT-VJXVFPJBSA-N	Pos	6.73	$[M+H]^+$	432.96	97.7
Orientin	PLAPMLGJVGLZOV-VPRICQMDSA-N	Pos	6.49	$[M+H]^+$	448.97	97.6

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1-Palmitoyl-sn-glycero-3-phosphocholine	ASWBNKHCZGQVJV-HSZRJFAPSA-N	Pos	10.76	$[M+H]^+$	496.26	96.7
Arachidonoylthiophosphorylcholine	NA	Pos	15.24	$[M+H]^+$	784.59	99.9
1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine	IHNKQIMGVNPMTC-RUZDIDTESA-N	Pos	11.69	$[M+H]^+$	524.39	99
Salicylic acid ethyl ester	GYCKQBWUSACYIF-UHFFFAOYSA-N	Pos	10.15	$[M+H]^+$	167.06	92.5
Loganic acid	JNNGEAWILNVFFD-CDJYTOATSA-N	Pos	7.91	$[M+H]^+$	399.07	90.1
3-Hydroxyphenylacetic acid	XQXPVVBIMDBYFF-UHFFFAOYSA-N	Pos	0.43	$[M+H]^+$	153.09	83
Resveratrol 3-O-D-glucuronide	QWSAYEBSTMCFKY-XQIXDYBNSA-N	Pos	6.13	$[M+H]^+$	427.03	79.7
(+)-Pinoresinol	HGXBRUKMWQGOIE-AFHBHXEDSA-N	Pos	14.02	$[M+H]^+$	341.16	77.3
Nigerose	YGEHCIVVZVBCLE-NIKVEEOSSA-N	Pos	0.98	$[M+H]^+$	364.96	76.5
Loganin	AMBQHHVBBHTQBF-UOUCRYGSSA-N	Pos	11.4	$[M+H]^+$	413.12	73.2
.beta.-Carboline-1-propionic acid	CNUHEVWYPKFJHH-UHFFFAOYSA-N	Neg	6.52	$[M-H]^-$	239.23	70.3
.DELTA.2-cis-Eicosenoic acid	FIKTURVKRGQNQD-HNENSFHCSA-N	Neg	9.64	$[M-H]^-$	309.31	86
1,4-D-Xylobiose	LGQKSQQRKHFMLI-WSNPFVOISA-N	Neg	13.7	$[M-H]^-$	281.38	79.9
1-Oleoyl-2-hydroxy-sn-glycero-3-phospho-	FQQQKGAFQIIGLQ-VYFUBJQISA-N	Neg	12	$[M-H]^-$	509.39	89.3

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(1'-rac-glycerol)						
2-Linoleoyl-1-palmitoyl-sn-glycero-3-phosphoethanolamine	HBZNVZIRJWODIB-XZBBILGWSA-N	Neg	12.18	$[M-H]^-$	714.57	95.5
3-Hydroxyoctadecanoic acid	POMQYTSPMKEQNB-UHFFFAOYSA-N	Neg	3.68	$[M-H]^-$	299.16	72.4
7-Hydroxy-4-methylcoumarin-3-acetic acid	OOGKDHCQSZAEQI-UHFFFAOYSA-N	Neg	3.76	$[M-H]^-$	233.25	85
cis-4,7,10,13,16,19-Docosahexaenoic acid	MBMBGCFOFBJSGT-KUBAVDMBSA-N	Neg	8.22	$[M-H]^-$	327.29	82.1
cis-5,8,11-Eicosatrienoic acid	UNSRRHDPHVZAHH-YOILPLPUSA-N	Neg	11.45	$[M-H]^-$	305.28	79.5
11-(Dansylamino)undecanoic acid	CEPGVMDMVJGHFQ-UHFFFAOYSA-N	Neg	11.4	$[M-H]^-$	433.32	77.8
.DELTA.2-cis-Eicosenoic acid	FIKTURVKRGQNQD-HNENSFHCSA-N	Neg	9.64	$[M-H]^-$	309.31	86
(Z)-6-Octadecenoic acid	CNVZJPUDSLNTQU-SEYXRHQNSA-N	Neg	5.58	$[M-H]^-$	281.24	83.8
12(13)-Epoxy-9Z-octadecenoic acid	CCPPLLJZDQAOHD-FLIBITNWSA-N	Neg	10.9	$[M-H]^-$	295.33	79.8
16-Hydroxyhexadecanoic acid	UGAGPNKCDRTDHP-UHFFFAOYSA-N	Neg	12.4	$[M-H]^-$	271.35	95.5
Citric acid	KRKNYBCHXYNGOX-UHFFFAOYSA-N	Neg	1.55	$[M-H]^-$	191.13	98
Heptadecanoic acid	KEMQGTRYUADPNZ-UHFFFAOYSA-N	Neg	8.3	$[M-H]^-$	269.17	100

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Hyperoside	OVSQVDMCBVZWGM-DTGCRPNFSA-N	Neg	6.77	$[M-H]^-$	463.14	100
Isosteviol	KFVUFODCZDRVSS-XGBBNYNSSA-N	Neg	9.7	$[M-H]^-$	317.17	97.9
Jasmonic acid	InChI Key ZNJFBWYDHIGLCU-HWKXXFMVSA-N	Neg	7.21	$[M-H]^-$	209.21	77.2
Orientin	PLAPMLGJVGLZOV-VPRICQMDSA-N	Neg	6.47	$[M-H_2O-H]^-$	447.17	93
Pygenic acid C	NFPZOORPDJBGME-LCRJMXSQSA-N	Neg	1.08	$[M-H]^-$	503.25	84.1
Robinin	PEFASEPMJYRQBW-HKWQTAEVSA-N	Neg	6.47	$[M-H]^-$	739.24	93.8
Salicin	NGFMICBWJRZIBI-UJPOAAIJSA-N	Neg	10.84	$[M-H]^-$	571.38	83.3
Vitexin	SGEWCQFRYRRZDC-VPRICQMDSA-N	Neg	6.72	$[M-H]^-$	431.22	100
Asp-Ala	DVUFTQLHHHJEMK-UHFFFAOYSA-N	Neg	5.45	$[M-H]^-$	203.26	95.7
Gly-Pro-Ala	GGLIDLCEPDHEJO-BQBZGAKWSA-N	Neg	8.38	$[M-H]^-$	242.31	93.9

3.5 Contaminants Detected in Rice and Black Gram Daal

Contamination of drugs such as Glibornuride, Saluamine, Carbocyclic thromboxane A2, Leukotriene B4 ethanolamide, 17-Trifluoromethylphenyl-13,14-dihydrotrinorprostaglandin F2.alpha., 3-Indoleacrylic acid, 17-Phenoxyprostaglandin F2.alpha. Minaprine, lactame, Olanzapin, Clofibric acid acyl-.beta.-D-glucuronide were detected during the analysis. All the contaminants detected in LC-MS analysis of Rice grains and black gram pulse are mentioned in **Table-5**.

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Table-5: Contaminants detected in rice and black gram pulses

Contaminant Detected in Rice grains						
Contaminants	**InChIKey**	**Mode**	**RT (min)**	**Adduct / Charge**	**Pre Mass (Da)**	**Lib Score**
Leukotriene B4 ethanolamide	DQLVVNIIINUTUIU-XLFGVTECSA-N	Pos	6.43	$[M+H]^+$	362.06	100
17-Trifluoromethylphenyl-13,14-dihydrotrinorprostaglandin F2.alpha.	NA	Pos	14.87	$[M+H]^+$	441.24	97.7
3-Indoleacrylic acid	PLVPPLCLBIEYEA-AATRIKPKSA-N	Pos	5.53	$[M+H]^+$	188.10	97.6
Glibornuride	RMTYNAPTNBJHQI-LLDVTBCESA-N	Pos	7.08	$[M+H]^+$	366.88	97.2
Minaprine, lactame	SGOJRGZBIVIGIS-UHFFFAOYSA-N	Pos	12.93	$[M+H]^+$	313.16	91.2
Salicylic acid ethyl ester	GYCKQBWUSACYIF-UHFFFAOYSA-N	Pos	10.17	$[M+H]^+$	167.06	89.9
Olanzapine	KVWDHTXUZHCGIO-UHFFFAOYSA-N	Pos	12.93	$[M+H]^+$	313.2	87.6
Clofibric acid acyl-.beta.-D-glucuronide	LCJVUUVMVSSIGZ-HNRZYHPDSA-N	Pos	11.33	$[M+H]^+$	413.17	71.3
Carbocyclic thromboxane A2	ZIWNJZLXPXFNGN-GXTQQWMXSA-N	Nege	8.06	$[M-H]^-$	347.28	79.9
Hexamethylcyclotris	HTDJPCNNEPUOO	Pos	0.1	$[M+H]^+$	223.02	86.2

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iloxane	Q-UHFFFAOYSA-N					
1,3-Dicyclohexylurea	ADFXKUOMJKEIND-UHFFFAOYSA-N	Pos	9.26	$[M+H]^+$	225.12	79.3
2,3,5-Trichlorobenzoic acid	CGFDSIZRJWMQPP-UHFFFAOYSA-N	Neg	8.99	$[M-H]^-$	223.22	91
2-Bromo-4-sec-butylphenol	ZAINGYFRUOVIGY-UHFFFAOYSA-N	Neg	12.56	$[M-H]^-$	227.33	73.8
Contaminants Detected in Black Gram Daal						
Contaminants	**InChIKey**	**Mode**	**RT (min)**	**Adduct / Charge**	**Pre Mass (Da)**	**Lib Score**
Leukotriene B4 ethanolamide	DQLVVNIINUTUIU-XLFGVTECSA-N	Pos	6.39	$[M+H]^+$	362.06	100
Perindopril	IPVQLZZIHOAWMC-QXKUPLGCSA-N	Pos	7.58	$[M+H]^+$	369.05	98.4
15(R),19(R)-Hydroxyprostaglandin E2	WTJYDBMHYPQFNJ-JTBTZFADSA-N	Pos	8.2	$[M+H]^+$	351.04	93.3
Hexamethylcyclotrisiloxane	HTDJPCNNEPUOOQ-UHFFFAOYSA-N	Pos	1.23	$[M+H]^+$	222.98	89.9
Clofibric acid acyl-.beta.-D-glucuronide	LCJVUUVMVSSIGZ-HNRZYHPDSA-N	Pos	11.4	$[M+H]^+$	413.17	86.9
Bisphenol A bis(2,3-dihydroxypropyl) ether	NISVZEWKUNUGQQ-UHFFFAOYSA-N	Neg	7.91	$[M+H]^+$	394.17	91.6
5-O-Desmethylomeprazole	TWXDTVZNDQKCOS-UHFFFAOYSA-N	Neg	7.68	$[M-H]^-$	312.23	94.5
Carbocyclic thromboxane A2	ZIWNJZLXPXFNGN-GXTQQWMXSA-N	Neg	8.08	$[M-H]^-$	347.28	82.3

.

2-Bromo-4-sec-butylphenol	ZAINGYFRUOVIGY-UHFFFAOYSA-N	Neg	12.57	$[M-H]^-$	227.33	73.7
Picloram	NQQVFXUMIDALNH-UHFFFAOYSA-N	Neg	6.56	$[M-H]^-$	239.23	80.7
Fenoprofen	RDJGLLICXDHJDY-UHFFFAOYSA-N	Neg	5.64	$[M-H]^-$	241.22	77.2
Desmethylverapamil	CAAKRPRWCVWYAO-UHFFFAOYSA-N	Neg	14.86	$[M+H]^+$	441.24	99.2
12-Ketodeoxycholic acid	CVNYHSDFZXHMMJ-VPUMZWJWSA-N	Neg	6.32	$[M-H]^-$	389.28	82.4
5-O-Desmethylomeprazole	TWXDTVZNDQKCOS-UHFFFAOYSA-N	Neg	7.69	$[M-H]^-$	312.23	89.8
Bisphenol C dichloride	PDRPQJYAPFFFAR-UHFFFAOYSA-N	Neg	13.01	$[M-H]^-$	279.37	90.7

LC-MS/MS analysis of rice (*Oryza sativa*) and black gram (*Vigna mungo*) revealed the tentative presence of several pharmaceutical-related compounds, including glibornuride, olanzapine, minaprine, saluamine, clofibric acid acyl-β-D-glucuronide, and synthetic prostaglandin derivatives. The potential occurrence of these substances in food grains raises concerns regarding unintended dietary exposure and associated health effects. Glibornuride, an antidiabetic sulfonylurea, may induce hypoglycemia, whereas olanzapine is associated with metabolic disturbances, including weight gain, hyperglycemia, and dyslipidemia[18,19]. Minaprine, an antidepressant, may produce neurological adverse effects[20]. Prostaglandin- and leukotriene-related compounds can modulate inflammatory and vascular responses, although their toxicity following dietary exposure remains uncertain[21]. The presence of pharmaceutical residues in food matrices may indicate possible environmental contamination through wastewater, irrigation, or agricultural practices[22]. However, these identifications require confirmation using authentic standards and quantitative analysis

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before establishing contamination, exposure levels, or potential health risks. Further investigation is necessary to assess their food safety implications

Conclusion

The LC-MS analysis of Black Gram pulse and Rice were performed on water and methanol. LC-MS screening indicated a solvent- and matrix-dependent metabolite pattern in black gram and Rice. In black gram, the negative-ion chromatographic output showed the presence of vitexin and isovitexin metabolites in the aqueous extract and methanolic extract as well. Hyperoside and additional flavonoid-like candidates, such as robinin and luteolin, were more evident in the methanolic extract, suggesting that both water and methanol recovered polar phenolic glycosides but with different relative responses. Rice showed fewer clearly interpretable phytochemicals in the aqueous extract, whereas the methanolic extract yielded a methoxylated-flavone candidate together with loganic-acid-related signals, indicating improved recovery of moderately polar secondary metabolites. Nevertheless, these assignments remain tentative because further analysis could be conducted using LC-MS TOF equipment to obtain a more detailed analysis of the food matrix. The results, therefore, support the healthy use of black gram pulse having vitexin, isovitexin, hyperoside, and enriched fatty acids as bioactive candidates and the methoxylated flavone and loganic acid as candidates for rice, but confirmation by diagnostic MS/MS fragmentation, authentic standards, replicate analysis, and feature-level blank subtraction is required before the compounds can be regarded as validated phytomarkers.

Authors' Contributions:

Ms. Sakshi Mahajan performed the sample extraction and analysis and drafted the manuscript. Dr. Pratibha Srivastava contributed to the conceptualization of the study, interpretation of the data, and technical writing of the manuscript.

Conflict of Interest

There is no conflict of interest between the authors.

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