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Quantitative analysis of bio-active phytochemical (s) in selected scented rice varieties (*Oryza sativa*) reveals its intake towards advantage against metabolic disorders

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NER, North-Eastern Region; HPLC, High Performance Liquid Chromatography; NER, North- East Region; RARS, Regional Agricultural Research Station; CE, Collision Energy; LCMS, Liquid Chromatography Mass Spectrometry; HRMS, High resolution mass spectrometry; p-NPG, 4-Nitrophenyl β-D-glucopyranoside; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TGA, Thermo Gravimetric Analysis; Based on ancestral knowledge towards health beneficial effect, the current study led to investigate the content of bioactive phytochemical (s) in the scented rice (variety "joha") grain indigenous to the NER-India and their effects on the enzyme involved in metabolic disorders and cellular glucose uptake efficacy. Results of both α-glucosidase inhibitory activity and the glucose uptake assay-guided fractionation based on column purification followed by spectral data analysis confirmed the presence of two major bioactive compounds as linoleic (ω-6-fatty-acid) and linolenic (ω-3-fatty-acid) acid. Quantitative analysis showed that linolenic acid content in scented rice verities was 2.1±0.3 mg/g, which is ~1.6 fold higher than its content in non-scented rice variety from the same region. The relative ratio (ω-6/ω-3) of these two essential unsaturated fatty acids content in the scented rice variety seeds was calculated as 23.5±0.6 while, in non-scented rice variety seeds, it was 31.6. Chemical fingerprinting by GCMS and HPLC of methanolic extract of selected rice samples revealed the presence of various fatty-acid along with other volatile and polar compounds. In conclusion, the scented rice seeds content a better ratio of two essential unsaturated fatty acid compare to non-scented rice seed, which may be an effective choice as diet towards controlling metabolic disorder.

Keywords: Diabetics, Essential fatty-acid, Joha, Phytochemicals, Scented rice

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Rice has been a staple food mainly as a rich source of carbohydrate for a major human population across different countries of tropical and subtropical region of the world[1,2]. The mouth feel aroma, taste and physical appearance are the attributes associated with preference for consumption of rice as the whole kernel by the majority of human population[1-3] Some other attributes associated with few special varieties among the 40000 germ plasm of *Oryza sativa* have made them delicacy food items among elites and wealthy within and outside rice producing regions[3]. For example, aromatic rice varieties with rich fragrance and taste are due to the presence of 2-acetyl-1-pyrroline (2AP) along with several aldehydes, alcohol and fetch high price in national and international market[3-6]. These varieties are also tested *in vitro* and reported to possess several nutraceutical benefits[4,7]. In general rice germplasms contain different phytochemicals including phenol, polyphenol and flavonoid which exhibit potent in vitro antioxidant, antihyperlipidemic and anticancer activities[8-10]. Flavonoids such as luteolin, apigenin, quercetin, isorhamnetin, kaempferol, myricetin, tricin, etc. are also reported in rice grain with pronounced antioxidant activity, free radical scavenging ability, coronary heart syndrome prevention capability along with anticancer potential[8-9,11-12] either *in vitro* or *in vivo* system. In view of these functional health related attributes, the large germplasm within *Oryza sativa* has been actively inspected globally for their medicinal values in various disease models[7-9,11,13]. North eastern region (NER) of India is also home to several endemic germplasm of scented rice[3-4]. Unlike the 'Basmati' rice which has slender size grain and rich aroma and worldwide market value, the scented

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rice varieties of NER have remained confined as a delicacy item only within the region[4,7]. Four scented rice varieties locally known as "joha" and grown in Assam and the indigenous community of this region believes that this rice has potential beneficial effects on health. Joha rice is mostly cooked in festivity by common people and the elite class of society, consumes them regularly. Due to high market price, common people cannot afford to eat regularly. Scientific studies on medicinal properties or nutraceutical benefit of this rice towards the human health have been scanty[3]. Therefore, it was imperative to evaluate the aromatic rice varieties of Assam for their potential health beneficial effects. The specific objectives of this study were to: (A) Determine the phytonutrient contents and (B) Carry out the bioactive guided fractionation based on α-glucosidase activity and glucose uptake assay for the presence of any active constituents exhibiting the health benefit.

Methodology

Rice samples collection

Seeds of four varieties of scented joha rice viz., Kola (KJ1), Kon (KJ2), Keteki (KK) and Maniki Madhuri (MM) and one non- scented variety of commonly cultivated in Assam, Ranjit (RR) were collected from the Regional Agricultural Research Station (RARS), Titabar, Assam, India. Seeds of the rice varieties were authenticated by RARS, Titabar, Assam, India. All the scented rice (joha) variety seeds have the "popcorn" like a smell, which was verified manually.

Samples preparation

Seeds were first de-husked manually (not milled) and grounded to make powder for extraction. Extraction was carried out through cold maceration process with methanol for 72 h [total moisture content was measured with the help of Thermo Gravimetric Analysis (TGA) Instrument (Perkin Elmer, TGA 4000). TGA analysis for the samples were done; as rice seeds were taken and inserted in the TGA with a temperature run from 35°C to 130°C and a final hold of 30 min[14] and shown in Supplementary Table S1. After 72 h, the extract was filtered through muslin cloth, Whatman filter paper, then concentrated using rotary evaporator and dried completely[15]. On the basis of availability and popularity, Keteki joha was further studied in details. Keteki joha MeOH extract was further fractionated in to EtOAc, BuOH and H_2O fraction to check the distribution of bioactivity. Standards of linolenic acid and linoleic acid were prepared in methanol and filtered through a 0.22 µm syringe filter for LCMS and HPLC operation. A serial dilution from 1 mg/mL (0, 15.63, 31.25, 62.5, 125, 250, 500, 1000 µg/mL) of linolenic acid and 2 mg/mL (0, 125, 250, 500, 1000, 2000 µg/mL) of linoleic acid was used to prepare the calibration curve for quantification of unsaturated fatty acids in rice samples.

Materials and Methods

Reagents

Cell culture media-DMEM, FBS and 0.25% Trypsin- EDTA solutions were purchased from Gibco. Antibiotic and pen-strep solutions were purchased from Himedia. Standards gallic acid and quercetin were purchased from Sigma Aldrich. Aluminium chloride, sodium nitrite, sodium hydroxide, sodium carbonate, silica gel 60-120 mesh, glacial acetic acid, formic acid, analytical grade solvents methanol, chloroform, butanol, petroleum ether, ethyl acetate, HPLC grade methanol were purchased from Merck. Cell culture flask, plate and plastic materials related to cell culture work were purchased from Eppendorf, India or Thermo Fisher Scientific. Besides these, the other reagents used in different experiments but not mentioned were purchased from Sigma Aldrich.

Cell culture

L6 rat myoblast cells were purchased from the National Centre for Cell Science (NCCS), Pune, India. The cells were maintained in 75 mm^2 cell culture flask containing DMEM complete media (supplemented with 10% FBS, D-Glucose, Sodium pyruvate and 1% Pen Strep solution) at 37°C in 5% CO_2[22].

DPPH activity assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay was performed to evaluate the antioxidant capacity of different extracts from different varieties of joha rice. Ascorbic acid was taken as standard (positive control) as it has free radical scavenging capacity[16,17]. The working concentration of DPPH was prepared as 0.2 mM in MeOH (1 mg/mL) from 10 mg/mL. Ascorbic acid stock was prepared as 100 µg/mL in MeOH. Samples (20 µL) were mixed with 180 µL of DPPH with different concentrations (100, 250, 500, 750, 1000 µg/mL) and incubated in dark for 1 hour. Finally the absorbance was measured at 517 nm[17,18].

GC-MS analysis

The GCMS analysis was carried out to understand the volatile component diversity in the rice samples[19,20]. GC–MS spectral data were recorded in a Shimadzu GCMS-TQ8030 instrument with AOC-20i auto-injector equipped with 30 m x 0.25 µm x 0.25 mm EB-5MS capillary column. The initial oven temperature was programmed at 80°C and was ramped to 300°C at a rate of 5°C/min, then held for 10 min. The injector temperature was set at 300°C with split less injection mode. The temperature of ion source and interface were 230°C and 310°C respectively. The mass spectrometer was operated in scan mode from m/z 45 to 800. Helium gas at a flow rate of 1 mL/min was used as GC carrier gas. A volume of 1 µL was injected for each scented and non-scented rice samples for the analysis.

Column chromatography

Column purification of the bioactive EtOAc fraction of Keteki joha was carried out with silica gel (#60-120 mesh size) packed column to separate the compounds on a large scale depending on their polarity or hydrophobicity[15]. Before column purification, the separation was first optimized using thin layer chromatography (TLC). The column was packed with silica gel, slurred with petroleum ether, poured to the column and the silica was allowed to settle down. When the column was packed to the desired height, the sample (EtOAc extract adsorbed with silica gel) was placed on top of the silica gel. The analysis was performed using different solvent system i.e., petroleum ether, chloroform and methanol with increasing order of polarity. Fractions were collected and again TLC-optimized to confirm the separation of compounds.

α-Glucosidase activity assay

Alpha-glucosidase breaks down starch and disaccharides to glucose. Acarbose is an inhibitor of α-glucosidase; it is semi-synthetic in origin and belongs to D-glucopyranose[21]. In this assay, first 50 µL phosphate buffer (100 mM, pH=6.8) was used and subsequently different concentrations of acarbose (20 µL) and 10 µL α-glucosidase (1 U/ mL) was added followed by incubation at 37°C for 15 min. After the incubation 20 µL of p-NPG (5 mM) was added in each test sample and incubated at 37°C for 20 min. The reaction was then stopped by adding 50 µL of Na_2CO_3. The absorbance was measured at 405 nm. The blank was prepared to contain 70 µL of buffer. Acarbose stock was prepared as 10 mg/ mL and from there sample volume was prepared (70 µL) by taking different concentration as (0, 20, 30 and 40 µg/mL). α-Glucosidase activity of the different fraction (EtOAc, BuOH and H_2O) Keteki joha (KK) were assayed. The experimental extract was added in stated concentration of 0, 2.5, 5 and 10 mg/mL.

Glucose uptake activity assay

Facilitative glucose uptake transport systems are ubiquitous in animal cells and are responsible for transporting glucose across cell surface membranes[22]. The insulin-stimulated activation of glucose transport mainly occurs by one of the two mechanisms: translocation of GLUT4 from intracellular vesicles to the plasma membrane and augmentation of the intrinsic catalytic activities of the transport[22]. L6 rat skeletal muscle cells were grown into myotubes by serum starvation over a period of 8 days (maintaining cells in DMEM with 2% FBS, 1% pen-strap in 5% CO_2 at 37°C) in a 96 well culture plate[22]. The cells were maintained in serum-free medium for 18 h before adding test reagent. The culture medium was then discarded and the cells were washed with glucose-free, serum-free medium. Then the test compounds ethyl acetate fraction of Keteki joha and two of its fractions F3-9 and F22-23 (500 ng/mL and 1000 ng/mL) were added. The 96 wells plate was then incubated in a 5% CO_2 incubator at 37°C for 8 h. As positive control 100 nM insulin was added for an incubation period of 30 min. The nonspecific uptake was determined by exposing the cells to 20 µM cytochalasin B for 3 h. At the end of incubation the cells were exposed to 2-NBDG (2-[N-(7-nitrobemz-2-oxa-1, 3-diazol-4-yl) amino]-2-deoxyglucose), a fluorescent indicator diluted to 80 µM with glucose-free, serum-free medium (100 µL per well and incubated in a 5%CO_2 incubator at 37°C for 30 min.). The cells were washed rapidly with 200 µL/well of ice-cold PBS to prevent 2-NBDG efflux. After the wash step, 70 µL of 0.1 M PBS, pH 10, containing 1% Triton X-100 was added per well and maintained for 10 min in the dark. Then 30 µL of DMSO was added and homogenized by pipetting in the well. The fluorescence was finally measured in a micro-plate reader at λ_{ex}=470 nm and λ_{em}= 40 nm[22].

HPLC analysis

Quantification of crude rice extracts and fractions were carried out with the instrument consisting of Waters 1525 binary HPLC pumps with Waters Breeze2 LC software and a Waters 2998 photodiode array detector. A Waters SPHERISORB 5 µM ODS2

(4.6 x 250 mm) reverse phase C18 analytical column was used for chromatographic analysis and the absorbance of the eluted compounds was monitored at 210 nm (as in this wave length the maximum absorbance were recorded for the eluted compounds) by PDA detector. Two HPLC grade solvents were used in this study (Supplementary Table S2). Solvent A was 0.1% formic acid in water, while solvent B was 100% methanol. For analysis of unsaturated fatty acids in standards and rice samples, a gradient elution program was used (Supplementary Table S2) The major compounds came at ~ 18.9 and ~ 22.5 min in HPLC of bioactive column purified fraction, were collected and concentrated for further identification.

LCMS analysis

The analysis was performed by using Thermo Scientific, Extractive plus Mass spectroscopy equipped with Dionex Ultimate 3000, UHPLC system and 0.1% formic acid in water and methanol solvent system. With collision energy 30 and 40, the high-resolution mass spectra (HRMS) of two essential fatty acids and collected samples at ~ 18.9 and ~ 22.5 min from HPLC of bioactive column fraction were recorded for identification of the compounds.

Results

GCMS finger printing analysis

The GCMS spectrum of MeOH extract of all experimental rice variety seeds is presented in Figure 1A. The major detectable peaks were identified as mainly well-known fatty acids but varying in quantity. Due to presence in very small amount (may be), the other reported polyphenols or flavonoids are not detected or quantified by GCMS at least in experimental concentration. The equipped library (NIST11.lib) suggested the presence of different fatty acids content but in varying amount (Table 1). Based on NIST11.lib library information, occurrence of different secondary metabolites in different rice variety seed is shown in Table 1.

Antioxidant activity determination

In vitro antioxidant capacity of the standard compound ascorbic acid increased linearly with the increase in concentration. The IC_{50} value of ascorbic acid was measured as 8.6±0.1 µg/mL, and IC_{50} values of all the rice varieties seeds were higher than this value. Among the different varieties, maximum antioxidant activity was recorded in the MeOH extract of Maniki Madhuri and the calculated IC_{50} value was recorded as 650.8±23.4 µg/mL (Fig. 2A). Overall, the scented rice variety seeds showed greater antioxidant activity than the non-scented rice variety seeds (with calculated IC_{50} value as 985.5±175.6 µg/mL (Fig. 2A). MeOH, MeOH+H_2O and H_2O extracts of all the samples were also checked for anti-oxidant activity and the maximum activity were observed in the MeOH and/or MeOH+H_2O extract (Supplementary Fig. S1). As the total antioxidant activity of MeOH and/or MeOH+H_2O extract were similar, the further studies were carried out using only the MeOH extracts of the seeds of the different varieties.

α-Glucosidase activity analysis

α-Glucosidase activity of Keteki joha rice seed extract decreased with the increasing concentration of standard acarbose. The IC_{50} value of acarbose was calculated as 1947.5±103.8 µg/mL against the α-glycosidase activity (data not shown). For each fraction (fractionation from the MeOH extract of Keteki joha) and column fractions, the α-glucosidase activity was measured with the stated dose (Fig. 2B-C). Among the extracts, the maximum activity was observed in EtOAc fraction and was calculated as ~ 30% inhibition of α-glucosidase enzymatic activity upon treatment with 10 mg/mL of EtOAc extract (Fig. 3A) and among the column fractions of the EtOAc extract, maximum activity was recorded in fraction 3-9 (F3-9), 10-15 (F10-15), 19-21 (F19-21) and 22-23 (F22-23) (Fig. 2C). For instance, fraction 3-9 showed ~27% and fraction 19-21 showed ~45% inhibition of α-glucosidase inhibitory activity only at 5 mg/mL of dose.

Glucose uptake analysis

The glucose uptake assay in L6 cell line was carried out to further confirm the activity of the identified fractions. 100 nM insulin treated L6 cell line for 30 min showed ~3 fold increase in glucose uptake, whereas a dose of 1 µg/mL of column fraction 3-9 and 22-23 was able to show a ~3.2 and ~4.2 fold increase in glucose uptake, respectively (Fig. 2D) at 8 h of treatment. This result further strengthened our findings i.e., the potentiality of the phytochemicals isolated from the selected scented rice variety grain against the metabolic enzyme activity, which may help in metabolic disorder including diabetics.

Identification and quantification of the active components

The bioactive-guided active fraction of Keteki joha was further analyzed by TLC or HPLC to understand the component (s) in the mixture. In the stated HPLC

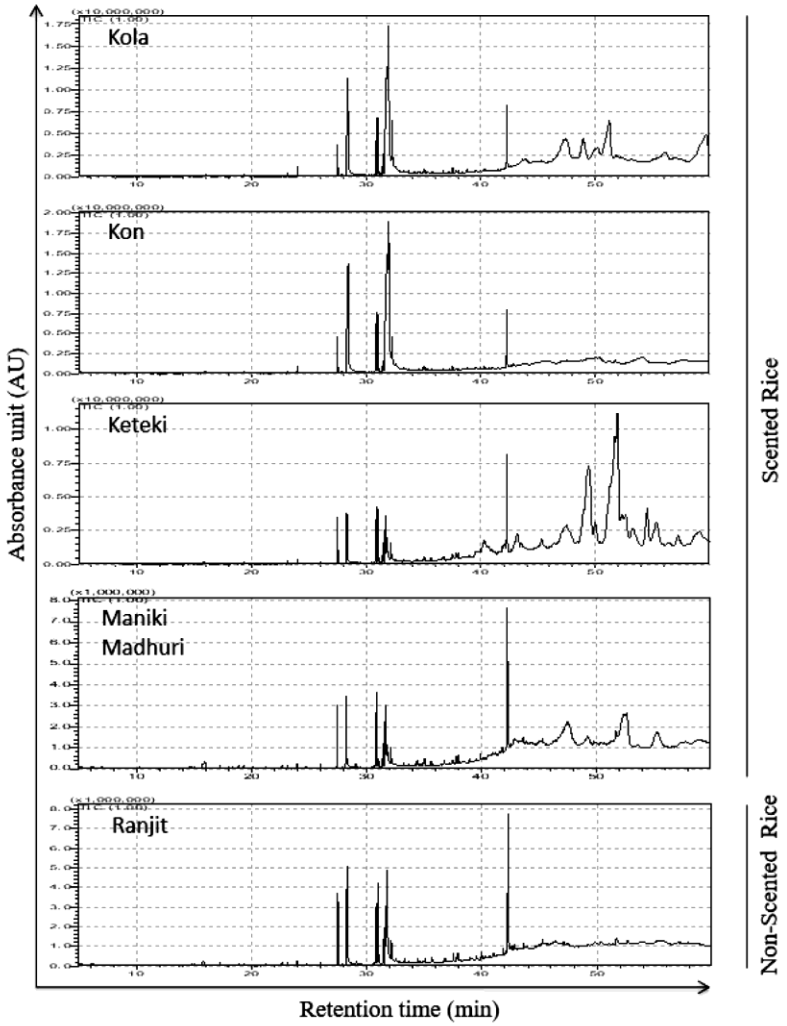

Fig. 1 — GCMS fingerprinting analysis: (A) GCMS fingerprinting was recorded for all the indicated rice varieties with a 30 m x 0.25 µm x 0.25mm EB-5 MS capillary column in a same concentration.

Table 1 — The suggested molecules from NIST11.lib by GCMS analysis

Average retention time (min)	Base peak (m/z)	Suggested molecule from NIST11.lib	% of major detected compound based on the area				
			Kola	*Kon*	*Keteki*	*ManikiMadhuri*	*Ranjit*
23.9	73.05	Tetradecanoic acid	0.6	0.6	0.2	0.9	0.6
27.4	74.05	Hexadecanoic acid, methyl ester	1.8	2.5	1.7	6.9	6.8
28.3	73.05	1-(+)-Ascorbic acid 2,6-dihexadecanoate	13.4	21.8	2.5	10.6	14.5
30.8	81.10	9,12-octadecadienoic acid(Z,Z)-, methyl ester	1.9	3.4	1.6	5.9	5.6
30.9	55.05	Methyl 9-octadecenoate	3.2	4.3	2.0	8.3	7.6
31.05	55.05	9-Octadecenoic acid	0.3	0.3	0.2	0.8	0.7
31.4	74.05	Methyl stearate	1.2	0.7	0.7	2.5	2.4
31.7	69.10	6-Octadecenoic acid	-	-	1.3	5.4	12.2
31.8	81.10	9,12-Octadecadienoic acid (Z,Z)	17.9	27.7	-	0.2	-
31.9	83.10	6-Octadecenoic acid	19.9	24.1	0.1	0.7	0.2
32.2	73.05	Octadecanoic acid	3.1	1.8	0.8	0.4	-
35.08	55.05	6-Octadecenoic acid, methyl ester	0.2	0.3	0.1	0.7	0.3
42.2	59.05	13-Docosenamide	4.3	4.9	4.3	20.3	16.7

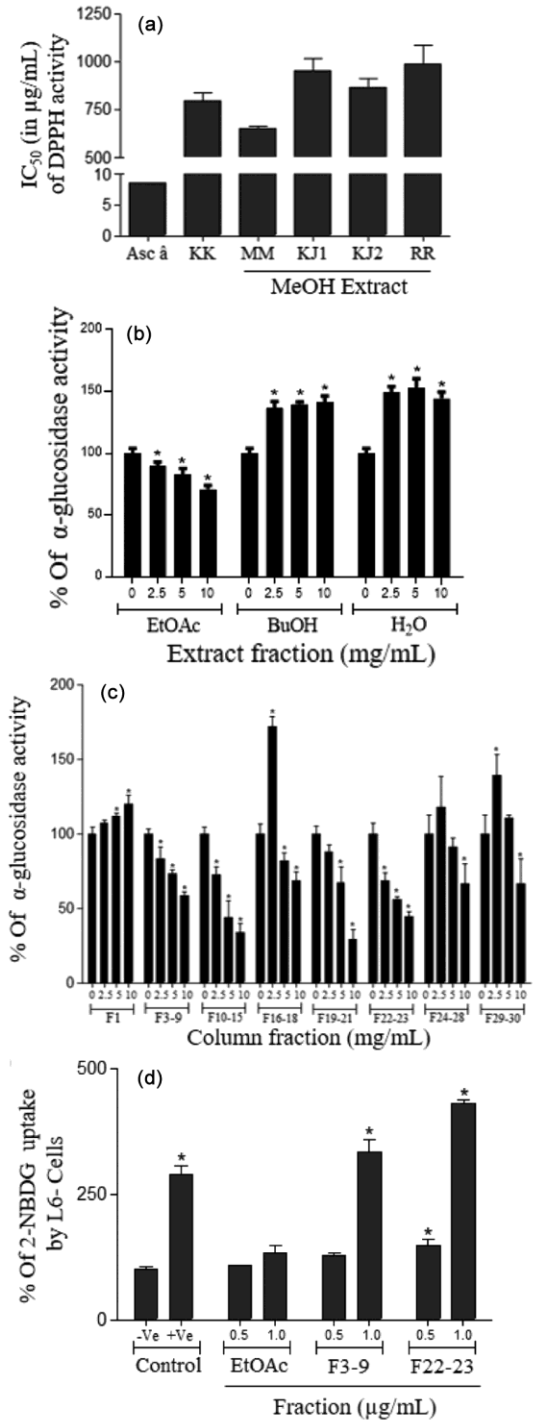

Fig. 2 — Antioxidant property of experimental rice grain and Bioactive guided fractionation: (A) The total anti-oxidant activity of the MeOH extract of different rice grain were measured. IC50 of DPPH scavenging activity of different varieties of scented rice (KJ1 = Kola joha, KJ2 = Kon joha, KK = Keteki joha, MM = Maniki Madhuri joha, RR = Ranjit). All the results were consistent in at least three independent experiments. Results are shown as mean ± SD (n =3). Ascorbic acid (Asc a) was used as positive control. (B) α- Glucosidase activity of different fraction of methanolic extract of Keteki joha (KK) at 0, 2.5, 5 and 10 mg/mL dose (C) α- Glucosidase of the different column purified fractions from Keteki joha (KK) at 0, 2.5, 5 and 10 mg/mL dose. (D) Glucose uptake assay of ethyl acetate fraction of KK and two column purified fraction F3-9 and F22-23 at 0.5 and 1.0 mg/mL of dose. Here insulin (100 nM) has used as positive control and 20 µM cytochalasin B has used as negative control. All the results were consistent in at least three independent experiments. Results are shown as mean ± SD (n =3).*Statistically significant ($p<0.05$) compared to corresponding DMSO-treated control and sample(s) treatment was calculated by one-way ANOVA followed by Dunnet's multiple comparison tests.

method, a single distinct peak was conspicuously visible in F3-9 and other two major peaks along with a common peak both in F3-9 and F22-23 fractions (Fig. 3A). Both the fractions were run through HPLC and the two peaks were separately isolated to record the mass spectra. The other active fraction F19-21 showed a chromatogram profile similar to that of F22-23 fraction (data not shown) but difference in amount of the components in the two fractions. The similarity between recorded mass peaks of the eluted bioactive fractions and that of standard solutions of the two unsaturated fatty acids was indication of the occurrence of the two unsaturated fatty acids in the scented rice variety seeds. Standard methanolic solutions of two essential fatty acids and two collected HPLC fractions were directly injected into the mass spectrometer to obtain a full mass spectrum required to identify the compounds.

Eluted fractions were collected based on the proposed optimized HPLC method, and their ionization pattern monitored against the two fatty acids at negative ion mode with collision energy (CE) 30 and 40. At CE 30, the observed mass of collected fraction and the linolenic acid were 277.21652 and 277.21634 and at CE 40, the parent mass was fragmented in sample and was 277.21628 for linolenic acid. For linoleic acid, the mass of fraction and standard were 279.23206 and 279.23209 at CE 30 and 279.23203 and 279.23193 at CE 40, respectively. The full mass patterns for standards as well as collected HPLC fractions are shown in Figure 3B. The experimental fragmentation pattern and HRMS data analysis confirmed that the two HPLC purified compounds were linolenic and linoleic acids.

To quantify the two essential fatty acids in methanolic extract of the scented rice variety seeds, HPLC analysis was performed. Figure 4A showed the proper separation of both the fatty acids in samples of scented rice and non-scented rice variety seeds under optimized conditions. To verify the eluted pure

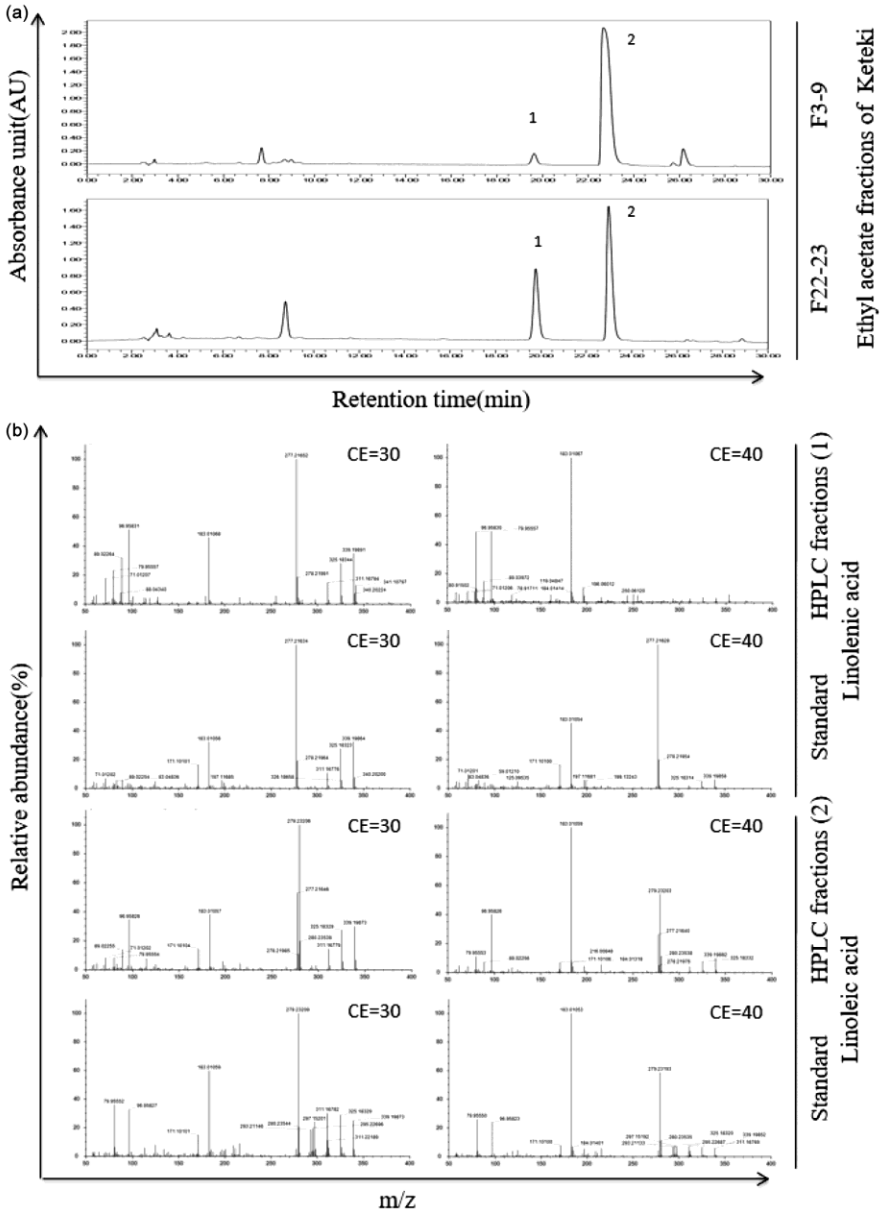

Fig. 3 — MSMS analysis: (A) HPLC chromatogram of the active column fraction of Keteki joha in gradient elution method as stated in the method section (B) MSMS ionization pattern of HPLC collected fractions and the standard linolenic acid and linoleic acid at CE = 30 and CE = 40.

compounds of rice grain, at the same chromatographic conditions, fatty acid standards linolenic acid and linoleic acid were also analyzed Figure 5A-B). At non- polar state of the gradient, the retention time increased and the linolenic acid and linoleic acid was eluted approximately at 18.9 min and 22.5 min, respectively which was equivalent to the retention time of all the rice varieties.

The calibration curve prepared using different concentrations of individual standards of two unsaturated fatty acids are shown in Figure 5A-B. Based on this calibration curve, the quantification of each fatty acids present in experimental rice varieties seeds shown in Figure 5C. From a 20 µL injection volume of 10 mg/mL of rice samples, the fatty acids content could be quantified in microgram level (Fig. 5C).

Discussion

Aromatic rice (especially joha) have been consider as one of the prime varieties of rice with ancient knowledge of health benefit and significant economic

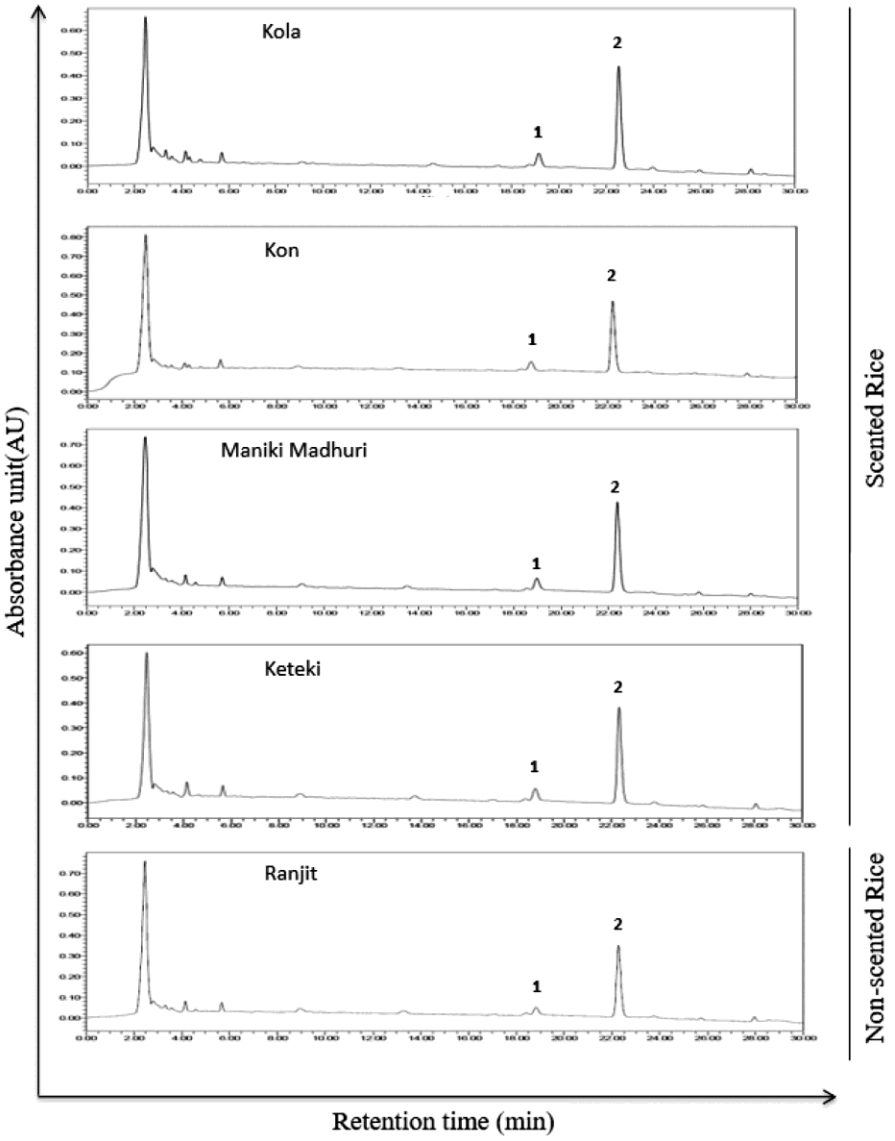

Fig. 4 — HPLC finger printing: (A) HPLC chromatogram of scented-rice (Kola, Kon, Keteki and Maniki Madhuri) indicates the presence of two fatty acids linolenic acid (1) and linoleic acid (2) respectively. The chromatogram was recorded in gradient elution method as stated in the method section.

interest worldwide due to excellent aroma and a decent physical appearance[1,3,4,16,23]. The present investigation was carried out to understand the nutraceutical benefit of these varieties. Research data on nutraceutical benefit of the joha rice has been very limited[3]. There are several scientific evidences which suggest the presence of essential bioactive compounds like phenols, flavonoids and fatty acid in rice grain with pronounced antioxidant and cholesterol lowering activity but in very trace amount[8,9,11,24,25]. Although rice contains a significant amount of starch and usually not recommended to the complicated diabetic patient, in spite of that being a staple food a majority of the population (even they have diabetics) prefer to take rice at least once in a day. For that reason, we had designed the study to evaluate the health benefit effect of the scented rice compare to non-scented rice towards the diabetes patient and subsequently to identify the major bio-active component (s) against the metabolic enzyme. In the first part of this study, we have performed the bioactive guided fractionation followed by column chromatography of ethyl acetate fraction of Keteki joha, which suggested the presence of two major components with potent α-glucosidase inhibitory activity. As α -glucosidase breaks down starch and disaccharides to glucose, it is very crucial

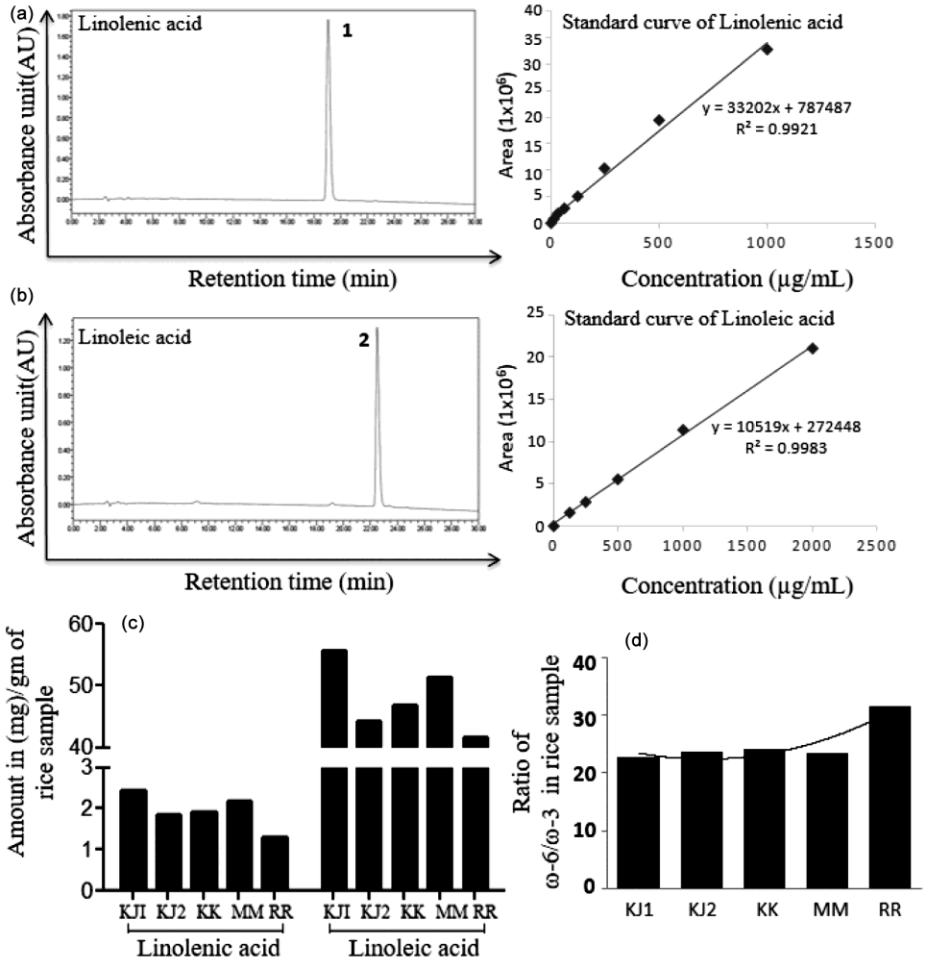

Fig. 5 — Quantification of the active component: (A) HPLC chromatogram of Linolenic acid (1) with the standard curve and (B) HPLC chromatogram of Linoleic acid (2) with the standard curve. (C) Quantification of linolenic acid and linoleic acid in scented rice in mg per gram of sample (D) The quantitative analysis of the ratio of omega-6 to omega-3 essential fatty acids in each individual rice grain. Here, KJ1 = Kola joha, KJ2 = Kon joha, KK = Keteki joha, MM = Maniki Madhuri joha, RR = Ranjit for both (C and D) the figure.

to inhibit its enzymatic activity in diabetes patient[26]. The commotions of those two isolated compounds have been further confirmed by in vitro glucose uptake assay. The HPLC purification was carried out to get the most pure compound for further spectral analysis. The GCMS fingerprinting was performed to check the difference of occurrence in the secondary metabolite and the integrated library had suggested the presence of different fatty acids. The HRMS and MSMS spectral data analysis confirmed the presence of two unsaturated fatty acids viz., linoleic acid and linolenic acid as the active components in the rice grain. Result of earlier studies also supports the importance of these two unsaturated fatty acids against different health disorder including diabetes. For example, a ratio of omega-6 to omega-3 essential fatty acids (EFA) of ~ 1 is required by human beings for maintaining the proper diet whereas the ratio in the developing country is more than 20[27,28]. A research article reported that a ratio of 4:1 (omega-6 to omega-3) in diet was associated with a 70% decrease in total mortality of cardiovascular disease[26,27]. As the diabetic patient more have a chance to get cardiovascular disorder, there might be a role of this ratio (omega-6 to omega-3) to control the diabetic also[27]. The diet of diabetic patients is a cause of concern, while rice being a prime food especially in Asian lifestyle[1-3]. Dietary intake of ω−3 and ω−6 fatty acid has to be regulated in a proper ratio as imbalance of ω−3/ ω−6 had been associated with diabetes and its related cardiovascular diseases[27]. It is important to know the ratio of ω−3: ω−6 fatty acids in food grains for balanced nutrition with other micro nutrients[27]. Thus a therapeutic dose of scented

rice extract as a rich source of omega-3/omega-6 essential fatty acid with other essential micro and macro nutrients may be developed to reduce occurrence of the different metabolic disorders. A quantitative analysis of the two isolated bioactive components was carried out for measuring the exact nutraceutical benefit of the rice varieties against metabolic enzyme. Accordingly, we had developed a facile HPLC method to determine the active component directly from the methanol extract. In general, fats, oils etc. cannot be analyzed without derivatization in HPLC due to unavailability of suitable UV chromophores[28]. But in rice grain, the amount of unsaturated fatty acids was adequate and therefore determined easily by HPLC from crude extract with methanol/water gradient method at 210 nm by PDA detector. The LCMS study suggested that this proposed method became selective and sensitive to these essential fatty acids; else the separation would have posed difficulty. The methanol/water solvent system with gradient, coupled with detector, played a crucial role in this separation process. A single injection to a HPLC system with a gradient elution even could detect the essential fatty acids in methanol extract of the rice grain even in very trace amount.

Conclusion

In conclusion, the ease in quantification of the two essential unsaturated fatty acids, with reference to standard, established the developed HPLC method as facile to detect those fatty acid in rice grain even presence in very trace amount. The ratio of omega-6 to omega-3 in food intake for a healthy individual also has a major concern and the result of this study on occurrence of the two fatty acids in better ratio clearly shown that the intake of scented rice verities over non-scented rice verity might provide health benefits to diabetes patients. Further study is required to understand the details molecular mechanism of total scented rice grain by which health benefit effect may be accrued.

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Conflict of interest

The authors declare no competing interest.

Author Contributions

PC, KND and PKD have performed the experiment. SKS, NCT and RD have conceptualized the study. SKS, PC and KND have analysis the data. All the authors have involved in the preparation of the manuscript. SKS has supervised the overall study and finalized in its present form.

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