In Vitro Antioxidant, Hypolipidemic and Anti-Lipase Potential of Joha Rice of Assam, India

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Present research accounts for *in vitro* antioxidant, hypolipidemic and pancreatic lipase inhibitory potential of two scented rice of Assam, India, namely ketoki joha and kola joha. Our result showed that ethanol extract of ketoki joha and kola joha (whole rice), particularly ketoki joha, exhibits a potent antioxidant potential when tested through DPPH•, NO•, H_2O_2 scavenging and lipid peroxidation inhibition assay. Among different whole rice extracts, ethanol extract of ketoki joha and kola joha at 100 µg/ml produced 76.67 % and 62.47 % inhibition of β-Hydroxy β-methylglutaryl-CoA reductase activity respectively. Ethanol and petroleum ether extracts potently inhibit cholesterol esterase. Ethyl acetate extract of ketoki joha showed the highest pancreatic lipase half maximal inhibitory concentration (IC_{50})= 254.28 µg/ml). In conclusion, the results showed that both rice varieties, mainly ketoki joha, possess potent antioxidant, β-Hydroxy β-methylglutaryl-CoA reductase, pancreatic lipase inhibitory effect, and cholesterol esterase inhibitory effect. Observations might suggest the role of whole joha rice in combating oxidative stress and obesity-related cardiovascular diseases, including atherosclerosis.

Key words: Whole Rice, antioxidant, hypolipidemic, antiobesity, Joha rice

Rice (*Oryza sativa*) is a staple food of most of the global population that is a rich source of carbohydrates and nutritive substances[1]. Assam, a northeastern state of India, produces different rice varieties that are categorized under sali (winter), boro (summer), ahu (autumn) and bao (deep water) rice. Peoples of Assam are cultivating different Joha rice cultivars (categorized under sali rice), which are known for their aroma, superfine kernel and excellent palatability[2]. Recently, joha rice has received the geographical indications tag from Govt. of India. Research from different parts of the world demonstrated that different rice germplasms exhibit antioxidant, anti-diabetic, anti-inflammatory, anti-hyperlipidemic, immunomodulatory and anticancer activities and contain bioactive phytochemicals like polyphenol, phytosterol, carotenoids and flavonoids[1,3]. It was also evident that intake of 90 g of whole grains daily reduces the risk of cardiovascular disease, cancer, and mortality[4]. Although much research has been undertaken to investigate the pharmacological activity of different rice cultivars, quality research on joha rice is still limited. Our present study, aimed to evaluate *in vitro* hypolipidemic, anti-obesity and free radical scavenging activity of two joha rice cultivars i.e., Ketoki Joha (KTJ) and Kola Joha (KJ). Seeds of two joha rice cultivars *viz.,* KTJ and KJ samples were collected from the Institute of Advanced Study in Science and Technology (IASST), Guwahati, Assam, India. The whole rice was processed for de-husking using a traditional instrument called "URAL" to get unpolished rice (rice with bran). Powdered whole rice sample was extracted by petroleum ether, ethyl acetate, ethanol and water using Soxhlet apparatus. Extracts were concentrated in reduced pressure using a rotary

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vacuum evaporator and stored in a cool place. *In vitro* antioxidant activity of extracts was completed using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), nitric oxide radical (NO•), hydrogen peroxide (H_2O_2) scavenging assay and Lipid Peroxidation (LPO) inhibitory assay method. Estimation of DPPH• scavenging effect of extracts was determined as DPPH solution (violet color) is reduced to diphenylpicryl hydrazine (yellow colored product), which was measured by taking absorption at 517 nm[5]. In NO• scavenging assay, Griess Illosvoy reaction is used to quantify nitrite generated from Sodium Nitroprusside (SNP). Different concentrations of extracts were mixed with SNP solution and incubated at 37° for 2 h followed by the addition of Griess reagent to generate a chromophore measured at 570nm[5]. In H_2O_2 scavenging activity, different concentrations of extracts were mixed with H_2O_2 solution (43 mM) in phosphate buffer and the absorbance was measured at 230 nm[6]. Prior approval from Institutional Animal Ethics Committee (IAEC) of Assam down town University, Guwahati has been taken to collect experimental animal blood and rat liver required for LPO assay and 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase assay. Malondialdehyde (MDA) is a cytotoxic product produced during lipid peroxidation. Rat liver homogenate was used as a source of polyunsaturated fatty acid in LPO assay. Chromophore produced by MDA and thiobarbituric acid reaction was measured at 532 nm against blank[5]. Ascorbic acid was used as a standard for *in vitro* antioxidant activities. The percentage of inhibition was estimated against blank using the formula

$$\text{Percentage inhibition}=(A_0-A_1)/A_0\times 100$$

Where, A_0= Absorbance of control and A_1 = Absorbance of test/standard. Antihyperlipidemic activity of extracts was investigated using the *in vitro* HMG-CoA reductase assay and Cholesterol Esterase (CEase) inhibition assay method. Rat liver homogenate was used to prepare microsomes using a standard protocol that acts as an HMG CoA reductase enzyme source. Different concentrations of whole joha rice extracts (25, 50, 75 and 100 µg/ml) were mixed with liver microsomes and HMG-CoA reductase activity of the extract was measured considering the relative oxidation of NADPH and release of NADP+[7]. Atorvastatin was used as standard. CEase inhibitory activity was performed in the presence of sodium taurocholate with p-Nitrophenyl Butyrate (p-NPB) as substrate. About 430 µl of sodium phosphate buffer (pH 7.0), 500 µl of taurocholate solution, 40 µl of acetonitrile, 10 µl of p-NPB solution, and 10 µl of extract were mixed and incubated for 2 min at 25° followed by the addition of 10 µl of enzyme solution. Absorbance was measured at 405 nm to measure *in vitro* CEase inhibitory potency of extracts[8]. *In vitro* pancreatic lipase inhibition assay, the ability of joha rice extracts was determined considering the hydrolysis of p-nitrophenyl butyrate (p-NPB) to p-nitrophenol using a standard method. Porcine pancreatic lipase was mixed with different extract concentrations and the volume was made up to 1 ml by adding Tri-HCl solution. The reaction mixture was incubated at 25° for 15 min, followed by adding p-NPB solution in acetonitrile. The reaction mixture was incubated for 30 min at 37°, and optical activity was measured at 405 nm. Orlistat was used as a standard[9]. IC_{50} value indicating the concentration at which an extract/standard would inhibit free radicals/reactive oxygen species/enzyme activity by 50 % was also calculated. Results obtained from *in vitro* antioxidant activity are presented in Table 1. Whole rice extracts of KTJ and KJ exhibited profound antioxidant activity. In the DPPH• scavenging assay, the IC_{50} value of the ethanol extract of KTJ, ethanol extract of KJ and ascorbic acid was found to be 102.77, 112.01 and 32.40 µg/ml respectively. DPPH• scavenging activity resulted in the reactivity of test substances with constant free radicals. In visible spectroscopy, DPPH• exerted prevailing absorption bands at 517 nm due to the odd electron quantity[10]. DPPH• scavenging activity of KTJ and KJ extracts indicated that extracts, particularly ethanol extract, contain potent phytochemicals that can effectively scavenge free radicals. Among all the extracts, ethanol extract of KTJ had the lowest IC_{50} value (752.13 µg/ml), followed by ethyl acetate extract of KTJ (938.90 µg/ml) and ethanol extract of KJ (962.61 µg/ml) when tested through NO• scavenging assay. NO• is an important pleiotropic mediator that regulates several physiological activities. Nevertheless, increased generation of NO• may induce oxidative damage and cause disorders like atherosclerosis, inflammation, cancer etc[11]. Nitric oxide synthase and reaction initiated by superoxide radicals can induce the

generation of peroxynitrite excessively, which may involve the pathogenesis of different degenerative diseases and oxidative damage[10]. Results showed the potent antioxidant activity of KTJ and KJ, suggesting a possible role of these extracts against free radical induced diseases. The concentration of the ethanol and water extract of KTJ required to scavenge H_2O_2 by 50 % was 80.53 and 106.87 µg/ml, respectively, while the IC_{50} value of ethanol extract of KJ was found to be 111.87 µg/ml. A lesser IC_{50} value indicates higher antioxidant activity. H_2O_2 can be highly reactive in nature and in the presence of Fe^{2+} or Cu^{2+}, and H_2O_2 induces the most reactive hydroxyl radical through the Fenton reaction[12]. Extracts effectively scavenge H_2O_2 in a concentration dependent manner. KTJ and KJ also effectively inhibit lipid peroxidation. The IC_{50} of ethyl acetate extract of KJ and KTJ was 109.78 and 132.61 µg/ml respectively, in lipid peroxidation inhibition assay, while IC_{50} of ascorbic acid was found to be 76.6 mg/ml. Lipid peroxidation causes degradation of the lipid bilayer of the cells membrane, thus inducing disruption of normal cell function and cellular homeostasis. End-products of lipid peroxidation can further induce mutagenesis or protein oxidation involved in the pathogenesis of different disease condition[5,13]. Extracts effectively inhibit lipid peroxidation and the generation of malondialdehyde. Hence, increasing interest in finding the level of protection can confer by an extract that may help avert cardiovascular disease. The result of the present study reports that whole joha rice extract exerts *in vitro* antioxidant and lipid peroxidation inhibitory activity in a dose-dependent manner. Previous studies suggested that rice contains diverse polyphenolic compounds[1,3]. These results indicated that whole rice could be helpful in the prevention of oxidative stress and its associated diseases. Endogenous cholesterol biosynthesis in the liver is mainly controlled by rate limiting HMG-CoA reductase, a rate limiting enzyme primarily responsible for controlling the biosynthesis of endogenous cholesterol in the liver. HMG-CoA reductase involves the Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-dependent conversion of HMG-CoA to mevalonate[14]. The inhibition of HMG-CoA reductase efficiently decreases cholesterol levels and is considered a key target for developing a hypolipidemic drug. Data presented in fig. 1 shows that KTJ and KJ extracts significantly inhibited HMG-CoA reductase activity in a concentration-dependent manner. At 100 µg/ml concentration, KTJ ethanol extract, KJ ethanol extract, and KTJ petroleum ether extract inhibit HMG-CoA reductase activity by 76.67 %, 62.47 %, and 61.43 %, respectively, while atorvastatin (50 µg/ml) inhibit enzyme activity by 61.5 % when compared with control. Thus, whole rice extract of KTJ and KJ can be considered potent inhibitors of this HMG-CoA reductase and can be considered lead in drug development targeting hyperlipidemia. Recent research supports that effective inhibition of digestion and absorption of dietary fat is a new approach to preventing hyperlipidemia. CEase induce hydrolysis of cholesterol ester in the small intestine to unesterified cholesterol and free fatty acids. Further, it also regulates the incorporation of cholesterol into mixed micelle[15,16]. CEase inhibitory activity of rice extracts is given in Table 2. The findings supported the strong CEase inhibitory activity of ethanol extract of KTJ (IC_{50}=87.11 µg/ml), pet ether extract of KTJ (IC_{50}=111.96 µg/ml), petroleum ether extract of KJ (IC_{50}=136.53 µg/ml). Several phenolic and flavonoids compounds, such as gallic acid, catechin, and epicatechin exhibited CEase inhibitory activity[16]. CEase inhibitors may confer as a solution to control the absorption of free cholesterol and reduce the bioavailability of dietary cholesterol resulting from cholesterol esters. Therefore, inhibition of CEase by whole rice extract of KTJ and KJ could play an essential role in managing hyperlipidemia and obesity by reducing dietary cholesterol absorption. Obesity results from an imbalance between energy intake and expenditure that increases the risk of cardiovascular, metabolic and endocrine disorders and has become a major global health concern[17]. Obesity is responsible for increasing triglycerides, low-density lipoprotein cholesterol, blood glucose and blood pressure, and reduction of high-density lipoprotein cholesterol[17]. Pancreatic lipase is synthesized and secreted by the pancreas and exerts a vital role in lipid digestion; almost 50 %-70 % of total dietary lipids are digested by lipase enzyme[9]. Therefore, the anti-lipase activity of phytoextract/phytochemicals is one of the most important mechanisms for finding an anti-obesity agent. Results obtained from our study showed that ethyl acetate extracts of KTJ and KJ have high

porcine pancreatic lipase inhibition potential with IC_{50} values of 254.28 and 259.05 µg/ml respectively, in comparison with the orlistat, which has an IC_{50} of 151.20 µg/ml. Many previous researches concluded that flavonoids and other phenolic compounds have porcine pancreatic lipase inhibitory activity by binding to the enzyme-substrate complex and causing a reduction in lipid absorption[9]. In our study, rice extracts showed porcine pancreatic lipase inhibition assay, which may be due to flavonoids and other phenolic compounds present in the extract. Recent studies investigated that joha rice contains diverse antioxidant phytochemicals, including apigenin, tricin, avenasterol, coumarin, coumaric acid, phenyl alanine, caffeic acid, and α-tocophenol[3,18]. An imbalance of linoleic acid (ω-6-fatty-acid) and linolenic acid (ω-3-fatty-acid) has been associated with the pathogenesis of metabolic and cardiovascular diseases. Presence of ω-6 and ω-3-fatty-acid are also reported in different joha rice cultivars[3]. Further, rice bran is an important source of dietary antioxidants that have been found effective against obesity and cardiovascular disease[1], though research on joha rice is limited. In our study, we have taken unpolished rice, and thus antioxidant molecules present in bran might be responsible for investigated pharmacological activities. The present study results concluded that both KTJ and KJ, particularly KTJ, produced strong antioxidant activity, hindering the enzymatic activity of HMG-CoA and CEase, and exhibiting an anti-lipase effect. Further studies are needed to isolate the phytochemicals and find the role of whole joha rice in managing or preventing hyperlipidemia, hypercholesterolemia, and cardiovascular diseases.

TABLE 1: *IN VITRO* ANTIOXIDANT ACTIVITY OF WHOLE RICE EXTRACTS OF KTJ AND KJ

Extract	IC_{50} value (µg/ml)			
	DPPH• scavenging assay	NO• scavenging assay	Lipid peroxidation inhibition assay	H_0O_2 scavenging assay
KTJ water extract	253.06±3.68	1009.42±24.58	224.95±6.19	106.87±1.59
KTJ ethanol extract	102.77±1.76	752.13±8.10	194.34±5.28	80.53±0.63
KTJ ethyl acetate extract	255.41±5.90	938.9±15.25	132.61±5.37	118.03±1.85
KTJ petroleum ether extract	320.09±4.12	1109.67±16.64	205.44±4.90	146.5±2.82
KJ water extract	226.4±1.80	1102.93±16.90	215.67±3.41	112.42±1.24
KJ ethanol extract	112.01±2.34	962.61±13.38	151.02±5.99	111.87±1.42
KJ ethyl acetate extract	290.17±9.15	1122.83±13.64	109.78±3.33	181.78±2.56
KJ petroleum ether extract	338.85±11.42	1293.36±6.79	178.21±0.15	166.4±4.76
Ascorbic acid	32.40±0.44	623.20±9.86	76.6±4.02	26.80±2.98

Note: Values are expressed as mean±standard error of mean (n=3); KTJ: Ketoki Joha; KJ: Kola Joha

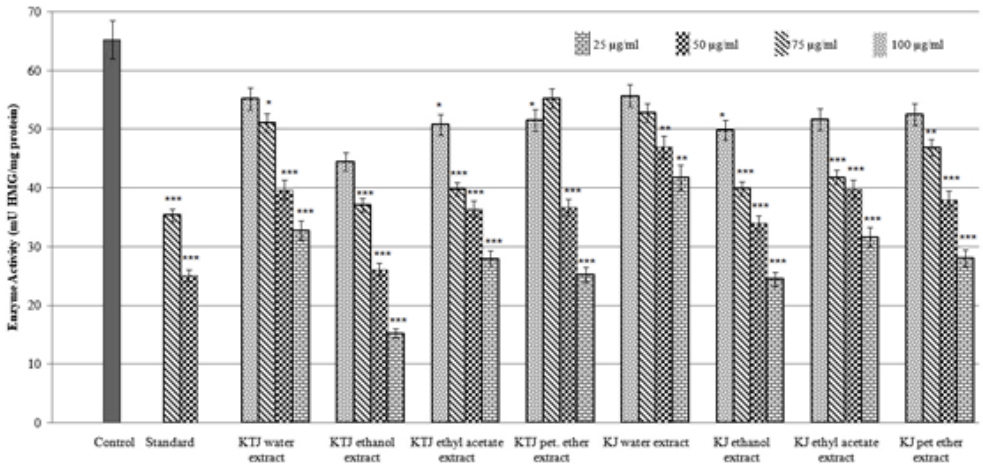

Fig. 1: Effect of whole rice extract of KTJ and KJ. Values were expressed as mean±standard error of mean (n=3); KTJ: Ketoki Joha; KJ: Kola Joha; Data were analyzed by one way analysis of variance, statistical significance was expressed as *p<0.05, **p<0.01 and ***p<0.001 in comparison to control group; (▒): 25 µg/ml; (▓): 50 µg/ml; (▒): 75 µg/ml and (▒): 100 µg/ml

TABLE 2: INHIBITORY EFFECT OF WHOLE RICE EXTRACT OF WHOLE RICE EXTRACTS OF KETOKI JOHA AND KOLA JOHA ON CHOLESTEROL ESTERASE AND PANCREATIC LIPASE

Extract	IC_{50} value (µg/ml)	
	Cholesterol esterase inhibitory activity	Pancreatic lipase inhibition assay
KTJ water extract	241.55±5.77	409.24±19.83
KTJ ethanol extract	87.11±3.36	354.55±2.90
KTJ ethyl acetate extract	240.73±7.65	254.28±2.11
KTJ petroleum ether extract	111.96±4.30	428.08±3.84
KJ water extract	260.44±7.04	450.92±5.72
KJ ethanol extract	136.87±2.33	323.39±1.19
KJ ethyl acetate extract	278.51±7.86	259.05±1.14
KJ petroleum ether extract	136.53±9.38	331.96±17.37
Standard drug	60.95±1.06 (simvastatin)	151.20±3.88 (orlistat)

Note: Values are expressed as mean±standard error of mean; n=3; KTJ: Ketoki Joha; KJ: Kola joha

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Conflict of interests:

The authors declared no conflict of interests

REFERENCES

1. Sen S, Chakraborty R, Kalita P. Rice-not just a staple food: A comprehensive review on its phytochemicals and therapeutic potential. Trends Food Sci Technol 2020;97:265-85.
2. Das A, Kesari V, Rangan L. Aromatic joha rice of Assam: A review. Agri Rev 2010;31(1):1-0.
3. Choudhury P, Dutta KN, Dev PK, Talukdar NC, Samanta SK, Devi R. Quantitative analysis of bio-active phytochemical (s) in selected scented rice varieties (*Oryza sativa*) reveals its intake towards advantage against metabolic disorders. Indian J Tradit Knowl 2021;20(1):210-20.
4. Halpern LW. Eating whole grains can reduce disease and mortality risks. Am J Nurs 2016;16:15.
5. Sen S, De B, Devanna N, Chakraborty R. Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of Meyna spinosa Roxb., an Indian medicinal plant. Chin J Nat Med 2013;11(2):149-57.
6. Kumari S, Elancheran R, Devi R. Phytochemical screening, antioxidant, antityrosinase, and antigenotoxic potential of Amaranthus viridis extract. Indian J Pharmacol 2018;50(3):130.
7. Durgaprasad MR. Anti hypertipidemic effects of various medicinal plant extracts on high fat diet induced obese rat models. SVKM's Narsee Monjee Institute of Management Studies; 2014.
8. Anandhi B. Study on *in vitro* cholesterol esterase Inhibitory activity and *in vivo* antihyperlipidemic activity of Anisomeles malabarica leaf extract. Tamil Nadu Dr MGR Medical University; 2017.
9. Jaradat N, Zaid AN, Hussein F, Zaqzouq M, Aljammal H, Ayesh O. Anti-lipase potential of the organic and aqueous extracts of ten traditional edible and medicinal plants in Palestine; a comparison study with orlistat. Medicines 2017;4(4):89.
10. Askin H, Yilmaz B, Gulcin I, Taslimi P, Bakirci S, Yildiz M. Antioxidant activity of the aqueous extract of iris taochia and identification of its chemical constituents. Indian J Pharm Sci 2018;80(5):802-12.
11. Sen S, Chakraborty R, Rekha B, Revathi D, Ayyanna SC, Hemalatha G, *et al.* Anti-inflammatory, analgesic, and antioxidant activities of Pisonia aculeata: Folk medicinal use to scientific approach. Pharm Biol 2013;51(4):426-32.
12. Chakraborty R, Biplab D, Devanna N, Sen S. Antiinflammatory, antinociceptive and antioxidant activities of Phyllanthus acidus L. extracts. Asian Pac J Tropic Biomed 2012;2(2):S953-61.
13. Félix R, Valentão P, Andrade PB, Félix C, Novais SC, Lemos MF. Evaluating the *in vitro* potential of natural extracts to protect lipids from oxidative damage. Antioxidants 2020;9(3):231.
14. Iqbal D, Khan MS, Khan MS, Ahmad S, Hussain MS, Ali M. Bioactivity guided fractionation and hypolipidemic property of a novel HMG-CoA reductase inhibitor from Ficus virens Ait. Lipids Health Dis 2015;14:1-5.
15. Heidrich JE, Contos LM, Hunsaker LA, Deck LM, Vander Jagt DL. Inhibition of pancreatic cholesterol esterase reduces cholesterol absorption in the hamster. BMC Pharmacol 2004;4(1):1-9.
16. Abeysekera WP, Arachchige SP, Ratnasooriya WD. Bark extracts of Ceylon cinnamon possess antilipidemic activities and bind bile acids *in vitro*. Evid Based Complement Alternat Med 2017;2017:7347219.
17. Klop B, Elte JW, Castro Cabezas M. Dyslipidemia in obesity: Mechanisms and potential targets. Nutrients 2013;5(4):1218-40.
18. Choudhury P, Dutta KN, Singh A, Malakar D, Pillai M, Talukdar NC, *et al.* Assessment of nutritional value and quantitative analysis of bioactive phytochemicals through targeted LC-MS/MS method in selected scented and pigmented rice varietals. J Food Sci 2020;85(6):1781-92.