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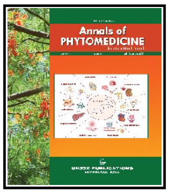

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Antidiabetic biomolecules and nutrient elements in Makhana (*Euryale ferox* Salisb.)

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Abstract

An experiment at ICAR-National Research Centre for Makhana, Darbhanga, identified Makhana seed endosperm as a source of quercetin (422 µg/kg) along with kaempferol (200 µg/kg) pure flavonoids that enhance rapid glucose metabolism in human cells. Seed endosperm contained 1.14 ± 0.02 mg/100 g of vitamin B1 and 5.4 ± 0.04 mg/100 g of vitamin C. Regarding antioxidant activities, makhana had the highest DPPH and FRAP values of 288.76 ± 0.09 mg AEAC/100 g FW and 99.14 ± 0.16 mg AEAC/100 g FW, respectively. Furthermore, a significant quantity of arginine (12.70 ± 0.21 g/100 g) as well as histidine (3.62 ± 0.11 g/100 g) was also detected in Makhana's protein content of dry endosperm. Makhana was found to be a rich source of several macro and micronutrients. Magnesium (20.00 ± 1.09 mg/100 g) and zinc (5.6 ± 0.24 mg/100 g), which regulate the secretion and storage of insulin, were also present in appreciable quantity. The Makhana seed kernel was also discovered to have significant amounts of iron (10.9 ± 0.13 mg/100 g), selenium (0.06 ± 0.02 mg/kg), and chromium (1.05 ± 0.06 mg/kg), which function as antioxidants. Our study reveals the highly nutritious properties of Makhana with ingredients having antidiabetic and immune-stimulating properties.

1. Introduction

Makhana (*Euryale ferox* Salisb.) is one of the most significant freshwater and nutritious crops in the wetland ecology. It is well-known for its economic importance, nutritional values and medicinal properties (Kumar *et al*., 2020; Kumar, 2023). Diabetes, a metabolic disorder or disease, is a growing epidemic and millions of people worldwide are affected. The diabetes treatment encompasses lifestyle modifications, oral antidiabetic medications, and insulin therapy. To combat diabetes, the incorporation of foods abundant in plant phyto-chemicals is recommended due to their extensive pharmacological properties. Persons with diabetes should eat a lot of Makhana, as it tends to lower blood sugar levels (Sodi and Kumar, 2019). Makhana has cardioprotective qualities and these qualities could be connected to Makhana's capacity to scavenge ROS and induce TRB-32 and Trx-1 proteins (Das *et al*., 2006). The secondary metabolites, flavonoids are an important class of phytocomponent, usually observed in fruits, vegetables and nuts. They are found to be promising to stabilise insulin transport. Flavonoids, alongside amino acids and minerals, significantly contribute to reducing diabetes. Flavonoids have antidiabetic potential primarily through modulating the glucose transporter by boosting GLUT-2 expression in pancreatic β-cells and encouraging GLUT-4 translocation through AMPK, CAP/Cb1/TC10, and PI3K/AKT pathways (Hajiaghaalipour *et al.,* 2015). By blocking the enzymes necessary for the digestion of carbohydrates and reducing intestinal glucose absorption, certain flavonoids can affect glucose metabolism. Barber *et al*. (2021) evaluated capacity of 4 structurally related flavonols like kaempferol, quercetin, galangin, and quercetagetin for inhibiting human α-glucosidases, isomaltase, sucrase and maltase. Antiobesity and antidiabetic properties of kaempferolare facilitated by regulation of SREBP-1c and PPAR-γ *via* AMPK activation (Zhang *et al*., 2015). Kaempferol may decrease elevated serum glucose levels and enhance glucose uptake in the rat soleus muscle with efficacy comparable to insulin. Apart from flavonoids, high value amino acids like arginine, histidine, leucine, isoleucine, proline and nutrients element including divalent cations, *viz.;* Ca^{2+} and Mg^{2+} play an important role in effectively reducing the condition of diabetes. Research indicates that arginine diminishes insulin resistance regarding amino acids and diabetes management. This indicates that insulin sensitivity may be enhanced. Arginine is essential for cells sensitivity in body to insulin. Arginine acts as a precursor to nitrogen oxide, signalling molecule that directly influences insulin sensitivity. In addition, endogenous nitric oxide (NO), a strong vasodilator that acts through intracellular second-messenger cGMP, is derived from arginine. Because it produces more NO, l-arginine causes peripheral vasodilation and prevents platelet aggregation in healthy persons. Vascular endothelial and immune cells use semi-essential amino acid l-arginine as a substrate for producing NO. These cells generate NO, essential for immune system function as well as blood pressure regulation (McRae, 2016). Arginine is important in management of diabetes-related nutritional disorders. The

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supplementation of amino acid l-carnitine enhances insulin sensitivity in diabetes management. In diabetic patients, higher carnitine intake may also lower LDL cholesterol levels. Moreover, carnitine preserves cellular integrity against oxidative stress. Oxidative stress results from free radicals generated by certain metabolic processes, environmental pollution, smoking, or ultraviolet radiation. Carnitine neutralizes free radicals, thereby protecting the heart, kidneys andeyes. An increase in blood plasma elevates lipoprotein A levels, thereby heightening the risk of coronary heart disease as well as circulatory disorders. The intake of carnitine diminishes the concentration of lipoprotein A in the patient's blood plasma, indicating its evident protective attributes. Diabetes disrupts calcium homeostasis, leading to impaired cellular regulation in erythrocytes, cardiac muscle, platelets, as well as skeletal muscle. Magnesium is an essential cofactor for metabolism of carbohydrates in addition to transport of glucose into cells. It contributes to insulin's cellular activity. Diabetes is associated with low magnesium intake.The onset of diabetes-related complicationsis accelerated when magnesium deficiency impairs cellular defences against oxidative damage, reducing resilience to oxidative stress caused by diabetes. It is acknowledged that micronutrients are essential nutrients required in trace amounts for enzyme regulation and glucose homeostasis (Kashiv *et al.,* 2016; Koekkoek and Van Zanten, 2016). Micronutrients serve a dual function: they maintain cellular structures at optimal levels; however, when deficient, alternative pathways are activated, potentially leading to illnesses. Crucial micronutrients including Zn, Fe, Se and Cr exert considerable physiological impacts and are directly associated with diabetes mellitus. As a micronutrient that controls over 100 enzymes that aid in protein folding, gene expression and generation as well as detoxification of reactive oxygen species (ROS), zinc is essential for metabolism. Zinc performs an essential function in cell; signalling and the cellular functions include apoptosis along with cell division. Diabetes as well as insulin resistance have been related to abnormalities in zinc homeostasis. This study emphasizes the importance of zinc in regulating immune function by detailing its influence on the activation of critical signalling molecules and epigenetic modifications (Wessels *et al*., 2017). Selenium is essential for the function of the glutathione peroxidase (GPx) enzyme. Diabetes elevates oxidative stress through the generation of ROS. GPx is crucial in cellular antioxidant defence. Oxidative stress, implicated in pathophysiology of diabetic nephropathy, represents an imbalance between ability of body to neutralize or detoxify the detrimental effects of free radicals *via* antioxidants. GPx one of the main enzymatic antioxidants, reduces the growing state of oxidative stress and transforms peroxides from harmful to non-toxic substances. This helps to prevent complications from diabetes (Altuhafi *et al.*, 2021). Since its identification as a vital trace metal in 1955, chromium (Cr) has demonstrated efficacy in enhancing glucose tolerance by diminishing insulin resistance. These two heavy metals in very low quantity act as antioxidant for diabetic patient. In the insulin cycle, Zn, a trace element, performs an important function. As a constituent of insulin, it is essential for its storage andfunction. Other than this, Zn is a component of many important enzymes that control glucose balance in body. As a cofactor for various enzymes, magnesium performs an essential function in carbohydrate metabolism, with adipocytes in low-magnesium environments exhibit decreased insulin-stimulated glucose uptake, potentially resulting in insulin resistance in diabetic cells. Micronutrients add free radicals and make them safe and can cause insulin resistance in diabetes cells. Malfunction of beta-cells diminishes insulin signalling, promotes chronic systemic inflammation, and modifies the activity of crucial Mg^{2+}-dependent signalling kinases as well as enzymes fundamental to carbohydrate and energy metabolism (Kostov, 2019). Magnesium deficiency can directly affect insulin signalling. Flavonoids, alongside amino acids andminerals, significantly contribute to reducing diabetes. Flavonoid has been demonstrated to enhance insulin uptake through modification of GLUT4. Recent research has suggested flavonoids may have hyperglycemic activity by preventing the activity of enzymes that break down carbohydrates, like amylase, glucosidase, and disaccharidases. Selenium in low quantity (1-2 mg/kg) acts as antioxidant which helps in glucose metabolism (Wang *et al*., 2017). Hence, the present research was conducted to quantify different biomolecules, *viz.*, flavonoids, vitamins, amino acids and nutrient elements including micro and macro-nutrients in Makhana to generate information about their importance in controlling metabolic disorders like diabetes and overall human nutrition.

2. Materials and Methods

Experiment was performed at ICAR-NRCM, Darbhanga, Bihar, during 2021-22. Dry gorgon nut (*E. ferox*) kernels were crushed and subsequently ground into powder with three separate replications for antioxidant and heavy metal analysis. Four replicated values for each were taken into consideration for Fisher's Protected LSD design. Superior Selection-1 Makhana genotype was used as the research material. Plant sample of Makhana (Herberium Accession No: 032) was authenticated by Department of Botany, B.N. Mandal University, Madhepura, Bihar, India (Letter No/VPG/Bot/64/24).

2.1 Flavonoid analysis

10 g of the sample was homogenized with 70% methanol. Samples were vortexed for 5.0 min and subsequently centrifuged at 4,500 rpm for 10 min. Supernatant was filtered utilizing a 0.45 µm syringe filter. Samples were subsequently injected into HPLC system with instrument conditions as follows: Column – Shimpack C18 (4.6 x 250 mm), 5 µm; Column oven temperature - 30°C; data acquisition time – 80 min; injection volume 20 µl; flow rate 1.0ml/min. Sample thermostat5°C; mobile phase A6% acetic acid in a 2 mM sodium acetate aqueous solution (v/v, final" pH 2.55); mobile phase B - aceto-nitrile; post run time -5 min (Tsao andYang, 2003). We performed two standards independently with column conditions prior to sample loading.

2.2 Vitamins

Vitamins were determined by as recommended by A.O.A.C (2016 20th Ed, 957.17 and 967.21) for B1and vitamin C. Thiamine (Vitamin B1) was determined by fluorometric method, whereas ascorbic acid

(Vitamin C) was determined by 2,6-Dichloroindophenol Titrimetric Method.

2.3 Antioxidant activity

The antioxidant activity of Makhana seed kernel samples was evaluated employing two assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity as well as FRAP (ferric reducing antioxidant potential). Both assays exhibit high reproducibility, accuracy, along with simplicity, that could be executed rapidly (Katalinic *et al*., 2006; Thaipong *et al*., 2006).

2.4 Amino acids

All essential amino acids were analysed using the methodologies outlined by Bidlingmeyer (1987). The method is based on the interaction of free amino acids with phenylisothiocyanate to produce stable derivatives that are then separated using liquid chromatography. Amino acid content of the sample was expressed as g/100 g of protein (p).

2.5 Proximate composition

The nutritive properties, including moisture, ash, crude fat, as well as crude protein contents, were assessed using AOAC methods (A.O.A.C., 2016). The analysis of crude protein was conducted utilizing Kelplus Elite Ex Micro Kjeldahl method with a conversion factor" of 6.25. The percentage of carbohydrates (by difference) by weight was determined according to standard method (Gopalan *et al*., 2004; Ranganna, 2017). Total dietary fiber (TDF) was quantified by enzymatic digestion of sample utilizing α-amylase (AOAC, 2016 method 991.43), using both fresh anddry Makhana kernel powder, with four replications of each biochemical analysis performed.

2.6 Minerals

For analysis of minerals content (N, P, K, Mn, Mg, Ca, Na, S, Cu, Zn and Fe), fresh Makhana seed kernel samples were washed initially employing tap-water followed by dilute hydrochloric acid (0.05N) and finally with double-distilled water. The samples were subsequently dried in an air oven at 65°C temperature until weight stabilized for three consecutive days. Dried samples were subsequently ground andpassed through an 80-mesh sieve (180 mm). An atomic absorption spectrophotometer (Analyst100, Perkin Elmer and Norwalk, CT, USA) was employed to estimate allthe mentioned elements (AOAC, 2016). The results are expressed as mg mineral/ 100 gFW for individual elements. All mineral values were added to get total minerals content and expressed as per cent.

2.7 Heavy metals

2.7.1 Selenium

Employing per-chloric acid (0.4 mol per lit.) low-molecular-weight compounds were extracted to produce a fraction that contained 3-15% of total selenium. After being dissolved in 0.1 mol per lit. sodium hydroxide, it was precipitated with acetone, proteins extracted from nut samples. They subsequently dissolved in pH7.5 phosphate buffer. The two possible states of selenium's binding to proteins-weakly and firmly-were the focus of protein fraction analysis. Employing size-exclusion chromatography along with online ICP-MS detection, selenium was identified. Proteinase K has been employed to hydrolyse the protein extracts enzymatically to obtain data regarding firmly bound selenium. The method employed for speciation had been ion-pairing HPLC-ICP-MS.Se-methionine primary compound was identified in Makhana following the method described by Kannamkumarth *et al*. (2002).

2.7.2 Chromium

The samples were dried in an air oven at 65°C temperature until weight stabilized for three consecutive days. The plasma solution was prepared by adding a 5 g sample of 20 ml (1+1) nitric acid and 20 ml (1+1) hydrochloric acid and diluted to 500 mlwith reagent water. Chromium content was measured by employing Inductively Coupled Plasma optical emission spectrometry method (Shwetaand Brar, 2020).

2.8 Statistical analysis

ANOVA employing a CRD (completely randomized design) with three replications wasapplied to all the data. Mean of triplicate measurements ± SD was usedto represent results. According to least significant difference, significant value was measured within column for each character (p<0.05). For antioxidant and heavy metal data analysis, Fisher's Protected LSD design was adopted, and four replicated values were taken into consideration.

3. Results

Observations made on the different nutritional and antidiabetic properties of the Makhana seed endosperm during the experiment were noted and are listed under the headings below:

3.1 Presence of flavonoids in Makhana

This experiment estimated pure flavonoids quercetin and kaemeferol employing UHPLC chromatography (Table 1). *E. ferox* seed endosperm's UHPLC chromatogram demonstrated quercetin content of 0.422 mg/kg as well as kaempferol content of 0.200 mg/kg as pure flavonoids.

Table 1: Quercetin and kaempferol in dry seeds endosperm of *E. ferox*

Sl. No.	Name	RT (min)	Area (Unit)	Results (mg/kg)
1.	Quercetin	64.50	1940.0	0.422
2.	Kaempferol	67.90	1405.0	0.200

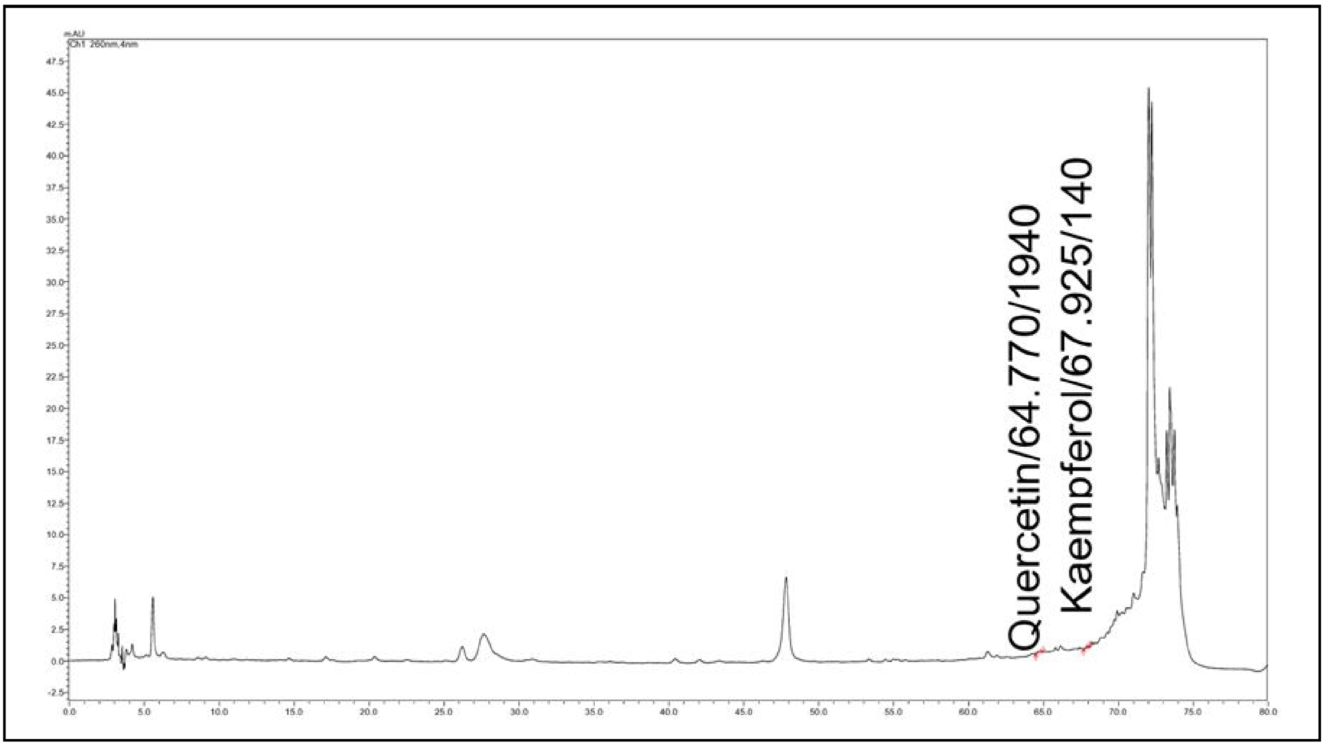

Figure1: Chromatogram of quercetin and kaempferol in dry seeds endosperm of Makhana.

3.2 Vitamins

Makhana seed kernel was found to contain 1.14 ± 0.02 mg/100 g of vitamin B1and 5.4 ± 0.04 mg/100 g of vitamin C, respectively (Table 2). The resistant starch, amylose wasalso present in appreciable amount (17.2 ± 0.15 %) (RS).

3.3 Antioxidant

Makhana has the highest DPPH and FRAP values, measuring 288.76 mg AEAC/100 g FW and 99.14 mg AEAC/100 g FW, correspondingly (Table3).

Table 2: Vitamins and resistant starch (RS) in dry seeds endosperm of *E. ferox*

S. No.	Parameters	Quantity
1.	Vitamin B1	1.14 ± 0.02(mg/100 g)
2.	Vitamin C	5.4 ± 0.04 (mg/100 g)
3.	Amylose content	17.2 ± 0.15 (%) (RS)
4.	Energy value	362.5 ± 1.19 (kcal /100 g)

Results were expressed as mean of triplicate measurements ± SD. Significance at (p<0.05).

Table 3: Antioxidant depiction in Makhana on fresh weight basis (FW), expressed as ascorbic acid equivalent antioxidant capacity (AEAC)

S. No.	Parameters	Quantity (mg AEAC/100 g FW)
1.	DPPH value	288.76 ± 0.09
2.	FRAP value	99.14 ± 0.16

Results were expressed as mean of quadruplicate measurements ± SD. Significance at (p<0.05).

3.4 Amino acids

Makhana seed endosperm contained maximum amount of amino acids as follows: aspartic acid (8.25 ± 0.19 g/100 g/p), glutamic acid (19.70 ± 0.06 g/100 g/p), serine (5.45 ± 0.24 g/100 g/p), histidine (3.62 ± 0.11 g/100 g/p), glycine (3.90 ± 0.03 g/100 g/p), arginine (12.70 ± 0.21 g/100 g/p), alanine (7.12 ± 0.26 g/100 g/p), tyrosine (2.40 ± 0.06 g/100 g/p), cysteine (0.12 ± 0.02 g/100 g/p), valine (6.24 ± 0.09g/100 g/p), methionine (3.43 ± 0.14 g/100 g/p), phenylalanine (5.60 ± 0.13 g/100 g/p), isoleucine (5.03 ± 0.17 g/100 g/p) and leucine (9.51 ± 0.07 g/100 g/p) (Table 4).

Table 4: Amino acid profiles of dry seeds endosperm of *E.ferox*

S. No.	Amino acids	Quantity of amino acids(g/100 g/p)
1.	Alanine	7.12 ± 0.26
2.	Aspartic acid	8.25 ± 0.19
3.	Arginine	12.70 ± 0.21
4.	Cysteine	0.12 ± 0.02
5.	Glycine	3.90 ± 0.03
6.	Glutamic acid	19.70 ± 0.06
7.	Histidine	3.62 ± 0.11
8.	Isoleucine	5.03 ± 0.17
9.	Methionine	3.43 ± 0.14
10.	Proline	3.25 ± 0.05
11.	Valine	6.24 ± 0.09

12.	Phenylalanine	5.60 ± 0.13
13.	Serine	5.45 ± 0.24
14.	Tyrosine	2.40 ± 0.06
15.	Leucine	9.51 ± 0.07
16.	Lysine	3.10 ± 0.11
17.	Tryptophan	n.d.

Results were expressed as mean of triplicate measurements ± SD. Significance at (p<0.05). (g/p= gram of protein).

3.5 Proximate composition

Makhana seed kernel contained 9.70 ± 0.62% protein, 0.7 ± 0.03% crude fibre, 0.31 ± 0.01% total fat and 75.7 ± 1.74% carbohydrates as shown in Table 5. The moisture and ash content of Makhana seed kernel were 11.15 ± 0.43% and 0.52 ± 0.02%, respectively.

Table 5: Nutritional composition of Makhana dry seeds endosperm

S. No.	Parameters	Proximate composition [(%) by weight]
1.	Crude fiber	0.7 ± 0.03
2.	Total ash	0.52 ± 0.02
3.	Protein (N*6.25)	9.70 ± 0.62
4.	Moisture	11.15 ± 0.43
5.	Carbohydrates (by difference)	75.7 ± 1.74
6.	Total fat	0.31 ± 0.01

Results expressed as mean of triplicate measurements ± SD.Significance at (p<0.05).

3.6 Nutrient elements content in Makhana

Data pertaining to Table 6 revealed that the magnesium content of gorgon nut seed was 20.00 ± 1.09 mg/100 g.The sulphur content was 32.00 ± 1.34 g/100 g.The calcium content was 342.00 ± 4.86 mg/100 g. Sodium and K content of the Makhana seed kernels were 45.0 ± 0.7 mg/100 g and 78.4 ± 0.05 mg/100 g, respectively. The important elements for insulin control, *i.e.*, Zn and Fe present in Makhana seeds were 5.6 ± 0.24 mg/100 g and 10.9 ± 0.13 mg/100 g, respectively (Table 6).

Table 6: Nutrient elements in dry seed endosperm of *E.ferox*

S. No.	Parameters	Quantity of nutrient elements
1.	Calcium (mg/100 g)	342.00 ± 4.86
2.	Magnesium (mg/100 g)	20.00 ± 1.09
3.	Sulphur (mg/100 g)	32.00 ± 1.34
4.	Potassium (mg/100 g)	78.4 ± 0.05
5.	Sodium (mg/100 g)	45.0 ± 0.7
6.	Zn (mg/100 g)	5.6 ± 0.24
7.	Fe (mg/100 g)	10.9 ± 0.13

Results expressed as mean of triplicate measurements ± SD. Significance at (p<0.05).

3.7 Presence of selenium and chromium as antioxidant

Significant quantities of selenium (0.06 ± 0.02 mg/kg) and chromium (1.05 ± 0.06 mg/kg) were found in dry kernel powder of Makhana (Table 7).

Table 7: Different antioxidant metals (selenium and chromium) in dry seeds endosperm of *E. ferox*

S. No.	Parameters	Quantity (mg/kg)
1.	Selenium	0.06 ± 0.02
2.	Chromium	1.05 ± 0.06

Results expressed as mean of quadruplicate measurements ± SD. Significance at (p<0.05).

4. Discussion

4.1 Presence of flavonoids in Makhana seed kernel

Quercetin, a polyphenolic compound, is most prevalent flavonoid that occurs in edible vegetables, fruits, wine, as well as nuts. The UHPLC chromatogram of *E. ferox* seed endosperm indicated the presence of quercetin at 0.422 mg/kg and kaempferol at 0.200 mg/kg in their pure forms (Table1), without any other derivatives of two flavonoids. As numerous other peaks have been identified in Figure 1, seed endosperm may contain additional glucosidic compounds as well as esters. Besides quercetin, these seeds may contain kaempferol glucosides, glucuronides, or ester compounds. With the help of modification of GLUT4, flavonoid has been proven to promote insulin uptake (Hajiaghaalipour *et al.,* 2015). GLUT4 (Glucose Transport Type-4) detected in skeletal muscle cells, adipocytes, along with cardiomyocytes (Giordano *et al*., 2014), is primarily accountable for insulin-stimulated glucose absorption in muscle as well as adipose tissues. Around eighty percent of glucose is transported into muscle cells. Recent research indicates that flavonoids may demonstrate hyperglycemic activity by inhibiting carbohydrate-hydrolyzing enzymes, including amylase, glucosidase, as well as disaccharidases (Barber *et al*., 2021). These results are corroborated by Zhu *et al.* (2017) in another aquatic nut; namely, lotus nut that had similar flavonoids. Kaempferol stimulates insulin secretion than enhances Akt activity by modulating mitochondrial calcium uptake (Yang *et al*., 2022). Kaempferol may improve glycoprotein anomalies associated with a higher risk of diabetes mellitus (Chandramohan *et al*., 2014). These two flavonoids increase insulin sensitivity and act as free radical scavengers in the cell. Thus, the amino acids in seeds are a good health indicator. Flavonoids can directly interact with proteins, which includes essential cellular receptors or signalling pathway components, affecting cell and tissue functions. Flavonoids reduce insulin resistance in insulin-sensitive tissues by regulating insulin signalling pathways. According to Sheikh *et al.* (2024), adding plants which have bioactive compound to one's diet or as part of a treatment plan may be a practical way to manage diabetes and its consequences. They suggested that phytochemicals or bioactive compound had antidiabetic effect.

4.2 Vitamins in Makhana seed kernel

Vitamin B1 and vit. C content were 1.14 ± 0.02 (mg/100 g) and 5.4 ± 0.04 (mg/100 g), respectively (Table 2). According to established research findings, vitamin B1 can help diabetics with their blood pressure and cardiac problems as well as their insulin and blood sugar levels (Beltramo *et al*., 2021). Additionally, thiamine can assist control blood sugar levels by increasing them and decreasing them when necessary. Indeed, vitamin C can lower the risk of diabetes and halt the disease's progression in those who already have type 2 diabetes. Vitamin C can assist in lowering HbA1c, fasting insulin, and blood glucose levels and helps lower oxidative stress indicators. People having diabetes typically have reduced amounts of vitamins A, C, and E, which are antioxidants (Samer, 2024).

4.3 Antioxidants in Makhana seed kernel

Antioxidants are primarily characterized as polyphenolic compounds capable of delaying, inhibiting, or preventing oxidation of susceptible materials by neutralizing free radicals (ROS), thus decreasing cellular oxidative stress. A potential antioxidant capacity of compound may be indicated by its reducing capacity. Antioxidant molecules would readily donate an electron or hydrogen to the DPPH free radical, converting it into a stable diamagnetic molecule (Tumilaar *et al*., 2024). However, high chlorogenic acid content along with decreased ferric ion might represent cause of FRAP values. Makhana had highest DPPH and FRAP values of 288.76 ± 0.09 mg AEAC/100 g FW, and 99.14 ± 0.16 mg AEAC/100 g FW, respectively (Table 3). Therefore, it can be concluded that Makhana is highly packed with nutrients and antioxidants which is helpful in maintaining good health. In general, DPPH had higher antioxidant concentration efficiency than FRAP (Lima *et al*., 2019). In a different study, Nair and Maheshwari (2024) found that the ethanolic extract of *Syzygium zeylanicum* (L.) DC, leaf extract exhibited the best antioxidant activity after the DPPH and FRAP testing. They suggested that both the methods for antioxidant analysis are valid.

4.4 Amino acids content in Makhana

From the present study, it was evident that makhana seed endosperm had different high value amino acids like arginine, histidine, proline, *etc*. Results were in conformity with the findings of Jana and Idris (2018). Research results from different experiments in life sciences have shown that arginine can reduce insulin resistance, *i.e.* Insulin sensitivity can be increased (Forzano *et al*., 2023). Arginine is essential for the insulin sensitivity of cells in the body. Arginine functions as a precursor to nitrogen oxide, signalling molecule that directly influences insulin sensitivity. Furthermore, arginine expands blood vessels and lowers blood pressure. The findings contradicted those of McRae (2016). Additional intake of carnitine may also reduce LDL cholesterol levels in diabetic patients. l-carnitine is a substance that is produced by the body from methionine as well as lysine that is also present in food, mostly in meat products. In the present experiment, the appreciable amount of lysine 3.10 ± 0.11 g/100 g/p and methionine (3.43 ± 0.14 g/100 g/p) were found in the seed endosperm of Makhana.

4.5 Proximate compositions of Makhana seed kernel

The proximate composition of Makhana seed kernels presented in Table 5 were similar as that of value reportedby Kumar *et al*. (2016), Jana *et al*. (2021); Kumar (2023). According to Kumar *et al*. (2016) Makhana seeds contain 8.7% protein, 0.5% crude fibre and 57.0% carbohydrates. Jana *et al*. (2021) also observed that Makhana seed kernel contained 10.5% protein and 0.5% fibre. The appreciable amount of protein and crude fibre and low amount of fat are prerequisite for balanced and nourished food.

4.6 Nutrient elements in Makhana seed kernel

Numerous antidiabetic biomolecules have been identified in gorgon nuts (*E. ferox*) including flavonoids, protein, vitamins and nutrition components, particularly Mg^{2+}. A high blood serum magnesium level increases insulin sensitivity in individuals in general. Foods that contain fermentable fructo-oligosaccharide (FOS) and vitamin B1 help human cells absorb magnesium more efficiently. High dietary Ca^{2+} intakes also have the same effect as these are divalent cations. As per the data in Table 6, the gorgon nut seed had a magnesium content of 20.00 ± 1.09 mg/100 g. The value was lower than in the previous research.This could be because the moist seed had higher moisture content. According to the current research, Makhana had a moderate level of Mg^{2+}, which could help treat diabetes and lessen magnesium deficiency. In majority of instances, the body's ability to absorb magnesium is dependent on the amount of thiamine present. Thiamine required ATP as well as magnesium to operate with the enzyme thiamine pyrophosphokinase to produce TDP (Maguire *et al*., 2018). Human obesity is associated with a deficiency in serum magnesium (Lu *et al*., 2020). The amount of amylose (resistant starch), which functions as FOS in the large intestine, was 17.2 ± 0.15% (RS) in the current investigation. In another experiment, Syed and Singh (2013) observed that lotus seed's amylose content varied from 18.75-20.84 per cent. It has been reported that fermentable dietary fibre, that includes fructo-oligosaccharides (FOS), increases absorption of Mg^{2+} in humans by 10-25%; however, underlying mechanisms are still uncertain. The high concentration of total divalent cations (Ca and Mg) in Makhana seeds improves human health and lowers the complications associated with diabetes (Jayanthi *et al*., 2017). Minerals as well as trace elements, considered micronutrients that are only present in trace amounts, are necessary for human health. They do, however, have distinct biological roles. Numerous health issues in humans are linked to deficiencies in certain micronutrients (Dubey *et al.,* 2020). Black wheat (endosperm) eating as part of the dietary regimen is advised for health advantages which lowers diabetes problems due to its high INQ for protein and dietary fiber and its strong nutritional content (Goel *et al*., 2024). As per Kumar *et al.* (2016), Makhana is incredibly nutrient-dense super food. Regarding mineral composition, Makhana had the highest phosphorus content (79.1 mg/100 g) and the highest potassium content (56.0 mg/100 g).

4.7 Presence of selenium and chromium as antioxidant in Makhana

The occurrence of heavy metals that consist of selenium as well as chromium is essential, as they operate as antioxidants that enhance insulin sensitivity in diabetic patients. The appreciable limit of selenium (0.06 ± 0.02 mg/kg) and chromium (1.05 ± 0.06 mg/kg) were present in dry kernels of Makhana (Table 7). Selenium and chromium as antioxidants increase insulin sensitivity in the cell. The generally permissible limit for heavy metals is 1-2 ppm (WHO,1996). Soil andwater must be devoid of heavy metal contamination, *i.e.*, toxicity. zinc, copper, as well as iron constitute vital minerals necessary for the proper structure, function and proliferation of cells through various biomolecules. Abnormal Zn, Cu, and Fe metabolism can result in a variety of chronic pathogenesis, including diabetes and diabetic complications. In cases of Cu and Fe overload as well as Zn and Cu deficiency, these pathogenic conditions appeared to be more prevalent. Significant amounts of Zn and Fe have been detected in the Makhana seed endosperm, with concentrations of 5.60 ± 24 mg/100g and10.9 ± 0.13 mg/100 g, respectively (Table 6). Human health depends on minerals and trace elements, which are micronutrients that can be obtained in trace amounts. Nonetheless, they play unique biological roles. Numerous health issues in humans have been associated to deficits of specific micronutrients (Sarma and Saha, 2024).

5. Conclusion

Makhana kernel powder is very effective against diabetes and its complications by virtue of being the rich source of flavonoids, vitamins, amino acids and antioxidants. Nitric oxide is produced by the breakdown of arginine in human cells, which enhances blood flow. Through improved insulin sensitivity and reduced LDL cholesterol, the secondary amino acid carnitine protects cells from oxidative stress and glucose transport. The amino acids valine, leucine, and isoleucine lower blood sugar levels. A diet high in fiber, low in sodium, and high in potassium (antihypertensive), $Mg^{2+}$and selenium (antioxidant) is highly desirable. Furthermore, quercetin and kaempferol, which are pure flavonoids that affect the rapid metabolism of glucose, are relatively prevalent in the Makhana seed endosperm. Due to its nutrients and antidiabetic compounds, Makhana (*E. ferox*) is a nutrient-dense medicinal superfood.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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