Original Article

Chemical Composition, Biochemical Activity of Black Rice Yeast Extract, and Their Potential as Anti-diabetic

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Abstract

Background: Metabolic disorders are characterized by high glucose levels (diabetes mellitus/DM) and have a very high prevalence, globally. Utilization of natural ingredients having anti-diabetic potential is one solution to these disorders. The purpose of this research was to study the chemical and biochemical content of black rice yeast extract and its potential as an anti-diabetic by analyzing its activity as an α–amylase inhibitor. **Materials and Methods:** First, maceration technique was used with distilled water as a solvent to produce black rice yeast extract (BRY-E). This was followed by profiling of chemical components of BRY-E and antioxidant activity test using 2,2-diphenyl-1-pikrilhidrazil (DPPH method). Finally, α–amylase inhibition value was determined **Results:** Total 55 compounds were identified in black rice yeast extract using the liquid chromatography–mass spectrometry (LC-MS). These included flavonoids, phenolics, antioxidants and other components. The IC_{50} of BRY-E or inhibition concentration that can dampen 50% of free radicals was found to be 150µg/ml. The IC_{50} of BRY-E as α–amylase inhibitor or concentration that can inhibit 50% of α–amylase activity was 1903µg/ml. Acarbose was used as a positive control in this study (IC_{50}=0.24µg/ml). **Conclusion:** BRY-E has potency as an anti-diabetic, although the value is still small as compared to acarbose.

Keywords: α–amylase inhibitor, antioxidant, biochemical, extract of black rice yeast, antidiabetic

Introduction

Black rice is composed of 10.60% protein, 2.43% crude fiber, 11.00% water, 13.26% starch, 20x10^{-4}% chromium[1], anthocyanins, and pro-anthocyanidins that are concentrated in the bran layer.[2] Several studies reveal that bran black rice has anti-diabetic abilities.[3,4] Black rice yeast is grown or fermented using black rice flour media. To reduce its starch content, fermentation is carried out. Black rice yeast obtained from fermentation is extracted using distillate water as a solvent to produce black rice yeast extract (BRY-E). Studies have shown that fermented black rice or black rice yeast can lower glucose levels in mice; hence it has the potential to be used as an active compound in medicines to treat diabetes mellitus (DM).[1]

According to available data, the number of people with diabetes will reach around 700 million by 2045.[5] This disorder can be triggered by oxidative stress, a condition caused by an increase in production of free radicals or reduction of antioxidant defense activity or both.[6] This condition can affect the presence of macromolecules in the body which ultimately trigger the emergence of various degenerative diseases including DM.[7] This disease is characterized by blood glucose levels ≥ 126 mg/dl in fasting conditions.[8] One of the therapeutic approaches in diabetes is to reduce blood sugar levels i.e. by inhibiting the amylase enzyme that hydrolyzes carbohydrates in the digestive system so that glucose absorption becomes slow. This enzyme has the ability to hydrolyze α-(1-4) glycosidic bonds in polysaccharides, such as starch and produce short chains i.e., dextrins and maltose. In humans, this enzyme is produced by the pancreas,

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known as pancreatic amylase, and found in the small intestine. Acarbose having IC_{50} of 5.5µM, is one of the synthetic drugs that have been used as antidiabetic and have the ability to inhibit α–amylase.[9] Moreover, fermented beverages can also reduce or protect against hyperglycemic conditions by reducing blood glucose level.[10]

Several studies have revealed that many compounds that have the potential to act as α-amylase inhibitors have been isolated from natural ingredients, including metformin from *Galega officinalis*[11], endophytic bacteria from Annona muricata leaves[12] and mahogany seed (*Swietenia mahagoni* (L.) Jacq.[13] Several isolated phytochemical groups also have enzyme inhibitory activity including alkaloids, sesquiterpenes, polysaccharides, flavonoids, fiber, ferulic acid, tannins, limonene and oleuropeoside.[9] Similarly, brown rice yeast has also been reported to have anti-DM activity in experimental animals.[1] This is associated with the presence of bioactive compounds having phenolics, flavonoids and antioxidants. This study aimed to examine the activity of BRY-E as an α–amylase inhibitor to determine its anti-DM potential in vitro. As the potential of BRY-E as an antidiabetic depends on its chemical content, therefore this study also investigated its chemical profile.

Material and Methods

Material

To conduct this research, commercial bakery's yeast and black rice were purchased from supermarkets. In addition, 3,5-dinitro salicylic acid from Sigma-Aldrich, potassium sodium tartrate, soluble starch, maltose, $NaHPO_4$ and NaH_2PO_4 from Merck were also used. Besides, NaOH (Pa) α–amylase (China), glucoamylase (China) and 2,2-diphenyl-1-pikrilhidrazil (DPPH) were also utilized. (Moreover, 100ml of DNS (2-hydroxy-3,5-dinitrobenzoic acid) reagent was used which was composed of 1gm DNS powder, 20ml of 2N NaOH and 30gm Ka-Na tartrate.

Methods

Black rice yeast preparation

Black rice is used as a yeast growing medium by means of flouring (smoothing and sifting with a size of 100 mesh). Black rice flour was added in distilled water in a ratio of 1:4 and then gelatinized by heating and then cooling. The gel from black rice was hydrolyzed enzymatically to break down the starch. Next, yeast was added to the hydrolyzate formed in ratio of 1:1. It was then fermented for 14 days, after which freeze, dryer was used to reduce the water to obtain black rice yeast (BRY).

Extraction of the BRY-Extract

The BRY was extracted by the maceration method with distillate water solvent for 24 hours and was repeated 3 times. Later, the extract was filtered and concentrated using a freeze dryer to obtain pure extract of BRY (BRY-E).

Chemical profiling

LC-MS instrument was used to evaluate the chemical composition of BRY-E.. The column used in the procedure was Shimadzu Shim Pack FC-ODS (2mm × 150mm, 3µm), while the injection volume was 1 µL. the procedure was carried out at 35°C for 1 hour with the flow rate of 0.5ml/min. Moreover, 95% ethanol was used as a solvent. The LC-MS test resulted in a chromatogram profile based on which, the compounds were identified.

Determination of biochemical activity

Two biochemical activities of BRY-E were tested in this study i.e. antioxidant and α–amylase inhibitor, where the former activity was determined by usingDPPH.[14,15] The viscous extract was dissolved in methanol p.a in five different concentrations i.e. 10, 20, 40, 60 and 160µg/ml. Then 2ml of sample solution of each concentration was added in a dark vial followed by the addition of 0.004% DPPH solution (2ml). The sample solution was incubated at 37°C in a dark room for 30 minutes and its absorbance was measured using a UV-Vis spectrophotometer at λ 515 nm. For control solution, the same steps were carried out by replacing the sample solution with methanol p.a. Using absorbance values of control (A_c) and BRY-E sample (A_s), the inhibition percentage (%I) was calculated by the following equation 1.

$$\% I = \frac{A_c - A_s}{A_k} \times 100\%$$

The value of %I and the concentration of BRY-E were mapped to obtain a linear equation which was then used to calculate the IC_{50} value of BRY-E.

The activity of α-amylase inhibition was determined by dissolving α-amylase (0.025mg) into 1ml of phosphate buffer solution (pH 6.9; 0.025M).[16] Initially, three test tubes of sample solution, control and positive control were labeled. After that, 1ml of BRY-E (800-2500µg/ml) was put into the sample tube followed by the addition of 0.5ml α–amylase (1 unit/ml). The mixture was then pre-incubated at 37°C for 30 minutes and vortexed. Then, 1 mL of starch solution (1% w/v) was added and incubated at 37°C for 15 minutes. Later, 2ml of DNS reagent was added to the mixture and heated in a water bath for 5 minutes. After cooling, the solution was tested using UV-vis spectrophotometer at λ 540 nm. Preparation of the control solution was carried out with same protocol, only the α-amylase was replaced with distilled water. Positive controls were also prepared according to the previous procedure but BRY-E was replaced with 1% acarbose[17] using a variation of 5-60µg/ml concentration. The %I was calculated by the following equation 2:

$$Inhibition\ \%= \frac{Abs_{540}\ (\text{control}) - Abs_{540}(\text{BRY-E})}{bs_{540}\ (\text{control})} \times 100\%$$

Where Abs_{540} (control) and Abs_{540} (BRY-E) are absorbance without sample and with sample BRY-E, respectively, and were measured at λ 540 nm. After that, linear regression equation was created by mapping %I against concentration. By using the obtained equation, the IC50 value was determined. In these calculations, acarbose was used as a reference amylase inhibitor.

Results and Discussion

The profiling of chemical components of BRY-E

The results of the BRY-E chromatogram are presented in Figure 1 and chemical components of BRY-E are shown in Table 1.

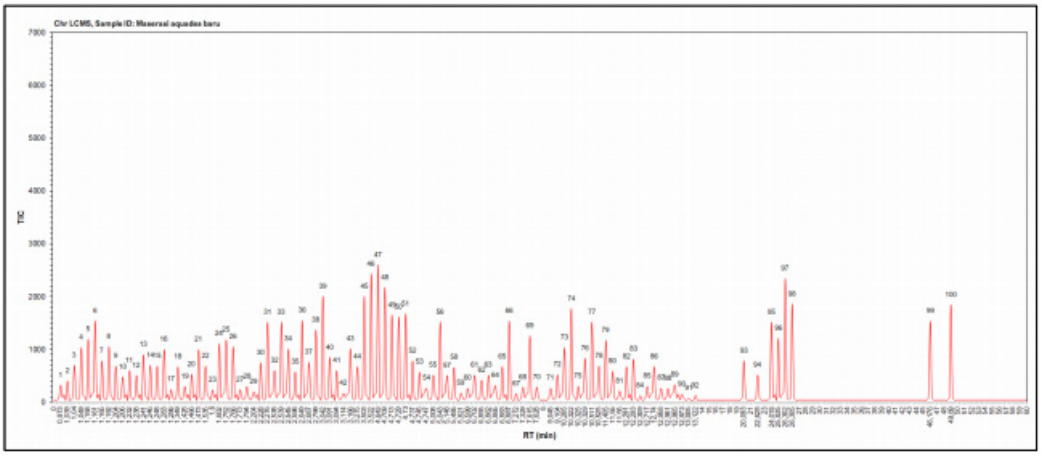
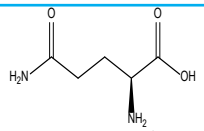
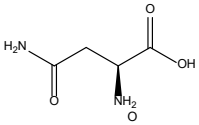
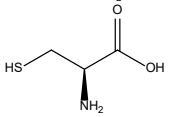
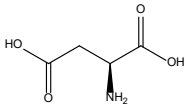
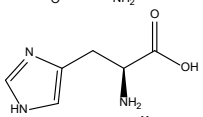
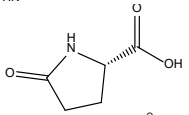
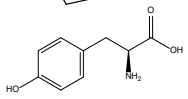
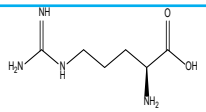
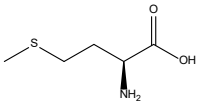
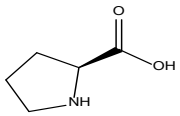
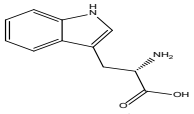
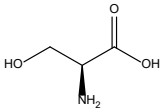
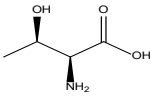
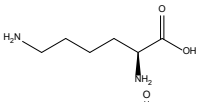
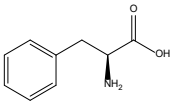
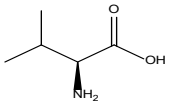
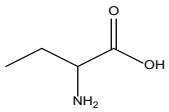
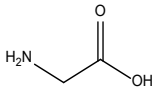
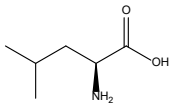
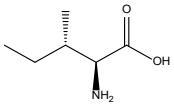
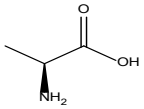
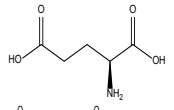
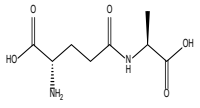
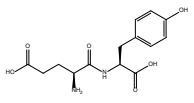
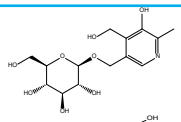
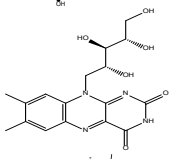
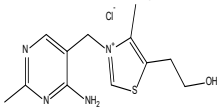
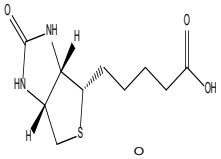
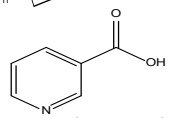
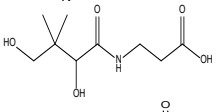
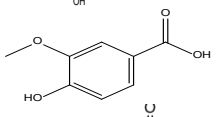
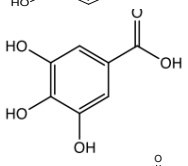
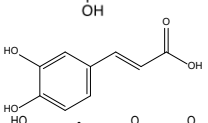
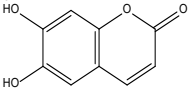
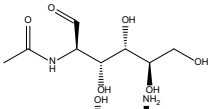
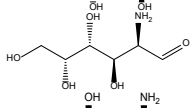
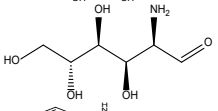
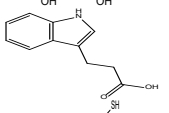
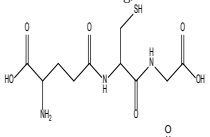
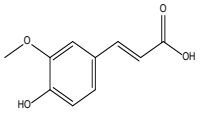


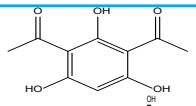
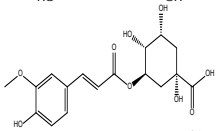
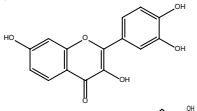
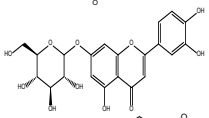
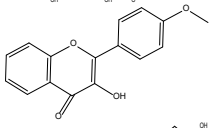
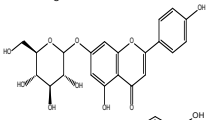
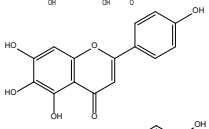
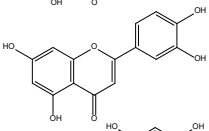
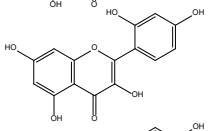
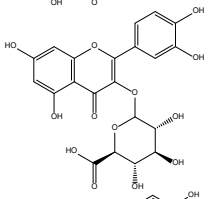
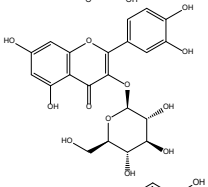
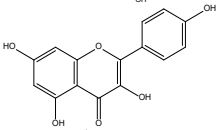
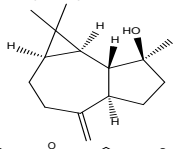
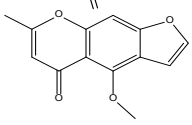
Figure 1 The chromatogram of BRY-E by Liquid chromatography–mass spectrometry (LC-MS) instrument. The abscissa is the retention time (RT): the time it takes for the compound to be excited, and the ordinates represent mAU (milli absorbance units).

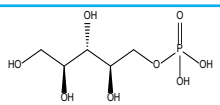
Table 1 The profile of BRY-E chemical components

Comp. No.	Retention time (Min.)	Composition (%)	Compound Result		Description
			Analysis (chemical formula/ EM/MW)	Identified Compounds	
	2.204	0.23873	Glutamine/$C_5H_{10}N_2O_3$/EM: 146.07/ MW: 146.14 m/z: 146.07 (100.0%)		Amino acids and their derivatives
	1.773	0.28464	Asparagine/$C_4H_8N_2O_3$/EM: 132.05/MW: 132.12 m/z: 132.05 (100.0%) and 133.06 (4.5%)		
	1.286	0.29074	Cysteine/$C_3H_7NO_2S$/EM: 121.02/ MW: 121.16 m/z: 121.02 (100.0%). 123.02 (5.0%) and 122.02 (4.5%)		
	1.794	0.35525	Aspartic acid/$C_4H_7NO_4$/EM: 133.04/ MW: 133.10 m/z: 133.04 (100.0%) and 134.04 (4.6%)		
	2.598	0.67870	Histidine/$C_6H_9N_3O_2$/EM: 155.07/ MW: 155.15 m/z: 155.07 (100.0%) and 156.07 (7.7%)		
	1.466	0.62799	Pyroglutamic acid/$C_5H_7NO_3$/EM: 129.04/MW: 129.11 m/z: 129.04 (100.0%) and 130.05 (5.6%)		
	4.745	0.67480	Tyrosine/ $C_9H_{11}NO_3$/EM: 181.07/ MW: 181.19 m/z: 181.07 (100.0%) and 182.08 (9.7%)		

Comp. No.	Retention time (Min.)	Composition (%)	Compound Result		Description
			Analysis (chemical formula/ EM/MW)	Identified Compounds	
	3.094	0.70805	Arginine/$C_6H_{14}N_4O_2$/EM: 174.11/ MW: 174.20 m/z: 174.11 (100.0%), 175.12 (6.7%), 175.11 (1.5%)		
	2.538	0.70806	Methionine/$C_5H_{11}NO_2S$/EM: 149.05/ MW: 149.21 m/z: 149.05 (100.0%), 150.05 (6.6%), 151.05 (4.6%)		
	1.232	0.70809	Proline/$C_5H_9NO_2$/EM: 115.06/MW: 115.13 m/z: 115.06 (100.0%), 116.07 (5.6%)		
	5.485	0.77631	Tryptophan/ $C_{11}H_{12}N_2O_2$/EM: 204.09/ MW: 204.23 m/z: 204.09 (100.0%), 205.09 (12.7%)		
	1.204	0.80545	Serine/$C_3H_7NO_3$/EM: 105.04/MW: 105.09 m/z: 105.04 (100.0%), 106.05 (3.4%)		
	1.248	0.80589	Threonine/$C_4H_9NO_3$/EM: 119.06/ MW: 119.12 m/z: 119.06 (100.0%), 120.06 (4.9%)		
	2.228	0.87966	Lysine/$C_6H_{14}N_2O_2$/EM: 146.11/ MW: 146.19 m/z: 146.11 (100.0%), 147.11 (6.7%)		
	2.687	0.89057	Phenylalanine/$C_9H_{11}NO_2$/EM: 165.08/ MW: 165.19 m/z: 165.08 (100.0%), 166.08 (10.2%)		
	1.241	1.05792	Valine/$C_5H_{11}NO_2$/WM: 117.08/ MW: 117.15 m/z: 117.08 (100.0%), 118.08 (5.9%)		
	1.283	1.17512	α-aminobutyric acid/$C_4H_9NO_2$/EM: 103.06/MW: 103.12 m/z: 103.06 (100.0%), 104.07 (4.5%)		
	1.049	1.21727	Glycine/$C_2H_5NO_2$/EM: 75.03/MW: 75.07 m/z: 75.03 (100.0%), 76.04 (2.3%)		
	1.766	1.23244	Leucine/$C_6H_{13}NO_2$/EM: 131.09/ MW: 131.17 m/z: 131.09 (100.0%), 132.10 (6.7%)		
	1.752	1.38719	Isoleucine/$C_6H_{13}NO_2$/EM: 131.09/MW: 131.17 m/z: 131.09 (100.0%), 132.10 (6.7%)		
	1.158	1.40213	Alanine/$C_3H_7NO_2$/EM: 89.05/: 89.09 m/z: 89.05 (100.0%), 90.05 (3.4%)		
	2.276	1.77766	Glutamic acid/$C_5H_9NO_4$/EM: 147.05/ MW: 147.13 m/z: 147.05 (100.0%), 148.06 (5.7%)		
	6.702	0.60315	γ-glutamyl alanine/$C_8H_{14}N_2O_5$/EM: 218.09/: 218.21 m/z: 218.09 (100.0%), 219.09 (9.6%), 220.09 (1.1%)		
	11.55	0.68852	Glutamyl tyrosine/$C_{14}H_{18}N_2O_6$/EM: 310.12/MW: 310.30 m/z: 310.12 (100.0%), 311.12 (15.6%), 312.12 (2.4%)		

Comp. No.	Retention time (Min.)	Composition (%)	Compound Result		Description
			Analysis (chemical formula/ EM/MW)	Identified Compounds	
	11.55	0.25568	Pyridoxine-β-glucoside/ $C_{14}H_{21}NO_8$/EM: 331.13/MW: 331.32 m/z: 331.13 (100.0%), 332.13 (15.7%), 333.13 (2.8%)		
	12.958	0.31839	Riboflavin/$C_{17}H_{20}N_4O_6$/EM: 376.14/ MW: 376.36 m/z: 376.14 (100.0%), 377.14 (20.3%), 378.14 (3.1%)		
	9.045	0.31845	Thiamin/$C_{12}H_{17}ClN_4OS$/EM: 300.08/ MW: 300.81m/z: 300.08 (100.0%), 302.08 (36.8%), 301.08 (15.3%), 303.08 (5.6%), 304.07 (1.5%), 302.09 (1.0%)		Vitamin
	7.329	0.33983	Biotin/$C_{10}H_{16}N_2O_3S$/EM: 244.09/ MW: 244.31 m/z: 244.09 (100.0%), 245.09 (12.7%), 246.08 (4.5%), 246.09 (1.3%)		
	1.428	0.35506	Niacin/$C_6H_5NO_2$/EMss: 123.03/ MW: 123.11m/z: 123.03 (100.0%), 124.04 (6.6%)		
	6.855	0.49925	Pantothenic acid/$C_9H_{17}NO_5$/EM: 219.11/ MW: 219.23 m/z: 219.11 (100.0%), 220.11 (10.3%), 221.11 (1.1%)		
	2.799	1.60717	Vanillic acid/$C_8H_8O_4$/EM: 168.04/ MW: 168.15 m/z: 168.04 (100.0%), 169.05 (8.9%), 170.05 (1.2%)		
	3,042	2,35322	Galic acid/ $C_7H_6O_5$/EM:170.0215/ MW:160.120 m/z (100.0%), 171.0249 (7.6%), 172.0258 (1.0%)		Fenolic
	4.643	3.04869	Caffeic acid/$C_9H_8O_4$/EM: 180.04/: 180.16 m/z: 180.04 (100.0%), 181.05 (10.0%), 182.05 (1.3%)		
	3.275	0.79199	Esculetin/$C_9H_6O_4$/EM: 178.03/MW: 178.14 m/z: 178.03 (100.0%), 179.03 (10.0%), 180.03 (1.3%)		Esculetin (derivad of Coumarin)
	6.887	1.80034	N acetylglucosamine/ $C_8H_{15}NO_6$/ EM: 221.09/MW: 221.21 m/z: 221.09 (100.0%), 222.09 (9.3%), 223.09 (1.3%)		
	3.503	2.35218	Galactosamine/$C_6H_{13}NO_5$/EM: 179.08/ MW: 179.17 m/z: 179.08 (100.0%), 180.08 (7.0%), 181.08 (1.1%)		Amino Sugar
	3.522	2.84977	Glucosamine/$C_6H_{13}NO_5$/EM: 179.08/ MW.0%), 180.08 (7.0%), 181.08 (1.1%)		
	4.747	0.31693	3-indolepropionic acid/$C_{11}H_{11}NO_2$/ EM: 189.08/MW: 189.21 m/z: 189.08 (100.0%), 190.08 (12.3%)		
	10.511	1.77665	Glutathione/$C_{10}H_{17}N_3O_6S$/EM: 307.08/ MW: 307.32 m/z: 307.08 (100.0%), 308.09 (11.2%), 309.08 (4.7%), 308.08 (1.9%), 309.09 (1.9%)		Antioxidant
	5.043	1.78084	Ferulic acid/$C_{10}H_{10}O_4$/EM: 194.06/ MW: 194.18 m/z: 194.06 (100.0%), 195.06 (11.1%)		

Comp. No.	Retention time (Min.)	Composition (%)	Compound Result		Description
			Analysis (chemical formula/ EM/MW)	Identified Compounds	
	5.821	0.21092	2.4-diacetylphloroglucinol/ $C_{10}H_{10}O_5$/ EM: 210.05/MW: 210.18 m/z: 211.06 (11.1%), 212.06 (1.6%)		2.4-diacetylphloroglucinol (Antibiotics)
	12.717	0.35934	3-O-feruloylquinic acid/$C_{17}H_{20}O_9$/ EM: 368.11/MW: 368.34 m/z: 368.11 (100.0%), 369.11 (18.7%), 370.11 (1.8%), 370.12 (1.7%)		3-O-feruloylquinic acid (derived of quinic acid)
	10.325	0.36032	Fisetin/$C_{15}H_{10}O_6$/EM: 286.05/MW: 286.24 m/z: 286.05 (100.0%), 287.05 (16.6%), 288.05 (2.5%)		
	22.628	0.60943	Luteolin 7 glucoside/$C_{21}H_{20}O_{11}$/EM: 448.10/MW: 448.38 m/z: 448.10 (100.0%), 449.10 (23.1%), 450.11 (2.6%), 450.10 (2.3%)		
	9.104	0.61803	3-hydroxy-4-methoxyflavone/ $C_{16}H_{12}O_4$/ EM: 268.07/MW: 268.26 m/z: 268.07 (100.0%), 269.08 (17.6%), 270.08 (2.3%)		
	20.093	0.92829	apigenin-7-glucoside/ $C_{21}H_{20}O_{10}$/ EM: 432.11/: 432.38 m/z: 432.11 (100.0%), 433.11 (23.3%), 434.11 (4.6%)		
	10.329	0.98030	Scutellarein/$C_{15}H_{10}O_6$/EM: 286.05/MW: 286.24 m/z: 286.05 (100.0%), 287.05 (16.6%), 288.05 (2.5%)		Flavonoid
	10.265	1.21529	Luteolin/$C_{15}H_{10}O_6$/EM: 286.05/: 286.24 m/z: 286.05 (100.0%), 287.05 (16.6%), 288.05 (2.5%)		
	11.405	1.37832	Morin/$C_{15}H_{10}O_7$/EM: 302.04/MW: 302.24 m/z: 302.04 (100.0%), 303.05 (16.6%), 304.05 (2.7%)		
	25.835	1.42219	Quercituron/$C_{21}H_{18}O_{13}$/EM: 478.07/MW: 478.36 m/z: 478.07 (100.0%), 479.08 (23.4%), 480.08 (5.3%)		
	24.018	1.78084	Isoquercitrin/$C_{21}H_{20}O_{12}$/EM: 464.10/ MW: 464.38 m/z: 464.10 (100.0%), 465.10 (23.4%), 466.10 (5.0%)		
	10.322	2.08331	Kaempferol/$C_{15}H_{10}O_6$/EM: 286.05/MW: 286.24 m/z: 286.05 (100.0%), 287.05 (16.6%), 288.05 (2.5%)		
	6.883	0.79600	Spathulenol/$C_{15}H_{24}O$/EM: 220.18/ MW: 220.35 m/z: 220.18 (100.0%), 221.19 (16.5%), 222.19 (1.5%)		a tricyclic sesquiterpene alcohol
	7.615	1.47467	Visnagin/$C_{13}H_{10}O_4$/EM: 230.06/ MW: 230.22 m/z: 230.06 (100.0%), 231.06 (14.3%)		derivative of chromone a furanochromone)

Comp. No.	Retention time (Min.)	Composition (%)	Compound Result		Description
			Analysis (chemical formula/ EM/MW)	Identified Compounds	
	7.628	0.34345	ribitol-5-phosphate/$C_5H_{13}O_8P$/ EM: 232.03/MW: 232.13 m/z: 232.03 (100.0%), 233.04 (5.9%), 234.04 (1.8%)		D-ribitol

The other compound Description:
EM: Exact Mass
MW: Molecular Weight

More than 55 bioactive compounds were identified in BRY-E including amino acids and their derivatives, vitamins, vanillic acid, phenolics, shikimic acid, coumarins and hydroxides. In addition, antioxidants, antibiotics, quinic acids, flavonoids, sesquiterpenoids, furanochromes and ribitol were also found. These bioactive compounds have remedial benefits; hence BRY-E has the potential to be used in therapeutic field for example, as anti-diabetic. Diabetes is characterized by hyperglycemia and is related to the presence or absence of insulin produced by β-cells.

Among the bioactive components identified in BRY-E, amino acids and their derivatives are known to be associated with insulin secretion. Glucose and amino acids influence insulin secretion by increasing the stimulus/secretion of coupling factors including ATP (Adenosin Triphosphate), GTP (Guanosin Triphosphate), cAMP (cyclic Adenosin Monophosphat) and NADPH (Nicotinamide adenine dinucleotide phosphate-hydrogen). Certain amino acids can affect insulin secretion and cell integrity;[16] for example arginine is involved in the secretion of insulin through a membrane depolarization process resulting in an increase in Ca^{2+} through the opening of Ca^{2+} channels.[17,18] Arginine can also be converted into L-glutamate and affect insulin secretion through the formation of metabolic coupling factors including ATP, NADPH, long-chain acyl coenzyme-A and diacylglycerol.[16] Glutamine is metabolized in the islets of Langerhans into L-glutamate which then donates its carbon skeleton to the tricarboxylic acid cycle and contributes to the availability of metabolic coupling factors.[19] Glutamate participates in insulin secretion by inducing stimulus-coupling factor. Moreover, co-transport of alanine and Na^+ affects depolarization of pancreatic cell membrane thereby increasing intracellular Ca^{2+}.[17]

BRY-E is also known to contain vitamin B including pyridoxine-β-glucoside (derivate of pyridoxine), riboflavin, thiamin, biotin, niacin and pantothenic acid (Table 1). Around 22% of people with T2DM are known to be deficient of vitamin B.[20] According to a study, plasma thiamine was decreased in both T1DM and T2DM.[21] Humans cannot synthesize thiamine and therefore need external source for it, such as by consuming vegetables, fruits or yeast. Table 1 shows that BRY-E contains 0.31845% thiamin which suggests it can be used as anti-diabetic. Thiamine and its derivatives are known to affect pancreatic function, improve endothelial function and reduce oxidative stress in both normal and hyperglycemic environments.[22]

Among other compounds, 0.33983% of biotin was also found in BRY-E in the present study. . Biotin is a water-soluble vitamin and acts as a prosthetic group for carboxylase, an enzyme that plays a role in carbohydrate metabolism. This vitamin has been developed as a new therapeutic alternative in the treatment of hyperglycemia.[23,24] Studies have reported biotin to decrease the expression of gluconeogenic genes in diabetic mice model. These genes are involved in the formation of glucose from non-carbohydrate carbon. Biotin not only exerts a beneficial effect on the pancreas gland, but also affects the expression of insulin and pancreatic glucokinase. Glucokinase is an enzyme that plays an important role in controlling the rate at which glucose enters the glycolytic pathway (glucose phosphorylation).[25] This enzyme also plays an important role in the storage of glucose in the form of glycogen in the liver.[26] The present study also found riboflavin (0.32%) in the composition of BRY-E.. Riboflavin (vitamin B2) acts as a precursor to flavin adenine mononucleotide (FMN) and flavin adenine dinucleotide (FAD), a coenzyme. This coenzyme plays a role in electron transfer in oxidation-reduction reactions.[27] Foods containing riboflavin can reduce the complications of T2DM triggered by oxidative stress and the formation of reactive oxygen species (ROS). Studies have depicted the effect of riboflavin on the reduction of oxidative stress, tissue damage, and cellular DNA damage in mice exposed to glucose uptake in the gut.[28] BRY-E is also known to contain niacin and pantothenic acid. Niacin is a vitamin that can reduce monocyte adhesion to endothelial cells and reducing cardiovascular risk[29], both of which are known to be associated with diabetes. Pantothenic acid functions as a substrate for the synthesis of CoA (coenzyme A) and ACP (Acyl Carrier Protein). L-serine affects the autoimmune system and blood glucose homeostasis. According to a study, blood glucose levels were found to be reduced in female diabetic rats which were given L-serine. This shows that lysine has anti-diabetic effects and its supplementation can be used in the therapeutic field for the prevention of T1DM.[30] In another study,

rats which were given streptozotocin injection were given lysine solution (2%) orally every day for 120 days. Interestingly, blood sugar levels of those rats decreased from 295 mg/dL to 99 mg/dL.[31] Similarly, isoleucine has also been reported to have an effect on blood plasma glucose in rats.[32] Likewise, alanine has also been found to have an anti-diabetic effect in alloxan-induced rats.[33]

The current study also showed that BRY-E contains 1.61% vanilic acid which according to some studies, has been reported to lower blood glucose in mice by inhibiting the activity of an enzyme known as protein-tyrosine phosphatase IB (PTPIB).[34] This enzyme functions as a negative regulator of insulin signaling by removing the phosphate group from the tyrosine residue so that the insulin receptor becomes active.[35] In addition, 0.79% esculetin compound was also found to be present in BRY-E in this study. It is a polyphenolic compound and a coumarin derivative which is widely used in the field of therapeutic and pharmacological applications. Esculetin acts as an antioxidant[36] and also inhibits adipogenesis in cells by reducing ROS. Over the past two decades, coumarins and their derivatives have attracted much interest because they have protecting effect on pancreatic cells, can increase abnormal insulin signaling, reduce oxidative stress/inflammation and inhibit α-glucosidase. Coumarins also exihibit antidiabetic activity by stimulating insulin production in pancreatic cells.[37] Studies have also shown esculetin to significantly decrease plasma glucose levels, HbA1c (hemoglobin A1c) and increase Hb levels.[38] Another study reported that oral supplementation of esculetin to diabetic rats for 45 days was effective in protecting hepatocytes and kidneys from oxidative damage.[39]

The present study also found several antioxidant compounds in BRY-E including 3-indolepropionic acid, glutathione and ferulic acid (FA) (Table 1). Amongst these, FA is a phenolic compound known to have anti-diabetic properties. Treating diabetic animals with FA can restore blood glucose, serum insulin, glucose tolerance and insulin tolerance to the normal range by stimulating glycogen synthase, increasing glucokinase activity and inhibiting glycogen phosphorylase. Hence, it can reduce hyperglycemia by suppressing gluconeogenesis and glycogenolysis.[40]

Other components of BRY-E identified in this study included gallic acid (2.35%) and phenolic acid (11.38%). These are polyphenols which have hyperglycemic effects and can reduce oxidative stress also.[41] Phenols contained in BRY-E include fisetin, luteolin 7 glucoside, 3-hydroxy-4-meth hoxyflavone, apigenin-7-glucoside, scutellarein, luteolin, morin, quercituron, isoquercitrin and kaempferol. Of these, kaempferol, quercetin and isoquercitrin are known antioxidants.[42] Kaempferol enhances the synthesis and secretion of insulin in beta cells. It also plays a role in protecting pancreatic beta cell function from the damage that leads to T2DM. Similarly, luteolin and luteolin 7 glucoside each have an anti-diabetic effect in mice through alpha-glucosidase inhibition.[43] Likewise, apigenin 7 glucoside not only stimulates insulin secretion but also acts as an antidiabetic agent through α -glucosidase inhibition. Another study showed that fisetin administration can reduce HbA1c levels, serum nitric oxide (NO), blood glucose and can inhibit cytokine production.[44]

Other compounds found in BRY-E are scutellarein (0.89%), morin (1.38%) and spathulenol (0.8%). According to a study, scutellarein can reduce hyperglycemia and hyperlipidemia in T2D rats.[45] Morin has also been proven as a bioflavonoid component that has anti-diabetic properties. Moreover, it also enhances insulin signaling and glucose metabolism.[46] Sesquiterpenoids also have a role in protecting pancreatic cells and increasing insulin secretion hence they can function as anti-diabetic agents. In liver, adipose tissue and skeletal muscle, sesquiterpenoids are known to increase insulin sensitivity by regulating glucose and protein transport in insulin signaling pathways. These compounds also protect target tissues from oxidative damage and slow down the progression of T2DM.[47]

α-amylase inhibitor activity of BRY-E

The use of α-amylase inhibitor is one of the approaches in the therapy of diabetes. It inhibits the enzyme α-amylase which hydrolyzes carbohydrates in the digestive system so that glucose absorption becomes slow. Table 2 shows the activity of BRY-E in inhibiting α-amylase (%I) in varying concentrations expressed in its IC_{50} value, a concentration required for inhibition of 50% of enzyme.[48]

Table 2: α-amylase inhibition activity of BRY-E in concentration variation and their IC50 value

No.	Inhibitor Concentration (μg/mL)	% Inhibition	IC50 (μg/mL)
1	800	2	
2	1000	6	
3	1500	36	1902.994
4	2000	57	
5	2500	64	

Table 2 shows that the IC_{50} value of BRY-E is 1903 μ/ml or 1.903 mg/ml. BRY-E was extracted from brewery yeast which is known to contain *Saccharomyces cerevisiae.* The IC_{50} value obtained for BRY-E was found much better than fermented beverage products with *Lactobacillus casei* in inhibiting α-amylase activity (35.65 mg/ml).[10] Acarbose was used as a positive control whose results are presented in Table 3. The IC_{50} value of acarbose in inhibiting α-amylase activity was found to be 0.24 μg/ml.

Table 3: α-Amylase inhibition activity of acarbose in concentration variation and their IC50 value

No.	Inhibitor Concentration (μg/mL)	% Inhibition	IC50 (μg/mL)
1	5	63	0.24
2	10	64	
3	15	65	
4	20	67	
5	40	71	
6	60	73	

Alkaloids, flavonoids and phenolics are active compounds which inhibit the activity of the α-amylase enzyme which is responsible for breaking down polysaccharides into glucose which takes place in the intestine.[27,49,50] Moreover, alkaloids and flavonoids are antidiabetic agents that play a role in lowering blood glucose levels by inhibiting glucose absorption. Flavonoids from *Nelumbo nucifera* are known to have an IC_{50} value of 2.20± 0.18 mg/ml in inhibiting α-amylase.[51] This ability is related to the presence of a hydroxyl group (-OH) of phenolics and amino acid side chains (such as aspartic acid/asp197 and glutamate/Glu233) at the active site of α–amylase.[52,53] Figure 3 shows the activity of BRY-E in inhibiting α -amylase expressed by %I for various concentrations and their IC_{50} values. This IC_{50} is dependent on the assay conditions.[48]

The results of LC-MS showed that BRY-E contains 11.38% flavonoid compounds and 2.35% phenolic compounds (Table 1). According to a study, although kaempferol has less hydroxyl groups in their molecular structures, yet it has high inhibitory activity IC_{50} value between 0.5 mM–6.0mM).[54] Kaempferol was able to inhibit pancreatic α-amylase with IC50>400M. However, another study showed contrasting results i.e. kaempferol showed almost no inhibitory activity against α-amylase.[55,56] BRY-E also contains luteolin which is involved in the inhibition of α–amylase.[38,39,57] Luteolin isolated from aloevera showed inhibition of procaine pancreatic α-amylase with IC_{50} of 50–500μ/ml.[58] Several factors that determine the IC_{50} value are competition between inhibitor and the substrate at the active site of the enzyme. The concentration of inhibitor and substrate affects this competition. BRY-E is also known to contain 1.78% of quercetin (isoquercetin). It is a flavanol which is known to have the ability to competitively inhibit α–amylase.[59] A study reported that pancreatic α-amylase was inhibited by quercetin (IC_{50}=146.8μM)[57], while intestinal α-amylase was inhibited by quercetin from *Hovenia dulcis* ($IC_{50=}$μg/ml).[60] Likewise, vanillic acid and caffeic acid present in BRY-E are known to have a relatively strong inhibitory activity against α-amylase ($IC_{50=}$0.4mM).[44,61]

For comparison, acarbose ($C_{25}H_{43}NO_{18}$) was used in this research. Acarbose reduces the absorption of carbohydrates in the small intestine by inhibiting the breakdown of carbohydrates, disaccharides, sucrose into glucose or fructose, thereby reducing postprandial blood glucose. According to a study, daily fluctuations in blood glucose and glycosylated hemoglobin concentrations in T2DM patients are controlled with acarbose.[62] Table 3 shows that the IC_{50} value of acarbose in inhibiting α-amylase activity is 0.24μ/ml. Another study showed that pancreatic α-amylase activity was inhibited by acarbose with an IC_{50} value of 5.3 μM.[57] Based on the -amylase inhibitor test of aqueous extract at various concentrations, the IC_{50} value of BRY-E was found greater than that of acarbose.

Antioxidant activity of BRY-E

The DPPH method was used to test the antioxidant activity of BRY-E. Results are shown in table 4.

Table 4: Data of antioxidant activity of BRY-E

Concentration (μg/mL)	% Antioxidant	IC50 (μg/mL)
10	16.026	150
20	19.016	
40	27.234	
80	36.936	
160	50.485	

In this study, the inhibition concentration or IC_{50} of BRY-E that can dampen 50% free radicals was found to be 150μg/ml. This suggests that BRY-**E can** be included in the moderate antioxidant group. This potential of BRY-E to reduce free radicals will help in reducing cell damage that leads to degenerative disorders such as diabetes. As shown in table 1, the antioxidants identified in BRY-E include 3-indole propionic acid, glutathione and ferulic acid. These compounds can inhibit the oxidation reaction of free radicals. In free radical compounds, some electrons do not have a partner and are unstable.[6,7,42]To achieve stability, electrons react with other nearby molecules to get a partner. If this reaction takes place continuously, it ultimately can cause cell damage and risk the body to diseases like diabetes, arthritis, atherosclerosis, cancer and other degenerative diseases.[63,64]

Conclusion

More than 55 compounds were identified in BRY-E including flavonoids and phenolics. Antioxidants and the other compounds were also detected using LC-MS instrument. The IC_{50} of antioxidant was found to be 150μg/ml and that ofα–amylase of BRY-E was 1.904mg/ml. On the other hand, IC_{50} of 0.24μg/ml was obtained for the positive control used in this study i.e. acarbose. Even though its IC_{50} value was less as compared to acarbose,

BRY-E has the potential to be used as anti-diabetic. Although BRY-E used in this study was not purified, it should be purified prior using it as it is sourced from natural ingredients.

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