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Chemical Properties of Black Rice Yeast Extracts as Pharmaceutical Ingredients for the Management of Type 2 Diabetes mellitus

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ABSTRACT

Black rice yeast has the potential to be used as an antidiabetic preparation in type 2 Diabetes mellitus. The study aims to identify the chemical composition of black rice yeast extract obtained from different polar solvents as pharmaceutical ingredients for type 2 Diabetes mellitus (DM). The Black rice flour was macerated using polar solvents (distilled water, methanol, and ethanol). The extracts were investigated for the following parameters: amylum, protein and chromium (III) contents, antioxidant activity using the DDPH free radical scavenging assay method and phytochemical profiling using LC-MS. Phytochemical profiling of the different extracts, ethanol, methanol, and water extract, showed 109, 104, and 107 compounds, respectively. The highest concentration of Cr^{3+} (0.39%) was found in the freeze-dried water extract. This extract also exhibited the most significant antioxidant activity compared to others. Freeze drying may have protected the bioactive constituents in the extracts from the photo-oxidation process resulting from drying under the sun. Black rice yeast extract obtained by maceration using polar solvents could be used as pharmaceutical ingredients for type 2 *diabetes mellitus*.

Keywords: Black rice yeast extract, Polar solvent, *Diabetes mellitus*, Maceration, LC-MS.

Introduction

Black rice yeast (BR-yeast) is yeast fermented in black rice nutrients rich media. Black rice or *Oryza sativa* L. is a group of cereals commonly found in nature and is known to be rich in anthocyanins.[1] Anthocyanins are unstable and easily damaged due to pH, light, oxygen, enzymes, ascorbic acid, temperature, sulfur dioxide or sulfite salts, and metal ions.[2] Anthocyanins in black rice are mainly cyanidin-3-glucoside and peonidin-3-glucoside.[3] This compound functions as an antioxidant. Antioxidants are molecules that can slow down or prevent the oxidation process of other molecules. Oxidation is a chemical reaction that can produce free radicals, thus triggering a chain reaction that can damage cells. Age-related diseases such as atherosclerosis, *diabetes mellitus* (DM), and cancer can occur due to exposure to reactive oxygen species, therefore an antioxidant diet deserves attention for the prevention of these pathophysiological conditions. The antioxidant potentials of black rice yeast obtained from DPPH free radical assay ranged from 13.0% to 76.4%.[4]

A fermentation process can increase the nutritional value of some foods. Fermentation involves chemical changes in an organic substrate through enzyme activity produced by microorganisms grown in media. It affects the chemical composition, amino acid profile and protein digestibility[5] of the material. Therefore, it is necessary to identify fermented compounds intended to be used as pharmaceutical preparations for diabetes mellitus.

Diabetes is a chronic degenerative disease characterised by an increase in fasting blood sugar levels above normal (above 126 mg/dL). Various metabolic disorders are associated with an irregular rise in blood sugar levels. *Diabetes mellitus* is related to hormonal disturbances and responsible for multiple complications in different body organs. DM is a significant health problem. Data from global studies show that the number of DM sufferers in 2011 was 366 million people, and these numbers are expected to increase to 552 million by 2030. The International Diabetes Federation (IDF) estimates that as many as 183 million people are not aware of their diabetic status. 80% of people with DM live in low-income and middle-income countries. There are two types of this disorder, namely, type 1 DM and type 2 DM.[6]

Ancient people have been using yeast for centuries to treat DM, and it is believed to reduce blood glucose levels. Yeast is rich in protein, lipids, RNA, vitamins and minerals. It also contains the B-complex vitamins thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (7) and folic acid (B9), as well as some minerals, including chromium. Types of yeast commonly used as food ingredients include Brewer's yeast, baker's yeast and torula yeast.[7]

The results showed that BR-yeast contained chemical components, including Cr^{3+} (103 x 10^{-4}%), protein (38%), crude fiber (5.89%), and starch (6.643%). The results also revealed that yeast grown in black rice (Yeast-BR) has the potential to reduce blood glucose levels (BGL) of mice induced with Type 2 diabetes mellitus by 130 mg/dL. BR-yeast can also repair liver cell damage due to high BGL, amounting to 41%. These reports validate BR-yeast potential in the management of type 2 diabetes mellitus and liver regeneration after exposure to type 2 diabetes. The presence of starch in antidiabetic preparations has been associated with increases in blood glucose levels. Hence, extraction with various solvents coupled with filtration is one method for reducing starch concentrations in preparations intended to lower blood glucose levels. Different solvents, both polar and non-polar, extract bioactive compounds from plant sources, depending on the desired product. Polar solvents mainly extract polar constituents. Commonly used polar solvents are methanol, ethanol and distilled water. Some literature reported that a decrease in blood glucose levels might be related to chromium (III). The regulation of blood glucose levels by chromium is not well understood. Recently it has been known that chromium interacts with the Low Molecular Weight Chromium (LMWCr) substance.[8]

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The study aims to identify BR-yeast's chemical contents, including the chromium (III) content and other chemical components of the BR-yeast extract from polar solvent maceration as pharmaceutical ingredients for use in type 2 DM.

Materials and Methods

Materials

The commercial baker's yeast and Black rice used for this study were purchased from supermarkets. All reagents, Liquid amylase and glucoamylase, 96% ethanol (Merck), 96% methanol (Merck), Bradford reagent, KI and I_2 (Merck), Bovine serum albumin (Sigma), chromium (III) chloride hexahydrate (Sigma), DPPH (1,1-diphenyl-2-picrylhydrazyl) (Merck) were of analytical grades.

Preparation of black rice yeast

The black rice material was grounded to a fine flour. The flour was gelatinised through a heating process and allowed to cool, then hydrolysed using commercial amylase and glucoamylase. The hydrolyzate was added to the bakery's yeast in a ratio of 1:1. The yeast in black rice was fermented for ten days. After that, it was dried using a freeze dryer. The product obtained is black rice yeast.

Black rice yeast extraction

To obtain the BR-yeast (Y_{br}) crude extract, about 50 g of the sample was macerated with 150 mL distilled water, ethanol (96%), and methanol separately for three days. The extracts were filtered using a filter paper assisted by a vacuum pump to obtain water extract (WYbr), ethanol extract (EYbr), and methanol extract (MYbr). Subsequently, the water extract freeze-dried for 24 h, while the methanol and ethanol extracts were concentrated to dryness using a rotary evaporator.

Determination of protein content

The protein content of BR-yeast extracts was carried out using the microsession method described by[9]. A total of 0.4 ml of BR-yeast extract was reacted with 4 ml of Bradford's solution, homogenised before measuring the absorbance at a wavelength of 595 nm. Total protein content was determined based on a standard protein curve. The standard protein used is BSA (Bovine serum albumin).[10]

Determination of starch content

Starch content was determined using the Fuwa method. In this method, a standard solution of starch (starch) with concentrations of 2, 4, 8, 10% is prepared. About 250 µL of each solution was added to 250 µL of iodine solution (containing 2% KI and 0.2% I_2) and diluted with distilled water to a volume of 5 mL. The absorbance read at a wavelength of 600 nm.[11] A mapping between the standard solution's concentration and the absorbance value was made, and the regression equation determined. Each BR-yeast extract was treated the same as the standard solution. The starch content in each extract was calculated from the regression equation obtained from the standard starch solution previously determined.

Determination of chromium (III) content

The chromium (III) content of the extracts was analysed according to the method of voltammetry cyclic. It involves preparing a standard Chromium (III) chloride hexahydrate (Sigma) solution with various concentrations 31.24, 62.5, 125, 250, 500, and 1000 ppm, then values determined. The concentration of each standard solution is mapped with the current value so that the linear regression equation $Y = ax + b$ will be produced. The sample solution's values to be tested are then determined using the regression equation obtained from the standard solution. The chromium (III) concentration of the sample solution is obtained.

Determination of antioxidant activity

The antioxidant activity of the extracts was determined using the DPPH immersion method. In this method, about 50 ppm DPPH solution was prepared by weighing 5 mg of DPPH reagent and dissolved with 100 ml of absolute methanol in a volumetric flask. The extracts' antioxidant activities were determined by preparing various concentrations 10, 40, 80 ppm and 160 ppm from which aliquots (0.5 mL) was transferred into marked test tubes. To each of these test tubes, a 3.5 mL DDPH solution was added. The tubes were vortex and incubated at 37 ºC for 30 minutes in a dark room. The absorbances were measured at a wavelength of 517 nm.[12]

Determination of phytochemical contents

The phytochemical contents of black rice yeast extract was carried out using the Shimadzu LC-MS-8040 LC / MS instrument, the Shimadzu Shim Pack FC-ODS column (2 mm x 150 mm, 3 um), 1 ul injection volume, 35^{o}C column temperature, 0.5 mL flow rate / min, solvent: ethanol 95%, run time 60 minutes.

Results and Discussion

Black rice has a higher protein, vitamin and mineral content compared to other rice.[13] The components of black rice can be used in the development of yeast through the fermentation process. Generally, black rice amylum is enzymatically hydrolysed to produce glucose. Upon fermentation in the presence of yeast, the glucose is converted to ethanol and carbon dioxide. The fermentation process is followed by autolysis resulting in lysed yeast cells and their components mixed with black rice media. The mixture forms black rice yeast extract (Ybr), which is widely used in the pharmaceutical industry. The macro-nutrient analysis of Ybr is shown in Table 1.

Protein functions as a source of energy and building blocks for various enzymes and hormones which support the immune system.[14] The protein contents of the water extract of BR-yeast from the sun drying process (WYbr *) compared to that from drying with a freeze dryer (WYbr **) shows that WYbr * is higher. This difference is thought to be due to photooxidized protein residues, which affect the primary, secondary, and tertiary protein structures. Photo-oxidation can also affect the solubility of proteins in water. The protein concentration in the water yeast rice extract using sun drying was the highest compared to ethanol extract (EYbr*) and methanol extract (MYbr*). Organic solvents, such as ethanol and methanol, can lower the dielectric constant, resulting in decreased protein solubility. Amylum can be hydrolysed to form glucose which increases blood sugar levels. It is therefore not recommended for type 2 anti-DM products. The amylum concentration in WYbr** (2.39%) was higher when compared to WYbr* (0.71%). Sun-dried black rice yeast extracted with distilled water, ethanol, and methanol showed that the ethanol extract (EYbr*) had the smallest amylum concentration (0.15%). The polarity of ethanol is lower than water, thereby reducing the amylum's solubility and hydrogen bonding. Besides, ethanol can dehydrate amylum. This can be a consideration for the development of yeast preparations extracted with ethanol, which can reduce amylum content, decreasing the anti-DM 2 activity of black rice yeast.

The Cr^{3+} ion reduces the concentration of glucose in the blood and increases insulin sensitivity. The black rice yeast water extract obtained by freeze dryer (WYbr**) showed higher Cr^{3+} levels when compared to the *sun-dried yeast extract. It is thought that sunlight can cause Cr^{3+} ions to undergo photo-oxid*ation to form Cr^{4+} so that the concentration of Cr^{3+} ions decreases.

Table 1: Results of chemical analysis of extracts

Extract name	% protein	% amylum	% Cr3+	antioxidant activity (IC_{50}) ppm
WYbr**	0.14	2.39	0.39	102.74
WYbr*	0.34	0.71	0.12	123.42
EYbr*	0.16	0.15	0.12	688.53
MYbr*	0.18	1.25	0.21	260.20

* (sun drying yeast) ** (drying yeast with a freeze dryer)
Measurements were made to the dry weight of the sample

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Antioxidants are substances that can prevent the oxidation process and reduce free radicals.[15] Free radicals such as reactive oxygen species (ROS) in the body can cause DNA damage, resulting in degenerative diseases such as diabetes. Antioxidant intake can help reduce ROS.[16] Indirectly, antioxidants are associated with the prevention and reduction of diabetes.

The drying process is believed to affect black rice yeast water extract (WY_{br}*). Our study results showed that the antioxidant activity of WY_{br}* is lower when compared to the freeze-dried extract (WY_{br}**). Drying in the sun can cause antioxidant compounds to be oxidised (photo-oxidation).[17] Sun-dried black rice yeast extracted with ethanol (EY_{br}*) and methanol (MY_{br}*) also showed less antioxidant activity than WY_{br}**.

The difference in extraction solvent will affect the extracted secondary metabolite compounds. Phytochemical profiling of black rice yeast extract showed that ethanol extract contained 109 compounds, 104 compounds in the methanol extract, and 107 compounds were identified in the water extract (Table 2). The drying process's effect does not affect the amount of the extracted compound in the water solvent. Water as a solvent can extract most vitamins. In contrast, ethanol and methanol cannot extract vitamins except C. Distilled water cannot extract fructose-6-phosphate, chrysin, coumestrol, 2-carboxyarabinitol, lignoceric acid, and steroids (dolichosterone, castasterone, sitosterol) compounds. The difference lies in the percentage content of each compound from each yeast extract. The highest percentage of compounds in the yeast extract was obtained by maceration techniques and methanol as a solvent, except for vitamins B and amino acids. On the other hand, methanol and ethanol solvents can extract fatty acids such as lignoceric acid. Apart from fatty acids, ethanol can extract non-polar steroid compounds that water solvents cannot take up.

The content of vitamins, microelements and amino acids in black rice is higher than other rice types.[18] The total amino acids in the water extract of black rice yeast obtained by sun-drying (WYbr *) were higher than the water extract (WYbr **) obtained by freeze dryer drying, with percentage values of 21.89% and 21.81% (Figure 1 and 2), respectively. This observation is also in tandem with the measurement of the total protein content of WYbr *, which is higher when compared to WYbr **. Amino acids act as precursors for structural proteins, defence proteins, transport proteins, hormones, and enzyme.The highest total vitamin B content is in the yeast extract of black rice dried with a freeze dryer. A freeze dryer will maintain the stability of nutrients such as vitamins. This shows that vitamin B's content in black rice yeast that is dried using a freeze dryer is more preserved. It was noted that vitamin B was not identified in the ethanol (EY_{br} *) and methanol (MY_{br} *) extracts (Figure 3 and 4). However, vitamin C was found in higher concentrations in both extracts.

The four black rice yeast extracts (WY_{br} **, WY_{br} *, EY_{br} *, and MY_{br} *) have relatively the same carbohydrate content, except WY_{br} ** and WY_{br} *. The two extracts did not express any fructose-6-phosphate compounds. The highest carbohydrate content was found in methanol extract (MY_{br} *), 21.13%. The whole black rice yeast extract contained purines such as xanthine, which plays a role in nucleic acid metabolism.

Glucosamine is one of the amino sugars identified in the four black rice yeast extracts. The glucosamine concentrations of WY_{br} **, WY_{br} *, EY_{br} *, and MY_{br} * were 7.81%, 7.62%, 7.80%, and 8.03%, respectively. Although this compound is sugar, it does not affect blood sugar levels or insulin sensitivity.

Coumarins are classified as phenolic compounds with a fused α-piron ring.[19] The highest coumarin content (0.81%) was found in MYbr *. The bioactivity of these compounds such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, anti-tubercular, anti-seizure, anti-adipogenic, anti-hyperglycemic, antioxidant, and neuroprotective has been reported.[20]

Glutathione is a natural antioxidant composed of intracellular tripeptides in the form of Gamma-Levo-glutamyl-L-cysteine-glycine. These compounds can prevent damage to cellular components caused by reactive oxygen species (ROS), such as lipid peroxides or heavy metals, and possess anti-diabetes, anticancer, anti-neurodegenerative, and anti-HIV activities.[21] The four black rice yeast extracts contain glutathione in various concentrations.

Vanillic acid, gallic acid, caffeic acid, and 3-O-feruloylquinic acid are phenolic compounds identified in black rice yeast extract. One of the functions of phenolic compounds as antioxidants involves the donation of hydrogen atoms to free radicals to become stable.

Flavonoids are antioxidant compounds that can protect the body from ROS[22]. The results of phytochemical profiling on black rice yeast extract showed that the concentration of flavonoids in ethanol extract (EYbr *) were the highest with 12 identified flavonoid compounds. Chrysin, coumestrol, 3-hydroxy-4-methoxyflavone, luteolin, kaempferol, fisetin, scutellarein, morin, apigenin-7-glucoside, luteolin 7 glucoside, isoquarcetin, and quarciturone compounds are flavonoids found in EYbr *. The flavonoids in methanol extract (MYbr *) and water extract (WYbr * and WYbr **) were lower than that of EYbr *. The MYbr *, WYbr * and WYbr ** extract contained ten flavonoid compounds identified as 3-hydroxy-4-methoxiflavone, luteolin, kaempferol, fisetin, scutellarein, morin, apigenin-7-glucoside, luteolin 7 glucoside, isoquarcetin, and quarciturone.

This study showed that black rice yeast extract contains phenolic and flavonoid compounds that function as antioxidants. The content of Cr^{3+} in black rice yeast can act as type-2 anti-DM. The highest antioxidant activity was found in the freeze-dried black rice yeast water extract.

Table 2: Classification of compounds in the yeast extract of black rice with maceration solvent variations

No	Compound Name	Maceration solvent/content in %				tR (min)	Molecular Formulas
		Distilled water *	Ethanol*	Methanol*	Destilat water **		
Carboxylic Acid							
1	Formic acid	0.37656	0.38530	0.39656	0.38576	0.973	CH_2O_2
2	Acetic acid	0.46561	0.47643	0.49035	0.47700	1.039	$C_2H_4O_2$
3	Valeric acid	0.55638	0.56932	0.58595	0.57000	1.206	$C_5H_{10}O_2$
4	Gluconic acid	0.58395	0.59753	0.61498	0.59824	5.148	$C_6H_{12}O_7$
5	Citric acid	0.59415	0.60797	0.62573	0.60869	5.008	$C_6H_8O_7$
6	2-O-caffeoylhydroxycitric acid	0.77752	0.79559	0.81883	0.79654	12.74	$C_{15}H_{14}O_{11}$
7	Propionic acid	0.80553	0.82426	0.84834	0.82524	1.040	$C_3H_6O_2$
8	Glucuronic acid	0.89957	0.92048	0.94737	0.92158	4.737	$C_{13}H_{24}$
9	Butyric acid	0.90052	0.92146	0.94838	0.92256	1.165	$C_4H_8O_2$

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No	Compound Name	Maceration solvent/content in %				tR (min)	Molecular Formulas
		Distilled water *	Ethanol*	Methanol*	Destilat water **		
10	Lactic acid	1.20692	1.23498	1.27105	1.23645	1.192	$C_3H_6O_3$
Dicarboxylic acid							
1	Tartaric acid	0.27091	0.27721	0.28531	0.27754	1.60	$C_4H_6O_6$
2	Fumaric acid	0.58617	0.59979	0.61731	0.60051	1.238	$C_4H_4O_7$
3	Maleic acid	0.77329	0.79126	0.81438	0.79221	1.349	$C_4H_4O_4$
4	Succinic acid	0.80581	0.82454	0.84863	0.82553	1.246	$C_4H_6O_4$
5	Malic acid	1.14722	1.17389	1.20818	1.17529	1.473	$C_4H_6O_5$
Amino acids and their derivatives							
1	Glutamine	0.34743	0.20567	0.16541	0.23873	2.204	$C_5H_{10}N_2O_3$
2	Asparagine	0.39224	0.25152	0.21261	0.28464	1.773	$C_4H_8N_2O_3$
3	Cystein	0.39820	0.25762	0.21888	0.29074	1.286	$C_3H_7NO_2S$
4	Aspartic acid	0.46116	0.32205	0.28519	0.35525	1.794	$C_4H_7NO_4$
5	Pyroglutamic acid	0.61299	-	-	0.62799	1.466	$C_5H_7NO_3$
6	Histidine	0.77688	0.64511	0.61769	0.67870	2.598	$C_6H_9N_3O_2$
7	Tyrosine	0.77308	0.64122	0.61368	0.67480	4.745	$C_9H_{11}NO_3$
8	Arginine	0.80554	0.67443	0.64787	0.70805	3.094	$C_6H_{14}N_4O_2$
9	Methionine	0.80554	0.67443	0.64787	0.70806	2.538	$C_5H_{11}NO_2S$
10	Proline	0.80557	0.67446	0.64790	0.70809	1.232	$C_5H_9NO_2$
11	Tryptophan	0.87217	0.74261	0.71804	0.77631	5.485	$C_{11}H_{12}N_2O_2$
12	Serine	0.90061	0.77171	0.74799	0.80545	1.204	$C_5H_9NO_2$
13	Threonine	0.90104	0.77215	0.74844	0.80589	1.248	$C_3H_7NO_3$
14	Lysine	0.97305	0.84583	0.82428	0.87966	2.228	$C_6H_{14}N_2O_2$
15	Phenylalanine	0.86930	0.73967	0.71501	0.89057	2.687	$C_9H_{11}NO_2$
16	Valine	1.14706	1.02388	1.00753	1.05792	1.241	$C_5H_{11}NO_2$
17	∝-aminobutyric acid	1.14705	1.17372	1.20800	1.17512	1.283	$C_4H_9NO_2$
18	Glycine	1.30259	1.18304	1.17134	1.21727	1.049	$C_2H_5NO_2$
19	Leucine	1.31741	1.19819	1.18693	1.23244	1.766	$C_6H_{13}NO_2$
20	Isoleucine	1.46846	1.35276	1.34601	1.38719	1.752	$C_6H_{13}NO_2$
21	Alanine	1.48304	1.36768	1.36137	1.40213	1.158	$C_3H_7NO_2$
22	Glutamic acid	1.84960	1.74276	1.74741	1.77766	2.276	$C_5H_9NO_4$
23	γ-glutamyl alanine	0.58875	0.60243	0.62003	0.60315	6.702	$C_8H_{14}N_2O_5$
24	Diaminopimelic acid	0.10054	0.10288	0.10588	-	4.825	$C_7H_{14}N_2O_4$
25	Glutamyl tyrosine	0.78648	0.80476	0.82827	0.68852	11.55	$C_{14}H_{18}N_2O_8$
Vitamin							
1	Pyridoxine-β-glucoside	0.24958	-	-	0.25568	11.55	$C_{14}H_{21}NO_8$
2	Riboflavin	0.31079	-	-	0.31839	12.958	$C_{17}H_{20}N_4O_8$
3	Thiamin	0.31084	-	-	0.31845	9.045	$C_{12}H_{17}N_4OS$*
4	Biotin	0.33172	-	-	0.33983	7.329	$C_{10}H_{16}N_2O_3S$
5	Niacin	0.34658	-	-	0.35506	1.428	$C_6H_5NO_{21}$
6	Pantothenic acid	0.48733	-	-	0.49925	6.855	$C_9H_{17}NO_5$
7	Ascorbic acid	1.16217	1.18919	1.22393	1.19061	3.166	C_6H8O_6
Carbohydrate							
1	Melibiose	0.77687	0.79493	0.81816	0.79588	12.291	$C_{12}H_{22}O_{11}$
2	Trehalose	0.93878	0.96061	0.98867	0.96176	12.293	$C_{12}H_{22}O_{11}$

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No	Compound Name	Maceration solvent/content in %				tR (min)	Molecular Formulas
		Distilled water *	Ethanol*	Methanol*	Destilat water **		
3	Arabinose	1.27180	1.30137	1.33939	1.30292	1.602	$C_5H_{10}O_5$
4	Xylose	1.73630	1.77666	1.82857	1.77879	2.539	$C_5H_{10}O_5$
5	Stachyose	1.75734	1.79819	1.85072	1.80034	46.176	$C_{24}H_{42}O_{21}$
6	Rhamnose	1.77228	1.81348	1.86646	1.81565	2.649	$C_6H_{12}O_5$
7	Mannose	1.85253	1.89559	1.95097	1.89786	4.729	$C_6H_{12}O_6$
8	Galactose	1.89060	1.93454	1.99106	1.93686	4.713	$C_6H_{12}O_6$
9	Raffinose	2.12824	2.17771	2.24133	2.18032	26.305	$C_{18}H_{32}O_{16}$
10	Glucose	2.48633	2.54412	2.61845	2.54717	4.709	$C_6H_{12}O_6$
11	Maltotriose	2.68060	2.74291	2.82304	2.74619	26.302	$C_{18}H_{32}O_{16}$
12	Fructose-6-phosphate	-	0.79103	0.81414	-	8.030	$C_6H_{13}O_9P$
Purin							
1	Xanthine	1.15771	1.18462	1.21922	1.18603	2.545	$C_5H_4N_4O_2$
Phenolic							
1	Gallic acid	2.29702	2.35041	2.41908	2.35322	3.042	$C_7H_6O_5$
2	Vanilic acid	1.56879	1.60525	1.65215	1.60717	2.799	$C_8H_8O_4$
3	Caffeic acid	2.95304	3.04504	3.13400	2.97587	4.643	$C_9H_8O_4$
4	Ferulic acid	1.73831	1.77871	1.83068	1.78084	5.043	$C_{10}H_{10}O_4$
Shikimic acid							
1	Shikimic acid	0.97390	0.99654	1.02565	0.99773	3.091	$C_7H_{10}O_5$
Amino Sugar							
1	N acetylglucosamine	1.75734	1.79819	1.85072	1.80034	6.887	$C_8H_{15}NO_6$
2	N acetyl muramic acid	0.78651	0.80479	0.82831	0.80576	10.525	$C_{11}H_{19}NO_8$
3	Galactosamine	2.29599	2.34937	2.41800	2.35218	3.503	$C_6H_{13}NO_5$
4	Glucosamine	2.78170	2.84636	2.92952	2.84977	3.522	$C_6H_{13}NO_5$
Phytohormones							
a.	Auxin						
1	3-indoleacetic acid	0.20305	0.20777	0.21384	0.20802	3.114	$C_{10}H_9NO_2$
2	3-indolepropionic acid	0.30936	0.31655	0.32580	0.31693	4.747	$C_{11}H_{11}NO_2$
b.	Cytokinins						
1	Zeatinriboside	0.14679	0.15020	0.15459	0.15038	12.309	$C_{15}H_{21}N_5O_5$
2	Kinetin glucoside	0.15419	0.15778	0.16239	0.15797	13.122	$C_{16}H_{19}N_5O_7$
3	Trans-zeatinglucoside	0.36639	0.18020	0.38586	0.18041	12.973	$C_{16}H_{23}N_5O_6$
4	6-benzylaminopurine	0.19532	0.19986	0.20570	0.20010	7.272	$C_{12}H_{11}N_5$
5	Kinetin-9-N-glucoside	0.30490	0.31199	0.32110	0.31236	12.961	$C_{16}H_{19}N_5O_6$
6	Kinetin	0.31029	0.31750	0.32678	0.31788	6.599	$C_{10}H_9N_5O$
7	Trans-zeatin	0.17611	0.37491	0.18546	0.37536	6.865	$C_{10}H_{13}N_5O$
8	Kinetin-7-N-glucoside	0.38264	0.39154	0.40298	0.39201	12.965	$C_{16}H_{19}N_5O_6$
9	Cis-zeatin	0.59540	0.60924	0.62704	0.60997	6.862	$C_{10}H_{13}N_5O$
Coumarin							
1	Esculetin	0.77308	0.79105	0.81416	0.79199	3.275	$C_9H_6O_4$
Phytic acid							
1	Myo inositol	1.91821	0.80479	2.02014	1.96515	4.730	$C_6H_{12}O_6$
Antioxidants							
1	Glutathione	1.73422	1.77453	1.82637	1.77665	10.511	$C_{10}H_{17}N_3O_6S$

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No	Compound Name	Maceration solvent/content in %				tR (min)	Molecular Formulas
		Distilled water *	Ethanol*	Methanol*	Destilat water **		
Antibiotics							
1	2.4-diacetylphloroglucinol	0.20588	0.21066	0.21682	0.21092	5.821	$C_{10}H_{10}O_5$
Quinic acid							
1	3-O-feruloylquinic acid	0.35076	0.35891	0.36940	0.35934	12.717	$C_{17}H_{20}O_9$
Flavonoid							
1	Fisetin	0.35171	0.35989	0.37040	0.36032	10.325	$C_{15}H_{10}O_6$
2	Luteolin 7 glucoside	0.59488	0.60871	0.62649	0.60943	22.628	$C_{21}H_{20}O_{11}$
3	3-hydroxy-4-methoxyflavone	0.60327	0.61729	0.63532	0.61803	9.104	$C_{16}H_{12}O_4$
4	Apigenin-7-glucoside	0.90612	0.92718	0.95427	0.92829	20.093	$C_{21}H_{20}O_{11}$
5	Scutellarein	0.95688	0.97913	1.00773	0.98030	10.329	$C_{15}H_{10}O_6$
6	Luteolin	1.18627	1.21384	1.24930	1.21529	10.265	$C_{15}H_{10}O_6$
7	Morin	1.34550	1.37667	1.41689	1.37832	11.405	$C_{15}H_{10}O_7$
8	Quercituron	1.38822	1.42049	1.46199	1.42219	25.835	$C_{21}H_{18}O_{13}$
9	Isoquercitrin	1.73830	1.77871	1.83067	1.78084	24.018	$C_{21}H_{20}O_{12}$
10	Kaempferol	2.03355	2.08082	2.14161	2.08331	10.322	$C_{15}H_{10}O_6$
11	Chrysin	-	0.31168	-	-	8.003	$C_{15}H_{10}O_4$
12	Coumestrol	-	0.43011	-	-	9.083	$C_{15}H_8O_5$
Sesquiterpenoids							
1	Spathulenol	0.77699	0.79505	0.81828	0.79600	6.883	$C_{15}H_{24}O$
Furanochrome							
1	Visnagin	1.43945	1.47291	1.51594	1.47467	7.615	$C_{13}H_{10}O_4$
Ribitol							
1	Ribitol-5-phosphate	0.33525	0.34304	0.35306	0.34345	7.628	$C_5H_{13}O_6P$
Results of glucose metabolism or something else							
1	Pyruvic acid	1.76700	1.80807	1.86089	1.81024	1.161	$C_3H_4O_3$
2	∝-ketoglutaric acid	0.76714	0.80543	0.82896	0.80640	1.535	$C_5H_6O_5$
3	Acetyl Co A	2.1095	2.15855	2.22161	2.16113	49.890	$C_{23}H_{38}N_7O_{17}P_3S$
4	2-carboxyarabinitol	-	0.31753	0.32681	-	5.147	$C_6H_{12}O_7$
Saturated fatty acids							
1	Lignoceric acid	-	0.33554	0.34534	-	12.719	$C_{24}H_{48}O_2$
Steroids							
1	Dolichosterone	-	0.31702	-	-	23.973	$C_{28}H_{46}O_5$
2	Castasterone	-	0.31361	-	-	24.047	$C_{28}H_{48}O_5$
3	β-sitosterol	-	0.56705	-	-	17.163	$C_{29}H_{50}O$

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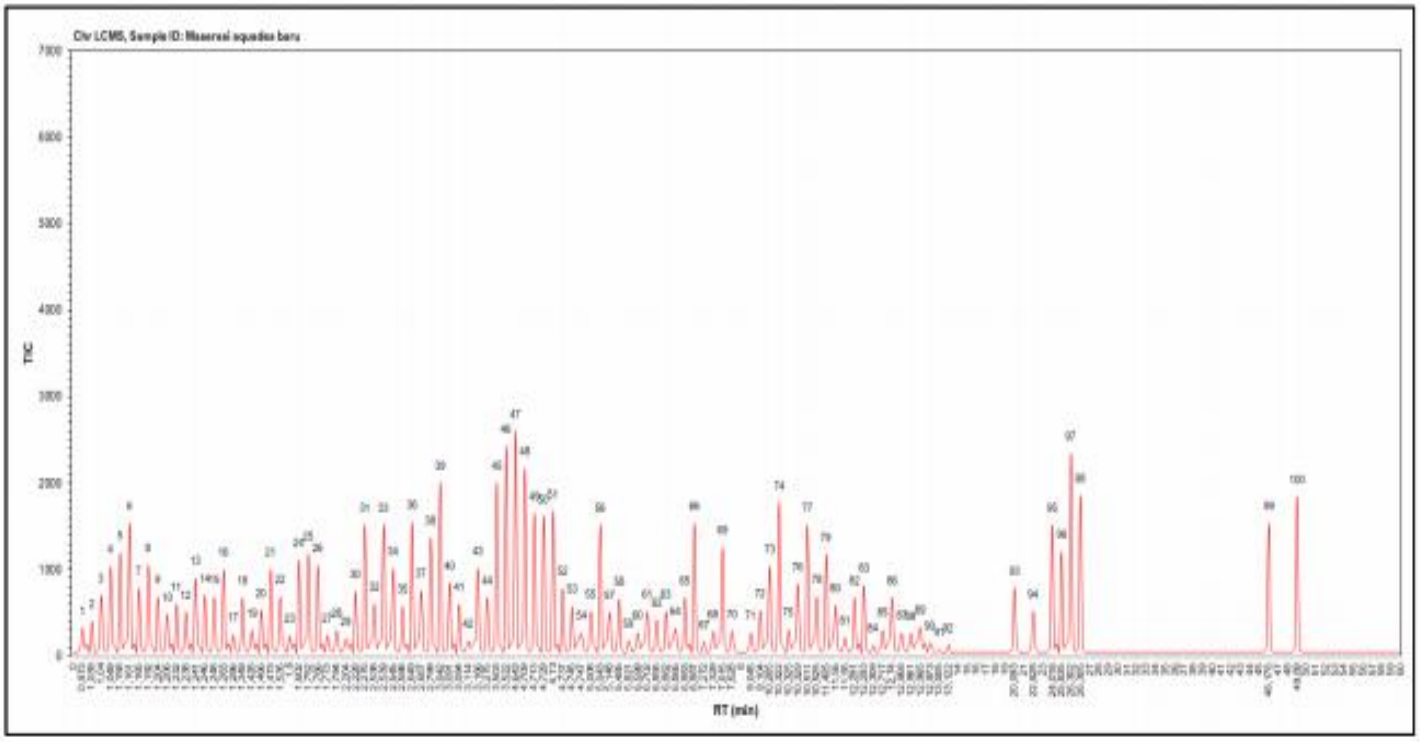

Figure 1: LCMS chromatrogram of black rice yeast water extract** (WY_{br}**)

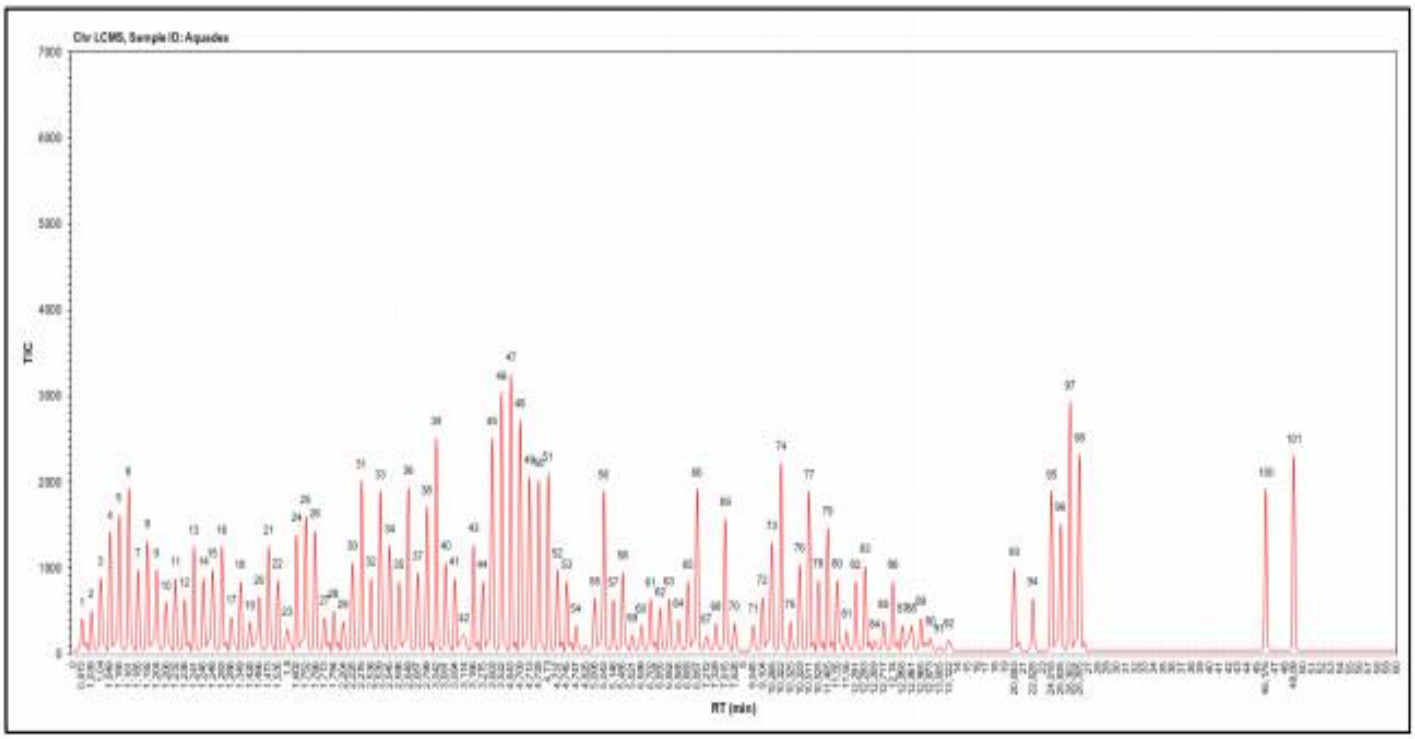

Figure 2: LCMS chromatrogram of black rice yeast water extract * (WY_{br}*)

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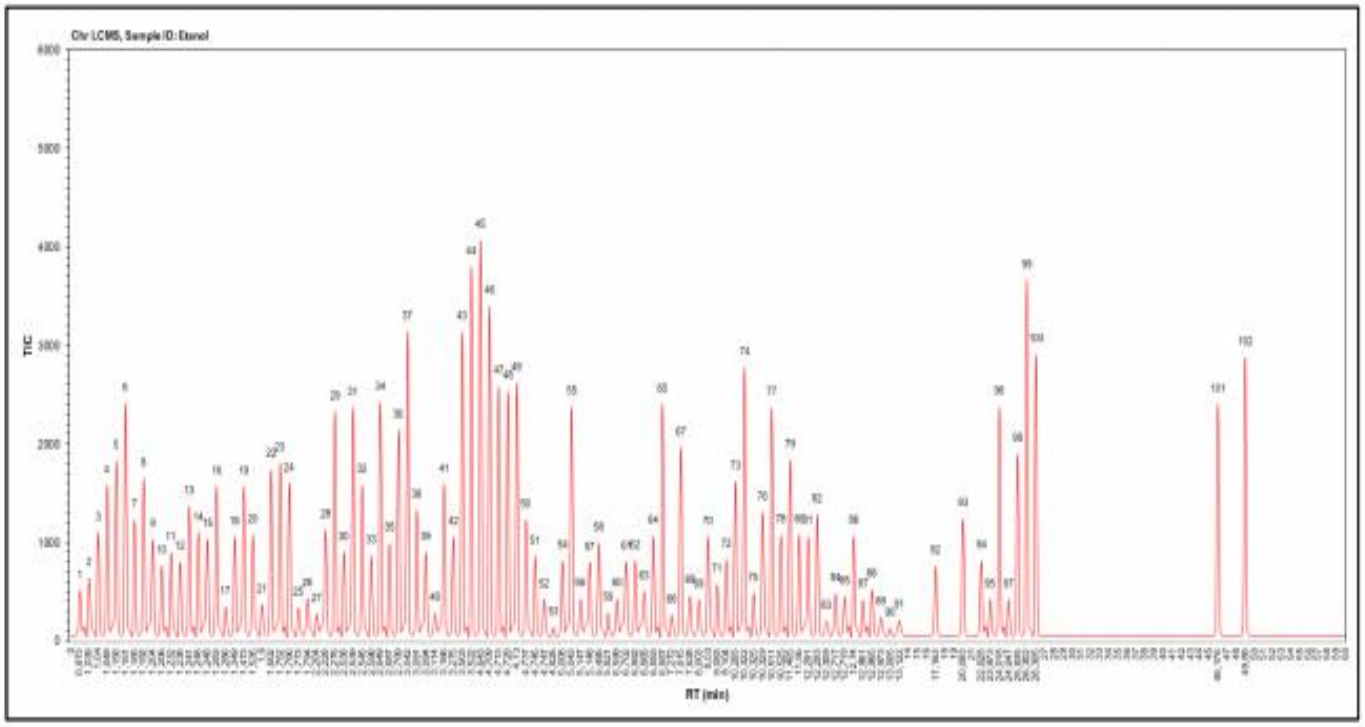

Figure 3: LCMS chromatrogram of black rice yeast ethanol extract * (EY_{br}*)

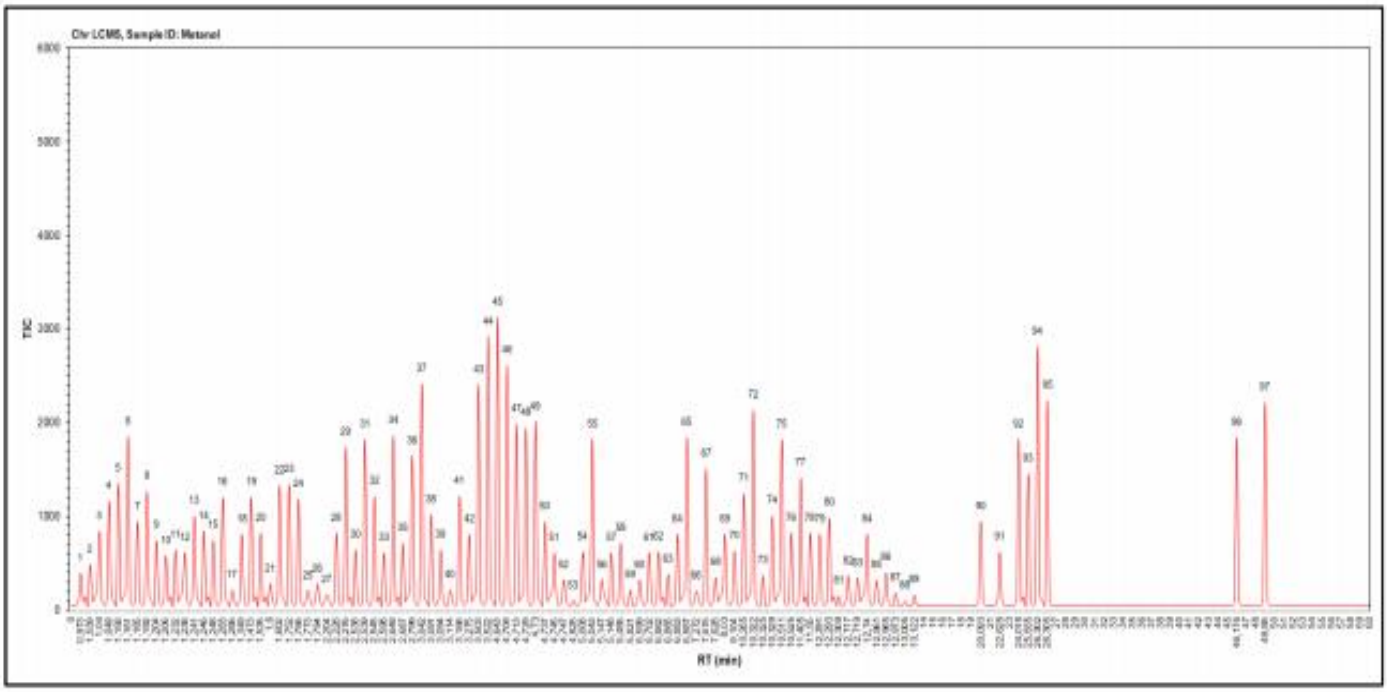

Figure 4: LCMS chromatrogram of black rice yeast methanol extract * (MY_{br}*)

Conclusion

The results showed that yeast without maceration had the highest percentage of protein, starch, and chromium (III). The highest antioxidant activity was exhibited by black rice yeast water extract obtained using a freeze dryer. The results of the LC-MS/MS analysis of black rice yeast water extract, both black rice yeast obtained by sun-drying process and freeze dryer, showed 107 compounds. In comparison, 109 and 104 compounds were identified in the methanol and ethanol extracts, respectively. Black rice yeast extract obtained from polar solvents by maceration has the potential to be used as type 2 anti-DM preparation.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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