Evaluation of the combination of black rice bran ethanol extract and glimepiride in reducing blood glucose and protecting kidney, liver and pancreatic cells

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INTRODUCTION

Glucose metabolism plays an important part in the process of insulin secretion from the pancreatic beta cell and metabolic dysfunction is considered to be a contributing factor to the decreased release of insulin in diabetes mellitus (DM) (Ashcroft et al., 2017). Diabetes mellitus (DM) is associated with metabolic disorders that contribute to the development of DM (Sola et al., 2015). Diabetes is associated with renal and liver impairment through an underlying mechanism involving elevated oxidative stress (Julián et al., 2015; Sullivan and Forbes, 2019).

Sulfonylureas (SUs) are frequently used in the treatment of type 2 diabetes mellitus (T2DM) as drugs that stimulate the secretion of insulin. Glimepiride (GLM) is a newly invented second-generation SU medication, it is used in more than 60 countries worldwide (Basit et al., 2012; Kalra et al., 2018). GLM enhances insulin sensitivity in peripheral tissues and promotes glycemic management by diminishing insulin resistance (Jayakrishnan, 2022). Hypoglycemia and weight gain are significant problems of SU treatment, GLM is generally well-tolerated and its distinctive characteristics may offer benefits compared to other insulin secretagogues. GLM could possibly be administered to patients with renal insufficiency, however in such cases patients should be closely monitored (Basit et al., 2012). SUs are metabolized in the liver and are predominantly excreted by the kidneys, so long-term use of SUs has been associated with various effects (Sola et al., 2015).

Combination therapy is frequently employed to prevent complications or improve outcomes. Supplementary treatment for diabetes may involve the administration of specific medicinal herbal remedies. Black rice bran (BRB) is one of the traditional herbs that has the potential to be used in oral hyperglycaemia treatments. The BRB extracts contain a high concentration of phenolic chemicals, including anthocyanins. Black rice has the highest concentration of anthocyanins (292.79±6.96 ug/g) and the outer pericarp (bran) of the black rice kernel contains higher quantities of anthocyanins. The primary anthocyanins found in black rice include cyanidin-3-o-glucoside, peonidin-3-o-glucoside, malvidin-3-o-glucoside, pelargonidin-3-o-glucoside and delphinidin-3-o-glucoside (Kumar and Murali, 2020; Wahyuni et al., 2020). Dietary anthocyanins and phenolic compounds, such as resveratrol, γ-orzanol and epicatechins, have demonstrated the ability to control the expression of genes

Abstract: Long-lasting hyperglycemia can potentially cause damage to organs such as the kidneys, liver and pancreas. Glimepiride (GLIM), as a drug of choice in the treatment of diabetes mellitus (DM), has the risk of decreasing the functioning of organs such as the kidneys, liver and pancreas. Black rice bran ethanol extract (EEBRB) with antioxidant content has been shown to protect the kidney, liver and pancreas organs. The aim of this study was to establish the effect of EEBRB on lowering fasting blood glucose (FBG) and protecting several organs after GLIM administration in alloxan (ALX)-induced hyperglycemic rats. A total of 20 rats were divided into 4 groups and treated for 21 days treatments using following preparations: normal control (NC), diabetic group (DC), GLIM 1 mg/ kgBW and combination of glimepiride 1mg/kgBW and EEBRB 50 mg/KgBW (GLBR). The results showed that the GLBR was able to lower blood glucose levels back to normal (<126 mg/dL) and protect kidney, liver and pancreas cells by increasing the amount in normal cells.

Keywords: Diabetes mellitus, black rice bran, glimepiride, alloxan, antioxidant.
that play a role in insulin release (Kang et al., 2020). Studies have shown that BRB, which contains cyanidin-3-glucoside-type aglycons, can effectively lower blood glucose levels by stimulating the release of insulin and improving insulin sensitivity (Kumar and Murali, 2020). The antioxidant properties of BRB have been demonstrated to protect cells in the liver and kidneys of hyperglycemic rats (Wahyuni et al., 2020). The combination of GLIM and BBR is predicted to have a synergistic effect on reducing blood glucose levels, also referred to as FBG. Moreover, the antioxidant properties of BBR facilitate the restoration of beta cells in the pancreas, kidneys and liver by protecting them from the harmful impacts of free radicals, which are responsible for the deterioration of these cells. The objective of this study is to demonstrate the efficacy of administering these two components together in reducing fasting blood glucose (FBG) levels and protecting the liver and kidneys from damage caused by hyperglycaemic conditions.

MATERIALS AND METHODS

Drugs and chemicals
Black rice bran (BRB) is harvested from Klaten, Indonesia; glimepiride is obtained from PT. Indofarma; alloxan (Sigma-Aldrich®); reagent kit Glucose GOD FS (DSi), Urea FS (DSi), Creatinine (DSi), ALAT (GPT) FS (DSi); aquadestilata; ethanol 96%, hydrochloric acid (Emerck), glucose and saline solutions (NaCl solution 0.9%).

Experimental animal
The research involved male Wistar Albino rats weighing between 140-180 g and aged 2-3 months. These rats were sourced from the animal housing facility at the Pharmacology Lab of Muhammadiyah University in Surakarta, Indonesia. The rats were kept in polypropylene cages furnished with soft woodchip bedding. They were subjected to a natural light-dark cycle with a period of that was of equal duration. The temperature of the environment was regulated at a constant 25±1°C. The rats were housed in the controlled setting in laboratory for a duration of one week, during which they were given unrestricted access to a diet consisting of standard pellets and water. Finally, they were chosen at random for the purpose of carrying out this experiment.

Extraction of BRB in preparation for anthocyanin enrichment
The dried black rice bran (BRB) was soaked in a mixture of ethanol, water and hydrochloric acid (0.1 N) in a ratio of 50:50:0.5 (v/v/v) for 2 hours. The resulting filter removed the solvent with a vacuum evaporator for six hours and continues with a dry exhauster for twenty-four hours to produce a thick extract enriched with anthocyanin (Hou et al., 2013; Wahyuni et al., 2020).

Hyperglycemic animal modelling with alloxan induction 150mg/kgBW
The rats were induced with alloxan (ALX) 150 mg/kgBW (0.3% in saline) intraperitoneally and hyperglycemia was developed within 72h. The animals with blood glucose levels above 200 mg/dL were selected for the study (Sutrisna et al., 2018; Zahid et al., 2014). The research protocol has also passed ethical clearance by KEPK (No. 3733/A-1/KEPK-FKUMS/X/2021).

Experimental study design
Twenty rats were divided into four treatment groups, one normal (group NC) and three hyperglycemic groups. The oral treatment is given for 21 days with preparation. The normal control (NC) group and diabetic group (DC) were given aqua destilata 1 mL/200 gBW, the GLIM group were given glimepiride 1 mg/kgBW and the GLBR group were given a combination of glimepiride 1 mg/kgBW and EEBRB 50 mg/kgBW. Blood samples were taken on days 0, 7, 14 and 21 after the retoroorbital plexus vein of the eye (0.5 mL), centrifuged at a rate of 3000 rpm for 10 minutes to obtain the serum and stored in the freezer at a temperature of -21°C.

Biochemical analysis
Colorimetry with the appropriate reagents was used to measure the levels of FBG, Blood Urea Nitrogen (BUN), Serum Creatinine (SCr) and Serum Glutamic Pyruvic Transaminase (SGPT). Test animals are subjected to a chemical blood test in order to identify the existence of liver problems as well as renal disorders (Rahayu et al., 2018).

Glycogen levels
A total of 25 mg of liver which has been dried in an oven at 50°C overnight, is extracted using 1 mL of a 30% KOH solution, heated in boiling water for 20 minutes. Subsequently, 1.5 mL of 95% ethanol was added and incubated at a temperature of 4°C for 30 minutes. Glycogen is obtained through the process of centrifugation at 2500 rpm, 20 minutes. The suspended solid was diluted with 1 mL of distilled water. A 100 μl mixture was added to 3 mL of a 0.2% Antron solution. The presence of glycogen was confirmed by the appearance of a green colour, indicating the detection of acid sulphate. Colour absorption measured with spectrophotometer at a wavelength of 620 nm (Suarsana et al., 2017; De Carvalho et al., 2022).

Histopathological examination
The rats were euthanized and several organs, including the pancreas, liver and kidney, were removed, cleaned of excess fat and connective tissue and dried to eliminate any blood residue. The specimens were placed in a 10% neutral buffered formalin fixative solution. The dehydration and clarification of the tissues were conducted as a routine method, followed by their embedding in paraffin wax. Sections with a thickness of
approximately 5 microns have been formed using a microtome. Subsequently, these sections were put into slides. The process of staining with HE involves the removal of paraffins with xylene. Subsequently, they were rehydrated using graded alcohol and stained with haematoxylin and eosin (HE) dye. The tissue sample was analyzed histopathologically under a microscope with a magnification of 1000x.

STATISTICAL ANALYSIS

The data was analyzed using GraphPad Prism version 10 licensed using non-parametric statistics, specifically the Kruskal-Wallis test, at a significance level of p<0.05.

RESULTS

Effects of GLIM and GLBR on fasting blood glucose (FBG) levels
GLIM treatment led to a substantial reduction in blood glucose levels by the seventh day, reaching normal values (149.8 ± 147.1) mg/dL with a statistically significant p-value of less than 0.05 (Table 1). On the 21st day, there were still significant decreases in fasting blood glucose (FBG) levels. The efficacy of GLIM and GLBR significantly decreased (p<0.05) after 21 days of treatment when compared to group-DC.

Effects of administration GLIM and GLBR on body weight
The weight of rats with diabetes was measured and the findings indicated that almost each of the experimental animals observed a decrease in body weight. This trend was also observed in the DC group until the 21st day, during which their weight continued to decrease from an initial weight of (186.4±13.1) g to a final weight of (165.4±13.6) g (Table 2). The treatment group exhibited weight gain and improvement in hyperglycemic state after receiving either GLIM or GLBR, as indicated in Table 1 and Table 2. The GLIM group showed a weight gain of 145.8 ± 32.9 g initially, which increased to 166.3 ± 34.8 g after 21 days. The GLBR group had a weight gain of 163.8 ± 24.1 g at first, which increased to 188.0 ± 33.2 g.

Effects of GLIM and GLBR on hepatic glycogen levels
In the GLBR administration, the liver glycogen levels showed no significant differences with GLIM 1 mg/kg body weight (p <0.05).

Effects of GLIM and GLBR on biochemical analysis
In this investigation, levels of blood urea nitrogen (BUN) and serum creatinine (SCR) were also measured to assess renal function. All groups had SCR monitoring readings that stayed within the normal range, with levels under 1 mg/dL. A significant difference (p < 0.05) was seen when comparing the initial levels of BUN with the values obtained after 14 days and 21 days in the GLIM and GLBR groups. Liver function was monitored for changes in SGPT levels and the results showed that in the GLIM group, GLBR results were in the normal range at rates <7-56 IU/L. The DC group and GLIM group showed relatively higher BUN, SCR and SGPT values than the other groups. A decrease in kidney and liver function should be confirmed by histopathological tests.

Histopathology of pancreatic, kidney and liver cells
Results of histopathological examination of kidney, liver and pancreas cells showed that the DC group had more damaged cells than other groups (Figs. 2, 3, 4).

The GLBR group showed a higher number of normal pancreatic cells than GLIM group, with normal cell numbers not significantly different from the NC group (p>0.05).

DISCUSSION

Antioxidants are crucial for maintaining good health as they have the ability to reduce glucose levels and provide protection to the pancreas, liver and kidneys (Wahyuni et al., 2020). The main characteristic of antioxidant compounds is their ability to scavenge free radicals. Anthocyanins are one of the antioxidants contained in black rice bran (BRB), which has several pharmacological activities, including being able to reduce glucose (Tantipaiboonwong et al., 2017) through inhibition of the alpha glucosidase enzyme, which results in delaying the absorption of glucose in the blood (Young et al., 2017). Glimepiride (GLIM) has the action of lowering glucose levels by increasing insulin secretion. Giving GLIM and GLBR for 14 days shows that FBG is relatively normal and up to day 21, it is maintained at normal FBG levels with relatively stable FGB levels at <126 mg/dL (Power, 2006). Black rice bran has been shown to have the ability to reduce FBG by inhibiting glucose absorption by inhibiting the alpha-glucosidase enzyme (Young et al., 2017) and increasing insulin secretion (Wahyuni et al., 2016).

One of the side effects of using GLIM is an increase in body weight (Sola et al., 2015). The body weight of the test animals was monitored before and after treatment up to the 21st day and was used as an indicator during treatment. Besides that, weight loss can be an indication of a hyperglycemic condition. Body weight during the GLIM and GLBR treatments gradually increased. Meanwhile, the DC group on day 21 showed no increase in body weight (Table 2).
In another study, it was stated that there was a relationship between hyperglycemia and weight loss in hyperglycemic rat models. Weight loss in this diabetic condition is the result of protein degradation or muscle wasting. This can happen in the absence of glucose and lipids, so protein will be used as the body's main source of energy. Structural proteins contribute to changes in body weight. Weight loss in diabetic conditions is caused by structural protein degradation (Song et al., 2019).

GLIM as an antidiabetic, will stimulate insulin by closing K+ATP channels in pancreatic cells. This inhibitory action of K+ATP channels also occurs in kidney epithelial cells, so it has the potential to reduce kidney function (Martin et al., 2017). In checking organ function parameters such as the kidneys, several parameters such as SCr (Serum Creatinine) and BUN (Blood Urea Nitrogen) levels are monitored. Urea is the end product of amino acid and protein catabolism, which is filtered by the glomerulus and some will be reabsorbed when there is a disturbance in kidney function. The measurement of serum urea can be used as a reference for indications, one of which is to evaluate kidney function. Meanwhile, the examination of SCr can be used as a reference and as a sign of a problem due to the breakdown of muscles and will be excreted in the kidneys.

### Table 1: Average fasting blood glucose levels (mg/dL) before and after treatment for 21 days in each treatment group (N = 5).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Fasting blood glucose levels (mg/dL) Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Induction</td>
</tr>
<tr>
<td>NC</td>
<td>80.0 ± 28.4</td>
<td>93.0 ± 31.6</td>
</tr>
<tr>
<td>DC</td>
<td>91.4 ± 4.4</td>
<td>412.2 ± 94.5</td>
</tr>
<tr>
<td>GLIM</td>
<td>98.0 ± 8.7</td>
<td>442.2 ± 101.6</td>
</tr>
<tr>
<td>GLBR</td>
<td>92.0 ± 7.2</td>
<td>390.0 ± 73.0</td>
</tr>
</tbody>
</table>

N = Number of rats in each group; * = p < 0.05 vs group-GLIM, # = p >0.05 vs Group-NC and ** = p >0.05 vs Group-DC

### Table 2: Average body weight before and after treatment for 21 days in different treatment groups (N = 5).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Body weight (g)</th>
<th>Before treatment</th>
<th>Induction</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>183.2 ± 10.1</td>
<td>183.2 ± 8.1</td>
<td>184.6 ± 12.5</td>
<td>187.2 ± 9.9</td>
<td>183.8 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>DC</td>
<td>186.4 ± 13.1</td>
<td>185.8 ± 12.7</td>
<td>174.6 ± 13.1</td>
<td>170.6 ± 11.0</td>
<td>165.4 ± 13.6</td>
<td></td>
</tr>
<tr>
<td>GLIM</td>
<td>142.0 ± 16.7</td>
<td>145.8 ± 32.9</td>
<td>153.3 ± 28.4</td>
<td>158.8 ± 31.0</td>
<td>166.3 ± 34.8*</td>
<td></td>
</tr>
<tr>
<td>GLBR</td>
<td>164.5 ± 10.1</td>
<td>163.8±24.1</td>
<td>170.8±25.2</td>
<td>175.2 ± 26.0</td>
<td>188.0 ± 33.2*</td>
<td></td>
</tr>
</tbody>
</table>

N = Number of rats in each group; * = Weight gain after treatment; # = Significant weight gain at 21 days treatment.

### Table 3: Data for BUN, SCr and SGPT on various treatment groups specified at baseline, induction, day 14 and 21 after treatment (N = 5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Induced</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>49.6±12.9</td>
<td>49.6±12.9</td>
<td>34.0±12.7</td>
<td>32.6±21.3</td>
</tr>
<tr>
<td>DC</td>
<td>58.3±8.7</td>
<td>84.8±52.6</td>
<td>84.4±59.4</td>
<td>105.4±58.3</td>
</tr>
<tr>
<td>GLIM</td>
<td>33.0±14.6</td>
<td>83.8±74.5</td>
<td>48.8±8.6</td>
<td>63.5±12.3*</td>
</tr>
<tr>
<td>GLBR</td>
<td>35.7±3.3</td>
<td>46.3±14.7</td>
<td>47.3±5.3</td>
<td>58.7±2.6*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SCr (Creatinin) (mg/dL)</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.84±0.3</td>
<td>0.84±0.3</td>
<td>0.86±0.3</td>
<td>0.78±0.2</td>
</tr>
<tr>
<td>DC</td>
<td>0.74±0.2</td>
<td>0.66±0.2</td>
<td>0.78±0.1</td>
<td>0.88±0.5</td>
</tr>
<tr>
<td>GLIM</td>
<td>0.82±0.2</td>
<td>0.88±0.2</td>
<td>0.54±0.2</td>
<td>0.96±0.3</td>
</tr>
<tr>
<td>GLBR</td>
<td>0.6±0.1</td>
<td>0.6±0.3</td>
<td>0.43±0.2</td>
<td>0.72±0.1*</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>SGPT (IU/L)</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>NC</td>
<td>20.8±6.8</td>
<td>20.8±6.8</td>
<td>42.6±20.6</td>
<td>42.2±17.4</td>
</tr>
<tr>
<td>DC</td>
<td>31.2±9.9</td>
<td>46.8±33.4</td>
<td>39.8±15.8</td>
<td>74.2±36.7</td>
</tr>
<tr>
<td>GLIM</td>
<td>38.0±9.5</td>
<td>38.6±20.6</td>
<td>50.2±13.3</td>
<td>43.2±8.7**</td>
</tr>
<tr>
<td>GLBR</td>
<td>39.0±6.2</td>
<td>38.0±23.0</td>
<td>44.0±8.1</td>
<td>52.2±12.7*</td>
</tr>
</tbody>
</table>

N = Number of rats in each group, * = significant DC group after 21 days (p<0.05), # = not significant NC- group after 21 days (p>0.05).
through the kidneys (Thammitiyagodage et al., 2020). The DC group and GLIM groups showed relatively higher BUN and SCR values than the other groups (table 3). Decreased kidney function in the DC and GLIM groups was confirmed by histopathological tests.

**Fig. 1**: The liver glycogen levels (%b/b) after 21 days of treatment of various groups (N = 5), *significant DC group (p<0.05), # not significant NC-group (p>0.05).

Histopathological results on the kidneys after administration showed that the DC group had the most damaged cells compared to the other groups (p<0.05) (fig. 2). The same damage was also experienced by the group given GLIM 1 mg/kgBW (p>0.05) (fig. 2). This cell damage is caused by free radicals released in mitochondria during insulin secretion. In hyperglycemic conditions that last too long, chronic exposure to relatively high levels of Reactive Oxygen Species (ROS) causes cell function disorders (Graciano et al., 2011), including kidney cells, endothelial cells, smooth muscle cells, mesangial cells, podocytes and tubular cells (Forbes and Cooper, 2013).

The GLBR combination group showed the number of damaged kidney cells did not differ from the normal group (p>0.05) (fig. 2). The anthocyanins contained in black rice bran, which have antioxidant activity, will reduce damage to kidney cells in hyperglycemic rats, decrease ROS production and increase antioxidant enzymes (Les et al., 2020). Cyanidin 3-glucoside (C3G), the anthocyanin is rapidly distributed to several organs, including the kidneys and eliminated in its intact form (Baek et al., 2023), so it is possible that it will have a protective effect on these organs.

**Fig. 2**: Graph of the number of normal and damaged kidney cells in the various treatment groups (N=5). *showed a significant difference with the alloxan group (DC) (p<0.05), # did not differ significantly with the normal group (p>0.05).

**Fig. 3**: Graph of normal and damaged liver cells in various treatment groups (N=5). *showed a significant difference with the DC group (p<0.05), # not significant with the NC group (p>0.05).

Anthocyanins, which also act as antioxidants, will be able to prevent and repair liver cell damage. C3G increases glucose uptake and also reduces glycolytic activity. The increase in 6-phosphogluconate instead of ribose 5-phosphate and ribose 1-phosphate shows that C3G only slightly increases the conversion of the glucose 6-phosphate pathway to pentose phosphate, thereby increasing NADPH production and can be used to
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increase GSH levels, which increase antioxidant capacity in the liver (Jia et al., 2020). This can also be seen from the results of liver function monitoring, the GLBR group showed higher normal cells than the group that was only given GLIM (fig. 3).

The results of the examination for pancreatic damage in the GLBR group showed that the number of normal pancreatic cells was higher than that given by GLIM, with the normal cell count not significantly different from the normal group (p>0.05) (fig. 7). In hyperglycemic modeling, using ALX as a diabetogenic agent, which can damage pancreatic beta cells through the formation of ROS, is the main factor causing damage to pancreatic cells. In addition, the use of GLIM, which is a sulfonyl urea compound, can also cause losses. Increased secretion by GLIM will trigger ROS production, but if ROS production is excessive or sustainable, it will have a negative correlation. These ROS can damage pancreatic β cells (Patley et al., 2012; Marin et al., 2011; Wahyuni et al., 2020).

Anthocyanin, which also acts as an antioxidant, will be able to prevent and repair damage to cells in the pancreas, kidneys and liver (Wahyuni et al., 2020). The results showed the ability of GLBR to protect the kidneys, liver...
and pancreas, as seen from the number of normal cells that did not differ from the normal group (figs. 2-4).

Glycogen will be formed by the process of glycogenesis, which involves the hormone insulin. This formation is a form of glucose storage in the liver. GLIM administration for 21 days was able to increase liver glycogen levels through stimulation of glucose production and lactate release (fig. 1). Besides that, GLIM is also able to inhibit glycogenolysis through the inhibition of oxidative phosphorylation. GLBR did not cause an increase in glycogen levels. C3G anthocyanin compounds can increase glucose absorption and reduce glycolysis activity by increasing G6P and decreasing phosphoenolpyruvate and lactic acid. The increase in glucose 6-phosphate to pentose phosphate will increase the production of NADPH. It is possible that the NADPH generated from the pentose phosphate pathway is used to maintain the cellular oxidative capacity of the liver, which increases GSH levels, implying that C3G increases the antioxidant capacity in the liver (Jia et al., 2020) through the formation of glycogen as glucose storage in the liver.

CONCLUSION

Black rice bran has a synergistic effect with glimepiride in lowering blood glucose levels in rats. GLBR combinations can protect against damage to kidney, liver and pancreas cells.

REFERENCES


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Rahayu L, Yanti H and Supomo Y (2018). Analysis of SGPT and SGOT on rats induced by isoniazid to determine dose and hepatoprotective characteristic of unripe pineapple juice (Ananas comosus L. Merr), JIFI, 16(1): 100-106.