Effect of Extract-Fractions of *Balanites aegyptiaca* Fruit-Mesocarp on Glucose Metabolizing Enzymes in Diabetic Rats

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ABSTRACT

Several antidiabetic medicinal plants has shown to exhibit one of more mechanisms to low blood glucose. *Balanites aegyptiaca* fruit extract has shown to low blood glucose via some of the mechanisms but whether the fruit extract could stimulate glycolysis, glycogenesis, and inhibit gluconeogenesis and glycogenolysis are not fully clear. Hence, the need to evaluate the plant extract on the activities of enzymes in glycolysis, gluconeogenesis, glycogenolysis and glycogenesis in streptozotocin-induced diabetic rats. Ethanol extract of defatted *Balanites aegyptiaca* fruit-mesocarp was petition with water and ethyl acetate (1:1 v/v) then separated. Aqueous and ethyl acetate fractions obtained were separately administered to streptozotocin-induced diabetic rats daily for 28 days period. Results of the study showed that treating diabetic rats with either aqueous or ethyl acetate fractions of ethanol extract of *Balanites aegyptiaca* fruit-mesocarp exert significant regulatory effect on the activities of some key hepatic enzymes of glucose metabolism. The aqueous fraction (AFF) in particular enhances glucokinase activity (from 2.22±0.02 to 3.58±0.05 U/min/mg protein) and G6PDH activity (from 1.45±0.02 to 2.10±0.02 U/min/mg protein) but suppressed glucose-6-phosphatase (from 1.44±0.05 to 0.17±0.00 U/min/µmole Pi liberated) among others. In conclusion, ethanol-aqueous fraction of *Balanites aegyptiaca* fruit-mesocarp exert glucose enzymes regulatory effect by enhancing glucokinase and G6PDH activity but suppressed gluconeogenic enzymes possibly to low blood
glucose in diabetic rats. Further research is needed to explore the plant extract (ethanol-aqueous fraction) in order to ascertain the bioactive hidden compounds.

**Keywords**: *Balanites aegyptiaca*, Fruit-Mesocarp, Ethanol Extract-Fractions, glucose metabolizing enzymes

### 1. INTRODUCTION

Hyperglycemia in diabetes mellitus is largely results from hepatic glucose over production associated with glucose metabolic disorder which occurs as a result of disturbance in the activities of enzymes involved in glycolysis, gluconeogenesis, glycogenesis and glycogenolysis [1]. Studies have shown that disturbance of carbohydrate metabolism has significant influences on glucose homeostasis [2,3]. Deficiency of insulin disrupts carbohydrate metabolism by suppressing the activities of glycolytic and glycogenic enzymes while promoting gluconeogenic and glycogenolytic enzymes [1,4].

Maintenance of normal glycemia requires matching of glucose utilization and endogenous production. This could be achieve via coordinated regulations of several metabolic pathways; glycolysis, gluconeogenesis, glycogenolysis, and glycogenesis [5,6]. Several regulatory enzymes like glucokinase, phosphofructokinase, pyruvate kinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, phosphoenolpruvate carboxlkinase, glycogen synthase and glycogen phosphorylase play key roles in these metabolic pathways [6].

According to Abdollahi et al [7], any agent with potential to reverse hepatic carbohydrate metabolism might have influence on enzymes involved in glucose and glycogen metabolisms. To this regard, Agius [8] has reported the inhibition of enzymes involved in gluconeogenesis and glycogenolysis which led to the lowering of fasting plasma glucose. Some antidiabetic medicinal plants has shown significant impacts by modulating the activities of glucose metabolizing enzymes [9,10].

Studies has reported that plants extract reverses phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase activities [11,12].

The plant *Balanites aegyptiaca Delile*, also known as ‘desert date’ in English, a member of *Zygophyllaceae* family, is a common plant specie of the dry land areas of Africa and Asia [13,14]. In Nigeria, it is found mostly in the Northern region. It is known as ‘Aduwa’ in Hausa, ‘Utazi’ in Igbo, and ‘Teji’ in Yoruba. *Balanites aegyptiaca* has a long history of traditional uses for wide ranges of disease [15].

The fruit extracts of *Balanites aegyptiaca* was reported to have exhibited prominent antihyperglycemic activity in diabetic-induced animals [16-18]. It was reported that the plant fruit extract stimulated insulin secretion [16,19], increased muscle basal glucose uptake [20] to lowered blood glucose level. It was also reported that it retards the activities of some enzymes of carbohydrate metabolism such as intestinal α-amylase [21]. In a recent studies, the plant leaves extract was reported to have inhibited alpha amylase activity *in vitro* [22] while in our study it was found to reverse the activities of some key hepatic enzymes of glucose metabolism in diabetic rats [23].

It was reported that the antidiabetic activity of medicinal plants depends upon a variety of mechanisms which include: stimulation of insulin secretion, inhibition of insulin degradative processes and reduction of insulin resistance [24], regenerating or repairing
pancreatic β-cells and protecting the destruction of the β-cells [25], stimulation of glycogenesis and hepatic glycolysis [26], inhibition of α-amylase/α–glucosidase enzymes [27], inhibition of gluconeogenesis [8] and preventing oxidative stress in pancreatic β- cell dysfunction [28]. *Balanites aegyptiaca* fruit extract has shown to low blood glucose via some of the mechanisms but whether the fruit extract could stimulate glycolysis, glycogenesis, and inhibit gluconeogenesis and glycogenolysis are not fully clear. Hence, the need to evaluate the plant extract on the activities of enzymes in glycolysis, gluconeogenesis, glycogenolysis and glycogenesis in streptozotocin-induced diabetic rats.

2. MATERIALS AND METHODS

2.1. Materials

a) Chemicals/reagents

All chemicals/reagents used were of analytical grade and were obtained from Sigma Aldrich, USA; British Drug House, England; Randox laboratory, UK.

b) Plant collection

*Balanites aegyptiaca* fruit-mesocarp was collected from Gubi village (latitude 10° 45’ N & longitude 9° 82’ E) in Bauchi, Bauchi state. It was identified and authenticated at the Herbarium Unit, Department of Biological Science, Ahmadu Bello University Zaria. A voucher specimen (voucher no: 900175) was deposited in the herbarium of the Department.

2.2. Experimental animals

A total of twenty-five (25) male wistar albino rats were used for the study. The rats were obtained from the Animal House, University of Jos, Plateau State, Nigeria and kept in clean cages with 12 hours / 12 hours light/dark photoperiod. Water and feed ‘growers mash’ (Vital feeds, Jos) were supplied *ad libitum*. The rats were allow to grow attaining a weight between 180-230g before used. All experimental protocol was in conformity with the institutional guidelines that are in compliance with national and international laws and guidelines for care and use of laboratory animals [29].

2.3. Methods

a) Plant extraction/fractionation

Plant fruit-mesocarp was defatted as performed by Jung *et al* [30] and extracted as done by Govorko *et al* [31] with little modification in the choice of the extraction temperature (60 °C). Seven hundred and fifty gram (750g) powdered of plant fruit-mesocarp was defatted for 2 hours with 1200 ml hexane on a mechanical shaker.

The hexane solvent was discarded, then the defatted sample air-dried. Exactly 200 g of the defatted plant fruit-mesocarp was mixed with 2000 ml of 80 % ethanol and heated to 60 °C for 2 hours. The extraction continued for an additional 10 hrs at 20 °C. The mixture was filtered through a cheese cloth and resulting ethanol extract was air-dried. The procedure was repeated twice with same amount of defatted plant fruit-mesocarp.
Ethanol fruit-mesocarp extract was dissolved in water (500 ml) and partitioned with ethyl acetate (500 ml) at 20 °C for 2 hours then separated using a separating funnel (1000 ml). Fractions were concentrated using a rotary evaporator at 40 °C and air dried. The dried aqueous (AFF) and ethyl acetate (EFF) fractions of *Balanites aegyptiaca* fruit-mesocarp were stored in air-tight containers and kept in a refrigerator at 4 °C until used.

**b) Induction of diabetes mellitus**

Diabetes mellitus was induced in rats by intra-peritoneal injection of Streptozotocin (STZ) at a dose of 60 mg/kg body wt dissolved in 0.1 M citrate buffer (pH 4.5). Rats were given 10 % glucose solution in their drinking water for 48 hours after STZ injection in order to prevent severe hypoglycemia. After 72 hours, blood glucose levels were checked and subsequent 1-week intervals to identify the onset and continued presence of diabetic hyperglycemia; rats with fasting blood glucose levels ≥200 mg/dl were considered diabetic and selected for the study [32].

2. 4. Experimental design

Effects of ethanolic extract-fractions of *Balanites aegyptiaca* fruit-mesocarp on the activities of glucose metabolic enzymes was assessed in the streptozotocin-induced diabetic rats. Rats were randomly allocated into groups of 5 rats each as follows;

- **Group A**: Diabetic + Aqueous fruit-Mesocarp fraction (AFF)
- **Group B**: Diabetic + Ethyl acetate fruit-Mesocarp fraction (EFF)
- **Group C**: Diabetic + Metformin at 200 mg/kg body weight (kolawole and Akanji [12])
- **Group E**: Diabetic control
- **Group F**: Normal control

At the end of the experiment, animals were sacrificed, liver were excised, homogenized and was used for the assay of the glucose metabolic enzymes. The extract-fractions were administered orally using oral gastric tube. Exact 400 mg/kg body weight of plant extracts were administered to various diabetic rats’ groups daily for 28 days period. The extract-dose used was determined following our previous acute toxicity report on the AFF and EFF of *Balanites aegyptiaca* [33].

2. 5. Assay of glucose metabolic enzymes’ activity

Hepatic key glucose metabolic enzymes like Glucokinase [34], Phosphofructokinase [35], fructose-1,6-bisphosphatase [36], Phosphoenolpyruvate carboxylkinase [37], Glucose-6-phosphate dehydrogenase [38], Glycogen phosphorylase activity [39], Glucose-6-phosphatase activity [40], Glycogen synthase activity [41], Pyruvate kinase [42] were all assayed following standard procedures.

2. 6. Statistical analysis

Data from the experiments were expressed as mean ± standard deviation (SD). Means were analyzed by one way analysis of variance (ANOVA) and compared by Duncan’s multiple range test (DMRT) [43]. Significant difference was accepted at $P < 0.05$. 

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3. RESULTS

3.1. Effect of EFF and AFF on glycolytic enzymes in diabetic rats

Change in the activities of glucokinase, phosphofructokinase and pyruvate kinase assayed from liver of non-diabetic, diabetic treated and diabetic untreated rats is presented in Table 1. *Balanites aegyptiaca* fruit-mesocarp extract-fractions showed a stimulatory effect on the glycolytic enzymes activities particularly the aqueous-fraction (AFF) which significantly (P<0.05) enhances glucokinase (from 2.22±0.02 to 3.58±0.05 U/min/mg protein).

Table 1. Effect of Ethanol Extract-Fractions of *Balanites aegyptiaca* Fruit-Mesocarp on Glycolytic Enzymes Activities in Liver of Streptozotocin-induced Diabetic Rats

<table>
<thead>
<tr>
<th>Animal Grouping</th>
<th>Diabetic+AFF</th>
<th>Diabetic+EFF</th>
<th>Diabetic Control</th>
<th>Diabetic+Metformin</th>
<th>Normal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucokinase (U/min/mg Protein)</td>
<td>3.58±0.05</td>
<td>3.10±0.02</td>
<td>2.22±0.02</td>
<td>2.72±0.02</td>
<td>3.53±0.01</td>
</tr>
<tr>
<td>Phosphofructokinase (U/min/mg Protein)</td>
<td>2.30±0.57</td>
<td>1.25±0.03</td>
<td>2.06±0.07</td>
<td>3.34±0.01</td>
<td>4.43±0.08</td>
</tr>
<tr>
<td>Pyruvate Kinase (U/min/mg Protein) ×10^{-1}</td>
<td>0.30±0.03</td>
<td>0.09±0.03</td>
<td>0.04±0.01</td>
<td>0.15±0.02</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>LDH (U/min/mg Protein) ×10^{-2}</td>
<td>6.07±2.05</td>
<td>7.13±2.69</td>
<td>4.36±1.58</td>
<td>6.12±2.19</td>
<td>7.10±1.60</td>
</tr>
</tbody>
</table>

Values are Mean ± SD of 5 determinations. Values with different superscript across the rows are significantly different (P<0.05) 
AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate fruit-mesocarp fraction, LDH = Lactate Dehydrogenase

3.2. Effect of EFF and AFF on gluconeogenic enzymes in diabetic rats

Changes in the activities of hepatic gluconeogenic enzymes namely glucose-6-phosphatase, fructose-1,6-bisphosphatase, and phosphoenol pyruvate carboxyl kinase in diabetic untreated, diabetic treated and non-diabetic rats are shown in Table 2. The diabetic untreated rats showed an increase in gluconeogenic enzymes activity. However, these were significantly (P < 0.05) suppressed in the diabetic treated animals.
Aqueous fruit-mesocarp fraction (AFF) suppressed glucose-6-phosphatase (from 1.44±0.05 to 0.17±0.0 U/min/µmole P\(_i\) liberated) and phosphoenol pyruvate carboxylkinase (from 0.81±0.15 to 0.38±0.04 U/min/mg protein) while ethyl acetate fraction (EFF) suppressed fructose-1,6-bisphosphatase (from 2.19±0.25 to 1.20±0.03 U/min/µmole P\(_i\) liberated) among others.

Table 2. Effect of Ethanol Extract-Fractions of *Balanites aegyptiaca* Fruit-Mesocarp on Gluconeogenic Enzymes Activities in Liver of Streptozotocin-induced Diabetic Rats

<table>
<thead>
<tr>
<th>Glucose-6-Phosphatase (U/min/µmole P(_i) liberated)</th>
<th>Animal Grouping</th>
<th>Diabetic+AFF</th>
<th>Diabetic+EFF</th>
<th>Diabetic Control</th>
<th>Diabetic + Metformin</th>
<th>Normal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.17±0.00</td>
<td></td>
<td>0.70±0.01</td>
<td>1.44±0.05</td>
<td>0.12±0.02</td>
<td>0.07±0.01</td>
<td></td>
</tr>
<tr>
<td>Fructose-1,6-Bisphosphatase (U/min/µmole P(_i) liberated)</td>
<td></td>
<td>1.41±0.06</td>
<td>1.20±0.03</td>
<td>2.19±0.25</td>
<td>1.02±0.02</td>
<td>1.40±0.07</td>
</tr>
<tr>
<td>Phosphoenol-pyruvate Carboxyl kinase (U/min/mg Protein)</td>
<td></td>
<td>0.38±0.04</td>
<td>0.46±0.01</td>
<td>0.81±0.15</td>
<td>0.11±0.04</td>
<td>0.09±0.01</td>
</tr>
</tbody>
</table>

Values are Mean ± SD of 5 determinations. Values with different superscript across the rows are significantly different (P<0.05)

**AFF =** Aqueous Fruit-mesocarp fraction, **EFF =** Ethyl acetate fruit-mesocarp fraction

### 3.3. Effect of EFF and AFF on glycogenolytic and glycogenesis enzymes in diabetic rats

Activities of glycogen metabolic enzymes; glycogen synthase (GS) and phosphorylase in liver of diabetic untreated, non-diabetic and diabetic rats treated with ethyl acetate and aqueous fractions of *Balanites aegyptiaca* fruit-mesocarp are presented in Table 3.

A significant (P<0.05) decreased in glycogen synthase activity was recorded in the diabetic control rats group (9.41±0.34 ×10\(^2\) U/min/mg protein) compared with metformin treated rats group (15.51±0.42 ×10\(^2\) U/min/mg protein) and the plant ethanol extract-fractions; aqueous fraction (12.24±0.22 ×10\(^2\) U/min/mg protein) and ethyl acetate fraction (11.75±0.11 ×10\(^2\) U/min/mg protein). However, there was no significant difference (P>0.05) in the activity of glycogen phosphorylase from the diabetic treated and untreated diabetic rats.
Table 3. Effect of Ethanol Extract-Fractions of Balanites aegyptiaca Fruit-Mesocarp on Glycogen Content and Glycogen Metabolic Enzymes Activities in Liver of Streptozotocin-induced Diabetic Rats

<table>
<thead>
<tr>
<th>Animal Grouping</th>
<th>Diabetic+ AFF</th>
<th>Diabetic + EFF</th>
<th>Diabetic Control</th>
<th>Diabetic + Metformin</th>
<th>Normal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic Glycogen (mg/g liver)</td>
<td>20.62±0.44</td>
<td>14.54±0.32</td>
<td>10.69±0.32</td>
<td>17.77±0.32</td>
<td>15.85±0.32</td>
</tr>
<tr>
<td>Glycogen Phosphorylase (U/min/mg Protein)</td>
<td>3.75±0.00</td>
<td>3.46±0.01</td>
<td>3.82±0.21bc</td>
<td>2.04±0.01a</td>
<td>2.07±0.01</td>
</tr>
<tr>
<td>Glycogen Synthase (U/min/mg Protein)</td>
<td>12.24±0.22</td>
<td>11.75±0.11</td>
<td>9.41±0.34</td>
<td>15.51±0.42</td>
<td>29.25±0.88</td>
</tr>
</tbody>
</table>

Values are Mean ± SD of 5 determinations. Values with different superscript across the rows are significantly different (P<0.05)
AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate fruit-mesocarp fraction

4. 4. Effect of EFF and AFF on enzyme of hexomonophosphate pathway in diabetic rats

Activity of glucose-6-phosphate dehydrogenase (G6PDH) was determined in the liver tissues of diabetic treated rats in order to assess the impact of ethyl acetate and aqueous fractions of Balanites aegyptiaca fruit-mesocarp (Figure II). Treatment with metformin and ethanol extract-fractions of Balanites aegyptiaca fruit-mesocarp enhanced G6PDH activity by varying degrees. The aqueous fraction effective enhanced G6PDH activity (from 1.45±0.02 to 2.10±0.02 U/min/mg protein) compared with values obtained from diabetic rats group treated with the ethyl acetate fraction.

5. DISCUSSION

Balanites aegyptiaca fruit extracts has been reported to exert potential antihyperglycemic activity [20,44]. Studies to explain how Balanites aegyptiaca fruit extract lowered fasting blood glucose suggested stimulation of insulin secretion [16], inhibition of intestinal α-amylase activity [21], increased muscle basal glucose uptake [16] as well as antioxidant activity [45]. Antidiabetic medicinal plants has been reported to exhibit variety of mechanisms to low blood glucose. The mode of actions exhibited by Balanites aegyptiaca
fruit extract might be attributable to the variety of different biologically active chemicals in the fruit [16,46,47].

In a recent studies, the plant leaves extracts was reported to have inhibited alpha amylase activity in vitro [22] and also reverses the activities of some key hepatic enzymes of glucose metabolism in diabetic rats [23]. Shafik et al [48] has reported that seed kernel of *Balanites aegyptiaca* enhanced glucokinase activity in diabetic rats. Activities of glucokinase, phosphofructokinase and pyruvate kinase has been shown to be sensitive signs of the glycolytic pathway and these are decreased in the liver of diabetic state [49].

Reduced activities of these enzymes in this study are consistent with other studies on glycolytic enzymes [1,50]. Administration of fractions of the ethanolic extract of *Balanites aegyptiaca* fruit induced significant increase in the activities of glycolytic enzymes supporting the notion that part of the therapeutic potential of several putative antidiabetic plants can involve the modulation of enzymes in carbohydrate metabolism [51,52].

The activity of hepatic glucokinase is reported to be reduced in diabetes mellitus and can be activated by an activator [53]. Increased glucokinase activity in diabetic rats treated with extract-fractions of *Balanites aegyptiaca* fruit-mesocarp might have improve glycolysis leading to the reduction in blood glucose. Shafik et al [48] have shown that extract of *Balanites aegyptiaca* seed-kernel promotes the activity of hexokinase, suggesting glycolysis stimulatory effect of the plant.

Glucconeogenesis is a main cause of the elevated hepatic glucose production contributing 50-60 % of the released glucose [54]. It has been noted that metformin inhibit

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Figure I. Effect of Ethanol Extract-Fractions of *Balanites aegyptiaca* Fruit-Mesocarp on Glucose 6-phosphadehydrogenase Activity in STZ-induced Diabetic Rats

Bars with different letters are significantly different (P<0.05)

AFF = Aqueous Fruit-mesocarp fraction, EFF = Ethyl acetate fruit-mesocarp fraction

G6PDH Activity (U/min/mg protein)

Diabetic +AFF  Diabetic +EFF  Diabetic + Met  Diabetic Control  Normal Control

Animal Groups (n=5)
hepatic gluconeogenesis to achieved anti-diabetic effect [55, 56]. *Balanites aegyptiaca* might have lowered blood glucose in part by inhibition of hepatic gluconeogenesis. Plants like *Eugenea jambolana* [57], *Centaurea bruguie-rana* ssp. *Belangerana* [58], and *Juglans regia* [59] have been reported to inhibit hepatic gluconeogenesis as part of their anti-diabetic mechanism.

In this study, extract-fractions of *Balanites aegyptiaca* fruit-mesocarp suppressed the activities of the gluconeogenic enzymes. This is in line with other studies where several plants extract were reported to have suppressed the activities of the gluconeogenic enzymes in diabetic animals [12,27,60,61]. Shafik *et al* [48] have reported that extract of *Balanites aegyptiaca* seed-kernel suppressed glucose-6-phosphatase activity.

Glycogen synthase (GS) catalyzes the rate limiting step in glycogen synthesis and is thus responsible for the storage of glucose as glycogen in the liver. Fractions of ethanolic extract of *Balanites aegyptiaca* fruit-mesocarp appear to have no effect on glycogen phosphorylase activity but activate synthase activity significantly in the diabetic treated rats. This is supported by the relative change in glycogen content in liver of the diabetic treated rats. Some plants extract have been reported to regulated glycogen enzymes leading to increased hepatic glycogen content [62,63]. According to Gutierrez [64], activation of glycogen synthase by plant suggested insulinogenic character; going by this statement one may propose that *Balanites aegyptiaca* contains component that exhibits insulin like effect.

Glucose-6-phosphate dehydrogenase is the first rate limiting enzyme of the pentose phosphate pathway which results in the production of ribose-5-phosphate and the reducing equivalent NADPH [65]. The study observed suppressed activity of G6PDH in liver of STZ-diabetic rats similar to reports by Karuna *et al* [66] and Saralakumari *et al* [67]. Diaz-Flores *et al* [68] have reported that decreased hepatic G6PDH activity depends on the severity of hyperglycemia. Decreased activity of G6PDH indicated low level of NADPH produced by hexose monophosphate pathway (HMP) which is unable to meet the cellular requirement for the enzymes that continuously maintain glutathione (GSH) in its reduced state or accumulation of glucose-6-phosphate which is a potent glycosylating agent that causes GSH depletion and thereby boosts glycation and it may also promote the final step of gluconeogenesis [69].

*Balanites aegyptiaca* fruit extract-fractions administration increased G6PDH activity in the diabetic treated rats, accompanied by increase in NADPH levels, a product of the HMP pathway. This might suggest that the ethanol-aqueous fraction of *Balanites aegyptiaca* fruit-mesocarp has the capacity to modulate hexose monophosphate pathway for alternative glucose oxidation. In addition, Atangwho *et al* [70] reported that glucose oxidation via gluconate pathway is associated with increased glucokinase activity. In this study, it is suggested that activation of G6PDH in the diabetic rats groups particularly that received the ethanol-aqueous fraction of *Balanites aegyptiaca* fruit-mesocarp may be a reflection of the increased activity of their glucokinase.

6. CONCLUSION

In conclusion, ethanol extract-fractions of *Balanites aegyptiaca* fruit-mesocarp could exert enzymes regulatory effect as recorded in this study from liver of diabetic rats. Aqueous fraction shows to be the most potent, it enhances glucokinase and glucose-6-
phosphatedehydrogenase (G6PDH) activity suggesting that the plant fruit extract could promotes glucose oxidation to low blood glucose. Further research is needed to explore the fruit-mesocarp ethanol-aqueous fraction for its pharmaceutical importance.

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