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RESEARCH ARTICLE

Phytochemical screening and antidiabetic activities test of ethanol extract from *Syzygium cumini L.* seeds in male Wistar rats induced by alloxan

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Abstract

Introduction: The ethanol extract of the jamblang fruit seed (*Syzygium cumini L.*) is known to have an antidiabetic effect and this is further confirmed by the high intensity of its α-amylase inhibitory effect. **Aim**: The purpose of this study was to determine the antidiabetic activity of jamblang fruit seed extract in reducing blood sugar levels. **Method**: Phytochemical screening was carried out on the ethanol extract from jamblang fruit seeds. Twenty-eight male wistar rats were given alloxan to induce diabetes. The rats were divided into four groups: normal control, negative control (CMC-Na), positive control (Glibenclamide), and a group that was given ethanol extract of jamblang seeds. Measurement of blood glucose levels was carried out on day 0 and day 15. **Results**: The phytochemical screening results show that the ethanolic extract of jamblang fruit seeds (*Syzygium cumini L.*) contains alkaloids, flavonoids, saponins, tannins, and polyphenols, as well as steroids and triterpenoids. The group being treated with ethanol extract from jamblang seeds showed decreased blood sugar levels on day 15, indicating the antidiabetic effectiveness of the extract. **Conclusion**: Ethanol extract from jamblang seeds (*Syzygium cumini*) contains alkaloids, flavonoids, saponins, tannins, polyphenols, steroids, and triterpenoids; and is able to reduce blood glucose levels of male wistar rats induced with alloxan.

Introduction

Diabetes mellitus (DM) is a chronic and progressive disease characterised by an increase in blood sugar levels (hyperglycemia). This disease is caused by pancreatic beta cells failing to secrete insulin and insulin resistance (Bhat *et al*., 2011). Long-term use of oral antidiabetic drugs can cause various side effects. Herbal medicine is an alternative choice because it has a lower-cost therapy and fewer side effects.

One of the herbal plants that have the potential to act as an antidiabetic drug is *Jamblang* seeds. *Jamblang* fruit is a tropical plant of the Myrtaceae family, where the plant stem and leaves have been widely used in Ayurveda and traditional Indian medicine to treat diabetes (Perera *et al*., 2017). *Jamblang* fruit (*Syzygium cumini L.*), known as *Juwet* fruit in Balinese society, has also been known to have various uses in medicine. *Jamblang* stem infusion has been shown to reduce blood glucose levels and increase insulin production in pancreatic epithelial cells with a high antioxidant effect (Perera *et al.,* 2017). Not only does it have an antidiabetic effect, but the *Jamblang* plant is also known to have antimicrobial, gastroprotective, antiulcerogenic, anti-inflammatory, hypolipidemic, immunomodulatory, antioxidant, and various other effects (Proma *et al*., 2018*). Jamblang* fruit seeds contain alkaloids, flavonoids, glycosides, steroids, glycosides, saponins, resins, phenols, tannins, and terpenoids.

The high number of flavonoids in the methanol extract of *Jamblang* seeds is known to have an antidiabetic effect. An in vitro study has shown that it has a large inhibitory activity on the α-amylase enzyme (Prabakaran & Shanmugave, 2017). An *in vivo* study of the antidiabetic effect of *Jamblang* seeds has never been reported in Indonesia; therefore, it is necessary to

test the antidiabetic activity of *Jamblang* seed extract using rats induced by alloxan.

Aim

The purpose of this study was to determine the antidiabetic effect of *Jamblang* seed extract in reducing blood sugar levels.

Materials and method

Materials

Jamblang fruit seeds, methanol 96% (Merck), alloxan monohydrate (Merck), glibenclamide (Indofarma), CMC-Na (Merck), aquabidest (Bratachem), 2N HCL (Merck), H_2SO_4 (Merck), Dragendroff's reagent (Merck), Mayer's reagent (Merck), Acetone P (Merck), P Oxalic Acid (Merck), ether (Merck), $FeCl_3$ (Unilab), chloroform (Merck), glucometer (Accu-Chek), glucometer test strip (Accu-Chek).

Sample collection and preparation of the Jamblang seeds

Jamblang seed samples were sorted from fresh and ripe *Jamblang* fruit. The sample used was *Jamblang* fruits harvested from the Banyuatis area, Buleleng, Bali. Fresh *Jamblang* seeds were dried in the sun and dried for further processing into powder. The Simplicia powder was then prepared for maceration in a 96% ethanol solvent in a ratio of 1:3 for three days. The extract was then evaporated using a vacuum rotary evaporator (Biobase RE-201D) at 40°C to obtain a thick extract.

Dosage determination

Jamblang seed extract (*Syzygium cumini L.*) was administered orally once a day to the test animals. *Jamblang* seed extract (*Syzygium cumini L.*) was given in a concentration of 10% in a 1% CMC-Na suspension.

Dosage determination of glibenclamide

In this study, the antidiabetic drug used as a positive control was glibenclamide. The dose of glibenclamide given to the test rats was 0.126 mg/200g BW.

Antidiabetic activity test

The antidiabetic activity test was initiated by adapting the male Wistar rats under standard conditions for one week. The initial blood glucose levels of male Wistar rats were measured after 12 hours of fasting. The animal diabetic model was made by inducing rats with 120 mg/kg BW of alloxan. Blood glucose was measured 72 hours after alloxan induction (day 0). The model used were rats with glucose levels > 200 mg/dl (Hery *et al*., 2013). From day 1 to day 15, the test animals were treated with ethanol extract from *Jamblang* seeds and glibenclamide. The blood glucose levels of the test animals were measured again on the 15th day. The enzymatic measurement method was Glucotest EZ Smart, and blood samples from the lateral tail vein were used.

Data analysis

The data obtained in the form of blood sugar levels of test animals were compared using bar charts, accompanied by the percentage decrease in pretest and posttest levels. The statistical analysis was performed using the *t*-paired test on SPSS 16.0 for Windows application with $p = 0.05$.

Results

Table I shows the phytochemical screening of 96% ethanol extract from Jamblang fruit seeds (*Syzygium cumini L.*).

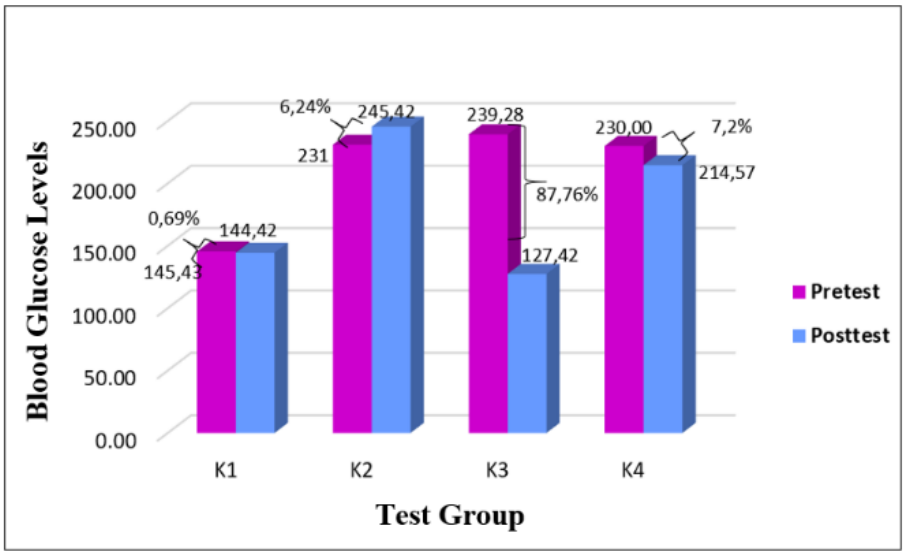
Descriptive analysis

Based on Figure 1, there is a decrease in blood sugar levels in the positive control group (K3) and the *Jamblang* seed extract group (K4) after being given treatment (posttest). The highest percentage decrease was seen in the positive control group (K3), with a decrease in blood sugar levels of 87.76%. Followed by a decrease of 7.2% in the group given *Jamblang* seed extract (K4), a decrease of 0.69% in the normal control group, while the negative control group (K2) had an increase of 6.24%. Comparing test results from the t-paired test on pretest and posttest blood sugar level data showed that there was an effect of treatment on the negative control group (K2), positive control (K3), and the *Jamblang* seed extract treatment group (K4) with $p < 0.05$.

Table I: Phytochemical screening of 96% ethanol extract from *Jamblang* fruit seeds (*Syzygium cumini L.*)

No.	Phytochemical test	References	Results	Conclusion	
1.	Alkaloids	†An orange precipitate formed (Dragendroff's reagent)	An orange precipitate formed	(+)	
		†A yellow precipitate formed (Mayer's reagent)	A yellow precipitate formed	(-)	
2	Flavonoids	†Intensive yellow fluorescence at UV 366 nm	intensive yellow fluorescence	(+)	
3	Saponins	†The presence of foam that lasts < 10 minutes as high as 1 - 10 cm and the foam does not disappear after the addition of one drop of HCL 2N	Foam is formed as high as three cm	(+)	
4.	Tannins and polyphenols	Tannins	†Dark blue/greenish black	Greenish black	(+)
		Polyphenols	†Dark blue/greenish black	Greenish Black	(+)
5.	Steroids and triterpenoids	Steroids	†A turquoise ring formed	A greenish blue ring formed	(+)
		Triterpenoids	†A brownish or violet ring formed	Formation of a brownish/violet ring	(+)

†(Tiwari *et al.,* 2011)



Analysis of the results from the t-paired test blood sugar level data pretest and posttest; Blood sugar levels (K1 pre - K1 post); $p = 0.538$ (no difference); Blood sugar levels (K2 pre - K2 post); $p = 0.0001$ (significantly different); Blood sugar levels (K3 pre - K3 post); $p = 0.0001$ (significantly different); Blood sugar levels (K4 pre - K4 post); $p = 0.0001$ (significantly different)

Figure 1: Comparison of blood sugar levels pretest and posttest

Discussion

Phytochemical screening was carried out to determine the class of compounds thought to be present in the ethanol extract of *Jamblang* fruit seeds (*Syzygium cumini L.*) The phytochemical test results in Table I showed that the ethanolic extract of *Jamblang* seeds (*Syzygium cumini L.*) contains alkaloids, flavonoids, saponins, tannins, and polyphenols, as well as steroids and triterpenoids. The screening results had similarities with the screening results conducted by Prabakaran and Shanmugavel (2017). Based on this research, the ethanol extract of *Jamblang* seeds (*Syzygium cumini L.*) was reported positive for containing alkaloids, flavonoids, glycosides, saponins, tannins, phenols, steroids, and triterpenoids.

In this study, antidiabetic activity was measured by decreasing the blood sugar levels of male Wistar rats induced by alloxan. The treatment group was divided

into four groups, namely the normal control group (K1), the negative control group (K2), the positive control group (K3), and the group with *Jamblang* seed extract (K4).

The results of measuring blood sugar levels after treatment (posttest) in the negative control, positive control, and *Jamblang* seed extract group showed that there was a significant change when compared to the blood sugar levels of the test animals before treatment (pretest).

The highest percentage decrease in blood sugar levels was 87.76% in the positive control group (K3). The treatment group that was administered with *Jamblang* seed extract had a percentage decrease of 7.2%. The normal control group experienced a decrease of 0.69%, which was not significantly different, while the negative control group experienced an increase in blood sugar levels by a percentage decrease of 6.24%.

The ethanolic extract of *Jamblang* seeds used in this study positively contained alkaloids, flavonoids, saponins, tannins, polyphenols, steroids, and triterpenoids. The content of carbohydrates, steroids, and alkaloids in *Jamblang* seeds was able to significantly reduce blood glucose levels in a rat model of type 2 diabetes (Proma *et al.,* 2018). The antidiabetic mechanism possessed by *Jamblang* fruit seeds is thought to be due to the high content of flavonoids, phenols, and antioxidants that are able to inhibit the α-amylase enzyme (Gajera *et al.,* 2017; Prabakaran & Shanmugavel, 2017).

Based on several studies that have been described above, it can be said that the antidiabetic effect caused is due to several metabolites contained in the ethanol extract of *Jamblang* seeds. This effect gave quite good results within 15 days of the administration, although it had not been able to provide a greater percentage of lowering blood sugar levels than the positive control (glibenclamide).

Conclusion

Based on the results of the research that has been carried out, the following conclusions can be drawn: ethanol extract from *Jamblang* fruit seeds (*Syzygium cumini*) contains alkaloids, flavonoids, saponins, tannins, polyphenols, steroids, triterpenoids and can reduce blood glucose levels of male Wistar rats induced by alloxan.

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