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α-Glucosidase and α -Amylase Inhibitory Activities of Jambolan (*Syzygium cumini* (L.) SKEELS) Fruit and Seed

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ABSTRACT
Jambolan (*Syzygium cumini* (L.) Skeels) is well known as a medicinal plant, this commodity is rich in compounds containing phenol and flavonoid that is able to medicate a lot of diseases, especially diabetes mellitus. This research aimed to investigate antidiabetic potential of jambolan fruit and seed extract in vitro. White jambolan flesh of fruit and seed extracts were prepared using binary solvent of aqueous ethanol (50% v/v) by adding solvent to 50 g sample in volumetric flasks. After that, the flasks were agitated at 280 rpm on 50°C for 45 min and then extracts were filtered. The filtrate evaporated using rotary evaporator at 400C under reduced pressure for the removal of solvent. Later on, the extracts were stored in sealed bottles. The antioxidant activity was analyzed used DPPH, while anti-diabetic activity analyzed α-glucoside enzyme inhibition activity by measuring the p-nitrofenol as enzymatic reaction product at wavelength 400 nm and α-amylase enzyme activity inhibition was determined using wavelength at 540 nm. The result of α-glucosidase, n-Heksan extracts of fruit and seed showed inhibition of IC50 = 50,35 ± 2,13 mg/mL and IC50 = 52,42 ± 1,60 mg/mL, respectively; ethyl acetat extracts of fruit and seed showed inhibition IC50 = 41,51 ± 0,27 mg/mL and IC50 = 59,85 ± 0,95 mg/mL, respectively; and ethanol IC50 = 55,79 ± 4,11 mg/mL and IC50 = 42,98 ± 0,80 mg/mL, respectively. Meanwhile the result of α-amylase, n-Heksan extracts of fruit and seed showed inhibition IC50 = 75,28 ± 1,04 mg/mL and IC50 = 74,72 ± 0,52 mg/mL, respectively, Ethyl acetat extracts of fruit and seed showed IC50 = 64,06 ± 0,20 mg/mL and IC50 = 67,92 ± 0,52 mg/mL, respectively, and Ethanol IC50 = 75,85 ± 0,34 mg/mL and IC50 = 64,63 ± 1,18 mg/mL, respectively. α-Glucosidase inhibition was lower than acarbose inhibition activity 63,40%, meanwhile, α-Amylase inhibition at fruit and seed of white Jambolan was stronger than gallic acid 36,36%.

Keywords: *Jambolan (Syzygium cumini (L.) Skeels), α-Glucosidase, α-Amylase*

1. INTRODUCTION

Jambolan (*Syzygium cumini* (L.) Skeels) belongs to the genus Syzygium of the myrtle family of *Myrtaceae*. Sometimes, it was called as *E. jambolana* Lam., *M. cumini* Linn., *S. jambolana* DC and *E. cumini* (Linn.) Druce. This fruit is recognized for its abundant compounds with medicinal properties such as volatile oils, anthocyanin, phenol, flavonoid, isoqercetin, myrecetin, etc. which makes this fruit widely used in traditional medicine [1].

Diabetes mellitus (DM) prevalence in Indonesia is significantly increasing. Based on the data of Health Research and Development Ministry, in 2013 the prevalence was 6.9%, and it enhanced up to 8.5 % in 2018. Diabetes mellitus is a persistent metabolic disorder disease, that is distinguished by high blood sugar along with carbohydrate, protein, and lipid interference as the consequences of insufficient insulin function [2]. Diabetes occurs because of insufficiency in insulin (a hormone that regulates blood sugar level) produced by the pancreas or inability of the body to effectively use the insulin it produces [3]. This condition could be controlled by inhibiting α-amylase and α-glucosidase enzyme activity. Amylase is an enzyme that play roles in hydrolysis process of alpha-1,4-glycosidic polysaccharides into dextrin, oligosaccharides, and monosaccharides [3], [4]. Inhibition of those enzyme activities was intended to delay glucose adsorption so that postprandial blood glucose is decreased.

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The common medicines that have been used to control blood sugar through the inhibition of digestive enzymes are reported to possess side effects e.g. gastrointestinal unrest, liver disorder, and other complications [5]. Candidates for medicinal raw materials derived from natural plant extracts are expected to be safer and more effective to use. The common materials used in inhibition of those enzyme activities are containing phenol and flavonoid. *Syzygium cumini* (L.) Skeels is well known as a plant with medicinal properties, this commodity is rich in compounds containing phenol and flavonoid that is able to medicate a lot of diseases, especially diabetes mellitus (antidiabetic plant). Various parts of jambolan, e.g. stem bark, leaves, fruits, and seeds, are commonly exploited in folk medicine to treat diabetes mellitus and hyperglycaemic [1].

This research aimed to investigate antidiabetic potential of white jambolan (*Syzigium cumini* (L) Skell fruit and seed in vitro.

2. MATERIALS AND METHODS

White jambolan fruit and seed were taken from Kersikan Villages, Godang Wetan, Pasuruan. White Jambolan fruit and seed were extracted using binary solvent i.e. aqueous ethanol (50% v/v). Samples of 50 g jambolan fruit and seed were put into volumetric flasks then added into the solvent. After that, the flasks were agitated at 280 rpm and 50 °C using orbital shaker for 45 min, followed by filtration of the extracts. To remove the solvent, the filtrate was evaporated facilitated by rotary evaporator at 40 °C under reduced pressure. Later on, kept the extracts in sealed bottles.

The antioxidant activity was analyzed used DPPH assay, while antidiabetic activity analyzed α-glucoside enzyme inhibition activity by measuring the p-nitrofenol as enzymatic reaction product at wavelength 400 nm. The α-amylase enzyme activity inhibition determined used wavelength of 540 nm. Statistical analysis was performed with three replications and the data was displayed as the mean value and standard deviation.

3. RESULTS AND DISCUSSION

The result of total phenolic content in fruit flesh was about 0,208-0,907 µg GAE/mg, while the content in seed was 0,259-1,537 µg GAE/mg. Phenolic compounds are substantial plant contents which are responsible for antioxidant activity and related to redox properties. Meanwhile, the hydroxyl groups in plant extracts function in the scavenging of free radical [6]. The total flavonoids content (TFC) in fruit was 0,605-6,189 µg GAE/mg, and in seed was 5,385-7,240 µg GAE/mg. Flavonoids are well-known as secondary metabolites with antioxidant properties, which the potential depends on free OH groups numbers and positions [7].

The antioxidant activity in fruit was 313,65-492,17, and in seed was 351,16-408,42. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) is a free-radical molecule, which is stable and losses its absorption spectrum on a range of 515–528 nm when it takes up an electron or a free radical species. According to Chithiraikumar [8] the DPPH assay is the most widely employed method due to its simplicity and reliability to investigate the radical scavenging capability of plant extracts which showed visually as a change of stable purple-coloured DPPH radical into the yellow-coloured DPPH. The detailing result of TPC, TFC, and Total DPPH were showed at Table 1.

Table 1. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant potential of white jambolan fruit and seed extracts

Extract	TPC (mg GAE/g)	TFC (mg QE/g)	Total DPPH (µg/mL)
Fruit			
n-Heksan	0.208 ± 0.010	6.189 ± 0.091	386.43 ± 7.60
Ethyl Acetate	0.907± 0.017	0.605 ±0.051	492.17 ± 4.24
Ethanol	0.246 ± 0.013	0.909 ± 0.420	313.65 ± 3.10
Seed			
n-Heksan	0.259 ± 0.006	7.240 ± 0.070	351.16 ± 6.57
Ethyl Acetate	1.537 ± 0.014	5.385 ± 0.102	354.57± 10.27

GAE: Gallic Acid Equivalents; QE: quercetin equivalents; DPPH: DPPH radical

The results of this study are consistent with previous studies which reported the phytochemical content of jambolan. Jambolan seed is reported to be rich in flavonoids and phenolics, while jambolan fruit is also rich in citric acid, gallic acid, diglycoside, cyanidin, and anthocyanins [1]. Rohadi [9] asserted that the powder of Jambolan dry seed containing total phenolic content of 10.77-45.99%, total flavonoid 0.33-2.28%, and total tanin of 6.32-26.90%, depending on the type of extractant. Flavonoid compounds are known to inhibit the activity of the α-glucosidase enzyme, e. g flavonoids from groups 3',4',7-trihydroxiflavone; 5,6,7,3'-tetramethoxy-4'-hydroxy-8-C-prenilflavone and quercetin-3-O-β-D-galactopyranoside, as well as strong inhibitor of α-amylase enzyme activity, namely from the miricetin-3- group O-ramnocide and europetin-3-O-ramnocide. Apart from flavonoids, tannins which are found in stem bark and jambolan leaves, are also reported to inhibit enzyme activity of α-amylase and α-glucosidase. Inhibition of enzyme activity by tannins was achieved through the interaction of complex formation or insoluble sediment [5].

α-amylase inhibition in fruit flesh and seed extract of white jambolan was stronger than gallic acid at 36,36%. Meanwhile, α-glucosidase inhibition was lower than acarbose inhibition activity at 63,40%. The result of α-amylase and α-glucosidase inhibition was showed at Table 2. Inhibiton of α-glucosidase and α-amylase postpones the carbohydrate digestion in the small intestine, hence reduces postprandial blood glucose levels in diabetic suffering patients [8], [10], [11]. The inhibition mechanism of these two notable enzymes has been thought to have better efficacy in lowering the levels of postprandial hyperglycemia [12-13]. This inhibition is caused by the flavonoid content in jambolan. Djamil [2] reported that the flavonoid content of 8-Glucopyranosyl-4 ', 5,7-trihydroxyflavone, also known as 8-glucopyranosylapigenin, 8-glucosylapigenin, can lower blood sugar and inhibit α-glycosidase enzyme.

The inhibition of jambolan fruit and seeds on α-amylase and α-glucosidase (IC50) activities in this study was greater than that of gallic acid (Table 2) and ethanol extract of *Syzygium cumini* (L.) skeel leaves [14] although still lower than acarbose, the commercial drug of DM (Table 2).

It was also reported that gallic acid from peel of jambolan fruit showed inhibition IC50 about 5.96 ppm [15]. However, the ability of jambolan fruit and seed extracts to inhibit α-amylase and or a α-glucosidase (IC50) was still higher than other extracts of natural ingredients such as Dayak onion ethanol extract (*Eleutherine palmifolia*) [16]; acetone and ethanol extract of *Picralima nitida* [10]; extracts of methanol, ethyl acetate and n-hexane of buni leaves (*Antidesma bunius* L.) [17]; and ethyl acetate extract of binahong leaves (*Anredera cordifolia*) [2]. In general, this inhibitory ability is supported by the phytochemicals of the materials which contain phenols, flavonoids, saponins, triterpenoids, and tannins [5].

The highest inhibition of α-amylase activity was showed in ethanol extract of jambolan fruit while the highest inhibition of α-glucosidase was obtained from ethyl acetate extract of jambolan seed. So, it is suggested that these treatments are the best inhibitor of α-amylase and α-glucosidase activity based on the results of this research.

Table 2. Inhibitory activities of while jambolan fruit and seed extracts as showed by IC50 values which showed α-Amylase and α-Glucosidase inhibitory potential

Extract	IC_{50} (mg/ml)	
	α-Amylase	**α-Glucosidase**
Fruit		
n-Heksan	75.28 ± 1.04	50.35 ± 2.13
Ethyl Acetate	64.06 ± 0.20	41.51 ± 0.27
Ethanol	75.85 ± 0.34	55.79 ± 4.11
Seed		
n-Heksan	74.72 ± 0.52	52.42 ± 1.60
Ethyl Acetate	67.92 ± 0.52	59.85 ± 0.95
Ethanol	64.63 ± 1.18	42.98 ± 0.80
Gallic Acid	36.37 ± 3.03	36.37 ± 3.03
Acarbose	-	63.40 ± 0.46

4. CONCLUSION

White jambolan's fruit and seeds extracts have the potential to control diabetes. It was supported by the ability of ethanol extract of jambolan fruit to inhibit the activity of α-amylase at 75,85 ± 0,34 mg/ml and ethyl acetate extract of jambolan seed to inhibit α-glucosidase at 59.85±0.95 mg/ml.

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