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α-Glucosidase Inhibitory Activities of *Rhizophora mucronata* FruitPowder

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Abstract : One of approach methods used to control blood sugar level is inhibiton of of α-glucosidase enzymeactivity. In this research, the α-glucosidase inhibitory activity of extracts of *Rhizophora mucronata*powder processed from immature and maturefruit were studied. *R. mucronata* fruit powder was extracted with hexane, ethyl acetate and ethanol.Phytochemical screening and inhibition of the α-glucosidase activity (IC_{50})analyses were done to the ethanol extract of the fruit powder. Analysis of total phenoliccontent was conductedto the immature and maturefruit rind powder.The results showed that the extractof*R.mucronata* fruit powder contained flavonoids, saponins, tannins and steroids, while the *R.mucronata*rind containedflavonoids and tannins.Extract of unpeeled mature*R. mucronata*powder showed the highest inhibitory activity against α-glucosidase and can be considered as an antidiabetic candidate.The inhibition activity of α-glucosidase from unpeeled mature*R. mucronata*powder extract was assumed to be related to the presence of flavonoidsand tannins, as well as high levels of total phenols ofthe rind.

Keywords: *R. mucronata* fruit, inhibitory activity, α-glucosidase, rind, phytochemical.

Introduction

Diabetes mellitus (DM) type 2 is more common in diabetic. Treatment of type 2 DM mostly is inhibition of α-glucosidase to delay glucose absorption postprandial[1].This enzyme is involvedin carbohydrate metabolisms to form glucose as final product[2]. The ability of the material to inhibit glucose absorption and reduce blood glucose level can be used as a guideline for antidiabetic type 2 drug. One of inhibitors developed as a drug is acarbose[3].

Mangrove is known as new bioactive source for drug and functional food.development. *Rhizophora mucronata*is one of the flora found in mangrovein Indonesian coastlines. Hypocotyl of *R. mucronata*shows antibacterial activity[4]. The bark of *R.mucronata* is astringent andused to cure diabetic[5]. Diabetic rat consuming*R.mucronata* leaf extract and glibenclamide was able to maintain body weight. In contrast, diabetic rat that did not consumed either *R.mucronata* leaf extract and glibenclamide experienced loss body weight[6]. The antidiabetic activity of *R. mucronata* extract was detected by insulin presence in the extract[7].Numerous natural bioactives may functionas antidiabetic and other therapeutic treatment, thus its exploration is important to provide safe products for human consumption[8].

The role *R. mucronata* extract made from bark, hypocothyl and root as hepatoprotective is related with the presence of phytochemical compounds namely flavonoids, alkaloids, coumarins and polyphenols[9]. Furthermore, *R. mucronata* contained tannin as catechin and epigalocathecin gallate[10]. Acetone (70%) extract of the bark of *Cynomorium songaricum* contained 18.3% tannin. The polyphenol flavan-3-ol oligomers showed inhibitory activity ona-glucosidase[3]. It is reported that polyphenol-rich extracts of berries inhibited α-glucosidase activity in vitro[11]. In addition to the bioactive compounds, both immature and maturefruit of *R.*

mucronata contained more than 20% total dietary fiber[12]. Dietary fiber can reduce blood glucose or insulin level postprandial[13].Fruit of *R. mucronata*can be harvested mature and immature, so the inhibitory activity by α-glucosidase of *R. mucronata*fruit flour prepared from mature and immature fruit should be studied.

Materials and Methods

Sample collection

R.mucronata fruit both mature and immaturewerecollected from mangrove areas Penunggul village, Pasuruan, East Java, Indonesia in August 2013.Themature fruit is marked by a yellow line on the hypocotyl.

Preparation of *R.mucronata fruit* flour

R.mucronata fruit powder were made from peeled and unpeeled fruit. Size reduction began with cutting of fruit (10 cm length), peelingand soakingin distilled water. Furthermore, fruit was drained, soaked in citric acid solution (0.5%)for 10 minutes, and followed by immersion in water for 3 days. After that, the fruit was drained, cut to reduce its size and dried in oven at a temperature of 75°C for 3 hours or until the moisture content of the material below the 14.5%[14].The dried fruit pieces were milled by discmill and sieved (80 mesh) in order to obtain *R. mucronata* powder. The rindof fruit, byproduct of the process of stripping *R.mucronata* fruit, also prepared to support the analysis by reducing the size around 5 cm.

Preparation of ethanol extracts of *R. mucronata* fruit flour

R. mucronata fruit flour wasfirst macerated in hexanefor3 days. Residue and filtrate were separated by Whatman filter paper. The residue was thenmacerated again in ethyl acetate for 3 days. On the fourth day, residue and filtrate were separated by Whatman filter paper.The residue of ethyl acetate was macerated again for the third time in ethanol for 3 days. On the fourth day, residue and filtrate were separated by Whatman filter paper. The filtrate of hexane, ethyl acetate and ethanol extracts were each concentrated by rotary evaporation. The extract obtained was properly labeled and stored in the freezer at 4°C until it was used.

Phytochemical screening

Phytochemical screening of *R. mucronata* fruit flourwas done qualitatively to analyze the presence of natural compounds namely flavonoids, alkaloids, steroid, tannin and saponin[15].

In-vitro α-glucosidase inhibitor activity assay

α-glucosidase inhibitory activity assay was conducted by test tube method[16]. One mg of α-glucosidase was dissolved in 100 ml of phosphate buffer (pH 7.0) containing bovine serum albumin. The enzyme mixture was diluted with the same buffer until 1/50 before being tested. Mixture containing 500 μL of 20 mM p-nitrophyenyl α-D-glucopyranoside, 990 μLof 100 mM phosphate buffer and 10 mL of samplesolution or DMSO was made. After incubation(5 min,37°C), the reaction was initiated by adding 500 μL of enzyme solution and incubated for another 15 minutes. Reaction was stopped by adding 2000 μL200 mM Na_2CO_3. Absorbance of p-nitrophenol was measured at 400 nm wavelength by using a Hitachi U-2000 spectrophotometer. The inhibitory activity (%) was calculated with the formula [(CS) / C] x 100, where C is the absorbance of DMSO without sample and S is the absorbance of the sample. Quercetin solution of 1% was used as a positive control.

Total Phenolics Content

Total phenolics content was measured by spectrophotometry using Folin–Ciocalteau reagent method[17],with slight modification. Ethanol extract was diluted with distilled wateruntil 10,000 ppm and then pipeted 0.1 mL. Ethanolextract was then reacted with Folin–Ciocalteau reagent (1mL reagent added with2 mL distilled water). Reaction was stopped by homogenization with 7.5% (w/v) sodium carbonate and incubation at room temperature for 30 minutes. The absorbance oftotal phenol was measured with specthrophotometer at a wavelength of 760 nm. Gallic acid was used as standard and the content of phenol total was determined based on the regression gallic acid formula. Total phenol was measured as total gallic acid equivalent per 100 g extract (g GAE/100 g).

Results and Discussion

Phytochemical Screening

Phytochemical screening was conducted to prove an assumption that there was a correlation between phytochemical of the ethanol extract of *R. mucronata* fruit powderand antidiabetic activity. Both mature and immature fruit powder, peeled and unpeeled, contained saponin, flavonoid, steroid and tannin (Table 1). The immaturefruit rindcontained flavonoid, steroid and tannin. In addition, *Terminalia kaerbachi*contained alkaloid, flavonoid and catechin tannin and hadα-glucosidase inhibitory activity[18].Flavonoid groups of tea leaves was used as antidiabetic[19].

Table 1. Phytochemical Screening of Ethanol Extract of *R.mucronata* fruit powder

Compound	*R. mucronata* ethanol extract					
	Peeled mature-fruit	Unpeeled mature-fruit	Rind of mature fruit	Peeled immature-fruit	Unpeeled immature-fruit	Immature fruit rind
Alkaloid	(-)	(-)	(+)	(-)	(-)	(-)
Saponin	(+)	(+)	(-)	(+)	(+)	(-)
Flavonoid	(+)	(+)	(+)	(+)	(+)	(+)
Steroid	(+)	(+)	(-)	(+)	(+)	(+)
Tannin	(+)	(+)	(+)	(+)	(+)	(+)

α-Glucosidase Inhibitory Activityof *R.mucronata* fruit flour

Utilization of some terestrial crude extract was used as antidiabetic drug. Parts of plant which have beenstudied arenamely leaves, fruit and bark. If the compound have α-glucosidase inhibitory activity, the compound is a potent drug forantidiabetic or antiobesity [20]. Table 2 showstheα-glucosidase inhibitory activity of *R.mucronata* fruit flour.

Table2. α-Glucosidase Inhibitory Activity of *R.mucronata* fruit powder

EthanolExtract	IC_{50} (µg/mL)
Unpeeled mature- *R.mucronata*powder	76.53
Unpeeled immature- *R.mucronata*powder	105.89
Peeled mature- *R.mucronata*powder	109.87
Peeled immature- *R.mucronata*powder	223.49

The highest inhibition activity against α-glucosidase was shown by unpeeled mature-*R.mucronata*powder with IC_{50} of 76.53 µg/mL. Extract of unpeeled*R.mucronata* fruit powderprepared from both mature and immaturefruit showed higher α-glucosidase inhibition activity compare to the IC_{50} value of peeled fruit. It indicated the relationship between phytochemical compounds in fruit rind, such as the presence of flavonoid and tannin, withα-glucosidaseinhibition activity. Tannin consistsof hydrolized tannin and condensed tannin. Hydrolized tannin does not have a role in reducing blood glucose of diabeticrat[21].Condensed tannin, called as proanthocyanidin, consisted of catechin and epigalocathecyn gallate.

Epicatechin-(4β,8)-epicatechin gallate (B2-3′-O-gallate),epicatechin gallate (ECG) and 2-(4-hydroxy phenyl) ethyl 3,4,5-trihydroxybenzoate (HETB) isolated from*Rhodia crenulata*root were potent drugs as α-glucosidase inhibitor. The inhibitory concentration of B2-3′-O-gallate, ECG and HETB were0.30 ± 0.03, 0.21 ± 0.04 and 3.10 ± 0.09 µM[22]. Catechin, called as proanthocyanidin, is one of flavonoid group[19]. Catechin reduced blood glucose level diabetic rats over 50%[23].Tannins on *R.mucronata* fruit powder acted as antidiabeticby inhibiting the digestive enzymes in intestine and glucose transporter[24]. Blackcurrant and rowan berry inhibited α-glucosidase IC_{50} 20 dan 30 µg GAE/ml[11].

One of standard drug widely consumed by diabetic patient is acarbose. Acarbose inhibitsα-glucosidase

enzyme, the digestive enzyme aids in glucose or carbohydrate absorption, and reduces blood glucose level. IC_{50} acarbose value was 117.20µg/mL[1]. IC_{50}value both of mature and immature fruit unpeeled werelower than IC_{50} value of acarbose. It showed that inhibition of α-glucosidase enzyme of mangrove fruit flour was higher that acarbose. Thus, *R. mucronata* fruitpowdercan be a candidate as functional food for antidiabetic.

Total Phenolic Content

Phenol has aromatic ring withone or two hidroxyl group. Flavonoid is the biggest of phenol group. Total phenol of *R. mucronata* fruit flour can be seen in Table 3.

Table 3.Total Phenol of *R. mucronata* fruit flour

Ethanol Extract	**Total Phenol (mgGAE/g)**
*R. mucronata*mature fruit	37.35
R. mucronata maturerind	459.14

Generally, total phenol of ethanol extract of mature *R.mucronata*rindwas higher than its fruit extract. Either the extract of fruit-rindor the extract of fruit tended todilute in polar solution. Phenol compound tend to dilute in polar solution[15]. Total phenol of mature *R.mucronata* fruit powder extract (37.5 mgGAE/g) was lower than mature *R. mucronata* fruit rind extract (459.14 mgGAE/g), both extracted by ethanol. It showed thatthere was a relation between total phenolic content of the fruit rindand the high α-glucosidase inhibitory activity. Linked with Table 1, total phenol could be flavonoid and tannin.

Conclusion

R.mucronata fruit flour contained flavonoid, saponin, tannin and steroid. Unpeeled *R.mucronata* fruit powder had the highest α-glucosidase inhibitory activitywith IC_{50}76.53 µg/mL,thus can be used as a candidate of antidiabetic.

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