



Exploration of Mango Fruits (*Mangifera indica*) as α -Glucosidase Inhibitors

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Abstract

Mango fruit (*Mangifera indica* L.) is the tropical fruit that grows easily in Indonesia with plenty varieties. This study aimed to determine the varieties of mango fruit and the most potent part of mango as antidiabetic agent through α -glucosidase inhibitory activities. Four types of mango fruit (*indramayu*, *manalagi*, *harum manis*, and *budiraja*) were used in this study. Each part of the mango fruit: peel, flesh, endosperm, and endocarp were extracted by maceration process with three different solvents (n-hexane, ethyl acetate (EtOAc), and ethanol (EtOH)). An ability of all 46 extracts in inhibiting the α -glucosidase at a concentration of 500 ppm were determined. Then 11 extracts with the high inhibition value were determined their IC₅₀ (concentration to inhibit 50% activity) values. EtOAc extract of *manalagi*, *indramayu*, and *budi raja* endosperm had the lowest IC₅₀ value which was not statistically significantly different (at 95%) with EtOAc extract of *budi raja* peel. The bioautographic Thin Layer Chromatogram showed that the most active band is characterized by white luminescence under UV 366 nm, yellow color under UV 254 and visible light. The band with R_f 0.93 from EtOAc endosperm extract of *indramayu* and *manalagi* and R_f 0.73 from EtOAc *budi raja* peel extract are the most active band which predicted as a flavonoid. The result adds the value of the peel and seed of mango, as well as an alternative in blood sugar control, which is easy to obtain, relatively cheap, and liked by the community.

How to Cite

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INTRODUCTION

Diabetes mellitus is a disease caused by the pancreas gland that can not produce enough insulin for the body or the condition when the body can not effectively use the insulin produced. Insulin plays a role in controlling blood sugar levels in the body. Approximately 8.5% of the total adult population in 2014 becomes diabetics, and according to WHO (2016), diabetes is one cause of death in 1.5 million people in 2012. One way to reduce the risk of diabetes mellitus is to control blood sugar levels by inhibiting the enzyme α -glucosidase which acts as a catalyst in the breakdown of polysaccharides or disaccharides into glucose in the small intestine.

Inhibition of this enzyme can inhibit the absorption of glucose in the blood (Cheng & Josse, 2004). Several types of drugs that work with the mechanism of inhibition of this enzyme are acarbose (Glucobay and Precose) and miglitol (Glycet). In addition, several types of plants that have been known to be antidiabetic agent include insulin leaf (Amanatie, 2015), brotowali, mahogany, pare and sambiloto (BPOM RI, 2004). Amir (2016) concludes that the mango fruit has the potential as an antidiabetic agent. Mangoes or *Mangifera indica* (L.), Anacardiaceae, contain polyphenol, flavonoids including quercetin and glycosylated xanthenes such as mangiferin (Singh *et al.*, 2004; Nãñez *et al.*, 2002; Ramanathan & Seshadri 1960; Nott & Roberts 1967). Quercetin has been shown to have antioxidant, antimicrobial, antitumor, antihypertensive, antiatherosclerosis and antiinflammatory effects. Mangiferin has antioxidant, antitumor, immunomodulatory, antiinflammatory and antiviral effects (Guha *et al.*, 1996; Lai *et al.*, 2003; Sarkar *et al.*, 2004; Dar *et al.*, 2005; Carvalho *et al.*, 2009; Noratto *et al.*, 2010; Hiraganahalli *et al.*, 2012). Mangiferin also exhibits antidiabetic properties (Muruganandan *et al.*, 2005, Daud *et al.*, 2010; Kumar *et al.*, 2013).

Mango fruit is one of the tropical fruit that grow easily in Indonesia with various varieties. During this time, the part of the mango that is used by the community is the flesh of the fruit. The peels and seeds are removed and become horticultural waste. Herbal medicines are often consumed by diabetics is relatively less favored because it has a bitter taste. Different mango cultivars from the Colombian Caribbean show the different content of active ingredient (Morales *et al.*, 2017), therefore it is important to find the best varieties of mango in Indonesia for specific purposes.

In this research, varieties of mango fruit

used were different. Cahyanto (2017) has conducted a study of characterization of mango characteristics based on peel anatomy and morphology in Subang, Indonesia against 21 mango varieties that showed different results for each variety. Differences in morphology and anatomy of each mango fruit varieties are expected to be associated with the active component compound content. Therefore, this study aimed to determine the varieties, namely *manalagi*, *harum manis*, *budi raja*, and *indramayu* and parts of the most potent mangoes as antidiabetic agent through inhibiting the activity of the α -glucosidase enzyme. The result of this study can add the value of all parts of the mango, especially the peel and seed, as well as an alternative in blood sugar control, which is easy to obtain, relatively cheap, and was liked by the community. In addition, the results of this study can be a reference for further research related to antidiabetic activity on mango fruit.

METHODS

Four varieties of mango fruit (*manalagi*, *harum manis*, *budi raja*, and *indramayu*) were used in this research. All sample were determined in LIPI Cibinong. Each mango fruit was separated into four parts (peels, flesh, endocarp, and endosperm), then dried to dry perfectly at 60 °C in the oven. After perfectly dried, all sample were grinded into powder.

All sample were extracted by maceration methods ranging from *n*-hexane, EtOAc, and EtOH. The extraction was performed with a sample and solvent ratio of 1: 5 and 1:10 adjusted to the sample condition. The extract obtained was then evaporated with a rotary evaporator at a temperature of 40-60 °C to obtain a crude extract.

A 50 μ L phosphate buffer pH 7.00 and 25 μ L substrate solution of p-nitrophenyl- α -D-glucopyranose (pNPG), and 25 μ L of α -glucosidase enzyme solution were added to a total of 10 μ L of the sample solution and incubated for 30 min at 37 °C. After completion of incubation, the mixture was added by 100 μ L Na_2CO_3 . The reaction product was a p-nitrophenol compound, which read its absorbance at 410 nm. The extract with the highest inhibitory applied to the TLC $\text{G}_{60}\text{F}_{254}$ plate was eluted using the best eluent.

Bioautography for inhibition α -glucosidase enzyme (Fauzi 2016) the crude extract was applied to the plate and then through the elution process with the best solvent that was determined before. The dried plates are then sprayed with a solution of the α -glucosidase enzyme and incuba-

ted for 60 min at room temperature. Subsequently sprayed a solution of α -D-glucopyranose p-nitrophenyl. The yellow band will appear after being left at room temperature. The band showed that the tested sample was active as a α -glucosidase inhibitor.

RESULTS AND DISCUSSION

Four varieties of mangoes from Indonesia was collected on March 2017 (Figure 1). The biggest size of fruits was found in *budi raja* mango, while the smallest size was *manalagi* mango. The peels, flesh, endocarp, and endosperm from the fruits were separated and dried. The moisture content of dried materials was summarized in Table 1.



Figure 1. Four varieties of mango fruits used on this study: *indramayu*, *harummanis*, *manalagi*, and *budiraja*

The moisture content of most of all part of fruit is below 10%. It means the dried materials could be storage for several time. The flesh part of all varieties of mango have moisture content around 20%. The flesh parts of all mango fruits are not dried enough, because of the high sugar content on the flesh. It needs other drying methods to get dried flesh from mango fruit such as freeze dryer.

Extraction process was performed prior to bio-activity test. Only the component which has similar properties with the solvent could be extracted. The extraction method used was maceration by increasing the polarity. Maceration process was chosen because maceration process is done on room temperature which will decrease

the destruction by high temperature. The yield of all extracts is shown in Table 2. Extract with the highest yield is on extract by ethanol as solvent with an average yield of more than 10%. It means that almost all part of mango fruits contained polar component which easily extracted by ethanol. EtOAc extract has a mean of yield that less than 10% and only *indramayu*'s peel extract with a yield that higher than 10%. The *n*-hexane extract also has a yield that less than 10% but there is one extract that has a yield higher than 10% (*budi raja* endocarp). High *n*-hexane extract yield indicates the presence of high non-polar compounds such as fat.

Determination of antidiabetic activity was started with a preliminary test at a sample concentration of 500 ppm for all extracts. A high percentage of inhibition shows that extract was able to inhibit enzyme's work, otherwise, the negative value means that the extract increases the activity of the α -glucosidase enzyme. Figure 2 shows that *budi raja* mango is the most potent mango fruit because it is seen that all parts of fruit and almost all kinds of extract are able to inhibit the work of α -glucosidase enzyme.

Part of mango commonly consumed is the flesh. Flesh sample extracted with ethanol from *indramayu*, *harum manis*, and *budi raja* along with *budi raja* sample extracted with ethyl acetate have a positive percent value of inhibition. This means by consuming this fruit, the activity of α -glucosidase enzyme will increase. The part of the mango fruit that has positive inhibitory activity is the endospermic part because all species of mango endosperm have positive inhibitory activity. This indicates that the seeds of mangoes that have not been utilized can be developed into inhibitors of α -glucosidase enzyme's work. Furthermore, the determination of the activity using IC_{50} was performed to extracts that possessing the inhibitory activity above 80%. IC_{50} is an extract concentration which inhibits enzyme activity up to 50%. The lower IC_{50} of an extract means the higher inhibitory power of the extract on the enzyme α -glucosidase so that the most potential as antidiabetic agent.

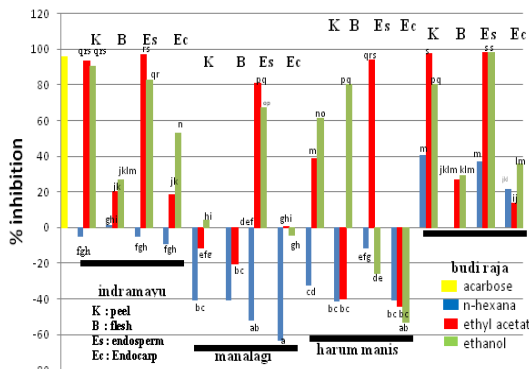
Table 1. Moisture content of dried mango fruits parts

Variety of mango	Moisture content (%) \pm SD			
	peel	flesh	endocarp	endosperm
<i>Indramayu</i>	10.37 \pm 0.29	22.78 \pm 0.08	8.43 \pm 1.22	9.57 \pm 1.31
<i>Budi raja</i>	7.44 \pm 0.13	22.78 \pm 0.08	6.38 \pm 0.37	9.95 \pm 0.24
<i>Harum manis</i>	5.88 \pm 0.16	22.55 \pm 0.31	4.99 \pm 0.28	6.70 \pm 0.24
<i>manalagi</i>	6.02 \pm 0.09	19.32 \pm 0.21	6.03 \pm 0.13	9.45 \pm 0.38

Table 2. Yield of extraction

Fruit part	Type of mango	Extract yield (%) with solvent		
		<i>n</i> -hexane	Ethyl acetate	ethanol
peel	<i>manalagi</i>	3.18	1.59	-
	<i>indramayu</i>	3.46	12.11	47.24
	<i>harum manis</i>	3.74	1.85	39.54
	<i>budi raja</i>	2.18	2.50	45.76
flesh	<i>manalagi</i>	0.65	0.61	-
	<i>indramayu</i>	1.02	1.43	91.78
	<i>harum manis</i>	0.37	2.20	-
	<i>budi raja</i>	0.42	2.44	-
endosperm	<i>manalagi</i>	1.57	3.15	36.53
	<i>indramayu</i>	1.92	2.76	24.57
	<i>harum manis</i>	0.77	1.20	11.19
	<i>budi raja</i>	0.80	1.47	16.35
endocarp	<i>manalagi</i>	9.14	2.34	34.08
	<i>indramayu</i>	6.76	3.04	30.08
	<i>harum manis</i>	2.30	2.52	34.41
	<i>budi raja</i>	15.42	3.11	34.92

- means the yield could not determined since the extract could not dry.



Description: The same small letters show no significant difference ($P < 0.05$), according to the Duncan multiple range test

Figure 2. Graph of screening of inhibition activity of α -glucosidase enzyme all extract at 500 ppm concentration

Only two types of varieties mango that active on peel part, namely *indramayu* and *budiraja*. The peel part of *harum manis* and *manalagi* is not active to inhibit α -glucosidase activity. The different activity of peel part from different mango varieties in India is also found on the antioxidant activity based on anti-scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,2-azinobis (3-ethylbenzothiazolin-6 sulphonic acid) (ABTS), and the reducing power. The different results from different varieties is found because of the different total phenolic and total

flavonoid content (Umamahesh *et al.*, 2016). The high content of total phenolic and total flavonoid gave the high activity of antioxidant.

The most potential antidiabetic extract is ethyl acetate extract of endosperm of three types of mangoes (*manalagi*, *indramayu*, and *budi raja*) because it has the lowest IC_{50} value and does not significantly different on statistical analysis (Table 3). The peel of *budi raja* mango has IC_{50} value that is not significantly different with the other three endosperms, so it can be utilized as well. This value is not as good as IC_{50} of acarbose value as a positive control. However, the waste of mango seeds and peel can be utilized as an inhibitor of α -glucosidase enzyme's work

Thin layer chromatography profiling of 9 extracts was performed by using bioautographic TLC. Through this method, active band can be determined as an α -glucosidase enzyme inhibitor (Macek 2006). The active band is defined as a yellow band (under UV light at 254 nm and visible light) and white band (under UV light at 366 nm) after spraying with substrate and enzyme. Figure 3.a is the appearance of the extract before being sprayed by enzymes and substrates. In chromatogram observations under 254 nm UV lamps and visible light, chromatograms before spraying enzymes and substrate (Figures 3. a.i and a.ii) did not show differences in bands color appearances on chromatograms after spraying (Figures 3 b.i and b.ii). Differences in the appe-

arance of band colors are seen during treatment under 366 nm UV lamp. The appearance of the band color on the chromatogram after spraying the substrate and enzyme more clearly than before spraying treatment. After spraying substrate and enzyme, there are three extracts that have an active band. *Manalagi* and *indramayu* endosperm ethyl acetate extracts have Rf 0.93, meanwhile *budi raja* peel ethanol extract have Rf 0.73 (Figure 3.b). The appearance color of bands with Rf 0.93 and 0.73 is bluish white under 366 nm UV lamp and more clearly after spraying treatment.

Table 3. IC₅₀ value of mango extracts

Extracts/Positive control	IC ₅₀ (ppm)
EtOAc <i>budi raja</i> peel	85.79 ^{bc}
EtOAc <i>indramayu</i> endosperm	70.48 ^b
EtOAc <i>manalagi</i> endosperm	64.71 ^b
EtOAc <i>indramayu</i> peel	138.85 ^d
EtOAc <i>budi raja</i> endosperm	72.79 ^b
EtOH <i>indramayu</i> peel	253.83 ^f
EtOH <i>budi raja</i> peel	183.58 ^e
EtOH <i>budi raja</i> endosperm	110.88 ^{cd}
EtOH <i>indramayu</i> endosperm	128.41 ^d
Acarbose (positive control)	0.064 ^a

Description: The same small letters show no significant difference ($P < 0.05$), according to the Duncan multiple range test

The prediction of compounds contained in the active bands was carried out qualitatively based on the luminescence under UV light at 254 and 366 nm and derivatisation using vanilin-H₂SO₄. The three samples produce the same band color that is black with 254 nm UV rays, bluish white under UV 366 nm, and purple color with derivatization with vanilin-H₂SO₄ (Figure 3 c). The appearance of those color refers to the appearance of flavonoid compounds. The Rf value produced by the EtOH extract of *budi raja* peel approximates the Rf value of the quercetin compound. Quercetin compound is a type of flavonoid compound contained in mango fruit and has been reported to inhibit the action of α -glucosidase (Carrascopozo *et al.*, 2016). Therefore, the resulting active band is suspected to be a quercetin compound. In addition, mangiferin is also a flavonoid compound, it is a group of glucosilxanthone which is reported by as antidiabetic agent contained in mango (Muruganandan *et al.*, 2005). The structure of quercetin and mangiferin is shown in Figure 4.

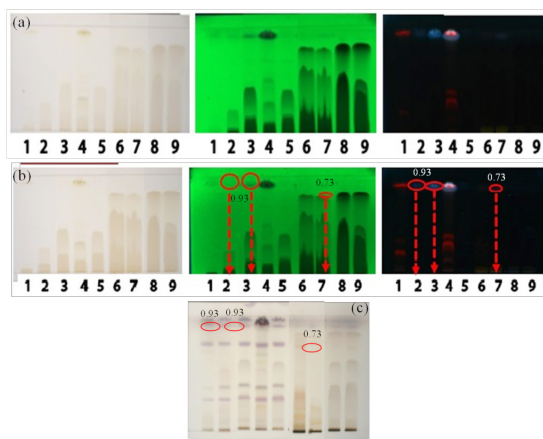


Figure 3. Chromatogram Bioautography of Alpha Glucosidase Inhibitor. (a) before substrate-enzyme spraying, (b) after substrate-enzyme spraying, (c) derivatization with vanillin-H₂SO₄ reagent (without spraying of the enzymes). EtOAc extracts of (1)*budi raja* peel, (2) *indramayu* endosperm, (3)*manalagi* endosperm, (4)*indramayu* peel, (5) *budi raja* endosperm, and EtOH extract of *indramayu* peel, (7)*budi raja* peel, (8)*budi raja* endosperm, (9)*indramayu* endosperm.

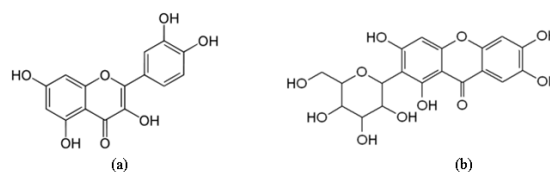


Figure 4. The structure of quercetin (a) and mangiferin (b)

This research showed that the peel and endosperm of mangoes fruit, which is usually not exploited by the public, has activity as an anti-diabetic agent. The result of this study can add the value of all parts of the mango, especially the peel and seed, as well as an alternative in blood sugar control, which is easy to obtain, relatively cheap, and liked by the community. In addition, the results of this study can be a reference for further research related to anti-diabetic activity on mango fruit.

CONCLUSION

Budi raja is a varieties of mango fruit that is most potential as an inhibitor of alpha glucosidase enzyme and the endosperm is the best part of the mango fruit developed as an alpha glucosidase enzyme inhibitor. Group of compounds suspected as active antidiabetic compounds in mango are flavonoid groups such as quercetin and mangiferin.

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