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by Tatas Hardo Panintingjati Brotosudarmo

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Saponin from purple eggplant (*Solanum melongena* L.) and their activity as pancreatic lipase inhibitor

Subandi^{1,a}, Lilik Zakiyaturroddiyah^{1,b,*}, Tatas H P Brotosudarmo^{2,c}

¹ Department of Chemistry, Universitas Negeri Malang, Malang, East Java, Indonesia

² Ma Chung Research Center for Photosynthetic Pigments (MRCPP), Universitas Ma Chung, Malang, East Java, Indonesia

E-mail: ^a subandi.fmipa@um.ac.id; ^b lxzakiya@gmail.com;

^c tatas.brotosudarmo@machung.ac.id

Abstract. Recently, obesity is one of the global problem, and orlistat is one of the known anti-obesity drugs, which works by inhibiting pancreatic lipase activity. Previous study has revealed that purple eggplant mesocarp, which contain alkaloids, flavonoids and saponins, has activity as pancreatic lipase inhibitor. On other hand, many class of saponin are inhibitor for the enzyme. Therefore, the aims of this study are to isolate and identify the saponins of purple eggplant (*Solanum melongena*) methanol extract and to test the activity as pancreatic lipase inhibitor. This study was conducted on four stages: 1) sample preparation, extraction and concentration of compounds from eggplant purple mesocarp; 2) saponin isolation by thin layer chromatography; 3) UV-Vis and FT-IR analysis to predict the class of the saponin isolates; and 4) pancreatic lipase inhibition assay. Extraction has been done by maceration using methanol as solvent, while the isolation was conducted by preparative thin layer chromatography using methanol: acetone (1:1) as eluent. Inhibition assay using titrimetric method with porcine pancreatic lipase (Sigma product) as enzyme, olive oil as substrate and orlistat as standard inhibitor. The result has showed that: the methanol extract of purple eggplant mesocarp contains two type of saponins that active as pancreatic lipase inhibitor, with the activity hundreds times higher than orlistat, at the same mass; and based on their UV-Vis and IR spectra, the isolates were predicted as steroid saponins.

Keywords: eggplant saponin, pancreatic lipase inhibitor, anti-obesity herbal

1. Introduction

Patients with obesity, both in children and adults, are increasing every year, so the World Health Organization decides that obesity is the most serious public health problem in the 21st century [1]. According to WHO data more than 1.4 billion adults (aged 20 years and over) are overweight. Among them there are more than 200 million men and nearly 300 million women are obese. Being overweight and obese causes 2.8 million adults to die each year due to the emergence of various chronic diseases such as diabetes and heart disease.

One of the most effective ways of treating obesity is by reducing the absorption of fat, by inhibiting pancreatic lipase activity [2]. Pancreatic lipase is an enzyme which responsible for breaking down fats in the human digestive tract, by converting triglyceride substrates present in dietary fat to monoglycerides and free fatty acids [3], so the fat can enter and be absorbed by the small intestine.

Currently, one of the known commercial anti-obesity drug is orlistat (Xenical®), and the drug is available as a non-prescription medication in many developed countries [4]. Orlistat works by inhibits gastric and pancreatic lipases in the lumen of the gastrointestinal tract to decrease systemic absorption

of dietary fat [5]. The use of orlistat, however, can cause side effects, such as hyperurisemia, diarrhoea, nausea, nyositis, gastric irritation, oily spots, flatulence, excessive farting, fecal incontinence and dry skin [2] and even acute pancreatitis with elevated pancreatic enzymes following orlistat therapy [4]. This unpleasant gastrointestinal side effect limits the patient's compliance with the use of this drug, thereby reducing its effectiveness [6]. In addition, orlistat is an imported drug, with relatively high price.

Purple eggplant (*Solanum melongena* L.) is mostly found in the tropics country, like Indonesia, and are widely consumed as vegetables that relatively cheap. Previous study has showed that some varieties of eggplant fruit (*Solanum anguivi* and *Solanum ningrum*) contain saponins which active as pancreatic lipase inhibitor. On other hand, some saponin from other plant also has activity as pancreatic lipase inhibitors [7]. Saponins are a class of compounds with a basic structure consisting of the steroidal and triterpenoid aglycone groups with one or more oligosaccharides [8]. The existence of saponin class in the eggplant and their possibility as pancreatic lipase inhibitor, has inspired the aims of this study, that are to isolate and identify the saponins from purple eggplant methanol extract and to test the activity as pancreatic lipase inhibitor.

2. Research Method

This descriptive laboratory research, was performed in 4 stages, namely 1) sample preparation, extraction and concentration of compound from purple eggplant, 2) saponin isolation using Thin Layer Chromatography (TLC) and phytochemical test, 3) sample inhibition test, and 4) UV-Vis and FT-IR analysis, to predict the identity/structure of saponin isolate. The extraction using methanol solvent by maceration method while saponin isolation has been done by preparative TLC using eluent of methanol: acetone of 1:1, and then the existence of saponin was confirmed by phytochemical test. Inhibitory activity test using titrimetric method with porcine lipase pancreas as enzyme, olive oil as substrate and orlistat as standard inhibitor [9].

2.1. Sample preparation

The purple eggplant (oval varieties) was peeled, washed thoroughly and mesocarp taken, was dried under the sun and then in the oven (60°C) to constant weight. Dried purple eggplant mesocarp obtained, then was smoothed in a way to be rendered, then sieved using distribution chamber 50 mesh to obtain purple eggplant mesocarp powder. The next stage were methanol extraction using maceration method, and then concentrated it using rotary evaporator.

2.2. Saponin Isolation Using TLC

Isolation of the compounds in the concentrated extract had been done using previous TLC metode [9]. Qualitative TLC first, to find the right eluent and then Preparative TLC. Each result of experiment stage were tested by phytochemical test of saponin and inhibition test against pancreatic lipase [9].

2.3. Pancreatic Lipase inhibitory assay of the samples

Pancreatic Lipase inhibitory power of an inhibitor sample can be calculated using both data of pancreatic activity assay, with and without inhibitor (equation 2 and 3).

2.3.1. Pancreatic Lipase activity assay. Lipase activity can be stated as the amount of substrate (triglyceride) that can be hydrolysed in one unit of time. One-unit activity of lipase (U/mL) indicates the amount of lipase enzyme that can release 1 μmol of free fatty acid per minute. Meanwhile the amount of the release free fatty acid can be determined by titration with Standard NaOH solution using phenolphthalein or change of pH (to 8.3) as indicator. Pancreatic Lipase activity (PLA) can be calculated by the equation 1.

$$PLA = \frac{(V_{os} - V_{ws}) \times N_{NaOH} \times 1000}{V_s \times t} \text{ (}\mu\text{mol/minute)} \quad (1)$$

Where:

V_{os} : the volume of required NaOH to titrate the oil substrate (mL)

V_w : the volume of required NaOH to titrate the water substrate (mL)

N_{NaOH} : Normalities of NaOH (mol/L)

1000 : conversion factor from mmol to μmol

V_s : substrate volume (25 mL)

t : incubation time (minutes)

2.3.2. *Pancreatic Lipase Inhibition Power (PLIP)*. PLIP of an inhibitor sample can be calculated using the equation 2.

$$PLIP = \frac{\text{Volume}_{\text{NaOH without inhibitor}} - \text{Volume}_{\text{NaOH with inhibitor}}}{\text{Volume}_{\text{NaOH without inhibitor}}} \times 100\% \quad (2)$$

Samples used as inhibitor were orlistat, juice of mesocarp, powder of cucumber mesocarp, cucumber mesocarp concentrated extract and saponin isolates from preparative TLC.

The sample inhibition power relative to orlistat (SIPRO) can be calculated using equation 3.

$$SIPRO = \frac{PIPL_{\text{sample}}}{PIPL_{\text{Orlistat}}} \times 100\% \quad (3)$$

3. Result and Discussion

In accordance with the stages of this research, the following results are presented according to that sequence.

3.1. The result of Sample Preparation

The result of sample preparation can be seen on table 1. According to table 1, the reducing mass of the sample from the whole fruit to mesocarp was and from the whole fruit to mesocarp powder was 1.84%.

Table 1. Mass of samples at each stages of sample preparation.

No	Procedure	Mass of Sample (grams)
1	Initial of purple eggplant	7000.0
2	Purple eggplant mesocarp	2500.0
3	Dry mesocarp powder	129.0
4	Dry mesocarp powder <50 mesh. (The remain >50 mesh was 34 grams)	95.0
5	After maceration of 95 grams of powder by 900 mL methanol (3x24 hours), be decanted/filtered and had been concentrated	23.6

3.2. Compound Separation Result by TLC

Analysis by qualitative TLC has shown that the right eluent was methanol: acetone of 1:1. By using the eluent, and UV lamp at 312 nM, were obtained 4 stains at TLC with each retention time can be seen at table 2. According to the research experience of this research group, one spot was one peak in the Chromatogram of LC/HPLC, so one spot is single compound. Therefore, the preparative TLC also used the same eluent to separate the saponin isolate in the purple eggplant methanol extract.

Table 2. Rf value and mass of each isolate/spot on preparative TLC using eluen of methanol:acetone 1:1.

Spot (Isolate) No	Rf Value	Mass of Isolate (mg)
1	0.1	11.0
2	0.6	12.0
3	0.8	19.9
4	0.9	18.0

Every spot on a preparative TLC plate then scraped off and dissolved in methanol, were filtered and then accommodated in a different vial bottle. The resulting filtrate is then evaporated at room temperature, to get rid of the solvent, be weighed and the mass of each isolate were shown in the table 2. All of the isolates, then were used for phytochemical test, inhibition test and spectroscopy (UV and FTIR) analysis.

3.3. Result of Phytochemical assay

The result of phytochemical assay has shown that all of the samples from purple eggplant mesocarp were saponin positive, unless for samples of isolate number 2 and 4 (table 3). All the positive samples were able to form foam if be shaken in water solution and the foam remain stable even be added by HCl. The foaming ability of saponin due to the nature of saponins that can lower the surface tension of the water and have the emulsifying properties. Saponin molecules contain sapogenin hydrophobic group and sugar hydrophilic groups (glycoside part). The presence of foam in this test, indicates the presence of glycosides that having the ability to form foams in water. The saponin-generated foam is not affected by the acid, so it remains stable when added with HCl.

3.4. Result of in vitro bioassay as pancreatic lipase inhibitor¹⁴

As described in the method, all of prepared samples were tested for bioactivity in vitro as pancreatic lipase inhibitors and the results are shown in table 4. Based on table 4:3 it can be seen that each mesocarp samples of purple eggplant: from juice, powder, concentrated extracts were having pancreatic lipase inhibitor activity. Even at the same mass, each isolate obtained has a much higher inhibitor activity than orlistat.

As can be seen in the table 2, that sample preparation has indeed reduced the quantity of mass obtained, but has significantly increased its bioactivity (table 3), this shows the successful of the preparation in increasing the concentration of bioactive substances in the sample. In fact, all isolates obtained had higher inhibitory activity (992 to 8372 times higher) against pancreatic lipase than orlistat, at the same mass. This shows their potency as an alternative anti-obesity herbs.

Interestingly, in the methanol extract of eggplant mesocarp there are also non-saponin compounds that active as pancreatic lipase inhibitors (isolate 2 and 4). This fact is in line with previous findings, that eggplant fruit contains saponin, flavonoid and alkaloid [10] and that *Solanum melongena* fruit contains flavonoids which can reduce blood fat levels [11]. However, to determine the types of two non saponin isolates, further identification is still needed.

Table 3. Data recapitulation of samples inhibition power and phytochemical test.

No.	Experiment Type	The amount of inhibitors	Lipase Activity (PLA) ($\mu\text{Mol}/\text{minute}$)	Inhibition Power to Pancreatic Lipase (PLIP) in %	Inhibition Power relative to Orlistat (SIPRO) in %	Inhibition power to orlistat at the same mass (120 mg) (%)
1.	Without Inhibitor	0	1.15	-	-	-
2.	With Orlistat as inhibitor	120 mg (1 tablet)	0.16	86	100	1.0x
3.	With mesocarp juice as inhibitor	22 mL (∞ 50 g mesocarp)	0.22	80.5	93.60	0.22x
4.	With mesocarp powder as inhibitor	2580 mg	0.86	25.2	29.30	1.35x
5.	With conc. extract as inhibitor	642 mg	0.35	69	80.23	15.0x
6.	With isolates 1 as inhibitor	5 mg	0.29	74.8	86.97	2087x (saponin)
7.	With isolates 2 as inhibitor	1.2 mg	0.32	72	83.72	8372x (non saponin)
8.	With isolates 3 as inhibitor	9.9 mg	0.34	70.4	81.86	992x (saponin)
9.	With isolates 4 as inhibitor	1.8 mg	0.80	30.4	35.36	2357x (non saponin)

3.5. Result of Saponin Identification using UV-Vis and FT-IR Spectroscopy

Because only isolates 1 and 3 were positively saponins, then the only two isolates were identified by UV and FTIR Spectroscopy.

3.5.1. *UV Spectra.* UV spectra of both isolates are shown in the Fig. 1 and its interpretation is in the table 4.

Table 4. UV spectrum interpretation of isolate 1 and 3.

Isolate	Peak at wavelength of (nm)	Shoulder at wave length of (nm)
1	204	269
3	205 and 291	326

Based on the UV spectrum (Fig. 1 and table 4), it was proven that both saponin isolates were different compounds.

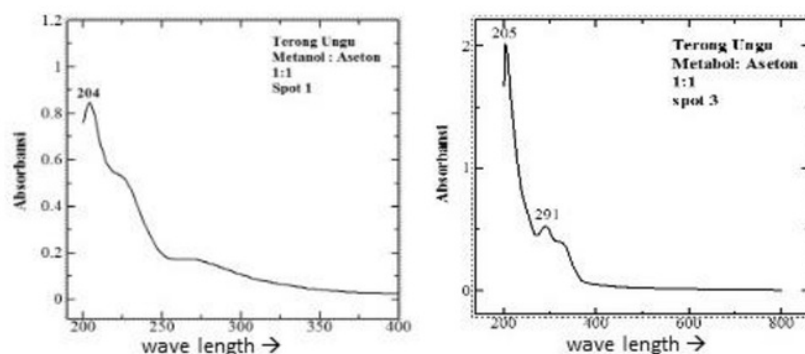


Figure 1. UV spectrum of isolate 1 (a) and isolate 3 (b).

3.5.2. *FTIR Spectra.* IR analysis was then carried out on both isolates to identify groups in each isolate, which produced spectra images and their interpretations as in Fig. 2 and table 5 (isolate 1) and Fig. 3 and table 6 (isolate 3).

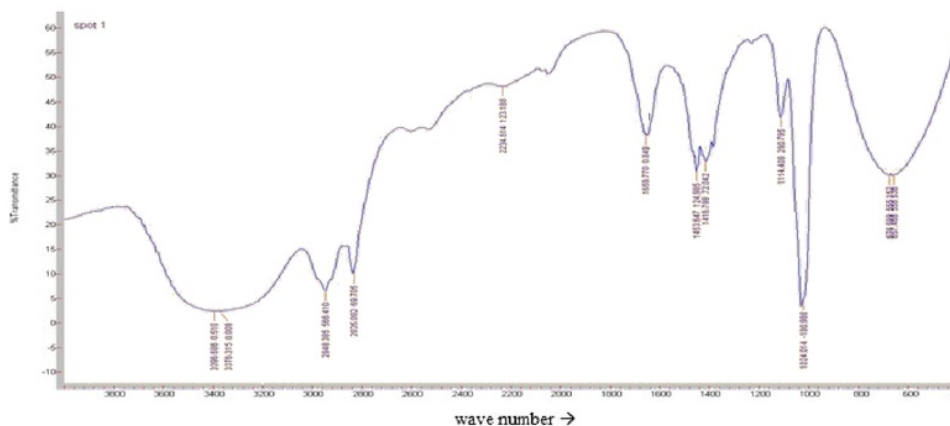


Figure 2. IR spectra of isolate 1.

Table 5. Overall results of FT-IR peaks from isolate 1.

No.	Wavenumber (cm ⁻¹)	Absorption Intensity	Typical Vibrations
1	3396.686 cm ⁻¹ ; 3376.315 cm ⁻¹	Widened	stretching of O-H
2	2948.412 cm ⁻¹ ; 2835.401 cm ⁻¹	Medium	stretching of C-H group
3	1651.469 cm ⁻¹	Medium	stretching of C=O group
4	1453.410 cm ⁻¹ ; 1416.740 cm ⁻¹	Medium	stretching of aromatic C=C
5	1114 cm ⁻¹	Moderately sharp	stretching of C-O group
6	1024 cm ⁻¹	Sharp	stretching of C-OH group
7	677.707 cm ⁻¹ ; 656.135 cm ⁻¹	Widened	stretching of aromatic C-H

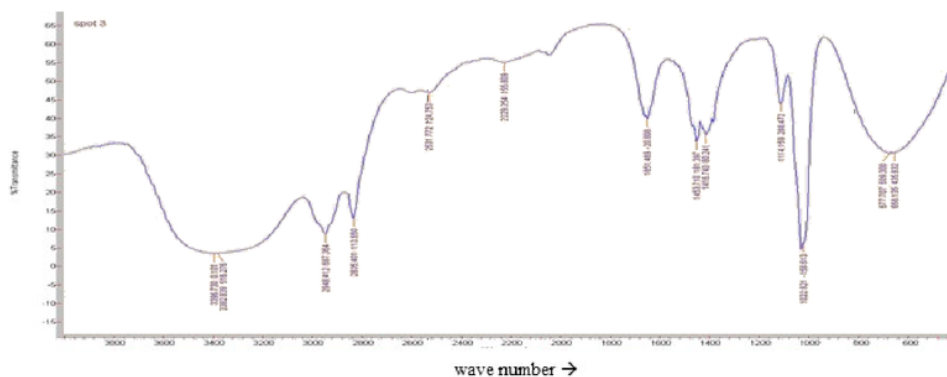


Figure 3. IR spectra of isolate 3.

Table 6. Interpretation of IR spectra of saponin isolate 3.

No.	Numbers of waves (cm ⁻¹)	Absorption Intensity	Typical Group Vibrations
1	3396.686 cm ⁻¹ ; 3376.315 cm ⁻¹	Widened	Stretching of O-H
2	2948.412 cm ⁻¹ ; 2835.401 cm ⁻¹	Medium	stretching of C-H group
3	1651.469 cm ⁻¹	Medium	stretching of C=O group
4	1453.410 cm ⁻¹ ; 1416.740 cm ⁻¹	Medium	stretching of aromatic C=C group
5	1114 cm ⁻¹	Moderately	stretching of C-O group
6	1024 cm ⁻¹	sharp	stretching of C-OH group
7	677.707 cm ⁻¹ ; 656.135 cm ⁻¹	Widened	stretching of aromatic C-H

3.5.3. Structure Prediction of Saponin Isolate from purple Eggplant Mesocarp. Recently, metabolite profiling can be used as a new tool to determine plant phenotype directly [12], so vice versa, plants that are closely related will produce similar metabolites. Therefore, to predict the structure of a saponin from the fruit of a plant, will be used saponin structures from the fruit of the plant species that are closely related, in this case the plant genus *solanaceae*. The structure of two types of saponins has been found from *solanacea* which has activity as an inhibitor for pancreatic lipase and can be seen in Fig. 4.

If the two known structure of saponins (Fig. 4 (a) and (b)) were compared to the result of IR spectra of the saponin isolates from purple eggplant mesocarp (c), then the comparison can be seen in table 7. Most of the compounds that have pancreatic lipase inhibitory activity have C-O group [7], so the existence of the C-O was predicted has important role to their inhibitory activity.

Based on the structure of their carbon skeletons, there are 11 main groups of saponin [13]. In accordance to the data on the table 7, can be suspected that the saponin isolates present in the purple eggplant have structures which more similar to the structure of saponin from the African eggplant (*Solanum anguivi*) (Fig. 4a) than saponin from *Solanum ningrum* (Fig. 4b). So the isolate 1 and isolate 3 were predicted as steroid class saponin [13]. However, further analysis is needed, including, for example, LC-MS and or NMR analysis, to confirmed their structures.

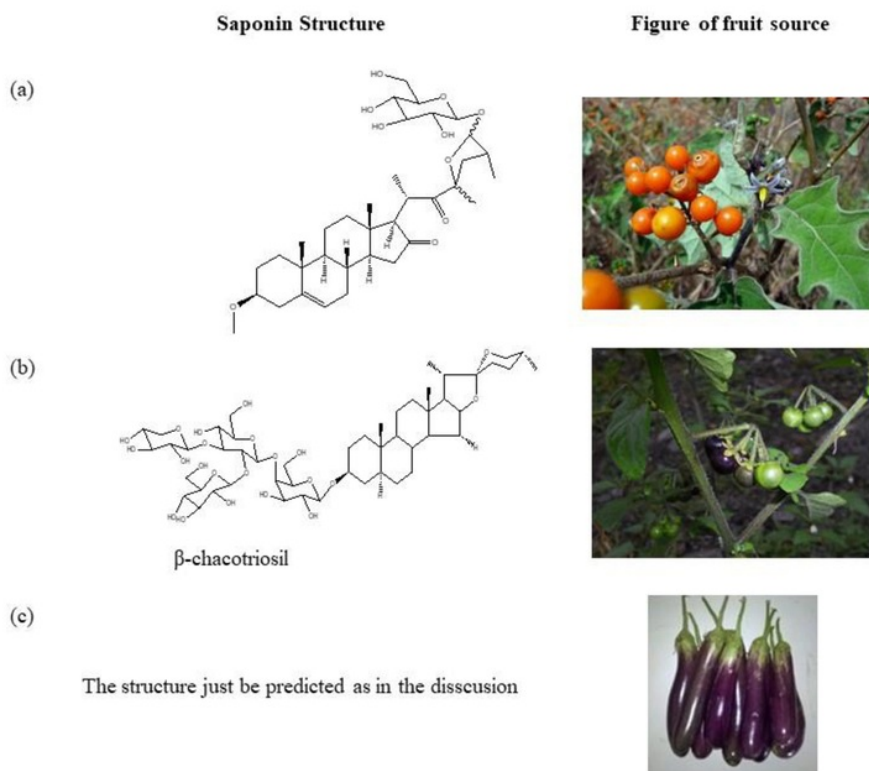


Figure 4. Structure of saponin compounds from (a) *Solanum anguivi* and (b) *Solanum nigrum* (leunca or ranti fruit) and (c) purple eggplant (this study).

Table 7. Structure comparison of saponin isolate of purple eggplant to known saponin structures from others sources at the genus.

No.	Source of Saponin	Existing of C-O group
1.	<i>Solanum anguivi</i> (African eggplant fruit)	Few
2.	<i>Solanum nigrum</i> (leunca fruit or ranti fruit)	Many
3.	Isolates of purple eggplant mesocarp (this study)	Few

18 Conclusion

Based on the results and discussion of this research, the conclusions are as follow. In the ethanol extracted of purple eggplant mesocarp there are two types of saponin isolates, that active as pancreatic lipase inhibitors. The isolates had an inhibitory power of 20 times (isolate 1) and 10 times (isolate 3) higher than orlistat at the same mass (120 mg). Based on UV-Vis and FT-IR spectra both saponin isolates were predicted as steroid saponins. So, both the juice or powder and especially saponin isolates from purple eggplant are potential to become anti-obesity drugs to replace orlistat.

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