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To cite this article: Subandi and Anis Nurowidah 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **515** 012098

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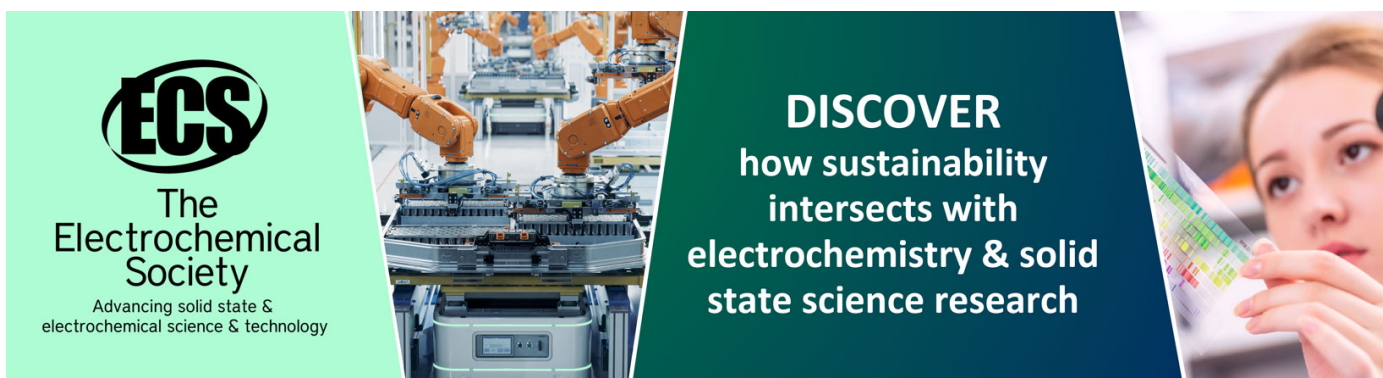
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The Potency of *Carica papaya* L. Seeds Powder as Anti-Obesity ‘Coffee’ Drinks

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Abstract: Previous studies have shown that papaya seed contained flavonoids, tannins, and saponins. Those compounds are potential as an inhibitor for pancreatic lipase, an enzyme that plays an important role for lipid absorption into the body. The aims of this study are to produce ‘coffee’ powder of papaya seeds for drinks; to test the organoleptic properties and the activity as a pancreatic lipase inhibitor. The seed was made into a powder by washing, sun drying, roasting, and then grinding. An organoleptic test was performed at 50 respondents and one trained respondent. Inhibitor activity for pancreatic lipase was measured relative to anti-obesity drugs of Orlistat (Xenical), using titrimetric method. The results showed that every 1.42 grams of papaya seeds powder have an inhibitory activity equivalent to 1 tablet (120 mg) of Orlistat. Most of the respondents like with the texture, color, and flavor of the drinks.

Keywords: Anti-obesity, pancreatic lipase inhibitor, organoleptic, papaya seed extract.

1. Introduction

Papaya (*Carica papaya* L.) is a plant that originally came from southern Mexico and northern South America, which is now widespread and widely grown throughout the tropic regions, including Indonesia. Based on reports of Central Statistics Bureau (BPS) on 2013, Indonesia belongs to the top 5 of papaya producing country in the world, with production reaching 871.3 tons [1]. Based on the report, the five provinces are the largest producer of papaya in Indonesia, that are East Java, Central Java, Lampung, West Java and East Nusa Tenggara with the details production shown at Table 1.

Table 1. Indonesia Production of papaya in 2013

Province	Production of Papaya (Ton)
East Java	281.943
Central Java	152.867
Lampung	97.579
West Java	69.930
East Nusa Tenggara	52.871



The abundant papaya production is supported by a tropical climate, as well as fertile Indonesian soil that suitable for cultivation of the plant. In addition, to the sweet taste, readily available at relatively low prices, papaya is also healthy because it contains beta-carotene (vitamin A) and high fiber. Almost all parts of the papaya plant, leaves, fruits, and seeds of economic value, either because of its nutritional value as well as its medical efficacy [2]. Nevertheless, the commonly used parts are the leaves and flesh alone. Papaya fruit flesh is usually used as a fruit dessert and raw materials of papain industry, the leaves are usually cooked as a vegetable, while the papaya seeds have not been widely used.

Generally, the papaya seeds, which contain about 40% of water [2], are only used as potential seeds to be planted again, but most are still regarded as trash and thrown away. In the case of papaya, the seeds contained in the cavity of the fruit with a mass about 15% of the fruit mass [3], contains antioxidants [4, 5], protein, carbohydrates, calcium, and beta-carotene [2]; fatty acids, benzyl isothiocyanate, benzyl glucosinolate, glucotropacolin, benzyl thiourea, beta-sitosterol, caricin, and myrosin enzyme [6, 7, 8].

Several studies have reported that the papaya seeds have the potential to be used in the medical field and pharmacology. Papaya seeds have antimicrobial activity against *Trichomonas vaginalis* and some pathogenic bacteria such as *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [9, 8]. Several other studies also report that papaya seeds have anthelmintic activity, namely the panacea to fight microbial parasites in the gut without causing side effects [8]. Anthelmintic activity is due to the Benzyl isothiocyanate in papaya seeds [10]. The extract of papaya seeds that have been macerated in water also has activity as an anti-amoeba against *Entamoeba histolytica* [2, 3, 11].

One of the anti-obesity drugs that are currently widely used is Tetrahydrolipstatin, isolated from Actinomycetes (*Streptomyces toxytricini*), which works as an inhibitor of pancreatic lipase and is available commercially under the name of "Orlistat" [17]. Nevertheless, the consumption of orlistat can cause some side effects such as hyperuricemia, diarrhea, nausea, myositis, gastric irritation, steatorrheas, oily spotting, flatulence, flatus with discharge, fecal incontinence, and dry skin [18]. Therefore, it has been widely studied some herbal remedies that can replace orlistat, as a pancreatic lipase inhibitor.

Pancreatic lipase is an enzyme that plays an important role in the absorption of fat into the body. This enzyme secreted by the pancreas into the intestine and hydrolyze 50-70% triglycerides into fatty acids [13, 14]. The results of previous studies have reported some plants that have activity as an inhibitor of pancreatic lipase. Some of them are Cassia Nomame [19], soybean [20], tea saponins [21], tea polyphenols [22], grape seed [23], *Eriochloa villosa*, *Orixa japonica*, *Setaria Italica* [24], *Platycodon grandiflorum* [25], and *Solanum stramonifolium* Jacq [26]. Mukherjee and Sengupta also reported that E. Jambolana stem, root A. Indica, leaves Cordifolia T. and T. foenum-graceum seeds also have inhibitory activity against pancreatic lipase [17]. On the other side, it has been revealed the presence of saponins, tannins, flavonoids, alkaloids, carbohydrates, phenolic compounds, and carotenoids in the ethanolic extract of *Carica papaya* seeds [12]. The presence of saponins, tannins, and flavonoids in the papaya seed suggested the seed allegedly also has potential as an anti-obesity drug.

The use of papaya seeds as a 'coffee' powder, which is efficacious as an inhibitor of pancreatic lipase in vitro, as well as organoleptic test of the beverages have not been reported. Therefore, the aims of this preliminary study are to produce 'coffee' powder of papaya seed at laboratory scale and to test the organoleptic properties as well as the inhibition power against pancreatic lipase.

2. Materials and Methods

2.1. General

Papaya seeds of Thailand types, which were used as powder by washing, dried in the sun, then roasted and milled. Organoleptic test conducted by test preference for texture, color, aroma and taste of coffee drinks papaya seeds using 50 respondents, and one expert respondent. The inhibition Power test against pancreatic lipase (Vitazym tablet, Kalbe Farma) was measured related to the anti-obesity drug of Orlistat (Xenical), using the titrimetric method.

2.2. Preparation of papaya seed extract

Some papaya fruits were cut open; the seeds were separated from the fruit to obtain 250 grams of papaya seeds, then washed thoroughly and dried in the sun for 3 days, then roasted over low heat, cooled at room temperature 5 minutes, then crushed into powder. The “coffee” powder was used for the organoleptic test, and 5 grams of papaya seed powder is ‘dissolved’ in water, beaten 1 minute and then centrifuged; filtrate used as a “coffee” papaya seeds extract for inhibition test of pancreatic lipase.

2.3. Pancreatic Lipase Activity Test

Olive oil emulsion consisting of 2.5 ml of olive oil and Arabic gum solution of 22.5 mL 10% (w / v) in water is homogenized for 10 minutes. Then added a solution consisting of 20 mL H₂O, 15 ml CaCl₂ 0.075 M, and 10 mL of 3 M NaCl, then homogenized back. The mixture was diluted with phosphate buffer to pH 7.5. until 100 ml, homogenized and was used as a substrate solution. Taken 25 mL substrate solution in a 100 ml Erlenmeyer and add 1 tablet Vitazym (which has been finely ground) as the source of lipase, shaken for 30 seconds, then incubated at 37 ° C for 25 minutes. Oil hydrolysis was stopped by heating Erlenmeyer into a boiling water bath for 10 minutes. The amount of liberated fatty acid was titrated with 0.1 N NaOH using pp as indicators. As a control substrate, has been using water instead of 2.5 mL of olive oil in the same procedures. The activity of Pancreatic lipase was calculated using equation (1).

2.4. Inhibition power Test of papaya seeds powder or Orlistat against Pancreatic Lipase

Taken 25 mL substrate solution as in 2.2, then add 1 tablet Vitazym (which has been finely ground) as the source of the lipase, and plus 2.5 ml of the of papaya seeds extract, shaken for 30 seconds. The mixture was incubated at 37 °C for 25 minutes (optimal incubation time). Hydrolysis was stopped by heating Erlenmeyer into a water bath of 100 °C for 10 minutes. The mixture was titrated with NaOH 0.1N using pp as indicators until the pink color arises, or until a pH of 10 (if using a universal indicator). As a control substrate was used water as a substitute of olive oil.

The power inhibition test of inhibitor (Orlistat or papaya seed extract) used the same procedure unless it has to be added fine powder of 1 tablet orlistat or 2.5 mL of papaya seed extract to the solution/ mixture before incubation at 37 °C. Lipase activity with or without inhibitor can be calculated by Equation 1.

$$\text{Lipase Activity} = \frac{(V_{SM} - V_{SA}) \times N_{NaOH} \times 1000}{25} \mu\text{Mol/tablet/minute} \quad (1)$$

Description:

VSM	= Volume of NaOH required to titrate the oil substrate
VSA	= volume of NaOH required to titrate 'water substrate ' (as control)
N _{NaOH}	= normality of NaOH used
1000	= conversion factor from mMol to μMol
25	= incubation time (minutes)

Inhibition power of an inhibitor can be calculated by equation 2 below,

$$\text{Inhibition power} = \frac{\text{lipase activity without inhibitor} - \text{lipase activity with inhibitor}}{\text{lipase activity without inhibitor}} \times 100\% \quad (2)$$

while power inhibition relative to the Orlistat can be calculated by equation 3, below

$$\text{Inhibition power relative to Orlistat} = \frac{\text{Papaya seed extract inhibition power}}{\text{Orlistat inhibition power}} \times 100\% \quad (3)$$

3. Results and Discussion

According to the aims of this study, this result and discussion were begun by the results of papaya seed powder preparation, results of the organoleptic test, and finally inhibition test result. Results of making coffee powder from papaya seeds are presented in Table 2. Data in Table 2 showed that the mass reduction of wet papaya to become powder of papaya seed is about 1/10-fold.

Table 2. Mass reduction papaya seeds during the process into a powder

Form of papaya seed	Mass (grams)
Before drying (wet papaya seed)	250.0
After drying	50.2
After roasting	25.3
After grinding (powder of papaya seed)	25.1

An organoleptic test was conducted by test preference for texture, color, aroma, and taste of 'coffee' drinks of papaya seeds, using 50 respondents and 1 trained respondent. The results of the organoleptic test by respondents and trained respondent can be seen in Figures 1, and Table 3, respectively.

Table 3. Organic Test Results by Trained Respondent

Organoleptic test of	Result
Texture and color	Similar to coffee bean powder
Aroma/ smell	Similar to Bali coffee, has a specific aroma like " <i>kemenyan</i> " (incense derived from gum benzoin)
Taste	delicious

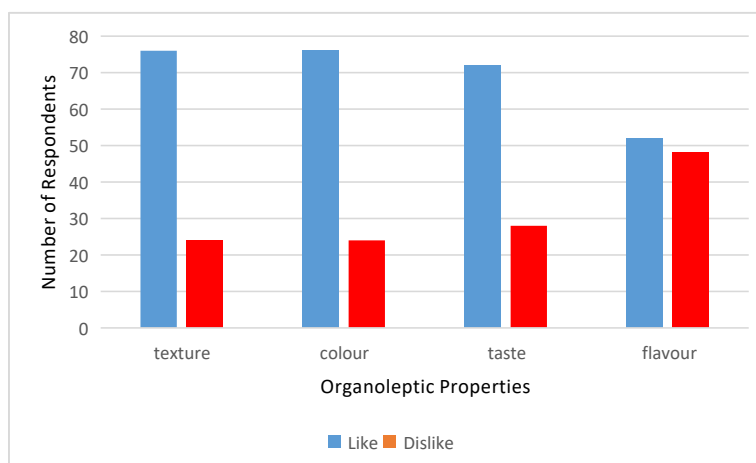


Figure 1. Results of the organoleptic test by 50 respondents

Based on data on Table 3, and Figure 1 can be concluded that the powder of papaya seed 'coffee' can be accepted by most of the panelists, so it fit for consumption as a drink of 'coffee' papaya seeds. Furthermore, the results show that if 5 grams of powdered papaya seeds was dissolved in 20 mL of water, was shaken 30 seconds and then centrifuged, can be obtained 10.5 mL of supernatant. 2.5 mL of this supernatant (equivalent to 1.25 grams of powdered papaya seeds) was used in trials against pancreatic lipase inhibition relative to orlistat, produces data as shown in Table 4. Inhibition test results against pancreatic lipase showed that water extract of papaya seed could reduce the activity of the enzyme (Table 4) significantly.

Tabel 4. The volume of titrant NaOH 0.1 N that were needed to neutralize the fatty acids released to calculated the Lipase activity without and with an inhibitor

Experiment no	Volume of NaOH 0.1 N was needed			
	Oil Substrate (V_{SM})	Water Substrate (V_{SA})	Substrate + Orlistat (1 tablet) (V_{SM})	Substrat + juice of papaya seeds (V_{SM})
1.	12.25 mL	8.63 mL	9.93 mL	9.97 mL
2.	12.70 mL	9.00 mL	10.00 mL	10.50 mL
3.	12.62 mL	8.17 mL	9.98 mL	10.34mL
Average of volume	12.52 mL	8.60 mL	9.97 mL	10.27 mL
Lipase activity (without inhibitor)	15.68 ($\mu\text{Mol/ tablet vitazym/ minute}$)		-	
Lipase activity (with Orlistat as inhibitor)	-	5.48 ($\mu\text{Mol/ tablet vitazym/ minute}$)		-
Lipase activity (with Papaya seed as inhibitor)	-	6.68 ($\mu\text{Mol/ tablet vitazym/ minute}$)		

Table 5. Inhibition Power of Papaya Seed Powder relative to 1 tablet of Orlistat

Reaction Condition	Lipase Activity ($\mu\text{Mol/tablet vitzym/minute}$)	Inhibition Power (%)	Inhibition Power relative to 1 tablet of Orlistat (%)
Without Inhibitor	15.68	0	0
With Orlistat as inhibitor (1 tablet of 120 mg)	5.48	65.05	100.00
With papaya seed, as an inhibitor (equivalent to 1.25 g of papaya seed powder)	6.68	57.40	88.24

By using equation (2) can be calculated inhibition power of papaya seed extract against pancreatic lipase, and inhibition power relative to Orlistat, as shown in Table 5. According to Table 5, 1.25 gr of papaya seed powder has inhibition power as big as 88.24 % of 1 tablet of Orlistat. So to get the same inhibition power as big as 1 tablet of Orlistat, the mass of papaya seed powder should be 1.42 gram, which equivalent to 14.2 gram of wet papaya seed. These results support the potency of papaya seeds as a herbal anti-obesity.

Based on the description above, both organoleptic and inhibitor test support the potentials of papaya seed powder as an anti-obesity drink. The results of this study are in line with the previous study, that aqueous extract of papaya seed could be useful in the management of Syndrome X, which is a consortium of obesity, type 2 diabetes mellitus, dyslipidemia, hypertension, and is relative save for oral consumption until the dose of 2000 mg/kg [26]. In addition, the consumption of papaya seed powder also allegedly has hepatoprotective and nephroprotective effects [27].

4. Conclusion

Based on the above explanation, it can be concluded that 25.1 grams of papaya seed powder can be obtained from 250.0 grams of wet papaya seeds. Most respondents like the texture, color, aroma, and taste of papaya seeds 'coffee'. The pancreatic lipase inhibition power of 1.42 gram of papaya seed powder is equivalent to 1 tablet (120.0 mg) of Orlistat. Therefore, using papaya seed powder as anti-obesity 'coffee' drink is reasonable.

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Acknowledgements:

The authors would like to thank Ms. Fenti Erlinda, Ms. Firda Afida Afni, and Ms. Lintang Iqnatus Suknah for experimental preparation. Special thanks to Mr. Afahlul Nur Faizin form PT Istana Kopi Tirtoyudo, Malang, East Java, Indonesia for his kind organoleptic testing, and also to Rector of Universitas Negeri Malang (UM), for the financial assistance to support this study, using PNBP 2018 fund.