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Research Article

PHARMACOLOGICAL SCREENING FOR POTENCY OF ANTIOBESITY ON FIVE PLANTS BASED ON ETHNOPHARMACOLOGICAL USE

FINNA SETIAWAN[1,2]*, KETUT ADNYANA I[1], ELIN YULINAH SUKANDAR[1], SUKRASNO[3], ARI YUNIARTO[1], RESNY PEBRITRINASARI[1]

[1]Pharmacology and Clinical Pharmacy Research Group, School of Pharmacy, Bandung Institute of Technology, Jalan Ganesa 10, Bandung 40132, West Java, Indonesia. [2]Faculty of Pharmacy, University of Surabaya, Jalan Kalirungkut, Surabaya, Jawa Timur 60293, East Java, Indonesia. [3]Pharmaceutical Biology Research Group, School of Pharmacy, Bandung Institute of Technology, Jalan Ganesa 10, Bandung 40132, West Java, Indonesia. Email: finna_florentina@yahoo.com

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ABSTRACT

Objective: The objective of this research was to screening potency of anti-obesity effect and antioxidant activity from *Sapindus rarak* Pulp., *Punica granatum* Leaves, *Jasminllum sambac* Flos., *Tamarindus indica* Pulp., and *Senna alexandrina* Leaves.

Methods: This study was performed through *in-vitro* method to determine the potency of anti-obesity, such as its ability to inhibit pancreatic lipase activity and radical-scavenging activity against free radicals like 1,1-diphenyl-2-picrylhydrazyl to determine the potency of these plants as antioxidant.

Results: The 50% inhibitory concentration of pancreatic lipase activity inhibitory and antioxidant activity consecutively, *P. granatum* Leaves (20.64 ppm; 77.89 ppm), *J. sambac* Flos. (53.46 ppm; 127.95 ppm), *T. indica* Pulp. (62.02 ppm; 159.16 ppm), *S. alexandrina* Leaves (94.69 ppm; 142.28 ppm), and *S. rarak* Pulp. (796.32 ppm; 147.56 ppm).

Conclusion: *P. granatum* Leaves showed the best potency of anti-obesity compared to other plants based on its ability to inhibit pancreatic lipase activity and antioxidant activity.

Keywords: Anti-obesity, Pancreatic lipase activity, Antioxidant.

INTRODUCTION

The prevalence of obesity increased in the last few decades and become problematic in the worldwide [1,2]. In 2013, 42 million children under the age of 5 were overweight or obesity. Furthermore, in 2014, more than 1.9 billion adults, 18 years and older were overweight, and over 600 million were obesity. Obesity condition increased morbidity and mortality risk. From this condition, need to develop effective strategies for weight management. Based on the World Health Organization, obesity defined as abnormal or excessive fat accumulation that presents a risk to health. Obesity can reduce productivity, confidence, and can increase the risk of several chronic diseases such as Type II diabetes mellitus, hypertension, stroke, atherosclerotic, arthritis, gout, respiratory failure, and cancer [3]. Risk factors of obesity are diet, age, physical activity, and genetic.

Indonesia is one of the biodiversity country in Southeast Asia which has many potential plants to treat several diseases, especially which used as anti-obesity. This study was performed to screening potency of anti-obesity from *Sapindus rarak* Pulp., *Punica granatum* Leaves, *Jasminum sambac* Flos., *Tamarindus indica* Pulp., and *Senna alexandrina* Leaves. The plants have shown potency as anti-obesity based on ethnopharmacological use. The study was performed using *in-vitro* methods through inhibition of pancreatic lipase activity and radical scavenging activity against free radicals such as 1,1-diphenyl-2-picrylhidrazyl (DPPH) to determine the potency of anti-obesity and antioxidant. The aim of pancreatic lipase activity assay was to know the ability plants to inhibit lipid absorption into the body by inhibit pancreatic lipase activity. Pancreatic lipase activity may influence the absorption of monoglyceride and free fatty acid into the body, which known as a cause of obesity. Inhibition of pancreatic lipase activity prevents lipid accumulation into the body. In addition, the oxidative stress in obesity condition associated with the atheroma plaques formation. Antioxidant activity from the plants can inhibit atheroma plaques production [4]. Therefore, present work was undertaken to study the potential anti-obesity of *S. rarak* Pulp., *P. granatum* Leaves, *J. sambac* Flos., *T. indica* Pulp., and *S. alexandrina* Leaves by *in-vitro* inhibitory pancreatic lipase activity and antioxidant activity.

METHODS

Plant material

S. rarak Pulp., *P. granatum* Leaves, *J. sambac* Flos., *T. indica* Pulp., and *S. alexandrina* Leaves were collected from Central Java, Indonesia and were identified in Herbarium Bandungense, School of Life Sciences and Technology, Bandung Institute of Technology, West Java, Indonesia.

Chemicals

DPPH, ascorbic acid, porcine pancreatic lipase, oleic acid, and bovine serum albumin (BSA) were obtained by Sigma-Aldrich. Orlistat (Xenical)® were purchased from Kimia Farma Pharmacy.

Preparation of extract

Plants were collected and extracted with ethanol 96% through maceration for *J. sambac* Flos., for 3 × 24 hrs and reflux for *S. rarak* Pulp., *P. granatum* Leaves, *T. indica* Pulp., and *S. alexandrina* Leaves for 3 × 2.5 hrs. Furthermore, the filtrates were concentrated using rotary evaporator and stored at the room temperature, protected from sunlight until further use.

Phytochemical screening of plants extract

Phytochemical screening was done to evaluate the presence of alkaloids, flavonoids, tannins, saponins, quinone, and steroid/triterpenoids in the plants extract.

Pancreatic lipase enzyme assay

Pancreatic lipase enzyme activity was determined by measuring the release rate of oleic acid from sesame oil. The method was conducted by

Han *et al.* (1999) [5] with slight modification. The substrate (5 ml in a 10 ml centrifuge tube) was prepared by sonication process for 5 minutes. The substrate containing 15 mmol/L sesame oil, 1 mmol/L NaCl, 1 mmol/L $CaCl_2$, 10 mg of BSA/ml, and phosphate buffer solution (pH 8.0). After sonication, the substrate was incubated with 50 µl of *porcine* pancreatic lipase enzyme and extract for 30 minutes at 37°C with various concentrations. About 30 minutes after incubated at 37°C were added to 3 ml of a 1:1 (v/v) mixture of chloroform and n-heptane extracted by shaking on the centrifuge tube for 10 minutes in a shaker. The mixture was centrifuged at 2000 rpm for 10 minutes. The upper aqueous phase was removed, and the lower phase was added with a copper reagent with volume 0.5 ml. The centrifuge tube was shaken again for 10 minutes, was centrifuged at 2000 rpm, and 0.5 ml of the upper phase (organic phase) was added with 0.5 ml diethyldithiocarbamate-sodium solution. The absorbance was then measured at 480 nm in a spectrophotometer ultraviolet-visible (UV-Vis). The percentage inhibition was calculated using following formula:

$$\%\text{Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Antioxidant assay

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple color). When antioxidant react with DPPH, which is a stable free radical, becomes paired off in the presence of a hydrogen donor (e.g., a free radical-scavenging antioxidant) and reduced to the DPPHH. Its consequence, the absorbance's decreased from the DPPH radical to the DPPH-H form (Fig. 1), its results is decolorization (yellow color) with respect to the number of electrons captured. This assay has been the most accepted as the model for evaluating the free radical scavenging activity of any new drug. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (diphenylpicrylhydrazine; non-radical) with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present) [6].

RESULTS AND DISCUSSION

Phytochemical screening of plants extract

Phytochemical screening of *J. sambac*, *S. rarak*, *S. alexandrina*, *T. indica*, and *P. granatum* as shown in Table 1. The results of phytochemical screening from these plants showed that the presence of alkaloids, flavonoids, saponins, quinones, tannins, and steroid/triterpenoids. Based on Pradono *et al*. [7] reported that alkaloids, flavonoids, saponins, quinones, tannins, and steroid/triterpenoids may inhibit the activity of pancreatic lipase enzyme.

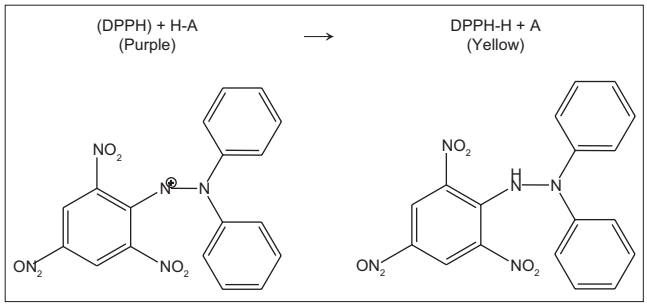

Fig. 1: Reaction of 1,1-diphenyl-2-picrylhidrazyl (DPPH) (free radical) to DPPH (non-radical)

Pancreatic lipase enzyme assay

As shown in Table 2, *P. granatum* Leaves, *J. sambac* Flos., *T. indica* Pulp, *S. alexandrina* Leaves, and *S. rarak* Pulp., exhibit the best potency to inhibit pancreatic lipase activity *in-vitro*. Furthermore, Table 2 also showed that *P. granatum* Leaves exhibit, the best inhibition activity with a value of 50% inhibitory concentration (IC_{50}) 20.64 ppm.

Pancreatic lipase is an enzyme, which produced by the acinar cell and secreted as an exocrine function of the pancreas. On the other hand, it's well known that dietary lipid is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase. The two main products formed by the hydrolysis of pancreatic lipase are fatty acid and 2-monoacylglycerol. Based on these facts, inhibition of these digestive enzymes is beneficial in the treatment of obesity [8].

Antioxidant assay

The scavenging reaction between (DPPH.) and an antioxidant (H-A) was shown in Fig. 1. DPPH 40 ppm in methanol; it was protected from light by covering the test tubes with aluminum foil. A volume of 750 µl DPPH solution was added to l50 µl methanol and absorbance were taken immediately at 512 nm for control reading. Each of the samples was then further diluted with methanol in various concentrations, and 750 µl DPPH solution was added. Absorbance was taken after 30 minutes at 512 nm using methanol as blank on spectrophotometer UV-Vis. The IC_{50} values for each drug compounds, as well as standard preparation were calculated. The DPPH free radical scavenging activity was calculated using the following formula:

$$\%\text{Scavenging} = \left[1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right] \times 100$$

Obesity showed the major risk factor for several disease including diabetes mellitus, cardiovascular disease, non-alcoholic fatty acid, osteoarthritis, cancer, and infertility. It is often accompanied by increased risk of mortality and became a serious health problem in the world. Recently, a study showed that oxidative stress could play an important role in the development of obesity by stimulating white adipose tissue deposition and altering food intake [9-11]. Oxidative stress also increased in obesity condition, which could be observed even in the childhood period [12].

Recent studies suggest that oxidative stress correlated with obesity condition and its complication. In obesity, condition showed lower antioxidant level compared to normal weight. Obesity is also characterized by enhanced levels of reactive oxygen or nitrogen species [13]. Antioxidant compound play an important role as a health protecting factor especially in the development of obesity by inhibiting level of oxidative stress. Table 3 showed that all plants have an antioxidant effect in different concentration. *P. granatum* Leaves showed the best activity of antioxidant compared to other plants with IC_{50} 77.89 ppm. Strategies to lower oxidative stress in obesity including

Table 1: Result of phytochemical screening of plants extract

S. No	Plants	Phytoconstituents					
		Alkaloids	Flavonoids	Saponins	Quinones	Taninns	Steroid/triterpenoids
1.	*Jasminum sambac*	+	+	+	+	+	+
2.	*Sapindus rarak*	+	−	+	+	+	+
3.	*Senna alexandrina*	+	+	+	+	+	+
4.	*Tamarindus indica*	−	+	+	−	−	−
5.	*Punica granatum*	−	+	+	+	+	+

Table 2: Result of IC_{50} from pancreatic lipase activity assay

Plant	Concentration (ppm)	Inhibitory pancreatic lipase activity (%)	IC_{50} of inhibitory pancreatic lipase activity (ppm)
Punica granatum Leaves	0.1	44.26±9.49	20.64
	1	63.92±4.43	
	10	49.65±10.52	
	100	65.78±5.69	
Jasminum sambac Flos.	0.1	51.36±16.64	53.46
	1	35.58±16.45	
	10	51.80±19.31	
	100	55.37±19.76	
Tamarindus indica Pulp.	0.1	19.62±0.43	62.02
	1	21.02±0.18	
	10	41.39±0.61	
	100	63.80±1.64	
Senna alexandrina Leaves	0.1	44.18±13.83	94.69
	1	56.57±15.45	
	10	35.83±26.11	
	100	50.89±15.25	
Sapindus rarak Pulp.	0.1	5.70±0.29	796.32
	1	10.99±0.61	
	10	15.86±5.95	
	100	29.73±8.88	
	1000	58.10±8.62	

IC_{50}: 50% inhibitory concentration

Table 3: Result of IC_{50} from anti-oxidant activity assay

Plant	Concentration (ppm)	Anti-oxidant activity (%)	IC_{50} of anti-oxidant activity (ppm)
Punica granatum Leaves	10	26.62±1.79	77.89
	50	44.06±1.53	
	100	71.08±1.27	
Jasminum sambac Flos.	1	38.34±1.37	127.95
	10	41.41±7.28	
	50	40.58±9.89	
	100	51.51±9.28	
	200	54.79±9.08	
Tamarindus indica Pulp.	1	35.89±3.52	159.16
	10	30.80±4.11	
	50	39.41±0.03	
	100	38.39±4.14	
	200	57.78±0.01	
Senna alexandrina Leaves	1	38.24±4.86	142.28
	10	39.86±4.08	
	50	43.74±4.18	
	100	46.01±4.98	
	200	54.62±5.80	
Sapindus rarak Pulp.	1	38.70±8.41	147.45
	10	34.38±6.39	
	50	44.82±9.40	
	100	42.86±10.57	
	200	55.24±4.92	

IC_{50}: 50% inhibitory concentration

weight loss, physical activity, and increased antioxidant became a key role in the treatment of obesity.

CONCLUSION

P. granatum Leaves showed the best antioxidant activity and pancreatic lipase inhibition activity compared to other plants, and this dual effect could be useful as strategies for obesity treatment.

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