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Zingiber cassumunar, Guazuma ulmifolia, and *Murraya paniculata* Extracts as Antiobesity: *In Vitro* Inhibitory Effect on Pancreatic Lipase Activity

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***Zingiber cassumunar* Roxb. (*Bangle*), *Guazuma ulmifolia* Lamk. (*Jati belanda*), and *Murraya paniculata* (*Kemuning*) have been used as slimming agents in *jamu*. A few researches have performed studies on their potency as antiobesity. The aim of this research was to investigate the potency of *Z. cassumunar* rhizome, *G. ulmifolia,* and *M. paniculata* leaf extracts as antiobesity agent based on *in vitro* inhibition activity of the extracts on pancreatic lipase activity. In this research, water content determination, phytochemical assay, toxicity assay and *in vitro* assay of inhibition activity on pancreatic lipase were performed toward single and mixture extracts of *Z. cassumunar, G. ulmifolia,* and *M. paniculata* resulted by water, ethanol, and saponin extractions. The results indicated that 100 ppm of ethanol extraction of *Z. cassumunar* had highest inhibition effect on the activity of pancreatic lipase (29.17%), followed by 100 ppm of water extraction of *M. paniculata* (25.66%), 60 ppm of ethanol extraction of *G. ulmifolia* leaves (25.13%) and ethanol extraction mixture of *Z. cassumunar, G. ulmifolia,* and *M. paniculata* leaves with ratio of 25:25:25 (21.58%). These inhibition effects were higher than inhibitory effect of 100 ppm of Xenical®/orlistat as the positive control, with the inhibition value of 17.53%. Saponin crude extracts had lower inhibitory effect than the other extractions. It was suggested that ethanol extraction of *Z. cassumunar,* and *G. ulmifolia* and water extraction of *M. paniculata* had potency as antiobesity agent.**

Key words: *Zingiber cassumunar* Roxb. (*Bangle*), *Guazuma ulmifolia* Lamk. (*Jati belanda*), *Murraya paniculata* (*Kemuning*), antiobesity, inhibitory effect, pancreatic Lipase

INTRODUCTION

Obesity is becoming one of the greatest threats to global health in this millennium, with more than 1 billion overweight adults and of those, at least 300 million are clinically obese (Arbeeny 2004). Obesity is primarily regarded as a disorder of lipid metabolism and the enzymes involved in this process could be selectively targeted to develop antiobesity drugs. Recently, newer approaches for the treatment of obesity have involved inhibition of dietary triglyceride absorption via inhibition of pancreatic lipase (PL) as this is the major source of excess calories. Natural products provide a vast pool of PL inhibitors that can possibly be developed into clinical products (Birari & Bhutani 2007). The market for antiobesity drugs is potentially huge, as it accounts for 2-6% of total health care costs in several developed countries. With its growing worldwide prevalence, the obesity market has been predicted to reach US$ 3.7 billion by 2008. At present, the potential of natural products for the treatment of obesity is still largely unexplored and might be an excellent alternative strategy for the development of safe and effective antiobesity drugs (Mukherjee 2003). There are many experiments to figure out the therapeutics for this critical public problem of obesity. One of the therapeutic approaches to preventing obesity is to retard absorption of fatty acid by the inhibition of lipase in the digestive organs (Ballinger & Peikin 2002; Yanovski & Yanovski, 2002). Pancreatic lipase is the most important enzyme in the digestion and absorption of dietary triglycerides (Embleton & Pouton 1997). The application of a lipase inhibitor was reported as a treatment for obesity. There are reports of established lipase inhibitors such as orlistat, a hydrogenated derivative of lipstatin, derived from *Streptomyces toxitricinin* (Ballinger & Peikin 2002), planclicins from *Streptomyces* sp., valilactone from *S. albolongus* (Birari & Bhutani 2007), and CT-II, isolated from Nomame herb (Shimura *et al.* 1992). In addition, some botanical foodstuffs such as the proteins in soybeans (Higaki 2003) and phosphatidyl choline and AR25_ from green tea extract (Juhel *et al.* 2000) had been reported as lipase inhibitors. The existence of lipase inhibitors was reported in various plant species, including *Cassia mimosoides* (Yamamoto *et al.* 2000), *Camelia sinensis* (Han *et al.* 2001), and *Salacia reticulate, Alpinia officinarum* (Shin *et al.* 2003), *Panax japonicus* rhizome (Han *et al.* 2005), *Platycodon grandiflorum* (Xu *et al.* 2005), *Gardenia*

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jasminoides (Lee *et al.* 2005) and triterpenenoids compound isolated from *Actinidia arguta*. Active compounds from medicinal plants which could inhibit pancreatic lipase were saponin, polyphenol, and terpenoids. In human clinical trials, lipase inhibitors effectively suppressed weight gain in obese patients by reducing the absorption of dietary fat (Drent *et al.* 1995; Yamamoto *et al.* 2000; Reaven *et al.* 2001). Several patents exhibited *Crataegus* and *Gingko biloba* extract had potent antiobesity (US Patent No. 6447818), patent of obesity care (US Patent No. 6737407), and pancreatic lipase enzyme (US Patent No. 7064122).

Indonesia is one of the countries with rich sources of medicinal plants. Its biodiversity is the second largest after Brazil. Thirty thousands types of plants, out of forty thousands, can be found in Indonesia and 940 of them are known to have restorative power. They have been used as traditional medicine by many generations of many ethnics in Indonesia. Three of them are *Zingiber cassumunar* Roxb. (in Indonesian is called *Bangle*), *Guazuma ulmifolia* Lamk. (in Indonesian is called *Jati Belanda*), and *Murraya paniculata* (in Indonesian is called *Kemuning*) which are potent as antiobesity. Our previous work showed that methanol extract of *G. ulmifolia* leaves could reduce the activity of lipase derived from *Rhizopus arrhizus*, while the chloroform extract increased the activity of the enzyme (Iswantini *et al.* 2003). Water and steroid extract could be acting as activator, while methanol, flavonoid, and tannin extract could be an inhibitor. However, these results were unclear, and were used as a motivation to continue the research by utilizing pancreatic lipase derived from human. The purpose of this work was to determine the inhibitory potency of single and mixture extracts of *Z. cassumunar*, *G. ulmifolia* leaves, and *M. paniculata* leaves on the activity of pancreatic lipase by *in vitro* method. Because one of active compound from medicinal plants which could inhibit pancreatic lipase were saponin, therefore we also determined the inhibitory potency of saponin crude extract.

MATERIALS AND METHODS

Plant Materials. *Zingiber cassumunar* rhizoma, *Guazuma ulmifolia*, and *Murraya paniculata* leaves were collected from their natural habitat in Bogor, west Java, Indonesia.

All of plant materials were extracted using water, ethanol and to obeserve saponin crude extract. Phytochemical assay of all extracts were performed to determine chemical compound content in all of extracts qualitatively. The toxicity assay of all extracts also were conducted to determine LC_{50} value of all extracts. The LC_{50} value were used as basic of concentration level determination for *in vitro* assay. The concentration of extracts for *in vitro* assay were lower than that of each LC_{50} value. The concentration of extracts lower than that of LC_{50} value were *in vitro* assayed on the activity of pancreatic lipase. The concentration of each single extract which have a highest inhibition on the activity of pancreatic lipase were mixed to be mixture extracts.

Preparation of Water, Ethanol, and Saponin Extractions. Water extraction was prepared as follows: 75 g of dried *Z. cassumunar* rhizoma, *G. ulmifolia*, and *M. paniculata* leaves were extracted continously by masseration using water (200 ml) which then evaporated at 60 °C to yield water extraction.

Ethanol extract were prepared similar to water extract preparation using ethanol 70% and evaporated at 40 °C. Saponin crude extract was prepared by modifying a method previously reported (Beutler *et al.* 1997): dried samples were refluxed with MeOH: dichloromethane (1:1) for 30 minutes with 3 repetitions. The dried extract was refluxed using four portions of ethylacetate: chloroform (1:1) to remove the flavonoids, alkaloids, and other un-wanting compunds, except saponins, from the desired product. After removing the ethylacetate- chloroform solvent, MeOH was added to the residue which then evaporated at 40 °C to yield saponin extract.

Phytochemical Assay. Phytochemical assay, based on an established procedure, was conducted to explore the secondary metabolites, such as: alkaloid, flavonoid, saponin, triterpenoid, steroid, and tannin (Harborne 1987).

Toxicity Test. Toxicity test was performed on shrimp larvae of *A. salina* to determine the LC_{50} value (Finney 1971). The LC_{50} value was determined by counting the number of shrimp larvae died upon applying the experiment condition and was let to sit for 24 hours.

Assay of Potent Inhibitory Effect on Pancreatic Lipase. Lipase activity was determined by measuring the rate of releasing of oleic acid from triolein. A suspension of 90 mmol triolein, 12.6 mmol lecithin, and 9.45 mmol taurocholic acid in 9 ml of 0.1M N-tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES), pH 7.0, containing 0.1 M NaCl was sonicated for 5 min. The assay system contained the following components in a total volume of 200 ml: 50 ml pancreatic lipase, 50 ml test compound solution, 0.5 mmol triolein, 0.053 mmol taurocholic acid, 0.07 mmol lecithin, 20 mmol TES, and 20 mmol NaCl. Incubation was carried out at pH 7.0 and 37 °C for 30 min. The released of oleic acid was determined by the method described above.

Data Analysis. Data analysis of inhibitory effect of single extract of *G. ulmifolia* leaves, *Z. cassumunar* rhizome, and *M. paniculata* leaves were performed by descriptive method, and data of inhibitory effect of mixture extracts were analyzed by statistical method.

RESULTS

Water Content and Extraction. The water content of *G. ulmifolia* leaves and *Z. cassumunar* rhizome were 13.66 and 11.17% (w/w), respectively. Meanwhile, the water content of dried powder of *M. paniculata* leaves was 12.34% (w/w). Yields collected from water, ethanol, and saponin extractions of *G. ulmifolia* leaves were 22.25, 5.77, and 0.90% respectively, and the yields for *Z. cassumunar* rhizome were 12.18, 11.04, and 5.01% respectively. The yield of ethanol 70% crude extraction of *M. paniculata* was 36.14%, It was higher than that of water crude

extraction (15.04%). Those extracts were assayed phytochemically, cytotoxicity using shrimp larvae and their inhibition on the activity of pancreatic lipase.

Phytochemical Assay. Results of phytochemical assay showed that ethanol extraction had more secondary metabolite compounds compare to those of water extraction. Water and ethanol extractions of *Guazuma ulmifolia* Lamk. contained flavonoid, saponin, and tannin, for *Zingiber cassumunar* contain flavonoid and saponin (Table 1 & 2). And all extracts of *Murraya paniculata* contained flavonoid and tannin (Table 3). However, all of water and ethanol extracts of three medicinal plants contained flavonoid.

Table 1. Phytochemical assay result of *Guazuma ulmifolia*

Compound	Dried sample	Extract		
		Water	Ethanol	Saponin
Flavonoid	++	++	+++	-
Alkaloid	-	-	-	-
Saponin	+	+	+	+
Steroid	+	-	+	+
Triterpenoid	-	-	-	-
Tanin	++	++	+++	-

+ = detected, - = non detected.

Table 2. Phytochemical assay result of *Zingiber cassumunar*

Compound	Dried sample	Extract		
		Water	Ethanol	Saponin
Flavonoid	++	++	+++	-
Alkaloid	-	-	-	-
Saponin	++	++	++	++
Steroid	-	-	-	-
Triterpenoid	++	-	+	++
Tanin	-	-	-	-

+ = detected, - = non detected.

Table 3. Phytochemical assay result of *Murraya paniculata*

Compound	Dried sample	Extract	
		Water	Ethanol
Flavonoid	+	+	+
Alkaloid	-	-	-
Saponin	-	-	-
Steroid	+	-	+
Triterpenoid	-	+	-
Tanin	+	+	+

+ = detected, - = non detected.

Table 4. LC_{50} value of extracts of *Guazuma ulmifolia*, *Zingiber cassumunar,* and *Murraya paniculata*

Sample	Extract	LC_{50} (ppm)
Guazuma ulmifolia	Water	672.65
	Ethanol	1070.93
	Saponin	1039.02
Zingiber cassumunar	Water	394.38
	Ethanol	81.099
	Saponin	920.64
Murraya paniculata	water	212.26
	ethanol	442.83

Toxicity Test. Results of toxicity test indicated that the LC_{50} value of all of extracts were less than 1,000 ppm except ethanol and saponin extracts of *Guazuma ulmifolia* Lamk (Table 4). The lowest and highest toxicity were observed from ethanol extract of *Guazuma ulmifolia* Lamk and water extract of *Murraya paniculata*, respectively.

***In Vitro* Inhibitory Effect of Single Extracts on the Activity of Pancreatic Lipase.** All tested extracts could inhibit the pancreatic lipase *in vitro* (Table 5 & Figure 1, 2 & 3). The highest inhibition effect of *G. ulmifolia* leaves, *Z. cassumunar*, and *M. paniculata* extracts were 60 ppm of ethanol extract (25.31%), 100 ppm of ethanol extract (29.17%), and 100 ppm of water extract (25.66%), respectively. The data showed that inhibition was higher

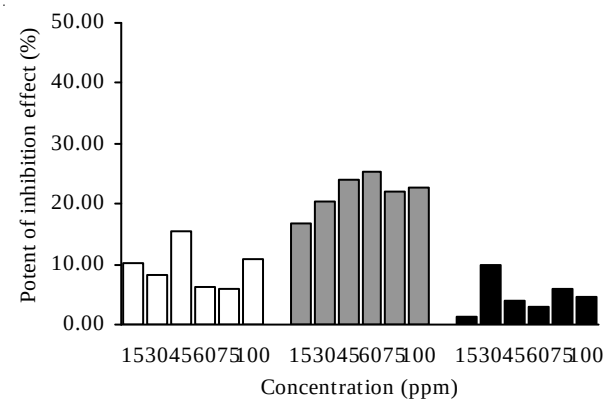

Figure 1. Potent of inhibitory effect of □: water extract, ▩: ethanol 70% extract, and ■: saponin extract of *Guazuma ulmifolia* leaves on the activity of pancreatic lipase.

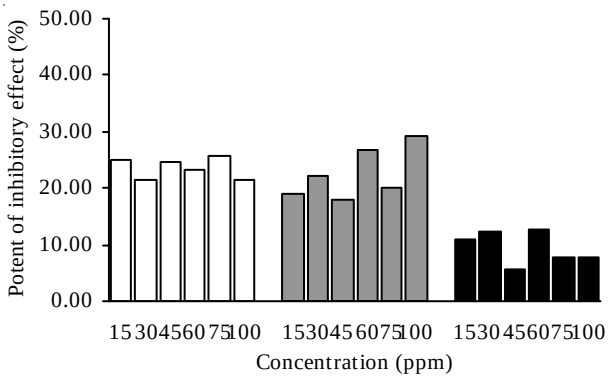

Figure 2. Potent of Inhibitory effect of □: water extract, ▩: ethanol 70% extract, and ■: saponnin extract of *Zingiber cassumunar* rhizoma on the activity of pancreatic lipase.

Table 5. Inhibitory effect of extracts at optimum concentration and positive control (Xenical®)

Extract	Sample				
	Guazuma ulmifolia	*Zingiber cassumunar*	*Murraya paniculata*	Control (-)	Control (+)
Water	15.48	25.80	25.66	-	-
Etanol	25.31	29.17	22.80	-	-
Saponnin	10.02	12.61	-	-	-
				0.00	17.53

than that of the positive control of Xenical® (17.53%). However saponin crude extracts had the lowest inhibitory effect.

***In Vitro* Assay of Potent Inhibitory Effect of Extract Mixtures on the Activity of Pancreatic Lipase.** The optimum inhibition concentration of *Z. cassumunar* rhizome, *G. ulmifolia*, and *M. paniculata* leaves extracted by water were 75, 45, and 100 ppm, respectively. Meanwhile, the extracts of *Z. cassumunar* rhizome, *G. ulmifolia*, and *M. paniculata* leaves from ethanol extraction showed that the optimum inhibition was performed by the concentration of 100, 60, and 30 ppm respectively.

In vitro assay of extract mixtures of 25 ppm and with varied in the ratio of the extracts showed that all mixtures could inhibit the activity of pancreatic lipase *in vitro* (Figure 4). The highest inhibition effect was showed by the extract combination of *Z. cassumunar:G. ulmifolia:M. paniculata* with ratio of 25:25:25, followed by mixture of *Zingiber cassumunar* and *Guazuma ulmifolia* extracted by water (45:75), extract mixture of *Zingiber cassumunar*, *Guazuma ulmifolia*, and *Murraya paniculata* (25:25:80) of water extration, extract mixture of *Zingiber cassumunar*, *Guazuma ulmifolia*, and *Murraya paniculata* (25:25:80) of water extraction. The highest inhibition effect of combined extracts (21.58%) was higher than that of Xenical® as positive control (19.13%).

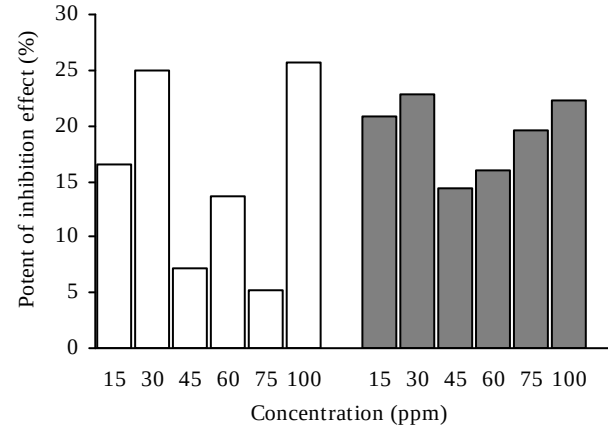

Figure 3. Inhibitory effect of □: water extract and ▩: ethanol 70% extract of *kemuning* leaves on the activity of pancreatic lipase.

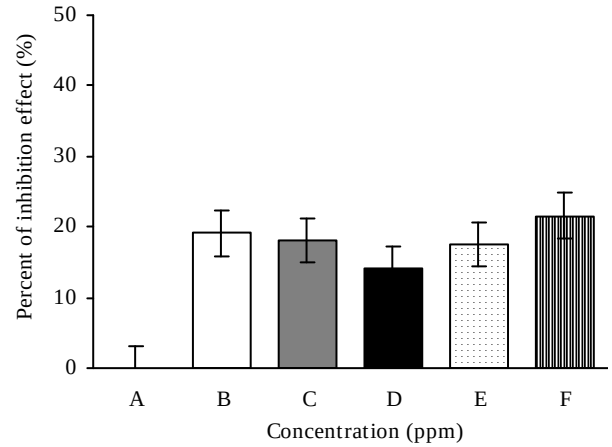

Figure 4. Inhibitory effect of several mixture extract on the activity of pancreatic lipase: A: control; B: Xenical®; C: mixture of water extract of *Zingiber cassumunar* and *Guazuma ulmifolia* (45:75); D: mixture of ethanol extract of *Zingiber cassumunar*, *Guazuma ulmifolia*, and *Murraya paniculata* (100:80:30); E: mixture of water extract of *Zingiber cassumunar*, *Guazuma ulmifolia* and *Murraya paniculata* (25:25:80); F: mixture of ethanol extract of *Zingiber cassumunar*, *Guazuma ulmifolia*, and *Murraya paniculata* (25:25:25).

DISCUSSION

The phytochemical assay toward water, ethanol, and saponin crude extractions was conducted to observe the type of secondary metabolite compounds contained in each extracts, qualitatively. The phytochemical assay of saponin extract of *Guazuma ulmifolia* showed that the detected compounds were not only saponin, but also steroid. This results are similar to those of the saponin extracts of *Zingiber cassumunar* where triterpenoid was also detected. This fact was due to the that saponin is a glycoside of steroid and triterpenoid (Wina *et al.* 2005), therefore, it gave positif results for steroid and triterpenoid test. Tannin compound was found in the extracts of *G. ulmifolia* extracted by both water and ethanol, but it was not found in the extract of *Z. cassumunar*. The amount of saponin compound detected on *Z. cassumunar* extract was more than that of *G. ulmifolia*. The alkaloid compound was not detected on either of their extracts. Meanwhile, the phytochemical assay showed no detected alkaloid and saponin compound on *M. paniculata* leaves (Table 3). Triterpenoid was also not detected on water and ethanol crude extraction of *M. paniculata*, however, they contained steroid. In contrast of this results, crude extracts of water extraction contained triterpenoid, but it did not contain steroid. The reason for the undetected compound could be due to the small content of that compound on each sample, or they are simply not present in the sample.

The toxicity test was used to determine the bioactive potency of a natural product, and to ascertain the lethal doses of a chemical compound found on a sample of herbal. The toxic secondary metabolites will cause the death of the larvae through two types of process, inhalation and diffusion. The obtained LC_{50} value was the minimum concentration level that can cause death to 50% of the tested animal population. Based on this test, it was suggested that all extracts had bioactive potency because they had significant effect on the life of the shrimp larvae. The extracts that had the highest bioactive potency and toxic were originated from the ethanol extraction of *Z. cassumunar*. This was shown by the lowest LC_{50} level of the extract of *Z. cassumunar* could kill 50% of the shrimp larvae. The LC_{50} value of each extract was used as basic of concentration level determination for *in vitro* assay. The concentration of extracts for *in vitro* assay was lower than that of each LC_{50} value.

Either single or mixture extracts had potential inhibition on the activity of pancreatic lipase. Single extract 100 ppm of *Zingiber cassumunar* of ethanol extraction had the

highest inhibition effect on the activity of pancreatic lipase (29.17) and *Murraya paniculata* leaves with ratio of 25:25:25 (21.58%). These inhibition effects were higher than inhibitory effect of 100 ppm of Xenical®/orlistat as the positive control, with the inhibition value of 17.53%. Shin *et al*. (2003) and Lee *et al.* (2005) mentioned that the *in vitro* inhibition effect of 3-methylether galangin from *Alpinia officinarum* and crocetin from *Gardenia jasminoides* toward the activity of pancreatic lipase using triolein substrate showed a lower inhibition than that of positive control, orlistat. This study showed that extracts of *G. ulmifolia*, *Z. cassumunar*, and *M. paniculata* have a higher effect to inhibit the activity of pancreatic lipase, therefore they can be used as antiobesity medicine, because inhibitor of pancreatic lipase activity was able to suppress dietary fat absorption from the small intestine of mice by *in vivo* inhibiting pancreatic activity (Martins *et al*. 2010). Although, its inhibition effect was less than grape seed extract of ethanol extraction with 80% inhibition of activity pancreatic lipase (Moreno *et al.* 2003).

All combined extracts, either water or ethanol extraction of *Z. cassumunar*, *G. ulmifolia*, and *M. paniculata* could inhibit the activity of pancreatic lipase. However, the inhibition effects of mixture extracts were less than that of their single extract. This result showed that extracts of *Z. cassumunar*, *G. ulmifolia*, and *M. paniculata* did not work synergistically, because inhibition of combined extract was low, it was only 0.85%. This result was less than the inhibition effect of 100 ppm extract of *M. paniculata* of water extraction, it was 25.66% (Figure 4). The inhibition activity of each extract became antagonistic toward each other when extracts of *Z. cassumunar, G. ulmifolia, M. paniculata* of water extraction were combined with the ratio of 75:45:100 ppm.

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