

INDONESIAN HERBALS REDUCE CHOLESTEROL LEVELS IN DIET-INDUCED HYPERCHOLESTEROLEMIA THROUGH LIPASE INHIBITION

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Water and ethanolic extracts of *Guazuma*, *Gynura*, *Muraya* and *Roselle* were evaluated for their antihypercholesterolaemia and antilipase activities in high fat diet (HFD)-induced rats. Ethanolic and water extracts were given on days 31–45. Total cholesterol and lipase activity in serum were measured. *Guazuma* water (Guw) and ethanolic (GuEt) extracts, *Gynura* water (Gyw) extract, *Muraya* ethanolic (MEt) extract as well as *Roselle* water (Rw) and ethanolic (REt) extracts caused reduction in cholesterol levels of HFD-induced rats significantly (17%–23%; $p < 0.05$). The potency of GuEt extract and Rw extract were similar to that of orlistat. Gyw extract, MEt extract and Rw extract reduced dietary fat absorption by inhibiting lipase activity.

Keywords: *Guazuma*, *Gynura*, *Muraya*, *Roselle*, Lipase activity

INTRODUCTION

Cholesterol, which is an important component for maintaining normal function of the cells is also associated with the incidence of atherosclerosis (Attie 2007). Nowadays, there has been a dramatic increase in obesity cases resulting from an excess of cholesterol in the human body (Kim *et al.* 2007). Traditional herbal medicines have been used in the treatment of obesity (Mochizuki and Hasegawa 2004; Sayama *et al.* 2000). Several studies reported that some herbal medicines such as *Garcinia* extract, hibiscus tea, garlic, turmeric and green tea exhibit potential antiobesity effect (Sukandar *et al.* 2010; Kim *et al.* 2007, 2003).

Several Indonesian herbal medicines such as *Guazuma ulmifolia* Lam, *Gynura procumbens* (Lour.) Merr., *Muraya paniculata* and *Hibiscus sabdariffa* L (Roselle) have been used as cholesterol lowering traditional herbal medicines in Indonesia. However, research on cholesterol lowering activity of these extracts has not been conducted yet. Polysaccharides, epicatechin (EP), and procyanidin oligomers, have been isolated from *Guazuma* extract (Rocha *et al.* 2007; Hor *et al.* 1996). *Guazuma* extract has been shown to exhibit antidiabetic activity in some studies (Alonso-Castro and Salazar-Olivo 2008; Alarcon-Aguilara *et al.* 1998) while the ethanolic extract of *Gynura* is shown to possess antitumour and anticancer activities (Sugiyanto *et al.* 2003, 1993). Several studies have found that *Gynura* contains flavonoid, sterol and steroid as well as some proteins such as peroxidase, catalase, carboxylase, osmotin-like protein and thaumatin-like protein (Hew and Gam 2010; Sudarsono *et al.* 2002). *Muraya* extract contains meranzin hydrate and has been used to reduce cholesterol levels in some Indonesian herbal medicine (Julaeha *et al.* 2010). Roselle phenolic compound, protocatechuic acid has been studied as an inhibitor of

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low-density lipoprotein (LDL) whereas Roselle anthocyanins are well known as antioxidants (Lee *et al.* 2002; Wang *et al.* 2000).

The purpose of this study was to investigate the effect of *Guazuma*, *Gynura*, *Muraya* and Roselle ethanolic and water extracts on the reduction of cholesterol level in serum and its inhibitory activity on lipase.

METHODS

The study was conducted in the Pharmacology Laboratory, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Gadjah Mada University. All experimental procedures were approved by the Animal Ethics Committee of the Integrated Research and Training Laboratory (*Laboratorium Penelitian dan Pengujian Terpadu*) of Universitas Gadjah Mada.

The plant materials were washed thoroughly with tap water and dried at 60°C in an incubator, diced into small pieces, powdered in a mixer grinder, and then the dried powders were macerated with 95% ethanol for 24 hours. The macerated extracts were subsequently filtered; the residue was macerated again with water for 24 hours. The ethanolic and water extracts were evaporated in a Rotavapor at 60°C under pressure. The plant extracts were kept at 20°C.

The research was conducted on 50 male Wistar rats aged 2 months with an average body weight of 150 g. Animals were divided into 10 groups of 5 rats. Rats were induced with high fat diet (HFD) containing 0.15% of lard and 0.05% of egg yolk. HFD was given for 45 days. On day-30, after 18 hours of fasting, blood was taken for initial cholesterol level and lipase activity. On day-31, either *Guazuma*, *Gynura*, *Muraya*, Roselle extracts or orlistat (obtained from P.T. Roche, Jakarta, Indonesia) were given to the rats. The doses of ethanolic or water extract were determined from what has been traditionally used in the community (Sudarsono *et al.* 2002). Doses of *Guazuma* water (Guw) extract, *Guazuma* ethanolic (GuEt) extract, *Gynura* water (Gyw) extract, *Gynura* ethanolic (GyEt) extract, *Muraya* water (Mw) extract, *Muraya* ethanolic (MEt) extract, Roselle water (Rw) extract and Roselle ethanolic (REt) extract are given in Table 1.

Table 1: Doses of ethanolic and water extracts of herbs.

Herb	Dose of extract (mg/kg body weight)	
	Ethanol	Water
<i>Guazuma</i>	59.6	69.1
<i>Gynura</i>	63.0	36.5
<i>Muraya</i>	44.6	32.8
Roselle	63.0	45.0

The extracts and orlistat were suspended in sodium carboxymethyl cellulose (CMC) 0.5%. After 15 day-treatments with either ethanolic extract, water extract or orlistat, blood were taken for serum cholesterol and pancreatic activity analysis. Total cholesterol level in serum was measured using Cholesterol Fluid Stable (DiaSys, Holzheim, Germany) whereas lipase activity in serum was analysed using Lipase Activity Assay Kit (Biovision, California, USA). Lipase activity test conducted in 7 groups indicated significant

cholesterol level reduction and control group. All experiments data were represented as mean±SEM of 5 replicates. One-way ANOVA was used to analyse the statistical significance followed by Fisher's Least Significant Difference (LSD) test. The statistical analysis was performed using SPSS 18.0 for Windows.

The formula to calculate reduction of cholesterol = $[C_{30}-C_{45}/C_{30}] \times 100\%$,

where,

C₃₀=cholesterol level at day 30

C₄₅=cholesterol level at day 45.

The formula to calculate reduction of lipase activity = $[L_{30}-L_{45}/L_{30}] \times 100\%$,

where,

L₃₀=lipase activity at day 30

L₄₅=lipase activity at day 45.

RESULTS

The present study showed that the HFD-induced hyperlipidaemia resulted in an increased total cholesterol level ($32.2 \pm 1.8\%$; $p < 0.05$). Guw and GuEt extracts, Gyw extract, MEt extract as well as both Rw and REt extracts reduced total cholesterol level significantly as compared to the HFD group. The effects of GuEt and Rw extracts were comparable to that of orlistat (Table 2). Furthermore, lipase activity decreased by 12%–41% ($p < 0.05$) after Gyw, MEt and Rw extracts treatment for 15 days. Rw extract significantly inhibited lipase activity higher than orlistat ($p < 0.05$) (Table 3).

DISCUSSION

Cholesterol level in the body is controlled by the biosynthesis of endogenous cholesterol, absorption of exogenous cholesterol and elimination through bile acids (Jones 1997). High cholesterol levels in serum are associated with greater risk of cardiovascular diseases (Klag *et al.* 1993). Diet and exercise have been recognised as non-pharmacological approaches in the management of hypercholesterolaemia. However, a non-pharmacological approach is not sufficient to lower cholesterol level for most people. Therefore, using traditional herbal medicine could be one of the alternatives for reducing cholesterol levels.

Some plants demonstrated the ability to reduce plasma lipids in both animal study and clinical trial (Duangjai *et al.* 2010; Sukandar *et al.* 2010; Martins *et al.* 2009; Birari and Bhutani 2007; Carvajal-Zarrabal *et al.* 2005; Kim *et al.* 2003). This study provides evidence suggesting the potential hypocholesterolaemic action of *Guazuma*, *Gynura*, *Muraya* and *Roselle* extracts. Guw, GuEt, Rw and REt extracts as well as Gyw and MEt extracts reduced cholesterol levels of HFD-induced hypercholesterolaemic rats. GuEt and Rw extracts exhibited similar potency on hypocholesterolaemic activity as orlistat [(–)-tetrahydrolipstatin, N-formyl-L-leucine (1S)-1-[[[(2S,3S)-3-hexyl-4-oxo-2-

oxetanyl]methyl]dodecyl ester], a pancreatic lipase inhibitor that acts locally in the gastrointestinal tract to inhibit lipase.

Table 2: Cholesterol levels in rats fed with experimental diets.

Treatments	Day 30 (mg/dL)	Day 45 (mg/dL)	Percentage (%)
HFD	94.4±5.3	100.8±4.5	+6 ^b
HFD+orlistat	94.0±2.5	72.2±4.6	-23 ^a
HFD+Guw	83.7±5.5	69.7±3.8	-17 ^{ab}
HFD+GuEt	93.8±2.2	72.2±5.7	-23 ^a
HFD+Gyw	93.0±6.2	76.2±3.7	-18 ^{ab}
HFD+GyEt	92.8±4.6	93.0±2.5	+0.2 ^b
HFD+Mw	91.8±7.3	87.2±8.2	-5 ^b
HFD+MEt	85.2±7.6	70.8±6.6	-17 ^{ab}
HFD+Rw	94.5±3.9	74.7±2.0	-21 ^a
HFD+REt	91.7±5.7	75.9±4.7	-17 ^{ab}

Notes: Data are expressed as mean±S.E.M (n=5)

Cholesterol levels on day 0 are 69.2±1.3 mg/dL (n=50)

^ap<0.05 compared with HFD

^bp<0.05 compared with HFD+orlistat

+ = increase

- = decrease

Table 3: Lipase activity in rats fed experimental diets.

Treatments	Day 30 (mU/mL)	Day 45 (mU/mL)	Percentage (%)
HFD	11.0±1.5	12.0±0.6	+9 ^b
HFD+orlistat	12.0±0.9	8.2±1.1	-32 ^a
HFD+Guw	11.7±1.4	10.7±2.1	-9 ^b
HFD+GuEt	10.9±2.2	12.7±2.5	+17 ^b
HFD+Gyw	12.0±1.7	9.4±0.6	-22 ^{ab}
HFD+MEt	12.6±2.1	11.1±1.4	-12 ^{ab}
HFD+Rw	11.2±2.9	6.6±1.2	-41 ^{ab}
HFD+REt	11.7±1.1	11.0±2.4	-6 ^b

Notes: Data are expressed as means±S.E.M (n=5)

^ap<0.05 compared with HFD

^bp<0.05 compared with HFD+orlistat

+ = increase

- = decrease

Lipase activity inhibition is one of the most widely studied mechanisms for the determination of the potential efficacy of herbal medicine as antihypercholesterolaemia (Birari and Bhutani 2007). In this study, Gyw and Rw extracts showed promising inhibitory activity against lipase. Pancreatic lipase is responsible for the hydrolysis of 50%–70% of total dietary fats (Birari and Bhutani 2007). Therefore, phytochemicals identified from traditional herbal medicine present an opportunity for the development of

newer therapeutics as antilipase agents (Duangjai *et al.* 2010; Martins *et al.* 2009; Birari and Bhutani 2007). Pancreatic lipase plays an important role in dietary cholesterol absorption as pancreatic lipase-mediated hydrolysis of the triacylglycerols was shown to be necessary for cholesterol transport to the intestinal cells (Huggins *et al.* 2003; Young and Hui 1999). As pancreatic lipase inhibition reduces dietary lipid digestion and absorption, Gyw and Rw extracts as well as MEt extract prevented long-term HFD consumption which was shown in the reduction of total cholesterol (Table 2) of high fat diet animals. Polyphenolics such as flavonoid obtained from rhizomes of *Alpinia officinarum* and from fruits of *Mangifera indica* showed moderate inhibition of pancreatic lipase (Moreno *et al.* 2006; Shin *et al.* 2003). Flavonoids, the main polyphenols in Gyw extract, might be responsible for antilipase activity. However, this does not rule out that other components present in Gyw extract might also be implicated in antilipase effect. Protocatechuic acid components present in Rw extract and meranzin hydrate have been shown to reduce cholesterol levels (Julaeha *et al.* 2010; Lee *et al.* 2002). However, there was no inhibition on lipase activity after the treatment of either Guw or GuEt extracts or REt extract. The reduction of cholesterol level which was caused by either Guw or GuEt extracts as well as REt extract might not be through the inhibition on cholesterol absorption which is regulated by lipase activity. Tannin and gel compound of *Guazuma* were hypothesised to reduce fat absorption in the gastrointestinal tract (Utomo 2008). Hibiscus acid being the main compound of REt extract inhibited pancreatic α -amylase function and oxidative stress in the human body (Hansawasdi *et al.* 2000; Tseng *et al.* 1997).

CONCLUSION

This study shows that *Guazuma* water, *Muraya* ethanolic and Roselle water extracts are able to reduce dietary fat absorption by inhibiting lipase activity suggesting that these herbs exhibit antilipase properties.

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