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FULL PAPER

HMG-CoA reductase and lipase enzyme inhibition from combination of *Gynura procumbens* and *Curcuma xanthorrhiza* aqueous extract

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Abstract

Lifestyle habits related to consuming excessive fatty foods lead to high levels of lipids in the blood. Some drugs are used to reduce lipids by inhibiting the action of the enzyme HMG-CoA reductase and the pancreatic lipase. The *Curcuma xanthorrhiza* rhizome and *Gynura procumbens* leaves were empirically used to reduce cholesterol and lipid levels. This study used the combination of aqueous extract of *G. procumbens* and *C. xanthorrhiza* rhizomes (4:1) and their single forms as herbs tested. Furthermore, this study aimed to determine the activity of the tested herbs in inhibiting the enzymatic actions of HMG-CoA reductase and pancreatic lipase in vitro, compared with quercetin, curcumin, and positive control. The results showed that the aqueous extract of *C. xanthorrhiza* with an IC_{50} value of 127.54 ppm has the highest activity in inhibiting the enzyme HMG-CoA reductase compared to the aqueous extract of *G. procumbens* and their combination. In the inhibition of lipase enzyme, *G. procumbens* aqueous extract had the most potent inhibitory activity compared to *C. xanthorrhiza* and their combination with an IC_{50} value of 100.08 ppm.

1. Introduction

Fatty foods that contain triglycerides and cholesterol are absorbed by mucosal cells. When food containing fat is consumed in excess, it leads to high levels of fat in the blood (Pebrianty, 2013). Excessive lipids in the body can be reduced by inhibiting the action of the HMG-CoA enzyme (3-hydroxy 3-methylglutaryl coenzyme A) reductase and pancreatic lipase (Last *et al*., 2011). Basic Health Research in 2018 showed that the Indonesian population used traditional medicine with 48% of herbal products, 31.8% of homemade herbs, and 24.6% of family medicinal plants (Ministry of Health Indonesia, 2018).

Statins are inhibitors of the HMG-CoA reductase, and it effectively inhibits this enzyme up to 95%, similar to Simvastatin, which is effective for reducing blood lipid levels even though it has several side effects, such as inflammation of the muscles (0.5%), myalgia (2-10%), rhabdomyolysis with acute kidney failure (0.1%), and impaired liver function (1-3%) (Varras, 2008; Burg and Espenshade, 2011; Lachenmeier *et al*., 2012). Lipase is an enzyme that breaks down and converts lipids to fatty acids (Shin *et al*., 2004). Increased pancreatic lipase activity can increase the absorption of monoglycerides and fatty acids (Joshita *et al*., 2000), and it causes the accumulation of fat in the body. Orlistat is an effective lipase inhibitor, with several side effects such as gastrointestinal disorders manifesting as oily feces, diarrhea, abdominal pain, and fecal stains (Filippatos *et al*., 2008). Synthetic drugs such as simvastatin and orlistat have some side effects on the body. Therefore, natural ingredients are needed as alternatives to inhibit the mechanism of HMG-CoA reductase and lipase enzymes. Natural ingredients that were empirically used to reduce cholesterol and lipid levels were *Curcuma xanthorrhiza* Roxb. (*C. xanthorrhiza*) and *Gynura procumbens* (Lour.) Merr (*G. procumbens*) (Achmad *et al*., 2009; Niaga Swadaya, 2010).

One of the contents of *C. xanthorrhiza* is curcumin, which can inhibit acyl-CoA activity in the liver (Aggarwal *et al*., 2004). Furthermore, it reduces peroxidase enzyme and total cholesterol, as well as increases high-density lipoprotein levels and Apolipoprotein A-1 levels (Yasni *et al*., 1993; Kertia and Sudarsono, 2005). *G. procumbens* leaf extract inhibits

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cholesterol synthesis, esterification, triglycerides, and HMG-CoA reductase activity in mice (Metwally *et al*., 2009). However, there is no in vitro study of the combination of the *C. xanthorrhiza* rhizome and *G. procumbens* leaves in inhibiting the enzymatic actions of HMG-CoA reductase and lipase.

In preclinical study showed that the combination of *G. procumbens* and *C. xanthorrhiza* aqueous extract (4:1) more effective decreases total cholesterol, triglycerides, and low-density lipoprotein, and then any other combination ratio (Damanik and Ikawati, 2015; Luthfia and Ikawati, 2015). Therefore, this study aimed to determine the inhibitory activity of aqueous extracts of *G. procumbens* leaves and *C. xanthorrhiza* rhizomes either in a combination (4:1) or in single form on lipase enzymes and HMG-CoA reductase through in vitro study.

2. Materials and methods

2.1 Materials

C. xanthorrhiza rhizomes and *G. procumbens* leaves were obtained from Nanggulan, Kulon Progo, and Gamagiri, Mangunan, Yogyakarta respectively. Conversely, curcumin was purchased from Nacalai, while quercetin, a lipid porcine from pancreas L3126-25G, orlistat PHR 1445-1G, simvastatin pharmaceutical secondary standard was obtained from Sigma Aldrich®. Furthermore, lipase activity was measured with a colorimetric assay kit K-722 (Biovision®), while the enzymatic actions of HMG-CoA were measured using the screening kit Colorimetric K-588 (Biovision®).

2.2 Preparation of G. procumbens leaf and C. xanthorrhiza rhizome water extract

G. procumbens dried leaves (40 g) was poured in 400 mL distilled water and boiled in an infusion pan. The dried rhizome of the *C. xanthorrhiza* weighed at 80 g was poured in 800 mL distilled water and boiled in separate infusion pan. Subsequently the aqueous extract of *G. procumben*s leaves and *C. xanthorrhiz*a rhizomes were freeze-dried (Virtis Bench Top Pro 3 ES USA).

2.3 Inhibition assay of HMG-CoA Reductase enzyme

The HMG-CoA reductase inhibition assay followed the HMG-CoA reductase activity/inhibitor screening kit Colorimetric (Biovision®) guidelines, while Simvastatin, quercetin, and curcumin were weighed using ultramicro scales (Radwag, sensitivity 0.1 µg). The concentration used of simvastatin, quercetin, and curcumin were 0.82-13.16 µg/mL, 2.63-42.11 µg/mL, and 2.63-42.11 µg/mL of ethanol, respectively. Samples of *G. procumbens* leaves, *C. xanthorrhiza* rhizome, and their aqueous combinations were weighed with Mettler Toledo scales (sensitivity 0.0001 g) at a concentration of 105.26-263.16 µg/mL, 52.63-157.89 µg/mL, and 52.63-157.89 µg/mL, respectively.

The test solution consisted of simvastatin, curcumin, quercetin, *G. procumbens* leaves, *C. xanthorrhiza* rhizome, and a combination of their aqueous extract in the ratio of 4:1. The test solution was made into 6 concentration series, and their reaction mixture contained 190 µL, consisting HMG-CoA of 12 µL substrate, 164 µL buffer, and 5 µL reductase enzyme, NADPH (Nicotinamide adenosine dinospotide hydrogen phosphate) 4 µL, and 5 µL test solution. In addition, it contains a mixture of the enzyme reaction, control, and the test solution. The reaction mixture was conducted in duplicate, incubated at 37°C for 10 mins, and the absorbance was measured at 340 nm wavelength.

2.4 Inhibition of lipase enzyme

The method used was based on the lipase activity colorimetric assay kit (Biovision®) guidelines that were modified by McDougall *et al.* (2009). Modifications made were the replacement of the enzyme mix with lipase and the absorbance was measured at a maximum wavelength of 400 nm. The lipase enzyme used was obtained from porcine pancreas (Sigma Aldrich®). Meanwhile, the concentration of orlistat was 0.39-6.25 µg/mL dimethyl sulfoxide. The sample used to confirm the obtained value was similar to the one used in the HMG-CoA reductase inhibitory test.

The reaction mixture of the test solution contained 100 µL of 88 µL buffer, 2 µL oxired probe, 2 µL lipase enzyme, 3 µL substrate, and 5 µL test solution. In addition, the mixture consists of the enzyme reaction, control, and the test solution. The reaction mixture was conducted in duplicate and incubated at 37°C for 60 mins, and its absorbance was measured at a wavelength of 400 nm.

The reaction mixture on inhibition of HMG CoA reductase and lipase was measured on the Corona SH-1000 Microplate Reader (Ibaraki-Ken, Japan) and the percentage of inhibition was calculated using the following formula where the difference between the absorbance delta of enzyme and the absorbance delta of the sample divided by the absorbance delta of enzyme than multiplied by 100%. The absorbance delta was the absorbance minus control. The IC_{50} value was obtained from the grade series linear regression with the percentage inhibition.

3. Results

Curcumin is the important ingredient in *C. xanthorrhiza* while *G. procumbens* is quercetin. Therefore, a comparison of the inhibition test of the enzyme HMG CoA reductase and lipase between curcumin and quercetin were conducted. Furthermore, the result was compared with the standard drug for each assay, i.e. simvastatin for HMG-CoA reductase, and orlistat for lipase enzymes assays. Enzyme inhibition is considered strong when the inhibitory activity is 34.49%, at a maximum concentration of 100 ppm, or with a percentage less or maximum of 2.89. While the inhibition of 10 - <34.49% and less than 10% are considered moderate and weak, respectively (Ong *et al*., 2014).

3.1 Inhibition assay of HMG-CoA reductase enzyme

This study used a combination of *G. procumbens* and *C. xanthorrhiza* aqueous extracts (4:1) because previous studies had shown that this combination was effective in lowering lipid profiles in rats (Damanik and Ikawati, 2015; Luthfia and Ikawati, 2015). The results of the IC_{50} inhibitory assay of the compounds on HMG-CoA reductase enzyme are shown in Figure 1. *C. xanthorrhiza*, *G. procumbens*, and their combined aqueous extract (4:1) water had 50% inhibitory potency at a concentration of 127.54 ppm, 180.86 ppm, and 150.13 ppm, respectively. The most potent of the samples, which can inhibit 50% of the HMG-CoA reductase enzyme is *C. xanthorrhiza* compared with *G. procumbens* and a combination of *G. procumbens* and *C. xanthorrhiza* (4:1) aqueous extract.

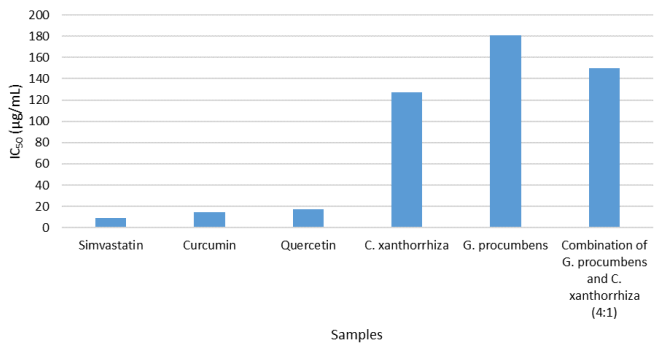

Figure 1 . IC_{50} result of positive control, comparators and samples on inhibition of the HMG-CoA reductase enzyme

Table 1 shows positive inhibitory potential, comparative and sample controls on HMG-CoA Reductase enzyme. The ratio is obtained from the division between concentration and percentage inhibition. *C. xanthorrhiza* extract has strong potential, while the mixture of *G. procumbens* and combination of *G. procumbens* and *C. xanthorrhiza* (4:1) extracts have moderate potential. Furthermore, IC_{50} was obtained from a sample divided by a comparison. The comparison of simvastatin with *G. procumbens*, *C. xanthorrhiza*, and the combination of their aqueous extract (4:1) were 20.62, 14.54, and 17.12, respectively.

3.2 Inhibition assay of lipase enzyme

The results of the IC_{50} inhibitory assay of the compounds on lipase are shown in Figure 2. *G. procumbens*, *C. xanthorrhiza*, and the combination of their aqueous extract (4:1) at respective concentrations of 100.08 ppm, 208.12 ppm, and 112.06 ppm can inhibit the activity of the lipase enzyme in hydrolyzing oleic acid by 50%. The most potent of the sample solution that can inhibit 50% of the lipase enzyme is *G. procumbens*. Lipase inhibitors inhibit enzymes that cause the lipolysis process to become inhibited and when reacted, the substrate will bind to a calibrator, which has high competition with the sample (Hidayat *et al*., 2014). In this study, orlistat has a 50% inhibitory potency at a concentration of 2.59 ppm. Several studies indicate that IC_{50} value of orlistat was 0.05-2 ppm (Hadváry *et al*., 1988; Lewis and Liu, 2012; Dechakhamphu and Wongchum, 2015; Padilla-Camberos *et al*., 2015).

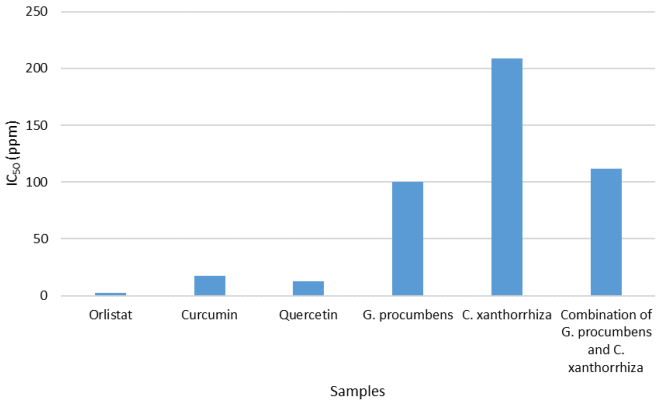

Figure 2. IC_{50} result of positive control, comparators and samples on inhibition lipase enzyme

Table 1. Inhibitory potential and comparison of positive control, comparators and samples on HMG-CoA reductase enzyme

Sample	Ratio Content ≤ 100 with	Potency	Comparison IC_{50} with	Comparison IC_{50} with	Comparison IC_{50} with
Simvastatin	0.18	Strong	-	0.61	0.50
Curcumin	0.59	Strong	1.64	-	0.83
Quercetin	0.62	Strong	1.99	1.21	-
C. xanthorrhiza	2.49	Strong	14.54	8.83	7.31
G. procumbens	3.22	Moderate	20.62	12.52	10.36
Combination of *G. procumbens* and *C. xanthorrhiza* (4:1)	3.17	Moderate	17.12	10.39	8.60

Table 2. Inhibitory potential and comparison of positive control, comparators and samples on lipase enzyme

Sample	Ratio Content ≤ 100 with percent of inhibition	Potency	Comparison IC_{50} with Orlistat (times)	Comparison IC_{50} with quercetin (times)	Comparison IC_{50} with curcumin (times)
Orlistat	0.80	Strong	-	0.21	0.15
Curcumin	0.55	Strong	6.49	1.39	0.72
Quercetin	0.52	Strong	4.67	-	-
C. xanthorrhiza	3.67	Moderate	37.48	16.75	12.05
G. procumbens	2.07	Strong	78.15	8.03	5.78
Combination of *G. procumbens* and *C. xanthorrhiza* (4:1)	2.24	Strong	41.97	8.99	6.47

Table 2 shows the inhibitory potential of positive control, comparative, and sample controls on lipase enzyme *G. procumbens* and its combination with *C. xanthhorrhiza* extracts have strong potential, while *C. xanthorrhiza* extract has moderate potential. Meanwhile, the comparison of orlistat with *G. procumbens*, *C. xanthorrhiza*, and their aqueous combination (4:1) were 37.48, 78.15, and 41.97, respectively. *G. procumbens* have strong potency inhibition compared to other samples because the amount of secondary metabolite compound content is more than *C. xanthorrhiza* and combination *G. procumbens* and *C. xanthorrhiza* (4:1) aqueous extract (Pradono *et al.,* 2011). Furthermore, the content of alkaloids in the leaves of *G. procumbens* results in decreased inhibition potency of extracts, while the content of flavonoids, saponins, and tannins result in increased inhibition potency of extracts (Pradono *et al*., 2011). Orlistat, curcumin, and quercetin have a strong inhibitory effect on the lipase enzyme.

4. Discussion

Acetyl CoA is an intermediate product from carbohydrates, fats, and some amino acids from proteins that cause animals and humans to convert food substrate to cholesterol. The HMG-CoA reductase enzyme can catalyze the process of converting acetate to mevalonate. Inhibition of cholesterol biosynthesis in the liver is performed by inhibiting the action of the HMG-CoA reductase enzyme. It is a unique enzyme that plays a role at the beginning of irreversible cholesterol biosynthesis (Burg and Espenshade, 2011). Cholesterol is synthesized from acetate under the influence of the HMG-CoA reductase enzyme. It becomes active when there is a deficiency of endogenous cholesterol. In the chain of reaction to produce cholesterol, Acetyl CoA is converted to HMG-CoA then converted to mevalonate. Furthermore, the mevalonate is converted to pyrophosphate, after that it becomes isopentenyl pyrophosphate, then converted to pyrophosphate geranyl and pyrophosphate faresil into squalent which eventually becomes cholesterol (Harikumar *et al*., 2013).

One of the contents of *C. xanthorrhiza* is curcumin, and it helps to maintain cholesterol homeostasis through the expression of the mRNA receptor (messenger ribonucleic acid) gene that codes for the enzyme biosynthesis of HMG-CoA reductase and farnesyl diphosphate. The expression of these genes causes increased protein-binding sterol elements due to synthesis and cellular absorption resulting in a reduction of protein-bound fatty acids translocation and mRNAs from alpha peroxisome proliferator receptors (Peschel *et al*., 2007).

Human pancreatic lipase is the main enzyme responsible for breaking down fat in the human digestive tract, and it converts the triglyceride substrate in food into monoglycerides and free fatty acids (Svendsen, 2000). Absorption of dietary fat in the body can be prevented by orlistat since it reduces triglycerides. Therefore, free fat can be reduced due to the orlistat mechanism in inhibiting lipase activity because of the binding ability of its catalytic site. These bonds show that the lipase enzyme cannot catalyze the hydrolysis reaction of triglycerides, which leads to a reduction in the absorption of the amount of free fatty acids by the intestine (Pebrianty, 2013).

Pancreatic lipase enzymes play a role in digestion and absorption of triglycerides from 90 to 95% of the digested fat (Ros, 2000). The ethanol extract fraction of *G. procumbens* leaves contain polyphenols and flavonoids which are used as anti-dyslipidemia by inhibiting the activity of lipase enzymes and play a role in lipid absorption (Setiawan, 2012). Furthermore, Dechakhamphu and Wongchum (2015) showed that flavonoid, phenolic, and alkaloid compounds are important in inhibiting lipase activity in vitro. However, phenolic compounds inhibit the lipase enzyme in the pancreas (Tiss *et al*., 2004) and it plays a role in catalyzing the hydrolysis of triglycerides to be absorbed by the body in order to reduce cholesterol levels (Sreerama *et al*., 2012; Onakpoya *et al*., 2015). Polyphenol compounds can inhibit enzymes involved in fat metabolisms, such as lipase and glycerophosphate dehydrogenase. Extracts containing polyphenols can reduce triglyceride and LDL levels, increase energy

expenditure, and reduce body weight and adipocyte cells (Yoshikawa *et al*., 2002).

The combination of *G. procumbens* and *C. xanthorrhiza* (4:1) aqueous extract on inhibition of HMG -CoA reductase enzyme showed smaller IC_{50} value than the single *G. procumbens* extract, as well as smaller value when compared with single *C. xanthorrhiza* extract. This study showed that *G. procumbens* extract can increase the effect of *C. xanthorrhiza* extract on inhibition of lipase enzyme, while *C. xanthorrhiza* extract can increase the effect of *G. procumbens* extract on inhibition of HMG-CoA reductase. Therefore, a combination of *Gynura procumbens* and *Curcuma xanthorrhiza* (4:1) extract is needed to obtain a synergistic effect as a dyslipidemia therapy. The results showed that the combination of *G. procumbens* and *C. xanthorrhiza* (4:1) aqueous extract was effective in the reduction of cholesterol levels by inhibiting the HMG-CoA and reducing triglyceride through inhibition of the lipase enzyme. Therefore, the combination of *G. procumbens* and *C. xanthorrhiza* (4:1) aqueous extract was effective as a therapy for dyslipidemia to reduce total cholesterol and triglyceride in vitro assay.

5. Conclusion

The results of the inhibitory analysis of HMG-CoA reductase showed that aqueous extract of *C. xanthorrhiza* has the most potent IC_{50} value than *G. procumbens* and a combination *G. procumbens* and *C. xanthorrhiza* (4:1), i.e 127.54 ppm. While the results of the lipase enzyme inhibition assay demonstrated that *G. procumbens* have the most potent IC_{50} value than *C. xanthorrhiza* and a combination *G. procumbens* and *C. xanthorrhiza*, i.e 100.08 ppm. Therefore, it is advisable to conduct in vivo to the efficacy and safety of lipid profiles. Suggestions for further study is that a combination of *G. procumbens* and *C. xanthorrhiza* (4:1) aqueous extract should be made for preclinical study of these products on the effectiveness of lipid profiles.

Conflict of interest

The authors declare no conflict of interest.

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References

Achmad, A.S., Hakim, H.E., Makmur, L., Juliawaty, D.L. and Mujahidin, D. (2009). Ilmu Kimia dan kegunaaan: Tumbuh – Tumbuhan Obat Indonesia. 1st ed. Bandung: Institut Teknologi Bandung. [In Bahasa Indonesia].

Aggarwal, B.B., Kumar, A., Aggarwal, M.S. and Shishodia, S. (2004). Cucurmin derived from turmeric (*Cucurma longa*): a Spice for All Seasons. In Bagchi, D. and Preuss, H.G. (Eds.). Phytopharmaceuticals in Cancer Chemoprevention. 1st ed. New York, USA: CRC Press.

Burg, J.S. and Espenshade, P.J. (2011). Regulation of HMG-CoA reductase in mammals and yeast. *Progress in Lipid Research*, 50(4), 403–410. https://doi.org/10.1016/j.plipres.2011.07.002

Damanik, F.S. and Ikawati, Z. (2015). Aktivitas Kombinasi Ekstrak Temulawak (*Curcuma xanthorrhiza* Roxb.) dan Sambung Nyawa (*Gynura procumbens* (Lour) Merr.) Terkuantifikasi dalam Penurunan Kadar LDL dan Trigliserida pada Tikus Wistar Jantan. Indonesia: Universitas Gadjah Mada, Unpublished. [In Bahasa Indonesia].

Dechakhamphu, A. and Wongchum, N. (2015). Screening for anti-pancreatic lipase properties of 28 traditional Thai medicinal herbs. *Asian Pacific Journal of Tropical Biomedicine*, 5(12), 1042–1045. https://doi.org/10.1016/j.apjtb.2015.09.012.

Filippatos, T.D., Derdemezis, C.S., Gazi, I.F., Nakou, E.S., Mikhailidis, D.P. and Elisaf, M.S. (2008). Orlistat-Associated Adverse Effects and Drug Interactions. *Drug-Safety*, 31, 53–65. https://doi.org/10.2165/00002018-200831010-00005.

Hadváry, P., Lengsfeld, H. and Wolfer, H. (1988). Inhibition of pancreatic lipase *in vitro* by the covalent inhibitor tetrahydrolipstatin. *Biochemical Journal*, 256(2), 357–361. https://doi.org/10.1042/bj2560357.

Harikumar, K., Althaf, S.A., Kumar, B.K., Ramunaik, M. and Suvarna, C. (2013). A Review on Hyperlipidemic. *International Journal of Novel Trends in Pharmaceutical Sciences*, 3, 59-71.

Hidayat, M., Soeng, S. and Prahastuti, S. (2014). Pengujian Aktivitas Inhibitor Lipase Ekstrak Etanol dan Hasil Fraksionasi dari Kedelai Detam 1 dan Daun Jati Belanda. *Chimica et Natura Acta*, 2, 76 – 82. https://doi.org/10.24198/cna.v2.n1.9146. [In Bahasa Indonesia].

Joshita, D., Azizahwati. and Wahyuditomo. (2000). Pengaruh daun jati belanda terhadap kerja enzim

lipase secara in vitro. *Warta Tumbuhan Obat Indonesia*, 6(2), 16-22. [In Bahasa Indonesia].

Kertia, N. and Sudarsono. (2005). Prospek Manfaat Rimpang Temulawak Bagi Kesehatan. *Majalah Obat Tradisional*, 10(34), 5–8. [In Bahasa Indonesia].

Lachenmeier, D.W., Monakhova, Y.B., Kuballa, T., Löbell-Behrends, S., Maixner, S., Kohl-Himmelseher, M., Waldner, A. and Steffen, C. (2012). NMR evaluation of total statin content and HMG-CoA reductase inhibition in red yeast rice (*Monascus spp*.) food supplements. *Chinese Medicine*, 7(8), 1-7. https://doi.org/10.1186/1749-8546-7-8

Last, A.R., Ference, J.D. and Falleroni, J. (2011). Pharmacologic Treatment of Hyperlipidemia. *American Family Physician*, 84(5), 551–558.

Lewis, D.R. and Liu, D.J. (2012). Direct Measurement of Lipase Inhibition by Orlistat Using a Dissolution Linked In Vitro Assay. *Clinical Pharmacology and Biopharmaceutics*, 1(3), 1–11. https://doi.org/10.4172/2167-065X.1000103.

Luthfia, E, and Ikawati, Z. (2015). Pengaruh Kombinasi Ekstrak Temulawak (*Curcuma Xanthorrhiza Roxb*.) dan Ekstrak Sambung Nyawa (*Gynura procumbens* (Lour.) Merr) Terhadap Kadar Kolesterol Total Tikus Wistar Jantan Yang Diinduksi Diet Lemak Tinggi. Indonesia: Universitas Gadjah Mada, Unpublished. [In Bahasa Indonesia].

McDougall, G.J., Kulkarni, N.N. and Stewart, D. (2009). Berry polyphenols inhibit pancreatic lipase activity in vitro. *Food Chemistry,* 115(1), 193–199. https://doi.org/10.1016/j.foodchem.2008.11.093.

Metwally, M.A.A., El-Gellal, A.M. and El-Sawaisi, S.M. (2009). Effects of Silymarin on Lipid Metabolism in Rats. *World Applied Sciences Journal*, 6, 1634–1637.

Ministry of Health Indonesia. (2018). Hasil utama Riskesdas 2018 Kementerian Kesehatan Badan Penelitian dan Pengembangan Kesehatan. Indonesia: Balitbangkes. [In Bahasa Indonesia].

Niaga Swadaya. (2010). Trubus Info Kit Herbal Indonesia Berkhasiat: Bukti Ilmiah dan Cara Racik. 2nd ed. Jakarta: Trubus Swadaya. [In Bahasa Indonesia].

Onakpoya, I.J., O'Sullivan, J. and Heneghan, C.J. (2015). The effect of cactus pear (*Opuntia ficus-indic*a) on body weight and cardiovascular risk factors: A systematic review and meta-analysis of randomized clinical trials. *Nutrition*, 31(5), 640–646. https://doi.org/10.1016/j.nut.2014.11.015

Ong, S.-L., Paneerchelvan, S., Lai, H.-Y. and Rao, N.K. (2014). *In Vitro* Lipase Inhibitory Effect of Thirty Two Selected Plants in Malaysia. *Asian Journal of Pharmaceutical and Clinical Research*, 7(Suppl. 2), 19–24.

Padilla-Camberos, E., Flores-Fernandez, J.M., Fernandez -Flores, O., Gutierrez-Mercado, Y., Carmona-de la Luz, J., Sandoval-Salas, F., Mendez-Carreto, C. and Allen, K. (2015). Hypocholesterolemic Effect and In Vitro Pancreatic Lipase Inhibitory Activity of an *Opuntia ficus-indica* Extract. *BioMed Research International,* 2015, 837452. https://doi.org/10.1155/2015/837452.

Pebrianty, Y. (2013). Aktivitas Sari Mesokarp Pepino (*Solanum muricatum*) dan Terong lalap Ungu (*Solanum Melongena*) Sebagai Inhibitor lipase Pankreas dan Potensinya Sebagai Minumam Kesehatan Penurun Kadar Lemak darah. Indonesia: Universitas Negeri Malang, Diploma Thesis. [In Bahasa Indonesia].

Peschel, D., Koerting, R. and Nass, N. (2007). Curcumin induces changes in expression of genes involved in cholesterol homeostasis. *The Journal of Nutritional Biochemistry*, 18(2), 113–119. https://doi.org/10.1016/j.jnutbio.2006.03.007.

Ros, E. (2000). Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. *Atherosclerosis*, 151 (2), 357–379. https://doi.org/10.1016/s0021-9150 (00)00456-1.

Setiawan, I.M. (2012). Uji Aktivitas Antidislipidemia Fraksi Air Ekstrak Etanolik Daun *Gynura procumbens* (*G. procumbens* (Lour) Merr) pada Tikus Jantan yang Diinduksi Diet Lemak Tinggi. Indonesia: Universitas Gadjah Mada. Thesis. [In Bahasa Indonesia].

Shin, J.-E., Han, M.J., Song, M.-C., Baek, N.-I. and Kim, D.-H. (2004). 5-Hydroxy-7-(4′-hydroxy-3′-methoxyphenyl)-1-phenyl-3-heptanone: A Pancreatic Lipase Inhibitor Isolated from Alpinia officinarum. *Biological and Pharmaceutical Bulletin*, 27(1), 138–140. https://doi.org/10.1248/bpb.27.138

Sreerama, Y.N., Takahashi, Y. and Yamaki, K. (2012). Phenolic Antioxidants in Some Vigna Species of Legumes and their Distinct Inhibitory Effects on α-Glucosidase and Pancreatic Lipase Activities. *Journal of Food Science*, 77(9), C927–C933. https://doi.org/10.1111/j.1750-3841.2012.02848.x.

Svendsen, A. (2000). Lipase protein engineering. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, Protein Engineering of Enzymes*, 1543(2), 223–238. https://doi.org/10.1016/S0167-4838(00)00239-9.

Tiss, A., Miled, N., Verger, R., Gargouri, Y. and Abousalham, A. (2004). Digestive Lipases Inhibition: an *In vitro* Study. In Muller, G. and Perry, S. (Eds.) Lipases and Phospholipases in Drug Development. United Kingdom: Wiley Online Library.

Varras, J. (2008). Managing Hyperlipidemia: An Evidence-Based Approach. *Journal of Managed Care Medicine*, 11, 17–22.

Yasni, S., Imaizumi, K., Nakamura, M., Aimoto, J. and Sugano, M. (1993). Effects of *Curcuma xanthorrhiza* Roxb. and curcuminoids on the level of serum and liver lipids, serum apolipoprotein A-I and lipogenic enzymes in rats. *Food and Chemical Toxicology*, 31 (3), 213–218. https://doi.org/10.1016/0278-6915(93) 90096-H.

Yoshikawa, M., Shimoda, H., Nishida, N., Takada, M. and Matsuda, H. (2002). Salacia reticulata and Its Polyphenolic Constituents with Lipase Inhibitory and Lipolytic Activities Have Mild Antiobesity Effects in Rats. *The Journal of Nutrition*, 132(7), 1819–1824. https://doi.org/10.1093/jn/132.7.1819.

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