Antidyslipidemia activity of Ethanol, Methanol and Ethyl acetate extract of Zingiber montanum rhizome

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ABSTRACT:
Excessive food consumption concerning to high calorie, dyslipidemia, obesity and cardiovascular disease. One alternative therapeutic approach in dyslipidemia patients is traditional use of herbal medicines such as Zingiber montanum rhizome. The aim of this study is to compare the anti-dyslipidemia activity of ethanol (EEZR), methanol (MEZR) and ethyl acetate extract of Zingiber montanum rhizome (EAEZR). EEZR, MEZR, and EAEZR were made by maceration method. The solvent extract was evaporated to obtain a viscous extract. Then a phytochemical screening for the compounds contained in each extract was performed. To induce dyslipidemia, Wistar male rats were given standard feed (80%), duck egg yolk (5%) and lard (15%) for 30 days, followed by administration of 1500 mg EEZR, MEZR and EAEZR dose for 30 days. On the 60th day, total cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL) blood measurements of the test rat were performed. Phytochemical screening result showed that EEZR, MEZR, and EAEZR contained flavonoid and terpenoid compounds. EEZR, MEZR, and EAEZR could decrease TC, TG and increased HDL in mouse blood significantly compared with dyslipidemia group (p <0.05). The TC and TG percentage decrease and the highest HDL level were owned by ethyl acetate extract of Zingiber montanum rhizome. EEZR, MEZR and EAEZR could decrease TC, TG levels and increase HDL in rat blood. The ability to decrease TC and TG levels from Zingiber montanum rhizome extract could be caused by the EEZR ability to inhibit pancreatic lipase enzyme activity so that it could suppress the fat absorption from the rats’ small intestine. The EAEZR ability was best amongst other because the active compound contained in non-polar Zingiber montanum rhizome is 2-methoxy-8-(3,4-dimethoxyphenyl)-1,4-naphthoquinone. EEZR, MEZR, and EAEZR have the ability to anti-dyslipidemia, where the best activity was owned by EAEZR.

KEYWORDS: EEZR, MEZR, EAEZR, Zingiber montanum rhizome, anti-dyslipidemia.

INTRODUCTION:
Dyslipidemia is a type of degenerative disease characterized by the increase of TC, TG levels and the decrease of HDL levels in the blood. Dyslipidemia that is not handled correctly will lead to other diseases such as diabetes, atherosclerosis, hypertension, and others. Unhealthy lifestyle became one of the trigger factors of the dyslipidemia occurrence. The dyslipidemia incidence keeps increasing in number1,2. Zingiber montanum is one of the nutritive rhizomes that are commonly used as carminative, anti-inflammatory, asthma, antioxidants and to overcome muscle pain. The Zingiber montanum rhizome ethanol extract is capable of inhibiting pancreatic lipase enzyme so that it can impede the lipid absorption in the small intestine3. In the Zingiber montanum rhizome, there are phenol, monoterpenes, sesquiterpenes, cyclohexane, curcuminoids, cassumunar A, B, C, naphthoquinone derivatives and phenylbutazone derivatives. The active compound contained in the Zingiber montanum rhizome is 2-methoxy-8-(3,4-dimethoxyphenyl)-1,4-naphthoquinone4.
Dyslipidemia can be treated with herbs. One plant that can be used to overcome dyslipidemia is the *Zingiber montanum* rhizome. *Zingiber montanum* rhizome contains efficacious compounds to overcome obesity. The solvent choice for extraction will affect the type of compound that can be extracted; therefore *Zingiber montanum* rhizome extraction is performed by using ethanol, methanol and ethyl acetate solvent. To determine the content of the compounds in each extract, phytochemical screening of each extract was performed, and its ability as an antidyslipidemic is being compared.

**MATERIAL AND METHODS:**

**Material:**

*Zingiber montanum* rhizome was collected during July 2012 from Ubud, Gianyar, Bali, Indonesia. Determination of *Zingiber montanum* rhizome was done at LIPI Kebun Raya Bedugul, Bali (855/IPH.UPT.04/AP/XII/2012). Cholesterol assay kits, triglyceride assay kits, HDL precipitant kits (Biovision Inc., Sanfransisco USA), boric acid, and citrat acid were purchased from PT. Kurniajaya Sentosa; 96% ethanol, methanol and ethyl acetate solvent were purchased from PT. Bratacho.

*Zingiber montanum* rhizome powder extraction:

*Zingiber montanum* rhizomes were cleaned, sliced thinly and then was dried. The dried *Zingiber montanum* rhizome was chopped, and then powdered. *Zingiber montanum* rhizome powder was macerated using ethanol, methanol and ethyl acetate solvent. Re-macerations were done twice; solvent was evaporated by using the rotary evaporator to get a thick extract of *Zingiber montanum* rhizome.

*Zingiber montanum* rhizome extracts phytochemical screening:

Each extract was spotted on GF254 TLC plate and then eluted with toluene: chloroform (1: 9). The TLC results were observed in visible light, UV 254 and UV 366. The plate was then sprayed with Liebermann-Burchard and sitroborat reagents. The terpenoid compounds presence would have purple appearance after being sprayed with LB reagents. The flavonoid compounds presence would have light blue appearance under UV 366 after being sprayed with a sitroborat reagent.

Preparation of test rat:

Wistar white rats weighing 120 ± 20 grams were given standard feed and drinking water. Male rats Wistar rats had an ethical clearance certificate (0135/KE-PH/V/2013) that was issued by Udayana University's Veterinary Faculty.

**Anti-dyslipidemia activity test of Zingiber montanum rhizome extract:**

Wistar male rats were acclimated for seven days, and they were grouped as followed:

a. Normal control group, rats were fed with standard feed and water
b. The negative control group, rats were fed with high-fat diet (80% standard feed, 15% pork oil, 5% duck egg yolk) for 30 days
c. The positive control group, rats were fed with high-fat diet (80% standard feed, 15% pork oil, 5% duck egg yolk) for 30 days then continued with simvastatin 7.2 mg/kg BB for another 30 days
d. EEZR group, rats were fed with high-fat diet (80% standard feed, 15% pork oil, 5% duck egg yolk) for 30 days then continued with EEZR 1500 mg/kg BW for another 30 days
e. MEZR group, rats were fed with high-fat diet (80% standard feed, pork oil 15%, 5% duck egg yolk) for 30 days then continued with MEZR 1500 mg/kg BW for another 30 days
f. EAEZR group, rats were fed with high-fat diet (80% standard feed, 15% pork oil, 5% duck egg yolk) for 30 days then continued with the EAEZR 1500 mg/kg BW for another 30 days

TC, TG and HDL levels measurements in the rats’ blood were performed on the 30th day after high-fat diet and the 60th day after the end of treatment.

**Lipid levels Measurements in rats’ blood:**

Rats’ blood was taken through the orbital sinus veins, and serum separation from whole blood was performed. TC, TG, and HDL level were then measured by using samples of rats’ blood serum. TC, TG and HDL levels measurement was done by using the spectrophotometric method.

**Analysis results:**

To know the existence of a significant difference between a normal group, negative control, and treatment with *Zingiber montanum* rhizome extract, tested with one way ANOVA statistic followed by LSD test. If p <0.05 then there is a significant difference between groups.

**RESULTS AND DISCUSSION:**

EEZR, MEZR, and EAEZR which contained flavonoid and terpenoid compounds were shown in the identification test by TLC and sprayed recording. EAEZR that was sprayed with LB reagents showed a more definite blue spot than EEZR and MEZR under UV light 254 and 366 observations (Figure 1). EAEZR extract that was sprayed with sitroborat was observed under UV light 254 and 366. It showed more evident purple spot than EEZR and MEZR (Figure 2).
Solvent polarity sequence from lowest to highest is ethyl acetate, ethanol, methanol. The difference of solvent polarity used for Zingiber montanum rhizome extraction causes variations in the extracted compound. The compounds that are obtained with ethanol, methanol and ethyl acetate solvents are usually identical with terpenoids and flavonoids. But in the extraction with ethyl acetate solvents, terpenoid and flavonoid compounds gave noticeable color intensity from the TLC identification result (Fig. 1 and 2). The active compounds contained in the Zingiber montanum rhizome are 2-methoxy-8- (3,4-dimethoxyphenyl) -1,4 naphtoquinone (Hartati et al., 2013) while the identity compounds contained in the Zingiber montanum rhizome is terpinen-4-ol⁸. The solvent used to extract the compound is a non-polar solvent. Thus it is more likely that the compound is derived by using ethyl acetate solvent.

TC levels in rats’ blood showed that there was an increase after being fed with high-fat diet for 30 days. The statistic test result showed that there was a significant difference of TC level in normal group blood and treatment group (negative control, simvastatin, EEZR, MEZR and EAEZR) where \( p < 0.05 \). By giving high-fat diet for 30 days, TC levels in rat blood could increase. EEZR, MEZR, EAEZR, and simvastatin were given for another 30 days, and then TC level in rats’ blood was measured. Decreased TC levels occurred in the treatment group (rats that were given simvastatin, EEZR, MEZR and EAEZR). There was a significant difference between TC level of rats’ blood in the treatment group and negative group.

TG levels in rats’ blood showed that there was an increase in a high-fat diet for 30 days. The statistic test result showed that there were significant TG levels differences in normal group and treatment group (negative control, simvastatin, EEZR, MEZR and EAEZR) where \( p < 0.05 \). High-fat diet administration for 30 days could increase TG levels in rats’ blood. Zingiber montanum rhizome extract was given for another 30 days then TG level in rats’ blood was measured. Decreased TG levels occurred in the treatment group (rats that were given simvastatin, EEZR, MEZR and EAEZR). There was a significant difference between TG levels of the treatment group (simvastatin, EEZR, MEZR, and EAEZR) compared to the negative group. Only TG levels in rats that were given EEZR did not differ significantly with negative controls.
HDL levels in rats’ blood showed that there was a decrease after high-fat diet administration for 30 days. The statistic test showed that there were significant differences in HDL levels in normal group and treatment (negative control, simvastatin and Zingiber montanum rhizome extract) where p < 0.05. High-fat diet administration for 30 days could reduce HDL level in rats’ blood. Zingiber montanum rhizome extract was done for another 30 days, and then HDL level in rats’ blood was measured. Increased HDL levels occurred in the treatment group (rats that were given simvastatin, EEZR, MEZR, EAEZR). There was a significant difference between HDL level of rats’ blood in treatment group and negative group.

The lipid levels difference in rats’ blood was calculated by reducing lipid levels after administration of Zingiber montanum rhizome extract to lipid levels before administration of Zingiber montanum rhizome extract (Table 1). The difference in rats’ blood lipid levels (TG and HDL) that were given EAEZR showed the greatest results. As for the difference of TC level between rats that were given EAEZR and EEZR, they showed almost the same results.

**Figure 4. Triglyceride levels in rats’ blood, a = significant difference to normal group (p < 0.05), b = significant difference to negative group (p <0.05), pre = TG level of rats’ blood after high-fat diet administration for 30 days, post = TG level of rats’ blood after 30 days treatment**

<table>
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<tr>
<th>Groups</th>
<th>KT</th>
<th>TG</th>
<th>HDL</th>
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<tr>
<td>EEZR</td>
<td>70.31</td>
<td>24.79</td>
<td>29.60</td>
</tr>
<tr>
<td>MEZR</td>
<td>45.55</td>
<td>76.14</td>
<td>24.06</td>
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<tr>
<td>EAEZR</td>
<td>69.60</td>
<td>117.74</td>
<td>40.64</td>
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</table>

The measurement results of TC, TG and HDL levels showed that Zingiber montanum rhizome extract could improve lipid profile which can decrease TC and TG levels and could increase HDL level in blood. The difference in rats’ blood lipid levels (TG and HDL) that were given EAEZR showed the highest results. This might be due to the active chemical content that was able to be extracted by the ethyl acetate solvent and made the ability to decrease TG levels and increase HDL levels was highest compared to the ethanol and methanol extract of Zingiber montanum rhizome. The ethanol extract of the Zingiber montanum rhizome is capable of inhibiting pancreatic lipase enzyme so that it can impede lipid absorption in the small intestine. The ability to inhibit the action of pancreatic lipase enzyme will impede the absorption of TG in the gut. Inhibition of pancreatic lipase enzyme is one way to overcome obesity. The tannins content in the Araucaria angustifolia extract is responsible for the ability to inhibit pancreatic lipase enzyme. Some plant extracts were rich in polyphenol, saponin and terpene contents are believed to have a role in the fat digestion inhibition. Gingko biloba extract which contained triterpenoid compound had a hypolipidemic effect by inhibiting pancreatic lipase enzyme. Flavonoid compounds that were contained in Armoracia rusticana root and left extracts were also able to inhibit pancreatic lipase enzymes. The ability of ethanol, methanol and ethyl acetate extract from Zingiber montanum rhizome in lowering total cholesterol, triglycerides and LDL were possibly caused by the terpenoid and flavonoid compounds.

**CONFLICT OF INTEREST:**

The authors declare no conflict of interest.
REFERENCES: