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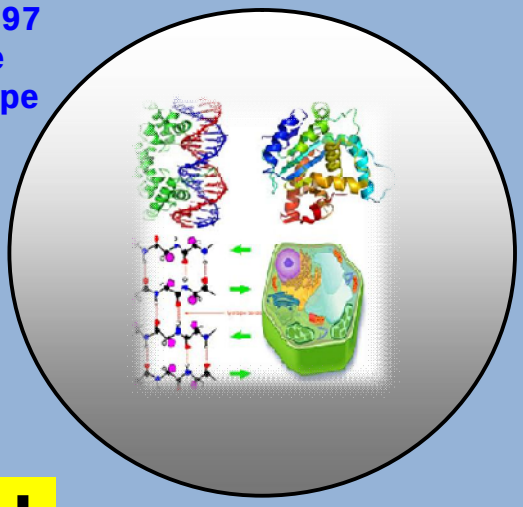

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RESEARCH PAPER

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Antioxidant Compounds of Gayam Seed (*Inocarpus fagiferus* Fosb) to Prevent Atherosclerosis through Increases of SOD Activity and Improvement of Lipid Profile on Wistar Rat

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This study aims to prove the potency of ethanol extract of gayam seed to prevent atherosclerosis through increase of SOD activity and improvement of lipid profile plasma blood of Wistar rat for 4 months. This was experimental study with randomized posttest only control group design. The samples were 25 Wistar rat, randomized into 5 groups: group K_1 (negative control), group K_2 (positive control, feed high fat diet), group P_1(high fat diet + ethanol extract dose 50 mg/kgbw), group P_2 (high fat diet + ethanol extract dose 100 mg/kgbw), and group P_3 (high fat diet + ethanol extract dose 150 mg/kgbw). This paper also describes the determination of the antioxidant activity and identification of the active compounds using gas chromatography-mass spectrometry (GCMS) analysis.

From the results it is evident that ethanol extracts in varied doses decrease the level of lipidsof total cholesterol, triglyceride, and LDL cholesterol, but do not affect the level of HDL cholesterol. They also increase SOD activity in blood plasm of Wistar rat. Thus the extract potential to prevent atherosclerosis disease. The ethanol extract which active as an antioxidant with IC_{50}= 280 ppm with contains thirteen compounds, i.e., one flavonoid compound, eight fatty acid and fatty acid ester compounds, and four unknown compounds according to Wiley 229 Library.

Keywords: Inocarpus fagiferus Fosb, Antioxidants, Fatty acid and Atherosclerosis.

INTRODUCTION

Screening of bioactive compounds from plant can be conducted with the phytopharmacologic approaches and phytochemical screening approaches. One of the phytopharmacologic approaches relies on etnobotany, i.e., screening bioactive compound of plant based on its use as traditional medicine by certain society (Farnsworth, 1996). Traditional medicine represents one of Indonesia's cultural assets and has been empirically proven from generation to generation (Kardinan and Taryono, 2003).

A plant which can be exploited as traditional medicine is gayam (*Inocarpus fagiferus* Fosb). The seed of this plant is applicable to heal the atherosclerosis and ischemic heart disease and potential as antioxidant agent based on empirical studies in Fiji Island (Sotheeswaran and Sharif, 1994; Segatri, 1995). This is possibly because of the chemical compounds content of the gayam seed in Fiji, especially those of secondary metabolic compounds such as phytosterol, diacylgliserol, α-tocopherol, linolenic acid, and linoleic acid as the major compounds (Sotheeswaran and Sharif, 1994). Nevertheless there is no research explaining the correlation between the compounds in the seed with the traditional healing, although many people have proven the effect and benefit of gayam seed, let alone scientific evidences of gayam seed in Bali. Only the statement of Agestia and Sugrani (2009) which explains that there is correlation between the structure of fatty acid or flavonoid and their possibility as antioxidant activity. Certain fatty acids like PUFA (polyunsaturated fatty Acids) have antioxidant activity as exogen antioxidant which can stimulate endogen antioxidant such as SOD (Superoxide Dismustase).

SOD is a catalysis reaction to convert anion superoxide (O_2^-) to hydrogen peroxide and oxygen that are not reactive. Expression and activity of SOD effect and response at vascular cells with oxidative stress (Chauhan *et al*., 2003; Faraci, 2003; Gunnett *et al*., 2003).Therefore, activity of SOD can be used as a biomarker of atherosclerosis disease (Didion *et al*., 2002). The other SOD activity, the level of lipid profile such as total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol in serum or plasma can also is used to determine damages of blood vessel like in atherosclerosis (Budiana, 2008). So that both SOD and levels of lipid profile a biomarkers to investigate the atherosclerosis disease, and the extract of gayam seed is expected to contain antioxidant compounds (Sukadana, 2012).

Therefore it is crucial to investigate the chemical contents of the gayam seed and their bio-activity as an antioxidant agent also to investigate whether ethanol extract of gayam seed can prevent atherosclerosis through increases SOD activity and improvement of lipid profile. In this paper the extraction process of the identification antioxidant compounds from the seed and the determination of secondary metabolite. The capacity antioxidant toward DPPH and its effect of atherosclerosis through measurement of SOD and level of lipid profileare described.

MATERIAL AND METHODS

Materials used in this research are: gayam (*Inocarpus fagiferus* Fosb) seed obtained from Tabanan Bali and taxonomically identified by LIPI's Kebun Raya "Eka Karya" Bali. Chemicals used are ethanol (p.a and technical), aquadest, DPPH (diphenylphicrylhydrazil), and phytochemical reagent.

Animal used is Wistar rat and with ethical clearance No. 0144/KE-PH/IX/2013 dated: 4 September 2013. Chemicals that used to analysis lipid profile were provided by UPT. Balai Laboratorium Kesehatan Provinsi Bali. Other material were Whatman No.1, ketamine and xylazine for anesthesia, blood plasm, standard feed BR1 CP511B, high fat diet feed consist of mixture BR1 CP511B and other materials with high cholesterol.

Equipments

Equipments used include a set of glass wares, extractor, analytical balance, blender, knife, rotary vacuum evaporator, desicators, and test tubes, testing dishes, volumetric pipettes, rat cage, syringe, micro haematocrit tubes (Cat. 7493 21), EDTA Eppendorf tubes, UV-vis spectrophotometer, and gas chromatographs-mass spectrometers (GCMS).

Procedure

Extraction of compounds from gayam seed (*Inocarpus fagiferus* Fosb).

About 5.85 kg dried powder of gayam (*Inocarpus fagiferus* Fosb) seed was macerated using 21.5 L ethanol 96% (Et OH). Maceration process was conducted 5 times at 24 hours each. The ethanol extract was evaporated using rotary vacuum evaporator to obtain a concentrated Et OH extract. It was tested for antioxidant activity with the DPPH test and it was compared with Vitamin E. Furthermore the concentrated Et OH extract was analyzed using Gas Chromatographs-Mass Spectrometers (GCMS) to determine its components. These Et OH extract was then was applied to animal model on Wistar rat 20 g/rat/days to prove the potency of concentrated Et OH extract of gayam seed to prevent atherosclerosis through increase of SOD activity and improvement of lipid profile using to randomized posttest only control group design with 5 groups.

Group K_1: negative control (rat group with feed standard)
Group K_2: positive control (rat group with feed high fat diet)
Group P_1: rat group with feed high fat diet + ethanol extract dose 50 mg/kg bw
Group P_2: rat group with feed high fat diet + ethanol extract dose 100 mg/kg bw
Group P_3: rat group with feed high fat diet + ethanol extract dose 150 mg/kg bw

The experiment was conducted for four months then the blood of each rat groups were analyzed for their SOD and lipid profile as total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol. These data were analyzed using one way Anova (SPSS version 19.0 for Windows).

RESULTS AND DISCUSSION

Antioxidant activity of ethanol extract from gayam seed (*Inocarpus fagiferus* Fosb).

The result of maceration of about 5.85 kg dried powder of gayam (*Inocarpus fagiferus* Fosb) seed using 21.5 L ethanol was about 87.272 g brown concentrated ethanol extract.

Antioxidant activity tests using DPPH showed that there was the ethanol extract was more active than the vitamin E (as antioxidant standard (see Figure 1). The value of IC_{50}which was less than IC_{50} =1000 means that the extract is potent active as antioxidant.

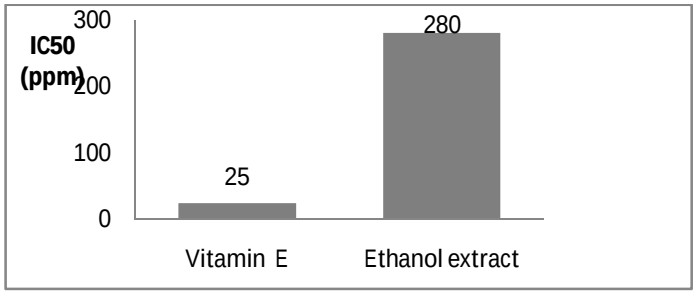

Figure 1. Antioxidant activity of ethanol extract.

Lipid Profile and SOD Activity of Wistar Rat Plasma

In the lipid profile of the rat plasma for 4th month experiment shown in Figure 2.The lipid profile and the one way Anova analysis show that all of the variable have normal distribution and homogenity ($p>0.05$) except SOD activity ($p<0.05$). The highest level of total cholesterol in shown by rat groups with high fat diet (K_2) with significant difference ($p<0.05$) if compared with other rat groups. Mean of the total cholesterol level for $K_1$is 50.00 ± 4.42 mg/dl, $K_2$is 82.60 ± 5.73 mg/dl, $P_1$is 66.20 ± 7.29 mg/dl, $P_2$is 70.80 ± 8.11 mg/dl, and P_3 is 70.40 ± 6.87 mg/dl.

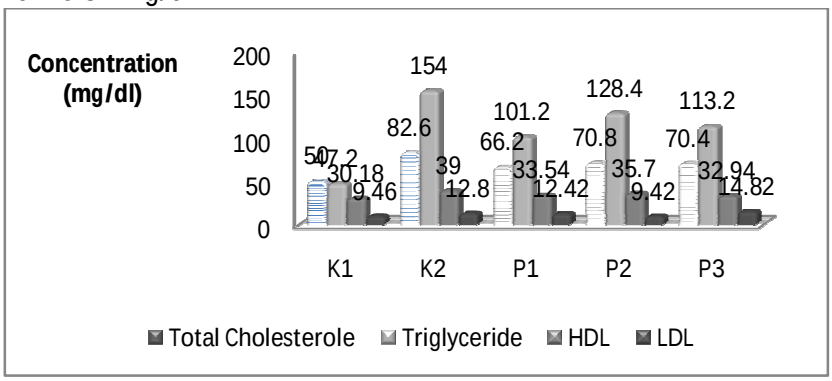

Figure 2. Lipid profile of Wistar rat blood plasma.

Significant decrease of total cholesterol level occured after the rat groups were given high fat diet and ethanol extract of gayam seed of 50 mg/kg bw (P_1, $p<0.05$). higher doses like in groups P_2 and P_3do not give significant difference in levels of total cholesterol compared to K_2 ($p<0.05$) but its level increase compared to P_1, while the level of total cholesterol $P_2$is not significantly different compared with P_3 ($p>0.05$). This is because by the compound components in gayam seed like saturated fatty acid in higher dose will affect the level of total cholesterol.The level of triglyceride decreases very significantly ($p<0.05$) after rat with high fat diet were given ethanol extract dose of 50 mg/kg bw (P_1, mean: 101.2 ± 7.623 mg/dl)) compared to K_2 (mean. $154,0 \pm 37.236$ mg/dl), while higher extract doses like in treatment group P_2 and P_3do not increase the level of triglyceride significantlly ($p>0.05$), but if the two groups were compared (P_2; mean. 128.4 ± 32.447 mg.dl and P_3; mean. 113.2 ± 33.094 mg/dl), they are not significantlly different ($p>0.05$).

Theoritically, level of HDL cholesterol should increases if antioxidant agent was given in higher dose, but the result of these experiment show that the level of HDL cholesterol tends to unchange (their means are not significantly different, $p>0.05$). The mean of HDL cholesterol for all of rat groups, K_1, K_2, P_1, P_2, and $P_3$are 30.18 ± 2.57 mg/dl, 39.00 ± 5.41 mg/dl, 33.54 ± 2.39 mg/dl, 35.70 ± 3.84 mg/dl and 32.94 ± 4.37 mg/dl respectively. The result of multiple comparisons test show that K_2 vs K_1 ($p<0.05$), P_1 vs K_2 ($p<0.05$), P_2 vs K_2 ($p>0.05$), P_3 vs K_2 ($p<0.05$), P_2 vs P_1 ($p>0.05$), P_3 vs P_1 ($p>0.05$), and P_3 vs P_2 ($p>0.05$). Mixture of many compounds that were contained in the ethanol extract of gayam seed possibly causes these contradiction.

LDL cholesterol is a bad cholesterol. Figure 2. shows that mean level of LDL cholesterol in all rat groups, K_1, K_2, P_1, P_2, and $P_3$are 9.46 ± 4.593 mg/dl, 12.68 ± 7.203 mg/dl, 9.42 ± 6.062 mg/dl, and 14.82 ± 7.626 mg/dl respectively. It means that all of them are not significantly different ($p>0.05$).

The result of analysis of SOD activity shows that concentrated ethanol extract in varied doses can increase activity of antioxidant endogen SOD. This means the compounds in ethanol extract can be expected to prevent atherosclerosis disease. In the normal rat (K_1) the percentage of rate inhibition ($48.746 \pm 5.303\%$) higher than the percentage of rate inhibition of high fat diet rat group (K_2), 17.254 ± 4.373 % ($p<0.05$). In hypercholesterolemia rat group (K_2) plak atherosclerosis occurs that decreasesthe SOD activity. Futhermore, if to hypercholesterolemia condition rat extract was given in varied doses the compounds in the extract will increase the endogen SOD activity as shown in Figure 3.

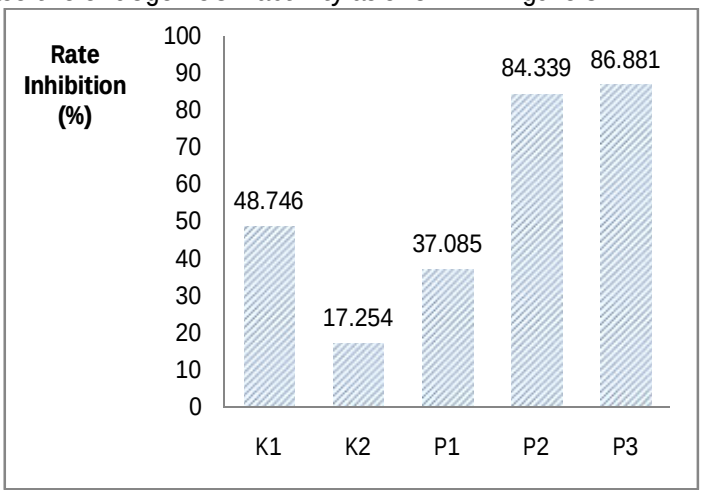

Figure 3. SOD activity of Wistar rat blood plasma.

In treatment group P_1, where rat with high fat diet a given ethanol extract at 50 mg/kg bw, there is increase in percentage of rate inhibition significantlly ($p<0.05$) compared to K_2, and when rats are given higher doses like in P_2, it drastically increase ($p<0.05$). Nevertheless, if rats were given ethanol extract with dose of 150 mg/kg bw (P_3) higher than that of P_2, percentage of rate inhibition is not significantly different ($p>0.05$), possibility because the compounds in gayam seed do not act as antioxidants.

Identification of ethanol extract

The result of phytochemical test indicates that the ethanol extract contains triterpenoids, steroids, flavonoids and phenolate compounds. Chromatograph of GCMS analysis indicates that the ethanol extract contains thirteen components (Figure 4). The WILEY229.LIB data base approach suggests that the extract contains one flavonoid compound (homopterocarpine, Rt. 28.042), eight fatty acids and fatty acid ester compounds (palmitic acid, Rt. 23.157; ethyl palmitic, Rt. 23.488; linoleic acid, Rt. 24.831; ethyl linoleic, Rt. 25.120; ethyl oleic, Rt. 25.181; ethyl stearic, Rt. 25.426; ethyl eicosanoic, Rt. 27.212;and ethyl docosanoic, Rt. 28.877) and four unknown compounds with retention times of23.319, 24.892, 25.317, and 30.647 minutes (see Figure 4).

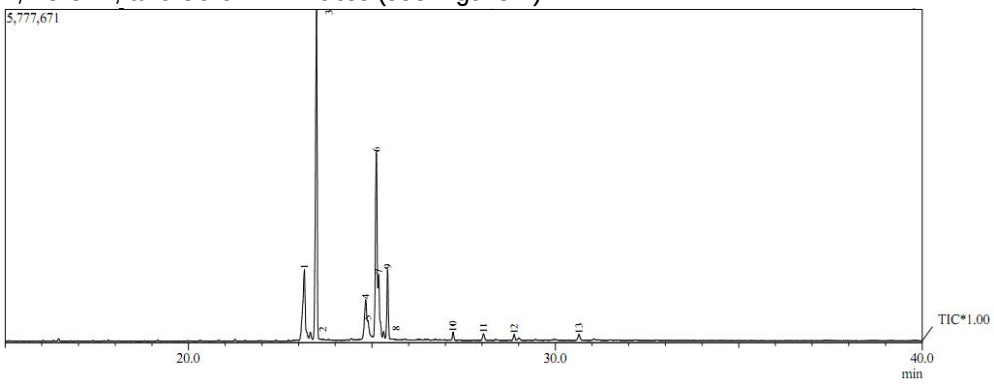

Figure 4. Chromatograph of ethanol extract.

The structures of all compounds are shown in Figure 5, while the unknown compounds are not shown.

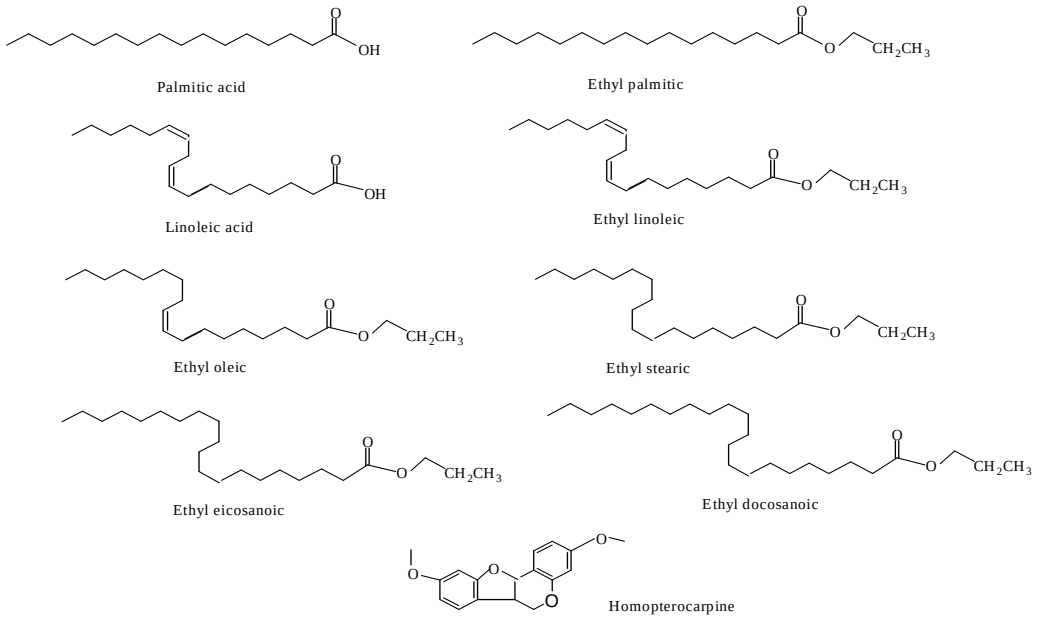

Figure 5.The structures of the compounds in the ethanol extract.

CONCLUSIONS

Conclusions of these researches are:

1. The ethanol extracts in varied doses decrease the levels of lipid profile i.e., total cholesterol, triglyceride, and LDL cholesterol, except the HDL cholesterol.
2. Concentrated ethanol extract increases SOD activity in blood plasma of Wistar rat so it is potential to prevent atherosclerosis disease.
3. The ethanol extract is identified as antioxidant compounds with IC_{50} = 280 ppm. It contains thirteen components; one flavonoids compound (homopterocarpine), eight fatty acids and fatty acid ester compounds (palmitic acid, ethyl palmitic, linoleic acid, ethyl linoleic ethyl oleic, ethyl stearic, ethyleicosanoic and ethyl docosanoic) and four unknown compounds.

Further research

In vivo experiment of ethanol extract to obtain its potential as anti atherosclerosis with relevant variables including ICAM-1, VCAM-1, SOD-2, SOD-3, MDA, etc.

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