Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.3, No.13, 2013

Hepatoprotective Activity Combination Between *Morinda Citrifolia* Linn (Mengkudu) Extract And Virgin Coconut Oil (Vco)

Rudi Alexander Repi[1], Mokosuli Yermia Semuel[2], Jantje Ngangi[2] and Harry Maurits Sumampouw[3]
[1]Manado State University Institute for Research
[2]Laboratory of Medicinal and Molecular Biology, faculty of mathematics and natural sciences, Manado State University
[3]Biology Department, faculty of mathematics and natural sciences, Manado State University
* e - mail : yere_bikipb@yahoo.co.id

ABSTRACT
This research aimed to determine phytochemical content combination between *Morinda Citrifolia* linn. extract and VCO, getting the best solution for their combining formulation and get the data of antioxidative and hepatoprotective activity in vitro and in vivo, the combination between VCO and *Morinda Citrifolia* Linn extract. Stages of the research consisted of phytochemical analysis with the combination between VCO and aqueous of *Morinda Citrifolia* linn extract , antioxidative activity analysis methods using DPPH and TBA and hepatoprotective in vivo test using wistar rats as test animals. The results showed that the phytochemical content of VCO combination with aqueous of *Morinda Citrifolia* Linn extract dominant are flavonoids, saponins and triterpenoids. Antioxidative activity: DPPH free radical capture with the combination of VCO and aqueous *Morinda Citrifolia* linn extract is very powerful with the effective concentration (EC50) 75.40 ppm compared with the positive control (BHT) the effective concentration is (EC50) 121.45 ppm. Based on the data-serum GPT activity and rat liver cell histology were found as well as the results of the analysis procedure is applied, it can be concluded that the combination of the VCO and the aqueous *Morinda Citrifolia* Linn extract has hepatoprotective characteristic with a dose range of 1.260 g / kg body weight up to 1,890 g / kg

Keywords: hepatoprotective, *Morinda Citrifolia* Linn+ VCO combination, antioxidants

1.INTRODUCTION

Consumption of coconut oil in a daily menu in reasonable amounts needed to maintain healthy heart and blood vessels. In contrast to western societies during this assumption that coconut oil is a saturated vegetable oil that can lead to heart and vascular disorders, a number of recent studies indicate, coconut oil actually able to protect health. 50 percent of the fatty acids in coconut oil is lauric acid, and seven percent are kapriat acid. Both are medium-chain saturated fatty acids (the number of carbon is 12) which is commonly called Medium Chain Fatty Acids (MCFA), easily metabolized and does not increase blood cholesterol levels. Kushwaha (2004) reported from his writing in vitro that dietary coconut oil gives good effects on cholesterol response.

Research Elisabeth (1997) concluded that the effect of increasing dietary concentrations of MCFA gives total cholesterol and LDL cholesterol were not significant and had no effect on HDL cholesterol compared with the oleic acid diet. On the other hand, a study conducted by Tholstrup (2004) 7 suggests that compared with oleic acid (LCFA), medium chain fatty triacyglycerol (MCT) gives an unfavorable effect of improving the plasma lipid profile of LDL cholesterol and triacylglycerol in the study subjects. There is no change in the activity of phospholipid transfer protein and cholesterol ester protein transfer discovery. VCO can lower serum and tissue lipid content as well as the oxidation of LDL (Nevin and Rajamohan, 2004).
North Sulawesi region known as "waving palm" because there were a lot of coconut tree in this area. Traditionally people make a lot of coconut cooking oil for its own consumption. On the other hand mengkudu plant (*Morinda Citrifolia* L.) grows well in this area. This plant has been used both as a traditional medicine such as cough medicine, asthma drugs, drug injury, worm medicine, relieving headaches, fatigue or as a mixture of food.

Mengkudu (*Morinda Citrifolia* Linn) has been widely used in traditional medicine. Reported that this plant has medicinal functions as diabetes, blood pressure, inflammatory, antiseptic, antibiotic, anthelmentik kidney stone crusher. (Dharma, 1985) and the rejuvenation of cells (terpenes), as a deterrent to the development of cancer cells, and antioxidants (free radical neutralizer) (Waspodo, 2000).
Mengkudu extract given at a dose of 10 g / day / kg bb has hepatoprotective effect it is characterized by decreased activity of the enzyme SGOT and SGPT in four treatments and significantly different when compared with the two treatments as well as histopathology seen the healing process and reduced liver tissue degeneration and necrosis (Suarsana and Budiasa, 2005).

Herbal medicine that originated in China, Korea and India are known and used almost all over the world because of efficacy against various diseases. The herbal medicines are generally a combination of botanicals herbs, so not only consist of one type of plant. Reductase activity and hepatoprotective stronger when combined various kinds

of herbs and this is due to the synergy effect of various bioactive than the use of pure compounds (Loh Suh In, 2007). Based on the setting in which it is necessary to study the combination of Mengkudu extracts and VCO as a hepatoprotective agent.

2. RESEARCH METHODS

Phase I research conducted in the Faculty of Biology Laboratorium UNIMA for 6 (six months) from May until November 2010. Tools and materials used include: Animal test used was a white male Wistar rats, uniform weights (± 150-180 g), aged 6-8 weeks. Obtained from laboratory test animals Pharmacology-Toxicology, Faculty of Pharmacy, University of Sanata Dharma, Yogyakarta. The tested material is a combination of the VCO and Mengkudu extract derived from North Minahasa. Material to form the model hepatotoxins paracetamol obtained from Kimia Farma Pharmacy Manado with 1% CMC carrier. Tool-kit to measure the activity of GPT GPT-serum, paraffin, formaldehyde 10%, xilol, alcohol, wax printing, dye haematoxylin-eosin (E. Merck, Darmstadt, Germany). Vitalab Micro (E. Merck, Darmstadt, Germany), an electric balance (Mettler Toledo, Model AB 204 made in Switzerland), microscope (Olympus, type BH.2, made in Japan), camera (Olympus, made in Japan), needle tuberculin, syringe injection, a set of surgical tools, glassware and tools infusion (list of equipment / materials complete in appendix). Stages of the research consisted of antioxidative activity assay using two methods: the method of reduction of free radical DPPH and TBA methods and hepatoprotective test using test animals mice. Research Procedure: 1. DPPH, ethanol extract samples prepared in various concentrations (10, 50, 100, 200 and 250 ppm). Each inserted into a test tube. Into each test tube was added 500 mL solution of 1 mM DPPH in methanol. The volume pressed until 5,0 ml and then incubated at 37 ° C for 30 min, and then absorbance was measured at a wavelength of 515 nm. Used as positive control BHT with concentrated adjusted. IC50 values calculated respectively by using the regression equation.

$$\% \text{ Inhibition} = \frac{[\text{Absorbance of control} - \text{Absrobansi sample}]}{[\text{Absrobansi control}]} \times 100\ \%$$

2 Tiobutirat acid method (TBA)

Ethanol extract of the sample was made in consentration 50 , 100 , 200 and 500 ppm . Each sample was taken as 1 mL and then dissolved in 2 mL of 0.1 M phosphate buffer pH 7.0 and 2 mL of 50 mM linolenic acid in ethanol 98.8 % . Positive control solution (control antioxidant) used α - tocopherol 1 mL , 2 mL of 0.1 M phosphate buffer pH 7.0 and 2 mL of 50 mM linolenic acid in ethanol 99.8 % . Negative control solution consisting of 1 mL of deionized water , 2 mL of 0.1 M phosphate buffer pH 7.0 and 2 mL of 50 mM linolenic acid in ethanol 98.8 % . All mixture placed in dark bottles threaded lid and incubated at 400C . One day after the maximum incubation time of Ferric thiocyanate method (FTC) conducted measurements of thiobarbituric Acid Reactive Substances (TBARS) by the method TBA (Kikuzaki & Nakatani , 1993) by taking as much as 1 mL of each test solution . Then added 2 mL of 20 % TCA and 2 mL of 1 % TBA in 50 % acetic acid . The reaction mixture was shaken and placed in water bath 1000C for 10 minutes . After chilling the solution centrifuged at 3000 rpm for 15 minutes . Then the absorbance was measured at a wavelength of 532 nm with 3 replications . Manufacture of standard curves using a solution of 1,1,3,3 - tetrametoksipropana (TMP) with consentration 0:15 , 0:30 , 0.60 , 0.75 . 1.50 and 3.0 lm . Each solution of various consentration respectively pipetted 1 mL and added 2 mL of 20 % TCA and 2 mL of 1 % TBA in 50 % acetic acid solvent . The reaction mixture was shaken and placed in water bath 1000 C for 10 minutes . Once cool , disentrufuse solution with 3000 rpm for 15 minutes . Absorbance then measured at a wavelength of 532 nm with two replications .

Hepatoprotective activity test

1) . The combination solution of making the VCO and Mengkudu (*Morinda Citrifolia* Linn) Extract: VCO and Mengkudu extract mixed in different proportion here in after referred to as the test solution.

2). Preparation of CMC 1%, 1% CMC solution was prepared by dissolving approximately 1.0 g of CMC that has been weighed carefully into the water up to a volume of 100 ml. This solution is used as a carrier of paracetamol.

3). Manufacture paracetamol suspension and dose determination.

4). Suspend paracetamol in 1% CMC prepared by dissolving a gram of paracetamol has been weighed carefully into the CMC 1% up to a predetermined concentration, the hepatotoxic dose. Paracetamol dose selected based on rats hepatotoksiknya dose of 2.5 g / kg (Donatus, 1983).

5). Grouping and treatment of test animals; Thirty-five rats were divided into 7 groups, each 5 tails. Group I mice given hepatotoxic doses of paracetamol 2.5 g / kg as a positive control. Group II were given distilled water for 6 days in a row as a negative control. Group III were given a combination of the VCO and the highest dose of Mengkudu extract is 2.835 g / kg for 6 consecutive days. After 48 hours of treatment, the group I, II and III have blood drawn by venesection from the lateral tail vein.

Each blood sample drawn serum, then set-serum GPT activity by spectrophotometry. After blood sampling, each

rat was sacrificed to take his footage incorporated into the solution of 10% formalin for preparation of liver cell preparations. Group IV were given a combination of the VCO and the lowest dose of Mengkudu extract (first rank) is 0.840 g / kg. Group V were ranked second dose combination of VCO and Mengkudu extract is 1.260 g / kg. Group VI given boiled herb-shy daughter ranks third dose is 1.890 g / kg. Group VII was given a combination of the VCO and Mengkudu extract dose highest ranking (fourth) is 2.835 g / kg.
Combination given betweenVCO and the provision of a combination of Mengkudu extracts as ranked each dose for six consecutive days. On the seventh day, mice groups IV, V, VI and VII were given doses of paracetamol hepatotoxicity. after 48 hours, all rats in group IV, V, VI and VII are venesection blood samples taken from the lateral tail vein for serum taken, and serum-GPT activity measured by spectrophotometry. Shortly after blood sampling, the mice were sacrificed to take his heart, then put in 10% formalin solution for histopathological preparations preparation of liver cells (Linawati *et al.,* 2005).
6). Manufacture of serum. Male white rat tail blood drawn through by way of the lateral tail vein scratch. Blood collected into a centrifuge tube (Eppendorf vial) through the wall of the tube, allowed to stand for 15 minutes, then centrifuged at 3500 rpm for 10 min and the supernatant was taken (serum).
7). Determination of activity of serum GPT-instrument used to analyze serum GPT-is-micro vitalab. In the photometric analysis-serum GPT activity was carried out as there are a number of reactions in Table I.

Table I. GPT - activity analysis of serum

serum or plasma	50 ul
Reagent solution	500ul
Mixed and added to the pipette , after one minute	
Start reagent	50UL
Mix and decreased infiltration read in one minute	

Reagents used are as follows : KH2PO4 , K2HPO4 , L - alanine , 2 - Oxoglutarat , NADH , LDH , NaOH , Glycerol , NaHCO3 . Enzyme activity is read at a wavelength of 340 nm , temperature 370 C , by a factor of 1745. GPT activity is expressed in U / l (Bergmeyer and Bernt , 1971) . GPT - serum enzyme measurements carried out in the Laboratory Prodia Manado .

3. RESULTS AND DISCUSSION

1. Phytochemical analysis of the combination of the VCO and Mengkudu aqueos Extract

From the analysis of the aqueos extract of Mengkudu phytochemicals with Harborne method (1996) note that the dominant class of secondary metabolites are flavonoids, saponins and triterpenoids. The presence of saponin is characterized by the formation of a stable foam after heated filtrate, based on the amount of foam that formed categorized as very high (+ + +). The presence of flavonoids as much as 10 ml of the filtrate was added 0.5 grams of magnesium powder, 2ml of alcohol carbohydrate (37% and HCL mixture of 95% ethanol with a ratio of 1:1) and 20 ml of amyl alcohol and then shaken strongly. Formation of red, yellow and orange in the lining of amyl alcohol showed a flavonoid. High intensity yellow
showed that high flavonoid (+ +). Presence of triterpenoids known as much as 0.1 grams of extract plus 2 mL of 30% ethanol and then heated and filtered. Filtrate evaporated then add ether 1:1. Ether layer plus reagent Lieberman Burchard (3 drops of acetic acid anhydride and 1 drop of concentrated H2SO4). Red and green colors indicate the presence of triterpenoids and green colors indicate the presence of steroids. Based on the intensity of color that form the content of triterpenoids categorized as high (+ +). Other classes of phytochemicals are alkaloids and tannins in the sample implies that very little negative categorized

2. antioxidative activity

a. Examination Results Anti-Radical Activity

DPPH by spectrophotometry. DPPH free radical activity analysis by spectrophotometry performed by treating the extract with 0.004% DPPH solution. Activity was measured by counting the number of purple DPPH reduction of color intensity which is proportional to the reduction in the concentration of DPPH solution. The damping is produced by reacting diphenyl molecule Pikril Hidrazil (DPPH) with a hydrogen atom of one molecule is released components of the test material to form diphenyl Pikril Hydrazine compounds are colored yellow.
Analysis is performed by spectrophotometry by measuring absorbance of DPPH at a wavelength of 517 nm. Percent reduction calculated by absorbance reduction count DPPH absorbance blank with the test material. Examination begins with the search for the concentration of active experimentation. At a concentration of 500 ppm, it extracts immediately provide damping experiments revealed that concentrations up to 250 ppm. Concentration range of the experiment should illustrate effective concentration, meaning that in the

concentration range giving activity to 50% or between 30-70% so that the EC50 (Effective Concentration 50) can be determined by interpolation activity of 50% on the curve equation.

Table 2 . DPPH free radical activity of the combination of damping and extract VCO Mengkudu water

Treatment	Concentration (ppm)					
	10	50	100	150	200	250
I	10,23	33,56	45,35	49,23	65,25	74,34
II	12,45	34,35	43,24	50,23	68,35	76,34
III	12,47	35,24	44,24	53,24	67,56	75,35
IV	15,23	37,56	43,26	54,35	72,45	76,45
V	15,34	38,34	43,45	57.38	74,34	78,35

Table 3 Regression equations and EC50 combination of VCO and Mengkudu at 30 minute

Treatment	30 minute	EC_{50}	damping activity of free radicals
BHT	0,987	121,45	Medium
I	0,994	350,45	Medium
II	0,992	205,56	Medium
III	0,995	150,34	Weak
IV	0,985	126,34	Weak
V	0,972	78,45	Strong

In the 30-minute activity profile is achieved as can be seen in Figure 3. Profile of each treatment did not show any differences, the experimental concentration range comparable activity increased with increasing concentration. And the regression equation determined from the equation plotted for the next activity to obtain a price 50% effective concentration (EC50) of each extract as shown in table 3.

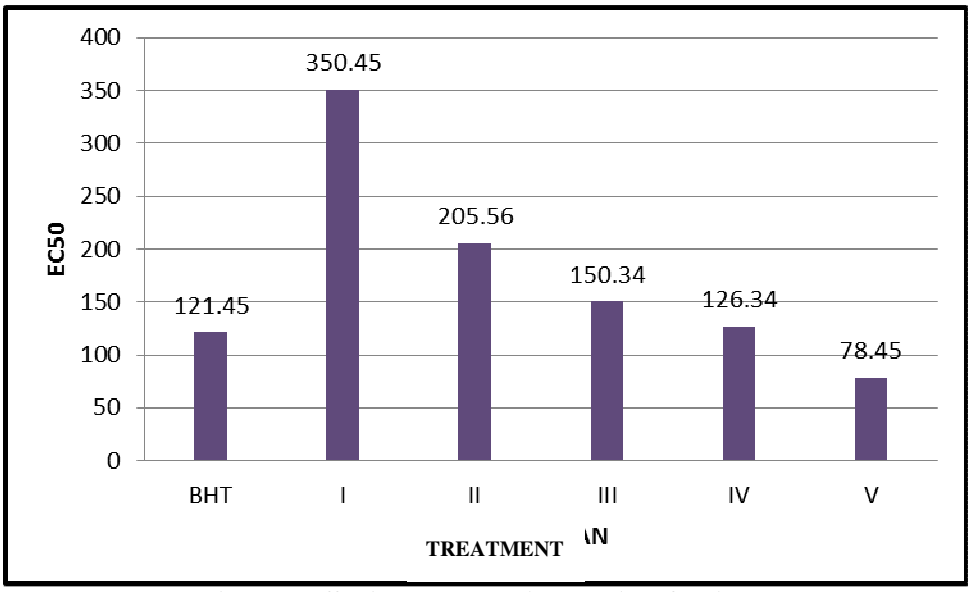

Figure 3. Effective Concentration (EC50) of various treatment

EC50 price can be interpreted that the combination of the VCO and the water Mengkudu extract has DPPH free radical activity higher than the positive control BHT, at a price EC50 = 78.45 ppm at 30 min (treatment V). VCO combination of water and extract mengkudu has the lowest activity with EC50 price = 350.45 ppm in 30 minutes (treatment I). Thus the combination of the water extract of mengkudu and VCO and potentially reduce free radicals.

b . TBA method
Measurement of MDA consentration

Based on the analysis of hydroperoxide with the FTC method, MDA consentration measurements performed on the 7th day in the hope of all hydroperoxide formed as a result of oxidation of linoleic acid has decayed into MDA. The intensity of the color formed on the samples showed antioxidative potential. The more faded red color means the better formed possessed antioxidative potential (Kikuzaki and Nakatani, 1993 in Mokosuli, 2008).
Without the addition of linoleic acid extract (control) had a more concentrated color intensity compared with the extract treatment at various consentration (attachment). MDA Consentration a high of 23.75 lm produced by linoleic acid without extract treatment combinations VCO and mengkudu extract water. Consentration MDA formed at day 7 (Table 8).

Table 4. MDA concentration of linoleic oxidation TBA method

Treatment	Average MDA (µM)
Asam linoleat	23,75
α – tokoferol 200 ppm	6,35
VCO+ Mengkudu (1:1) 50 ppm	13,56
VCO+ Mengkudu (1:1) 100 ppm	9,45
VCO+ Mengkudu (1:1)200 ppm	3,34
VCO+ Mengkudu (1:1)500 ppm	6,73
VCO+ Mengkudu (1:1)1000 ppm	7,45

Inhibition of oxidation
The inhibition of the oxidation of the best on consentration 200 ppm is 87.45%. Thus compared to the inhibition of the positive control α-tocopherol at 200 ppm consentration ie the VCO + 84.35% Mengkudu (1:1) at the same consentration have activity inhibition of linoleic acid oxidation better.
3 . Hepatoprotective activity in rats Test
Hepatotoksin paracetamol in mice studied of value-serum GPT activity and liver cell histology after determination of paracetamol dose of 2.5 g / kg (Table II, group I) compared with those found after treatment of distilled water alone (Table II, group II), as shown in the table-serum GPT activity after administration of paracetamol was found to be 2250.75 U / l. The activity value of 2673.50 ±% (± 28 x) greater than the group given distilled water alone (± 85.25 U / l, negative control). These findings suggest that paracetamol is likely to cause damage to the liver cells of rats. This situation supported by histopathologic picture is found in the form of occurrence of hyperemia and inflammatory cells and necrosis in central venous sentrolobuler.
In Table II (Group III) - serum GPT activity of rats after administration of a combination of water extract of mengkudu and VCO and 2,835 g / kg was found to be ± 386.23 U / l , when compared with the group given distilled water values ± 380.68 % (± 4.8 x) is greater than the normal values . Despite the activity of serum GPT - significant ($p < 0.05$) but histologically liver cells remain in a normal state . These findings suggest that the combination of the VCO and mengkudu extract water although it can enhance the activity of serum GPT - but does not affect the integrity of the liver cells.
In Table II (group IV to V) activity of serum GPT - stimulated mice were given paracetamol pretreatment herb stew - shy daughter dose 0.840 g / kg body weight up to 2,835 g / kg , respectively, were found to be 738.45 ; 568.78 ; 438 , 98 ; 438.45 U / l . When compared with mice given paracetamol alone (group I) the GPT activity was significantly ($p < 0.05$) ± 67.37 % to 80.65 % lower . These findings suggest that the combination of the VCO and mengkudu extract water could inhibit or prevent the increase in serum - GPT activity by paracetamol . Means likely a combination of the VCO and mengkudu extract water to prevent damage to the liver cells due to paracetamol . This situation is supported by the histological features of rat liver cells mainly groups V to VII (VCO combination of pretreatment and water extract of mengkudu 1,260 g / kg up to 2,835 g / kg) did not reveal any necrosis sentrolobuler picture (only found hyperemia) . In other words, the higher the pretreatment dose combination of water extract of mengkudu VCO and the greater the inhibition of liver cell damage .
Thus it can be stated that the combination of the water extract of Mengkudu VCO and shown to have hepatoprotective against paracetamol and kehepatotoksikan hepatoprotektifnya dose range between 1.260 g / kg

body weight up to 1,890 g / kg . Doses above 1.89 g / kg body weight is 2,835 g / kg had no greater effect than a dose of 1.89 g / kg . Doses below 1.26 g / kg ie 0.84 g / kg was found necrosis in rat liver cells .

Table 5 . Serum GPT activity - stimulated mice and histological toxic dose of paracetamol 48th hour due to a combination treatment of VCO and water extract mengkudu 1 x daily for 6 consecutive days (n = 5) .

Sample group	treatment	Dose(g/Kg BB)	serum GPT activity	Histology Preview
I	Paracetamol	2,5	2250,75± 57,25	- Hyperemia and inflammatory cells in the central venous - Necrosis sentrolobular (inflammatory cells erythrocytes) - Inflammatory cells to the area of central venous
II	distilled water	10	85,23 ± 6,35	Normal
III	VCO combination of water and extract mengkudu	2,835 g/kg BB	386,23 ±17,23	Normal
IV	The combination of water and extract mengkudu VCO + paracetamol	0,840	738,45±37,56	- Necrosis sentrolobular (erythrocytes and leukocytes near the central vein)
V	VCO combination of water and extract mengkudu + paracetamol	1,260	568,78 ± 16,43	- Inflammatory cells near the central vein - Hyperemia sinusoid and central venous
VI	VCO combination of water and extract mengkudu + paracetamol	1,890	438,98± 27,45	- Hyperemia in sinusoid
VII	VCO combination of water and extract mengkudu + paracetamol	2,835	438,45±37,34	- Hyperemia in sinusoids and central venous - Leukocytes in sinusoids - Necrosis has disappeared

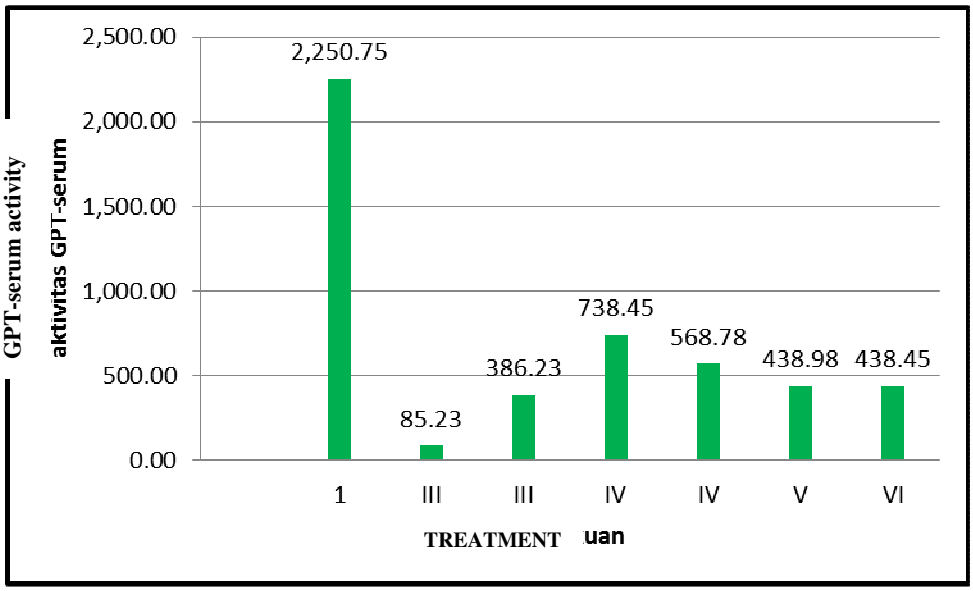

Figure 4 Diagram of serum GPT activity in the various treatment

4. CONCLUSION

From the results of the phase I study of the combination of water and extract mengkudu and VCO can be

concluded that:
1. Phytochemical content VCO combination with mengkudu extract water is the dominant flavonoids, saponins and triterpenoids.
2. Antioxidative activity: DPPH free radical capture VCO combination with the very powerful mengkudu extract water where consentration effective (EC50) 75.40 ppm compared with the positive control (BHT) is the effective concentration (EC50) 121.45 ppm.
3. Based on the data-serum GPT activity and rat liver cell histology were found as well as the results of the analysis procedure is applied, it can be concluded that the combination of the VCO and the water extract mengkudu has hepatoprotective properties with a dose range of 1.260 g / kg body weight up to 1,890 g / kg

5. Acknowledgements

Delivered to thank DP2M DIKTI through UNIMA Research Institute that has funded this research through competitive grants SKIM

References

Bergmeyer, H.U., dan Bernt, E., 1971*, Methods of Enzimatis Analysis*, Bergmeyer, H.U., (ed) vol 2, 755, 760-763, Academic Press Inc, New York.

Dharma, M. (1985). Tanaman Obat Tradisional Indonesia. Balai Pustaka Jakarta.

Donatus, I. A., Suyjipto, N. S., Wahyono, D., 1983, Pengaruh Cairan yang Keluar dari Batang Bambusa vulgaris Schard. terhadap Regenerasi Sel-sel Hepar Tikus Putih Jantan, *Risalah Simposium Penelitian Tumbuhan Obat III,* 105, Fakultas Farmasi Universitas Gadjah Mada, Yogyakarta

Elizabeth, *et al*., 1997, *Effect of MCFA, , Myristic Acid, and Oleic on Serum Lipoproteins in Healthy Subjects.*1997Journal of Lipid Research, volume 38,.

Kushwaha, *et al*., 2004, *Effect of Dietary Cholesterol With or Without Saturated Fad on Plasma Lipoprotein in Cholesterol Level in the Laboratory Opossum Model for Diet - Induce Hiperlipidemia.* Br J Nutr. 2004 Jul; 92 (1) : 63-70 5. Nevin KG,

Linawati Y, Apriyanto A, Susanti E, Wijayanti I, Donatus IA. Efek hepatorptektif rebusan herba putri malu (*Mimosa pigra* L.*)* Pada Tikus terangsang Parasetamol. Fakultas Farmasi UGM. Yogyakarta.

Nevin G dan Rajamohan T, 2004. Beneficial effects of vco on lipid parameters and in vitro LDL oxidation. *Clinial Biochemistry Vol. 37 (9) 830-835*).

Waspodo, I.S. 2000. Mengkudu: mengkudu jelek berkhasiat Obat. Majalah Inti Sari Edisi Maret 2000, Jakarta. Hal.55-60.