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Abstract

Moses in the cradle (*Rhoeo discolor* H.) is one of the two-colored (purple-green) plants containing alkaloids, flavonoids, and anthocyanin that have antioxidant activities. Paracetamol can cause liver necrosis because of the accumulation of free radicals in the body. It will trigger lipid peroxidation process distinguished by the increase of malondialdehyde level, the low activity of catalase enzyme, and the increase of SGPT enzyme level, the blood serum SGOT. This research aims to know the antioxidant effectiveness and activities of moses in the cradle ethanol extract over paracetamol-induced rats.

30 rats were divided into six groups, each group consists of five rats; Group I : as normal control, Group II : as negative control, Group III : as positive control where 52,92 mg/KgBB dose of Silymarin was given, Group IV-VI where 400 mg, 800 mg and 1.200 mg/KgBB dose of moses in the cradle ethanol extract was given consecutively. The antioxidant activity was performed by acknowledging the activities of the moses in the cradle extract, by measuring the totals of flavonoids and tannin, besides the levels of malondialdehyde serum and enzyme activity of catalase serum; antioxidant effectiveness was performed by measuring the levels of SGPT and SGOT serum. Measurement was performed on Day 1, Day 15, and Day 22.

The measurement result : the total of flavonoid was 499,5169 ppm, the total of tannin was 5,5701 ppm, affecting on lowering the levels of malondialdehyde, SGPT, SGOT serum, and increasing the catalase enzyme of male rat male strain Wistar-strain rats induced with paracetamol, which is comparable with silymarin positive control.

Keywords: level of total flavonoid, total of tannin, ethanol extract, moses in the cradle (*Rhoeo discolor* H.), paracetamol induction, malondialdehyde, activity of catalase enzyme, SGPT, SGOT, Wistar rats.

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I. Introduction

Paracetamol

Paracetamol is one of analgesic and antipyretic drugs that can cause liver damage. This can occur since paracetamol is metabolized in the liver, forming reactive n-acetyl-p-benzoquinone (NAPQI) metabolite and covalently interacting with liver macromolecules in the sistine section. The dosage of paracetamol that is used uncontrollably can cause damage in the liver (Khalisahnurjihany., 2019).

Liver is an organ that has a potential to experience damage because of various drugs and environment since it has a function in the process of metabolism and detoxification of chemical substances that get into the body (Lu, 2010).

One of the mechanisms that plays a role on liver damage is an over accumulation of free radicals that causes oxidative stress triggering lipid peroxidation process. The compound that becomes the sign of oxidative stress is the increase of malondialdehyde level and the low activity of catalase enzymes (Indahsari, et al., 2018).

Malondialdehyde is a highly reactive compound from the end result of lipid peroxidation and it is usually used as a biological biomarker of lipid peroxidation to assess the occurrence of oxidative stress. The high level of malondialdehyde shows the high level of free radicals within the body (Eka, et al., 2018). Catalase enzyme is one of the antioxidant defense system components in the body functioning to prevent the formation of hydroxyl radicals (Agustin, et al., 2021).

A standard to mark the damage of liver function is by the increase of Glutamate Oxaloacetate Transaminase (SGOT) Serum and Glutamate Pyruvate Transaminase (SGPT) Serum (Sasongko and Sugiyarto, 2018).

Antioxidant

Antioxidants can be used to neutralize free radicals that can damage the cells. Antioxidant is one of the compounds in moses in the cradle (*Rhoeo discolor* H.) that is included in the Commelinaceae family. Moses in the cradle has many uses, however, research on this, either pharmacology-wise or phytochemical-wise, is still limited (Ramesh et al., 2021). At the chemical analysis of moses in the cradle, there are compounds of phenolics, carotenoids, ascorbic acid, alkaloids, saponins, terpenoids, and flavonoids in the form of anthocyanin that has a strong usefulness as antioxidants (Sundhani, et al, 2016). Research of Sitorus et al., (2012) flavonoid compound identification on the moses in the cradle is measured by UV-Vis spectrophotometer, where anthocyanidin was found. Anthocyanidin is an anthocyanin aglicon that is formulated when anthocyanin is hydrolyzed with acid. According to the research of (Sánchez-Roque dkk., 2017), moses in the cradle extract contains saponins, phenols, tannins, flavonoids and coumarins.

Tannins are plant secondary metabolites that play a role as astringents, which specific taste is bitter. Tannins consist of highly complex polyphenol compound mixture and they are usually incorporated with short-chain carbohydrates.

Moses in the cradle (*Rhoeo discolor* H.) can be used as anti-diabetes, anti-inflammation, anti-bacterial, anti-aging, liver dysfunction prevention, anti-obesity, in-vitro research : anti-mucolytic, kidney stone disintegration, anti-mutagenic, and cancer resistor. This happens because flavonoid compound content has antioxidant effects by resisting various oxidation reactions (Sukohar et al., 2019).

According to Hisage (2018), ethanol compound administration of 400 mg/KgBB dose of moses in the cradle and 400 mg/KgBB dose on paracetamol-induced male Wistar-strain rats can show the improvement of the rat's liver damage level.

This research was performed by the level parameter of total flavonoids and total tannins on the extract, as well as the levels of malondialdehyde, catalase enzyme activity, SGPT and SGOT serum.

II. Research Method

Research objects : the antioxidant activity of extracts and the levels of malondialdehyde, catalase enzyme activity, SGOT, and SGPT.

Equipments : digital measurement, analytic measurement (Shimadzu), (Herma), chamber, centrifuge, micropipette, spectrophotometer, Microlab 300.

Materials : moses in the cradle simplisia, ethanol 96%, Mg powder, Amyl alcohol, gelatin solution 1%, dragendorff reactor, mayer reactor, bouchardat reactor, FeCl₃, parasetamol, CMC Na, Silymarin, aqua destilata, malondialdehyde reactor (mixture of Thiobarbituric acid 1% and TCA 10%), H₂O₂ (0,01M) solution, Buffer Phospat 0,05M pH 7,0 (NaOH, KH₂PO₄).

Research objects : the antioxidant activity of extracts, levels of total Flavonoids, total tannin, malondialdehyde and catalase enzyme activity, SGOT, and SGPT.

The samples of moses in the cradle was extracted with remaceration method, using ethanol 96% solvent which ratio is 1 : 5, concentrated into condensed extract.

Phytochemical screening of moses in the cradle, qualitative extract analysis includes:

1. Flavonoids : 0,5 ml of solution, added with Mg powder (±100 mg), 1 ml condensed HCl, amyl alcohol (±2 ml), shake them vigorously, let it separate, observe the transformation (Harborne, 1987). Red or orange color on the amyl alcohol compound is formed from positive flavonoid (Endarini, 2016)
2. Tannins : 0,5 ml solution was added with NaCl 10%, filtered, the filtrate was taken. Filtrate was divided into two, filtrate A and B. Gelatin 1% was added to Filtrate A. Positive result was when white precipitate was formed. FeCl₃ 10% was added to Filtrate B. Dark green or bluish green color occurs as the positive result (Endarini, 2016).
3. Saponins : 0,5 ml solution added with 10 ml hot water, let it cool, shaken vigorously for 10 seconds. Positive result was when a stable froth is formed for 10 minutes, which height is 1 until 10 cm. At the addition of 1 drop of 2N chloride acid, the froth does not disappear (Department of Health of the Republic of Indonesia, 1979).
4. Alkaloids : 0,5 ml solution was stirred with 1 ml 2N HCl and 9 ml hot aquadest, steam bathed it for 2 minutes, cooled, and filtered. Filtrate was divided into 2. Each was added with Dragendorff and Mayer reactors (Department of Health of the Republic of Indonesia, 1979). Positive result of alkaloids was the formation of reddish brown precipitate by the Dragendorff reactor and white precipitate by Meyer reactor (Endarini, 2016).

5. Steroids and Triterpenoids : 0,5 ml solution was added with ether, filtered. Filtrate was evaporated. 2 drops of anhydrous acetate acid and 1 drop of concentrated sulfate acid were added to the residue. Red color appeared on the positive terpenoids type and blue color appeared on the steroids type (Endarini, 2016).

Quantitative extract analysis includes:

The level of total flavonoid and total tannin were measured from the extract of moses in the cradle (Irianty et al, 2014).

Analysis of flavonoid level to know the level of total flavonoid used UV-Vis spectrophotometer, started with optimization of the wavelength which was performed to determine the maximum wavelength that will be used in the measurement using the standard solution.

Determination of Total flavonoid level :

1,5 ml methanol, 0,1 ml aluminum (III) chloride 10%, 0,1 ml sodium acetate 1 M, and 2,8 ml aquadest were added into 0,5 ml testing samples. After being incubated for 30 minutes, absorbance was measured using UV-Vis spectrophotometer at 436 nm wavelength.

Determination of Total tannin level :

1 mg ethanol extract of moses in the cradle was scaled and dissolved with aquades to 10 ml (100 ppm) and replicated for 3 times. Each replicate was pipetted for 9 mL and dissolved with aquadest to 10 mL (90 ppm). It was added with 1 mL Folin-Denis reagent, leave it for 3 minutes, added with 1,0 ml saturated Na₂CO₃ solution and incubated for 40 minutes, then its absorption was read during 649,9 nm wavelength (Irianty and Yenti, 2014).

Treatment on the tested animals :

330 male white Wistar-strain rats, aged 2-3 months old, weighed 150-250g, were divided randomly into 6 groups of 5. Group I : normal, Group II : negative control, Group III : positive control with 52,92mg/KgBB dose of Silymarin, Group IV-VI : 400 mg, 800 mg, 1.200 mg/KgBB dose ethanol extract of moses in the cradle. 250mg/KgBB dose of paracetamol induction (Merdana et al., 2019). On day 1, 15, and 22, the levels of malondialdehyde and catalase enzyme, SGPT, and SGOT activities were measured. The obtained data was analyzed using SPSS version 19.

SGPT and SGOT level measurement :

SGPT and SGOT level measurement used blood serum as a sample. The amount of serum was measured according to the Elitech Clinical System SOP. The reagent uses AST and ALT from Elitech Clinical System. Samples were analyzed using Spectrophotometric Microlab 30.

Measurement of MDA level :

200µl plasma was inserted in the centrifuge tube. 1,0ml TCA 20% was added and 1,0ml TBA 1%. TBA was made with glacial acetate acid solvent 50%. Divortex solvent was incubated for 45 minutes at the temperature of 95°C, cooled it down. The solvent was centrifuged at the speed of 6000 rpm for 15 minutes. Supernatant was measured using UV-Vis spectrophotometer at λ 532 nm (Momuat et al., 2011).

Determination of Catalase Enzyme Activity

0,05ml blood serum was added with 2000µl dapsyl phosphate 0,05M pH7,0 containing H₂O₂ 0,01M. Measurement at 240 nm wavelength was performed (Wahid & Safwan, 2019).

III. Result And Discussion

Ethics tests that have been performed to this research was Ethics from KEPK (Health Research Ethics Committee) of College of Pharmaceutical Sciences of Semarang Pharmaceutical Foundation by the protocol No. of 255/AHW-SW/KEPK/STIFAR/ EC/IV/2021.

Determination has been performed to the simplisis of Moses in the cradle (Rheo Discolor). Remaceration process was performed for 5 days by changing the solvent mixer every 1 x 24 hour Wahid and Safwan (2019). The obtained filtrate was then concentrated using a water bath at the temperature of 60°C. The use of water bath at the temperature of 60°C minimized the compound damage within the extract, 21,16% extraction yield was obtained.

Table 1. Phytochemical test result of moses in the cradle

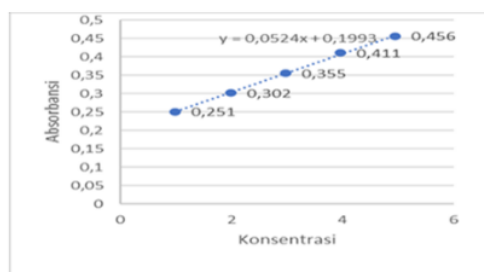
Sample	Flavonoid	Alkaloid	Tannin	Saponin	Terpenoid
Extract of Moses in the cradle	+	+	+	+	+

Description : (+) : within the extract

148,16 gram of moses in the cradle condensed extract was obtained, 21,16% extraction yield was obtained. At the phytochemical preliminary test, a result was obtained that ethanol extract of moses in the cradle contains the compounds of flavonoids, alkaloids, saponins, tannin, and terpenoids.

Result of Total Flavonoids Level Determination :

Regression equation can be obtained from the Graphic of level vs absorbance of total flavonoids level determination, the graphic can be seen at Graphic 2.



Graphic 1. Graphic of level vs absorbance of total flavonoids level determination

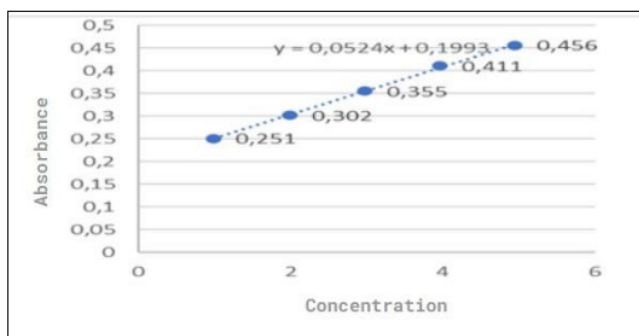
Flavonoids level can be measured from the regression equation with absorbance that can be seen at Table 2, the result of total flavonoids level was $499,5169 \pm 7,9971$ ppm

Table 2. Result of Total Flavonoids Level determination

REPLICATION NO	WEIGHING (Gram)	LEVEL (ppm)	LEVEL AVERAGE (ppm)	SD
1	0,0512	508,6155		
2	0,0509	496,3336	499,5169	7,9971
3	0,0500	493,6019		

Result of Total Tannin Level Determination

Regression equation can be obtained from the Graphic of level vs absorbance of total tannin level determination, the graphic can be seen at Graphic 2.



Graphic 2. Graphic of level vs absorbance of total tannin level determination

The total tannin level can be measured from a regression equation with absorbance that can be seen at table 4. The result of total Tannin level was $5,5701 \pm 0,5843$ ppm.

Table 3. Result of Total Tanning Level Determination

REPLICATION NO	WEIGHING (Gram)	LEVEL (ppm)	LEVEL AVERAGE (ppm)	SD
1	0,0503	5,856		
2	0,0500	4,923	5,5701	0,5843
3	0,0505	5,9870		

At the pharmacology test on ethanol extract of moses in the cradle.

Measurement of Malondialdehyde level

It used a UV-VIS Spectrophotometer, and the average result of malondialdehyde level measurement can be seen at table 4.

Table 4. Average $\pm$ SD Malondialdehyde Level (μ L)

Groups	Day 1 (μ /ml)	Day 15 (μ /mL)	Day 22 (μ /mL)
Normal C.	27,73 $\pm$ 0,73	27,91 $\pm$ 0,87	27,88 $\pm$ 0,94
Negative C.	26,66, $\pm$ 0,39	51,89 $\pm$ 2,76	51,95 $\pm$ 2,80
Positive C.	27,98 $\pm$ 1,48	51,42 $\pm$ 1,74	29,26 $\pm$ 1,05
P. 400	26,73 $\pm$ 0,86	52,53 $\pm$ 1,72	28,68 $\pm$ 1,65
P. 800	27,34 $\pm$ 1,36	48,66 $\pm$ 3,96	34,35 $\pm$ 4,91
P.1.200	27,16 $\pm$ 0,63	52,62 $\pm$ 2,46	34,78 $\pm$ 2,29

Description :

C : Control

T : Treatment of extract mg/kg BB dose

The measurement of MDA and catalase enzyme activity after paracetamol induction was given showed the result that there was a difference ($p < 0,05$) in all groups, except for the normal group. The tests of normality and homogeneity showed normal distributing data ($p < 0,05$) but it is not homogenous ($p < 0,05$). Different results among groups ($p < 0,05$) were obtained at Kruskal Wallis non parametric test, continued with Mann-Whitney analysis to know the difference among treatment groups.

Flavonoids and tannin compounds are contained in moses in the cradle, which bear a strong usefulness as antioxidants. In this research, 499,5169 ppm total flavonoids and 5,5701 ppm were analyzed. Flavonoids working mechanism can lower the MDA level which mechanism was giving hydrogen or electron atom donor to the free radicals. NAPQI was formed from the result of paracetamol metabolism in order to become a stable and non reactive compound, consequently, lipid peroxidation process can be hindered to be able to lower the MDA level and to increase catalase enzyme activity (Rahmawati., 2018). The result of catalase enzyme activity measurement can be seen at table 5.

Table 5. Average $\pm$ SD Catalase Enzyme Activity (μ L),

Groups	Day 1 (μ /ml)	Day 15 (μ /mL)	Day 22 (μ /mL)
Normal C.	201,11 $\pm$ 14,19	203,42 $\pm$ 15,64	203,200 $\pm$ 13,11
Negative C.	206,86 $\pm$ 10,60	56,00 $\pm$ 9,54	56,554 $\pm$ 9,96
Positive C.	209,35 $\pm$ 6,98	53,05 $\pm$ 7,09	299,02 $\pm$ 4,13
P.400	214,59 $\pm$ 3,01	52,12 $\pm$ 8,15	147,69 $\pm$ 4,51
P.800	208,55 $\pm$ 10,64	52,68 $\pm$ 12,37	164,185 $\pm$ 9,02
P.1.200	221,08 $\pm$ 10,31	56,37 $\pm$ 10,03	172,43 $\pm$ 3,41

Description :

C : Control

T : Treatment of extract mg/kg BB dose

It can be seen from table 5 that the positive group has an insignificant difference ($p > 0,05$) when compared to the group of 400 mg/KgBB dose ethanol extract of moses in the cradle. The positive control group and the group of 800 mg/KgBB dose ethanol extract of moses in the cradle, 1.200 mg/KgBB dose showed its

significance value ($p < 0.05$). A probability occurs in this result, because a dose that can no longer increase responses has been achieved. Pascila (2020) also explained that the dose increase of the drugs should be able to increase responses equivalent with the increased dose, however, such a thing occurred in this research, since there was an interaction with other chemical compounds between natural medicines and compound components which its contents are not single, consisting of various chemical compounds that work together to cause effects. In this research, 400 mg/KgBB ethanol extract of moses in the cradle showed an equivalent effect with 52,92 mg/KgBB silymarin.

Effectiveness as hepatoprotector at the assessment of SGPT and SGOT level using microlab 300 with the average result can be seen at table 6.

Table 6. Average $\pm$ SD SGPT - SGOT Level (μ /L)

Groups	Average $\pm$ SD SGPT - SGOT Level					
	Day 1		Day 15		Day22	
	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT
Normal C.	87,6 $\pm$ 30,6	86,6 $\pm$ 28,92	83,8 $\pm$ 22,3	85,0 $\pm$ 24,1	82,2 $\pm$ 20,6	84,2 $\pm$ 24,47
Negative C.	86,2 $\pm$ 36,98	106,2 $\pm$ 10,89	127,8 $\pm$ 42,65	177,6 $\pm$ 35,42	127 $\pm$ 36,06	177,8 $\pm$ 34,97
Positive C.	75,4 $\pm$ 15,79	91,4 $\pm$ 26,79	194,8 $\pm$ 43,76	193,8 $\pm$ 38,42	86,4 $\pm$ 18,39	104 $\pm$ 20,63
P.400	85,8 $\pm$ 3,56	98,0 $\pm$ 23,25	189,8 $\pm$ 30,11	221,2 $\pm$ 26,6	71,8 $\pm$ 12,62	99,2 $\pm$ 12,48
P.800	87,0 $\pm$ 30,94	88,2 $\pm$ 17,61	180,4 $\pm$ 44,57	199,4 $\pm$ 32,66	67,6 $\pm$ 19,32	106,4 $\pm$ 25,68
P.1200	81,8 $\pm$ 5,93	91,8 $\pm$ 22,31	190,0 $\pm$ 63,16	203,4 $\pm$ 40,47	93,2 $\pm$ 23,51	99,6 $\pm$ 11,93

Description :

C : Control

T : Treatment of extract mg/kg BB dose

On table 6, there was a significant difference ($p < 0.05$) if SGPT and SGOT level are compared among the groups of negative control, positive, ethanol extract of moses in the cradle dose of 400 mg/KgBB, 800 mg/KgBB, and 1200 mg/KgBB. This showed that ethanol extract of moses in the cradle in the three doses can have an effect to lower the SGPT level at the paracetamol-induced rats. At the positive control group, compared to the ethanol extract group of moses in the cradle, dose of 400 mg/KgBB 800 mg/KgBB, and 1200 mg/KgBB, showed insignificant difference ($p > 0.05$). This showed that 400 mg/KgBB 800 mg/KgBB, and 1200 mg/KgBB dose of ethanol extract of moses in the cradle showed an equivalent effect on 52,92 mg/KgBB silymarin.

As antioxidants, flavonoids, saponins, and tannin compounds can increase glutathione production, as endogenous antioxidants that can keep the free radicals because of the overdose giving of paracetamol. It also suppressed liver tissue damage and triggered liver cell regeneration by the increase of IL-10 production (Maulida et al. 2020). The working mechanism of antioxidant compound is by giving free radicals to its electrons, consequently, it can prevent continuous chain reaction of fatty peroxidation as well as proteins caused by the impact of the free radicals, in order that further cell damage can be prevented (Nofita et al., 2020).

One of the flavonoids at moses in the cradle is anthocyanin. It has a free hydroxyl group that is used to contribute hydrogen groups for the free radicals where at NAPQI formed from paracetamol metabolism, it will become unreactive (Sitorus et al., (2012).

Saponin compounds also have a role in repairing the liver damage, and lowers the enzyme level within the liver (Withawaskul et al., 2003).

Total phenolic, including flavonoids, tannin, has a correlation with the antioxidant activities. The correlation can be seen from the bigger total phenolic on the extract, the better its antioxidant activity (Rudiana,T.,dkk, 2018). In this research, considering flavonoids and tannin compounds within moses in the cradle, the total flavonoids is 499,5169 ppm, total tannin is 5,5701 ppm. It plays an adequately big role in improving the effect of antioxidants.

Tannin compound has an effect as hepatoprotector, by acting as free radical scavenger that is directly correlated to the free radicals as well as drug toxic metabolite, similarly NAPQI is the result of paracetamol metabolism (Anggraeny et al. (2021). Alkaloids compounds within the extract of moses in the cradle can protect the liver from the exposure of chemical compounds that causes the occurrence of the free radicals (Raj et al., 2010). They can also block tissue oxidative damage on the rats (Untari et al., 2014). Saponin compounds also play a role in repairing liver damage and lowering enzyme level at the liver (Withawaskul et al., 2003). In equivalent with silymarin as a comparative control, it has working mechanism by preventing the entrance of oxidant into the hepatocyte cell, stimulating protein synthesis needed for liver cell regeneration, as an antioxidant that neutralizes the free radicals and modulates the body immune responses (Zulkarnain et al., 2017).

The contents of flavonoids, tannin, alkaloids, and anthocyanin in moses in the cradle can lower the levels of SGPT, SGOT, and MDA, as well as improve the catalase enzyme activity. This is because the secondary metabolite content plays an active role in fighting the free radicals (scavenger free radicals) directly

correlated with toxic metabolite of paracetamol drug (NAPQI), protecting the body from cell damage, and possessing a high antioxidant activity because of the high levels of total flavonoids and total tannin within moses in the cradle.

IV. Conclusion

Moses in the cradle has antioxidant activities, as the levels of 499,5169 ppm total flavonoid extract and 5,5701 ppm total tannin that are effective as hepatoprotector that can fight NAPQI free radicals as the result of paracetamol metabolism. Moses in the cradle extract giving can lower the levels of SGPT, SGOT, and MDA and increase the catalase enzyme level at the paracetamol-induced rats.

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