LIFE: International Journal of Health and Life-Sciences
ISSN 2454-5872

Musdja et al., 2018

Volume 4 Issue 2, pp. 34-46

Date of Publication: 14th July, 2018

DOI-https://dx.doi.org/10.20319/lijhls.2018.42.3446

This paper can be cited as: Musdja, M. Y., Rahman, H. A., & Hasan, D. (2018). Antioxidant Activity of Catechins Isolate of Uncaria Gambier Roxb in Male Rats. LIFE: International Journal of Health and Life-Sciences, 4(2).34-46.

ANTIOXIDANT ACTIVITY OF CATECHINS ISOLATE OF UNCARIA GAMBIER ROXB IN MALE RATS

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Abstract

The aims of this study were to determine the antioxidant activity of catechins isolates of gambir by measuring the levels of Malondialdehid (MDA) in male white rats. Methods: Catechins of gambir were isolated by using partition method with ethyl acetate solvent. Gambir quality was determined based on National Standard of Indonesia: SNI 01-3391-2000. The yield of catechins obtained from the gambir isolate was determined as (+)-catechins by comparing with standard (+)-catechins and measured by a spectrophotometer UV-VIS at wavelength 279 nm. A total of 25 male rats were divided into 5 groups. Administration of catechins was suspended in 0.5% Na CMC with dose of 5 mg/kg b.w, 10 mg/kg b.w and 20 mg/kg b.w, as positive control was used

the suspension of vitamin E, 20 mg/kg b.w in Na-CMC 0.5% and as negative control was used 0.5% Na-CMC suspension. Administration of test preparations was performed per oral, 1 times per day for 7 days. On the eighth day, the rats were made become oxidative stress with swimming in water about 1 hour, until almost drowned. MDA levels of rats in each group were measured by comparing MDA levels, before were given test preparation on first day and after were given test preparations on the eighth day. The MDA level of serum rats were measured based on Wills method. Result: The statistical test of catechins isolate of gambir showed that all dosage of catechins isolate test of gambir had antioxidant effect and had significant difference to negative control ($p < 0.05$). Doses 5 and 10 mg/kg b.w did not show significant differences with vitamin A as positive control, while Dose 20 mg/kg b.w gave the strongest antioxidant effect and had significant difference with positive control ($p < 0.05$). Conclusion: Catechins of Gambir has stronger potential as an antioxidant than vitamin A.

Keywords

Antioxidant, Cancer, Catechins, Medicinal Plant, Natural Product, *Uncaria Gambir*, Male Rats

1. Introduction

In traditional medicine, gambir plants have long been used by most people in Southeast Asia and Southern Asia to treat various diseases. among others to treat: Diarrhea, gastric disease, antioxidants and prevent cancer, disorders mouth, treat burns, acne medication and one of the ingredients for betel quid chewing (Hanny 2017, Hamda 2014)

Taniguchi et al (2008) had conducted a study on the types of catechins present in Gambir, they had found 9 types of catechins in gambir namely "(+) - catechin, (-) - epicatechin Gambiriin A1, Gambiriin A2, Gambiriin B1, Gambiriin B2, Catechin-(4α-8)-ent-epicatechin, Gambirflavan D1 and Gambirflavan D2 " (Taniguchi et al, 2008).

Based on the results of research of some researchers, about the contents of catechins of some plants that contain catechins. Gambir is a plant containing the highest catechin. The results of the research of Amos (2010), total catechin content of the extract Gambir in Indonesia vary widely, with range 40-80%, Hilal and Engelhardt (2007) had obatained catechins of green tea in market with range 8.5 – 20.6 %, for black tea with range 0.74 – 10%. While according to Rangari the content of catechins from Acacia catechu is approximately 9-12%. Because the catechins content of the Plants of gambir, green tea, acacia very high, then these plants are widely used as antioxidants.

It is well known that natural antioxidants are plays an important role to prevent cancer growth (Sarafinovska and Dimovski 2013). Therefore Kaushik and Sahi (2017) have suggested to make a more complete Natural Medicine Database (NMD) for Drug Discovery, especially to treat diseases that cause higher mortality, such as cancer and cardiovascular diseases, so that medical personnels, researchers and the general public are easy to know and select the natural medicines that fit with their need (Kaushik and Sahi,2017). According to the results of Musdja, et al (2017) research, gambir also has efficacy as antibacterial (Musdja et al, 2017). Because gambir is a medicinal plant that contains very high catechins, higher than the catechins content of green tea leaf, where the catechins of green tea leaf have been proven efficacious as antioxidants (Armoskaite et al, 2011. Hilal and Engelhardt, 2007). Gambir is a medicinal plant that is very potential to be entered into Natural Medicine Database (NMD), for this we had done research on the efficacy of gambir as immunomodulator, (2012) as antibacterial (2017) and in this study as antioxidant.

In the *in-vitro* experiments, effects of antioxidants of gambir by using BPPH method already was done by some researchers, including by Angraini et al (2011) and Amir et al (2012). Both of them have got it, that gambir has a strong potential for antioxidants. However, the *in-vivo* experiment has not been done

In this study to determine the effects of antioxidant of catechins of gambir with *in-vivo* experiment was based on the reaction of MDA with thiobarbituric acid (TBA) in the bodies of rats :

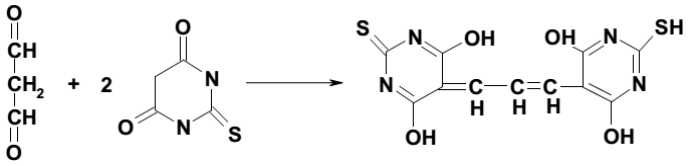


Figure 1: *Reaction between MDA with TBA*

The 2-thiobarbituric acid reactive substance (TBARS) test is one of the most frequently employed methods for assessing lipid oxidation in blood serum. Reacting with minor lipid oxidation products with predominantly MDA, TBA produces a colored complex with an absorption maximum at 532nm (Södergren, 2000).

In vivo antioxidant test with MDA measurement was already widely used by researchers, even now can also be purchased kits for these antioxidant measurements, such as Northwest Malondialdehyde Assay.

The process of formation of MDA is originated from poly unsaturated fatty acids that are rich in double bonds in cell membranes. In a state of oxidative stress, spikes in free radical blood levels exceed the threshold of endogenous antioxidants. In this state free radicals that have one or more unpaired electrons can bind to unsaturated fats on the cell membrane, so that the double bond becomes saturated and turns into an aliphatic bond. In these circumstances free radicals, such as reactive oxygen, reactive hydrogen, reactive peroxides will bind to the lipid bilayer on the cell membranes forming MDA.(Yagi, 1998).

2. Methods

Gambir was obtained from Payakumbuh area, Indonesia, This area is the largest gambir producer in the world. Compound and Phytochemical screening of gambir extract was done based on Harbone methods. (Harborne, 1998).

Catechins isolation was done based on National Standard Indonesia: SNI 01-3391-2000, "A total of 500 g of gambir powder was extracted with a water solvent at temperature of 90 - 96 ^{0}C for 15 minutes while stirring. Then the infusion was filtered in hot conditions using a funnel coated with filter paper. The extract obtained was partitioned with ethyl acetate, the ratio of extract with ethyl acetate (1 : ½.). Ethyl acetate phase was taken and the water phase was partitioned repeatedly with ethyl acetate until a clear solution was obtained. The ethyl acetate phase was condensed with an evaporator, then washed with cold water and filtered. Catechins that obtained was dried in an oven at temperature 70 0C". Gambir quality was determined based on National Standard Indonesia: SNI 01-3391-2000. The yield of catechins obtained from the gambir isolate was determined as (+)-catechins by comparing with standard (+)-catechins and measured by a spectrophotometer UV-VIS at wavelength 279 nm.

The eligible rats for the experiment were divided into 5 groups, each group consisting of 5 rats. Administration of catechins was suspended in 0.5% Na CMC with dose of 5 mg kg b.w, 10 mg / kg b.w and 20 mg / kg b.w, respectively, for positive control was used the suspension of vitamin E, 20 mg/kg b.w in Na CMC 0.5%. As a negative control was used 0.5% Na CMC suspension. Administration of test preparations was performed per oral, 1 times per day for 7 days.

On the eighth day, the rats were made become stress, that caused the rat's MDA levels to rise. Based on Wills method "the rats were treated with swimming in water about 1 hour, until

almost drowned. MDA levels of rats in each group were measured by comparing MDA levels, before were given test preparation on day 0 with after were given test preparations on day 8.

The MDA level of serum rats were measured based on Wills method. "A 200 µL serum rats was added 1 ml trichloroacetate (TCA) 20% and 2 ml of tiobarbituric acid (TBA) 0.67%. The solution was mixed homogeneously and heated on waterbath for 10 minutes. After cool, the solution was centrifuged at 3000 rpm for 10 minutes. The pink filtrate that formed was measured at wavelength 532 nm using a spectrophotometer.UV-VIS". The data that obtained were processed by statistical analysis using SPSS-16

3. Result and Discussion

The result of Compound and Phytochemical screening of gambir extract based on Harbone methods was obtained, that gambir extract containing flavonoids, phenolic and saponin.

Gambir quality requirements based on the National Standards of Indonesia: (Standar Nasional of Indonesia: SNI 01-3391-2000) were compared with quality of gambir for this research, as shown in table 1.

Table 1: *Comparison of Gambir Quality That Was Used For Research with Gambir Quality Requirements Written On the National Standards of Indonesia (Standar Nasional Indonesia: SNI 01-3391-2000)*

Type of test	Quality number 1	Quality number 2	Gambir for research
a. Physical condition : • shape • color • smell	 Intact Yellow to brownish yellow Specific	 Intact Yellow brown to yellow-black Specific	 Intact Yellow to brownish yellow Specific
b. Water content w/w	≤ 14%	≤ 16%	0.7%
c. Ash content w/w	≤ 5%	≤ 5%	2.6%
d. Catechin concentration w/w of dry weight	≥60%	≥50%	85.3%
e. Insoluble material content of : • water w/w of dry weight • alcohol w/w of dry weight	 ≤ 7% ≤ 12%	 ≤ 10% ≤ 15%	 3.8% 7.7%

The quality of the gambir that was used in this study, when compared to the requirements written on the National Standards of Indonesia: (National Standard of Indonesian: SNI 01-3391-2000), was a type of gambirr with the quality of number 1 or excellent.

This was indicated by Physical condition, Water content w/w, Ash content w/w, Catechin concentration w/w of dry weight and Insoluble content in water and alcohol are eligible, as shown in Table 1.

Table 2: *Comparison of Mda Concentrations in Rats, Between Before Administration Catechins With After Administration Catechins for 7 Days and And Rats Were Applied Swimming In Water For 1 Hour*

No	Group of rats	MDA concentration before administration Catechins	MDA concentration after administration Catechins	Percentage of decrease & increase of MDA concentration
1	Dose 5 mg/kg bw	0.592 ± 0.226	0.474 ± 0.182	↓ 20.19%
2	Dose 10 mg/kg bw	0.745± 0.248	0.523± 0.198	↓ 31.28%
3	Dose 20 mg/kg bw	0.497± 0.144	0.221± 0.101	↓ 57.63%
4	Vit. E (+ Control)	0.609± 0.001	0.453± 0.055	↓ 25.55%
5	Negative Control	0.533± 0.104	0.937± 0.126	↑ 77.79%

If, the data in table 2 above is made graph, as shown in Figure 1 below, it will be seen clearly. In the negative control group, MDA levels greatly increased sharply, after the rats were stressed by swimming for about 1 hour, so it nearly drowned, while in the group of rats treated with catechins and vitamin A. MDA levels decreased, because catechins had worked inhibits MDA formation in the body of rats. Catechins with a dose of 20 mg was the most powerful for lowering MDA levels

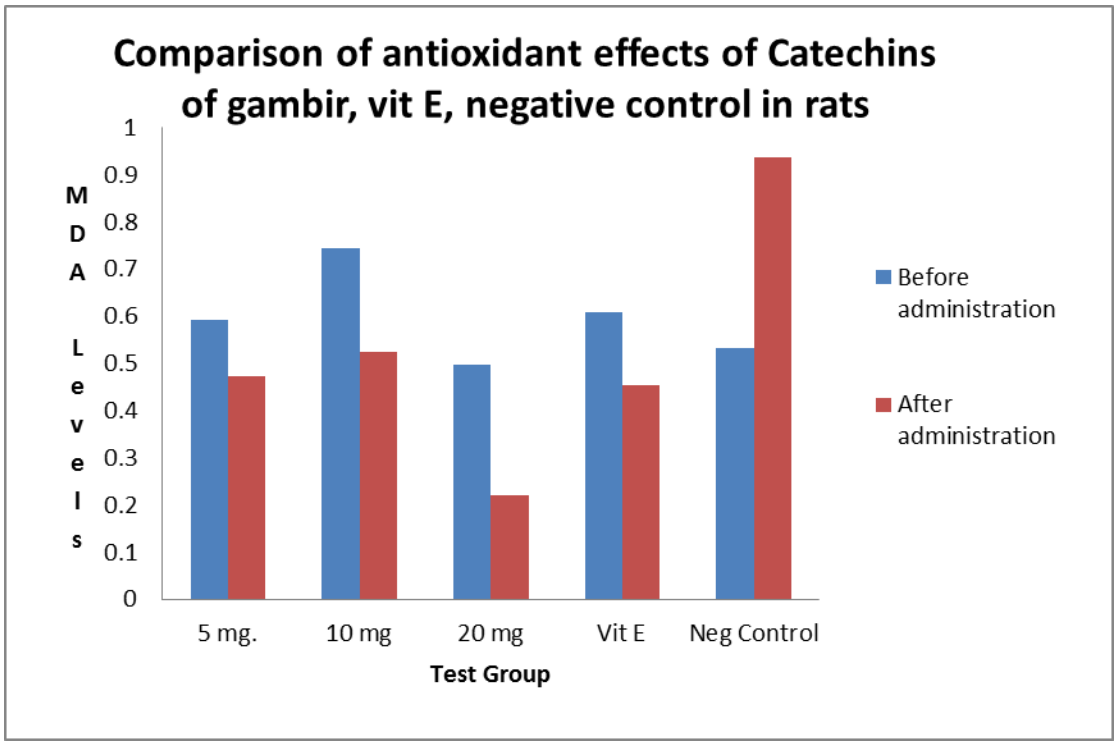


Figure 2: *Comparison of antioxidant effects of catechins of gambir between before with after administration for 7 days with doses, catechin 5 mg / kg b.w, 10 mg / kg b.w, 20 mg / kg b.w, vitamin E 20 mg / kg b.w and negative control*

In Table 2 and Figure 1. In the negative control group of rats, it is clear that there was an increase in MDA levels with oxidative stress after the rats were applied swimming in the water for 1 hour, in which the rats almost had drowned, resulting in increased levels of MDA in rats, due to oxidative stress. Where there was an increase in MDA levels of 77.79% compared with MDA levels on the first day, before the test preparation was given to rats.

While on the rats that was given catechins and positive control, the opposite happened, MDA levels decreased compared with MDA levels, before was given catechins and positive control (Vitamin E).

From the results of statistical tests was obtained, that the antioxidant effect of catechins dose 5 mg, 10 mg and 20 mg/kg b.w and vitamin E doses of 20 mg/kg b.e showed a significant difference to negative control ($P \leq 0.05$). While the antioxidant effect of catechins dose 5 mg and 10 mg / kg b.w did not show significant difference to vitamin E dose of 20 mg / kg b.w, but the antioxidant effect of catechins dose 20 mg / kg b.w stronger than antioxidant effect of vitamin E dose 20 mg / kg b.w and significantly different ($P \leq 0.05$).

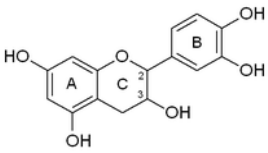
Gambir contains a number of biologically active compounds, especially catechins compounds. As research of Taniguchi et al (2008), the catechins compound of gambir are "(+)-catechin, Catechin - (4α-8)-ent-epicatechin, (-)-epicatechin Gambiriin A1, Gambiriin A2, Gambiriin B1, Gambiriin B2, Gambirflavan D1 and Gambirflavan D2". The main content of catechins of gambir is (+)- catechin. Catechins compounds are known to be powerful chain breaking antioxidants and are important constituents of plants. As are also many contained by the *Camelia sinensis* L and *Acacia catechu* L. It has been recognized as natural antioxidant. Activity antioxidant of catechins of gambir is estimated from (+)-catechin or the synergy work of catechins of gambir.

The result of Compound and Phytochemical screening of gambir extract in this research was obtained, that gambir extract containing flavonoids, phenolic and saponin. According to Kasote et al (2015) Flavonoids are classified into six major subclasses, flavones, flavonols, flavanones, catechins or flavanols, anthocyanidins and isoflavones. Flavonoids are commonly found in vegetables, fruits, nuts, seeds, stems, flowers, tea, gambir, acacia and wine. As shown in Table 3.

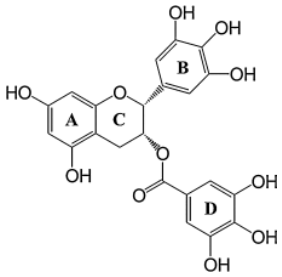
Table 3: *Classification of Flavonoids and food sources (Sandhar et al 2011)*

No	Flavonoids subclass	Representative flavonoids	food sources
1	Flavonol	Kaempherol, myricetin, quercetin, rutin	Onion, kale, broccoli, apples, cherries, berries, black tea, red wine
2	Flavone	Apigenin, rutin, luteolin	Parsley, celery, thyme, red wine, tomato skin
3	Flavanone	Naringin, naringenin, hesperidin, taxifolin	Citrus, lemon, orange, grapefruit
4	Isoflavone	Genistin, genistein, daidzein	Soybean and products
5	Flavanol	Catechin	Tea, gambir, acacia catechu
6	Anthocyanidin	Cyanidin, apigenidin	Cherry, raspberry, strawberry, colored fruits

Many studies have suggested that catechins of flavonoids group is an antioxidant to cure diseases caused by free radicals and for treatment of various diseases such as cancer, obesity, cardiovascular diseases, neurodegenerative diseases, diabetes mellitus, and so on (Banjarnahor and Artanti 2014, Luo et al, 2017). The main content of gambir catechins is (+)- catechin (Taniguchi et al, 2008) while the main content of green tea catechins is epigallocatechin3-Gallate (Luo et al, 2017), as shown in figure 3.



(+)- Catechin



Epigallocatechin3-Gallate

Figure 3: The Main Content Of Gambir Catechins Is (+) - Catechin And The Main Content Of Green Tea Catechins Is Epigallocatechin3-Gallate

The antioxidant capacities of flavonoids are much stronger than those of vitamins C and E. (Procházková et al 2000), when associated with the results of research of Amos (2010), total catechin content of the extract Gambir with range 40-80%, Hilal and Engelhardt (2007) had obatained catechins of green tea in market with range 8.5 – 20.6 %, for black tea with range 0.74 – 10%. While according to Rangari the content of catechins from Acacia catechu is approximately 9-12%. So that gambir is antioxidant strongest than all of vegetables, fruits, nuts, seeds, stems, flowers, tea, gambier, acacia and wine in Table 3.

According to Procházková et al (2011), the mechanism of action of flavonoids as antioxidants can be (1). Direct scavenging of reactive oxygen species (ROS), (2) activation of antioxidant enzymes, (3) metal chelating activity, (4) reduction of α-tocopheryl radicals, (5) inhibition of oxidases, (6) mitigation of oxidative stress caused by nitric oxide, (7) increase in uric acid levels, (8) increase in antioxidant properties of low molecular antioxidants.

Because there are at least 8 mechanisms of action of flavonoids as antioxidants (Procházková et al, 2011), whereas in gambir is already known there are 9 types of catechins. (Taniguchi et al, 2008). While Progranulin (PGRN) is working to stimulate the proliferation and survival of several types of cancer cells. And obesity can also be controlled with antioxidants.

(Donma & Donma, 2017). To know which type of catechin is most effective as an antioxidant for disease requires more and more extensive research on the benefits of catechins as an antioxidant.

On the other hand, Luo et al (2017) has explained about Mechanisms and Application on Hepatocellular Carcinoma of Epigallocatechin3-Gallate (EGCG) of Green Tea (Luo et al (2017). It would be better, if any researcher would conduct a study on the comparison of pharmacological activity between Epigallocatechin3-Gallate (EGCG) with (+)-catechin for treatment of cancer, obesity, cardiovascular diseases, diabetes mellitus, and so on.

4. Conclusion

Based on the description above, catechins of gambir is a natural product that potent for use as an antioxidant, as has been studied previously, that catechins in green tea have stronger antioxidant capabilities than vitamins C and E. Catechins from gambir also have more stronger as antioxidant than vitamin E.

Although the catechins content of gambir in nature is higher than the catechin of green tea, to know which one is better for antioxidants. There should be a study comparing (+)-catechin of gambir leaves with Epigalocatechin of green tea leaves.

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