Gambir (Uncaria gambir Roxb.) as A Potential Alternative Treatment for Hyperlipidemia

Gambir (Uncaria gambir Roxb.) sebagai Alternatif Pengobatan yang Potensial pada Hiperlipidemia

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Abstract

Gambir (Uncaria gambir Roxb.) is a member of family of Rubiaceae. In Asia including Indonesia, extracts of gambir are empirically used daily to weed out. The high content of catechin flavonoids in gambir has a pharmacological effect in the treatment of hyperlipidemia that potential to be developed into traditional medicine. This literature review aimed to examine the potency of pharmacological effect of gambir as hyperlipidemia treatment therapy based on the results of studies in silico, in vitro, in vivo pharmacological effects and its safety to provide evidence of scientific information to the community. The literatures used for analysis in this study including evidence-based articles on both pharmacology and safety which are available in Pubmed and Google Scholar. The results showed a very strong potency of gambir plants in the treatment of hyperlipidemia with catechin as bioactive compounds. In silico study revealed mechanism action of catechin as antihyperlipidemic using 2 pathways, inhibition of the enzyme HMG-CoA reductase and increase the LDL receptors. In vitro studies of catechin are able to inhibit lipid absorption in the intestine through inhibition of pancreatic lipase activity, lipid hydrolysis and emulsification, micelle cholesterol deposition. Pre-clinical tests on animals showed that the ethyl acetate fraction of gambir leaves was able to reduce the levels of total cholesterol, triglycerides, LDL and increase blood plasma HDL. The long-term use of gambir leaves has been proven to be safe, not mutagenic, no hematological, clinical biochemical abnormalities and no abnormalities in the vital organs of the animal models.

Keywords: Uncaria gambir Roxb., treatment, hyperlipidemia
INTRODUCTION

Gambir (Uncaria gambir Roxb.) is a plant from the Rubiaceae family which is a semi-vine herbaceous plant with an elongated branching, oval-shaped leaves with tapered ends, hairless (slippery) leaf surface, short leaf stalks, the flowers are compounded with a pink or green crown, short petals, funnel-shaped crown (like a coffee flower), five stamens, and a capsule with two chambers. The fruit is round like an egg, about 1.5 cm in length and black.¹

Gambir contains active compounds with pharmacological effects. Gambir extract is one of the industrial plant commodities that has high economic value and good prospects for farmers and foreign suppliers. In Indonesia, gambir is commonly used by people for betel nut, but actually the use of gambir itself exceeds that in its development gambir is often used for drugs in treating burns, headaches, diarrhea, dysentery, mouth sores, skin injury, as mouthwash, to facilitate the process digestion in the stomach and intestines.²³ Several recent studies reported that Gambir have potential effect as antioxidant, antibacterial and antihyperlipidemic.⁴⁵⁶

Hyperlipidemia is one of the main risk factors for cardiovascular disease. Until now, hyperlipidemia can be controlled by diet, exercise and synthetic drug therapy. Many synthetic drug therapies are known to have side effects. The hyperlipidemia drug from statin group that is widely used as the main choice of therapy is simvastatin, but this drug has side effects such as myopathy, hepatotoxicity, peripheral neuropathy, dizziness, diarrhea and allergies.⁷ Currently, there are many research on medicinal plants potencies which have the same effects as synthetic drugs, but with less side effects.

Gambir leaf extract contains catechin as the main compound as well as several other compounds such as catechu tannic acid, quercetin, red catechu, fluorescent gambir, fat and wax.⁸ Catechin is secondary metabolite compound derived from flavonoids which has antihyperlipidemic activity. The administration of tea extracts containing catechin significantly reduced cholesterol levels in blood serum and rat liver compared to those without the test sample.⁹ Catechin can reduce the formation of cholesterol from mevalonate through inhibition of the activity of HMG-CoA reductase.¹⁰ Catechin compounds contained in grape seeds have cholesterol-lowering activity by inhibiting pancreatic cholesterol esterase, binding of bile acids, and reducing the solubility of cholesterol in micelles to delay cholesterol absorption.¹¹

The high content of catechin in gambir leaves makes gambir a potential plant to be used as raw material for hyperlipidemia drugs. This review aimed to examine the pharmacological potential of gambir as a treatment for hyperlipidemia from the results of in silico, in vitro and in vivo studies of its pharmacological effects and safety so as to provide evidence of scientific information to the community.
METHODS
This literature review is a study of scientific articles on gambir plants that have been published in various scientific journals. The study starts with collecting literature and then conduct a literature review related to pharmacological effects and safety. The literature studied was obtained from journals, research reports, and proceedings, both national and international, published between 2010 and 2020, which are available on Pubmed and Google Scholar. The number of literatures reviewed is 28 research articles covering phytochemical compound (9 studies) and pharmacological effects (19 studies) based on the results of in silico, in vitro and in vivo study as well as their safety from the results of toxicity tests.

RESULTS
A large amount of information of gambir as a treatment for hyperlipidemia drugs has been obtained in this literature review. The information were grouped based on its phytochemical compound and pharmacological effects. The pharmacological effects in this study were detailed into in silico study, in vitro study, in vivo study, and safety test.

a. Phytochemical Compound
Previous study that identified the chemical content of gambir reported two active polyphenols of the flavonoid group, (+) - catechin and (+) - epicatechin, characterized from dried water extract. Kassim reported that the ethyl acetate extract has highest radical scavenging activity which is 88.63 % at 50 ppm followed by methanol extract. Other study reported that the DPPH radical scavenging activity of various gambir extract ranged from 92.0 to 93.1% and the catechin content ranged from 99.4 to 108.5 µg/ml. Catechin has been reported as the main bioactive compounds and has been used as metabolite biomarker to determine the quality of gambir. A comprehensive study by Nonaka found a new bioflavonoid compound, gambiriin. Gambir also contains the yellow flavonoid quercetin, as well as gambirdin and isogambirdin.

Andasuryani et al. also showed catechins as constituents of the most abundant content in gambir besides epicatechin and gambiriin. A further study isolated (+) - catechin, (+) epicatechin, and seven dimer flavins known as gambiriins A1, A2, B1, B2, procyanidin B1, procyanidin B3, and gambiriin C from aqueous extracts. Through rapid chromatography, four new indole alkaloids, namely gambirtannine, dihydrogambirtannine, oxogambirtannine, and neoxygambirtannine were identified from aqueous extracts of leaves and stems. Table 1 shows the phytochemical content of the active compounds identified from the gambir plant.

b. Pharmacological Effects
In Silico Study
The mechanism of catechins as an antidyslipidemia can be explored using a molecular docking study which is an in-silico model study used to screen compounds based on their mechanism of action against target proteins. In this study, catechin compounds were analysed molecularly using Molecular on Environment (MOE) software to knowing the power of affinity and interaction with the enzyme HMG-CoA reductase and LDL receptors which play role in cholesterol metabolism. The results of molecular docking showed that the catechin interaction with the HMG-CoA reductase enzyme and the LDL receptor had Gibbs values of -6.5758 kcal/mol and -16.1709 kcal/mol, respectively. The potential mechanism action of catechin as antidyslipidemia uses two pathways, inhibition of the enzyme HMG-CoA reductase and increase in LDL receptors.

An in silico study conducted by Isnawati et al. showed that the Gibbs energy values of simvastatin, atorvastatin, catechin gallate, epicatechin gallate, gallochatechin gallate and epigallocatechin gallate of -6.4974; -8.543; -9.5736; -10.6395; -10.4765; and -10.598 kcal/mol, respectively. The Gibbs energy value shows that the bond strength and inhibiton of catechin derivatives against HMG-CoA reductase are better than those of Simvastatin and Atorvastatin.
Another in silico docking study over flavonoids group including catechin were carried out using AutoDock 4.2. Three important parameters, i.e binding energy, inhibition constant and intermolecular energy were determined. The results showed that all the selected flavonoids binding energy were in range -6.98 to -5.06 kcal/mol, when compared with that of the standard (-4.11 kcal/mol). Inhibition constant (7.69 to 193.95 µmol/L) and intermolecular energy (-7.87 to -6.85 kcal/mol) of the flavonoids also coincide with the binding energy. In the cholesterol esterase assay, \( \text{IC}_{50} \) value of chalconewas found to be (18.30±0.31) µg/mL, whereas that of gallic acid was (759.69±31.56) µg/mL. All the remaining compounds exhibited \( \text{IC}_{50} \) values ranging from (32.90±0.06) µg/mL to (166.35±4.10) µg/mL.

### Table 1. Chemical Compounds Isolated from Gambir (Uncaria gambir Roxb.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Group</th>
<th>Extract</th>
<th>Part of Plant</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Catechin</td>
<td>Flavonoid</td>
<td>Water</td>
<td>Leaves and young twigs</td>
<td>HPLC</td>
</tr>
<tr>
<td>(-)-Epicatechin</td>
<td>Flavonoid</td>
<td>Water</td>
<td>Leaves and young twigs</td>
<td>Crystallization</td>
</tr>
<tr>
<td>Gambiriin A1, A2, A3, B1, B2, B3</td>
<td>Flavonoid</td>
<td>Water</td>
<td>Leaves and young twigs</td>
<td>NMR</td>
</tr>
<tr>
<td>Gambiriin C</td>
<td>Flavonoid</td>
<td>Water</td>
<td>Leaves and young twigs</td>
<td>NMR</td>
</tr>
<tr>
<td>Gambiriin A1, A2, B1, B2</td>
<td>Flavonoid</td>
<td>Water</td>
<td>Leaves and young twigs</td>
<td>HPLC</td>
</tr>
<tr>
<td>Procyanidin B1, B3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gambirine</td>
<td>Flavonoid</td>
<td>Water</td>
<td>Leaves and young twigs</td>
<td>HPLC</td>
</tr>
<tr>
<td>Isogambirine</td>
<td>Flavonoid</td>
<td>Water</td>
<td>Leaves and young twigs</td>
<td>HPLC</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Flavonoid</td>
<td>Water</td>
<td>Leaves</td>
<td>MS</td>
</tr>
<tr>
<td>Roxburghine A, B, C, D, E</td>
<td>Alkaloid</td>
<td>Water</td>
<td>Leaves</td>
<td>NMR</td>
</tr>
<tr>
<td>Gambirtannine</td>
<td>Alkaloid</td>
<td>Water</td>
<td>Leaves</td>
<td>HPLC</td>
</tr>
<tr>
<td>Dihydrogambirtannine</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Oxogambirtannine</td>
<td></td>
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<tr>
<td>Neoxygambitannine</td>
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All the selected flavonoids contributed cholesterol esterase inhibitory activity.\textsuperscript{20}

**In Vitro Study**

In vitro study on catechin was significantly inhibit lipid absorption in the intestine. The mechanisms include inhibition of pancreatic lipase activity, lipid hydrolysis, and emulsification in the intestine and precipitation of cholesterol micelle.\textsuperscript{21, 22}

Catechin administration can significantly reduce LDL levels. Catechin plays a role in regulating genes that can metabolize total cholesterol thus decreased LDL levels in blood plasma due to catechin activity in lipogenesis gene expression.\textsuperscript{23}

**In Vivo Study**

The catechin activity test of the gambir plant was conducted by Yunarto \textit{et al}, by administration ethyl acetate fraction to the rats that were previously made hyperlipidemic by feeding them with high lipids and cholesterol diets. The study used 36 white male rat strain Sprague Dawley aged 2.5 months which were completely randomized into six groups: normal, aquadest control, positive control (simvastatin 2 mg / kg BW), dose I (fraction 5 mg / kg BW), dose II (fraction 10 mg / kg BW) and dose III (20 mg / kg BW). Rats were induced with a diet containing cholesterol and saturated fat for 28 days, except for aquadest control. Furthermore, the rats were given the test material for 28 days. The results showed that compared to the aquadest control and positive control, the dose fraction of 20 mg / kg BW was significantly able to reduce the levels of total cholesterol, triglycerides, LDL and increase HDL (p < 0.05).\textsuperscript{24}

Another study also strengthened the pharmacological effects of the gambir plant as an antihyperlipidemic. Experimental research by Sari \textit{et al} (2018) uses a post test group design approach. The study was conducted on 25 rats which were divided into 5 groups, negative control group (K-), positive control (K +), as well as catechin groups of 20 mg/kg, 40 mg/kg and 80 mg/kg. All the quails were fed on fat-rich forage, except those in the control group. In the end of the sixth week of medication, lipid levels in serum, aorta and cardiac muscle were detected, and the livers were investigated. The result revealed that fatty degeneration in each catechin group was lower than that of the model group (H=42.98, q=6.90-10.95, p < 0.05), so did for the liver coefficient (F=17.15, q=3.00-4.92, p < 0.05).\textsuperscript{25}

The treatment group was then administered with catechin isolates at a dose of 10 mg / kg / day, 20 mg / kg / day, and 40 mg / kg / day for 14 days. The results showed a decrease in triacylglycerol levels after catechin isolates administration. Triacylglycerol levels in positive control group (K+) 147.8 ± 8.5 mg / dL were higher compared to the negative control group and the treatment group, 104.6 ± 15.3 mg / dL, 101.4 ± 15.7 mg / dL, 106.4 ± 17.6 mg / dL, and 110 ± 3.2 mg / dL, respectively. There were significant differences in the P1, P2, and P3 groups with the positive control group (p < 0.05).\textsuperscript{4}

An in vivo study examining the effect of catechin isolates on total cholesterol levels were conducted on 30 individuals which were divided into five groups, K-, K +, P1, P2, P3. Groups K+, P1, P2, and P3 were feeding with a high-fat diet for 14 days. Then administered with catechin gambir isolates at a dose of 10 mg / kg, 20 mg / kg, and 40 mg / kg for P1, P2, and P3 respectively for 14 days. The results showed a decrease in total cholesterol levels of serum after catechin isolates administration. The mean of total cholesterol level in serum of negative control was 70.03 mg / dl, positive control 82.03 mg / dl, treatment one 70.21 mg / dl, treatment two 69.4 mg / dl, and treatment three 70.4 mg / dl. There was a significant difference in the positive group, treatment 1, treatment 2, and treatment 3 (p < 0.05).\textsuperscript{26}

Catechin also shows an effect on regulation of blood fat and prevention of atheroschlerosis in an experimental atherosclerosis model over 120 male quails. They were randomized to control group, model group, lovastatin (79.5 mg/kg) group, as well as catechin groups of 20 mg/kg, 40 mg/kg, and 80 mg/kg. All the quails were fed on fat-rich forage, except those in the control group. In the end of the sixth week of medication, lipid levels in serum, aorta and cardiac muscle were detected, and the livers were investigated. The result revealed that fatty degeneration in each catechin group was lower than that of the model group (H=42.98, q=6.90-10.95, p < 0.05), so did for the liver coefficient (F=17.15, q=3.00-4.92, p < 0.05).\textsuperscript{26}
Another study of catechin against atherosclerosis in mice showed that the combined administration of catechins and caffeine has the inhibitory effect on the development of atherosclerosis in mice.\textsuperscript{27}

In vivo study of catechin effect inhibit the increase of cholesterol level also reported by Mawarti \textit{et al} in male wistar rats given high-fat diet. The results showed that EGCG lowers cholesterol levels (p<0.05). Cholesterol levels decreased significantly by 55\% (p=0.00) at a dose of 8 mg/kg BW when compared to the control group (+).\textsuperscript{28}

Several other studies were by Suzuki \textit{et al}. reported methylated catechins direct the strong lipid-lowering activity in mice; Labdi \textit{et al} confirmed the protective role of green tea intake in against atherosclerosis on hypercholesterol-fed rat; Ahmad \textit{et al} reported the drinks supplemented with catechins and EGCG are effective against obesity, hypercholesterolemia and hyperglycemia in rats.\textsuperscript{29, 30, 31}

\textbf{Safety Test}

A study by Hasti, Muhtar and Bakhtiar (2012) reported that gambir extract has activity as a protector of mice liver cells at doses of 30, 100, and 300 mg / kg BW.\textsuperscript{32} A mutagenicity test using mutant bacteria strains of \textit{Salmonella typhimurium} TA 98, TA 100, \textit{Escherichia coli} WP2 uvrA using Amest test method showed that gambir extract is not mutagenic based on mutagenic test results and calculation of the value of fold increase (over baseline).\textsuperscript{33} The acute toxicity test and long-term (sub chronic) toxicity test were carried out according to the BPOM guidelines. The results of a research conducted in 2016 showed that the results of the acute toxicity test for ethyl acetate fraction of gambir leaf extract up to a dose of 5500 (> 5000 mg / kg BW), thus it was declared safe and included in the practical non-toxic category.\textsuperscript{34} The acute toxicity test and long-term (sub chronic) toxicity test were carried out according to the BPOM guidelines. The results of a research conducted in 2016 showed that the results of the acute toxicity test for ethyl acetate fraction of gambir leaf extract up to a dose of 5500 (> 5000 mg / kg BW), thus it was declared safe and included in the practical non-toxic category.\textsuperscript{34}

\textbf{DISCUSSION}

Phytochemical analysis showed that the major polyphenolic compound of gambir is catechin, where the highest content is in leaves and young twig. Based on antioxidant activity test, catechins are capable of obstructing and scavenging free radicals.\textsuperscript{3, 12} The amounts of active ingredients in medicinal plants are always fairly low. There is an urgent need to develop effective and selective methods for the extraction and isolation of bioactive natural products. The selection of the solvent is crucial for solvent extraction.\textsuperscript{36} The ethyl acetate extract of gambir contain the highest catechin. Ethyl acetate has lower polarity index followed by methanol and water. Differences in the polarity of the extraction solvents could cause a wide variation in the level of bioactive compounds in the extract.\textsuperscript{37} Solvent with higher molecular weight has lower polarity which allows other substances of about the same molecular weight to be easily extracted.\textsuperscript{3} Catechin contains higher molecular weight, this can be attributable to higher solubility of catechin in ethyl acetate than the other solvents.

In silico study was carried out by computational simulation using two target proteins with the intention of comparing the ability of catechin to reduce total cholesterol levels in blood plasma through two possible pathways, namely inhibiting the action of the HMG-CoA reductase enzyme and increasing the action of the LDL receptor. The Gibbs energy value below zero (0) indicates the spontaneous reaction of the formation of a bond between the compound and the target protein. The lower Gibbs free energy value indicates that the reaction bond occurs more spontaneous and easier so that the bond is more stable. Thus, catechin bind more easily to LDL receptors compared to the enzyme HMG-CoA reductase.\textsuperscript{18}
It can also be seen that the group which plays a role in bonding is the hydroxyl group of the catechin, but the amino acids that play a role in the two bonds are different. The mechanism of action of natural compounds in increasing the activity of LDL receptors is by binding to ERE, Sp-1 or Ap-1 at the LDL receptor which acts as a promoter at LDL receptors. Increased expression of LDL receptors will trigger an increase in LDL metabolism so that LDL levels in plasma going decrease. The more bonds formed, the more stable the catechin binds to the HMG-CoA reductase enzyme and provides a stronger pharmacological effect. The presence of hydrophobic bonds in the catechin interaction with the enzyme HMG-CoA reductase makes the surrounding hydrogen bonds loosen so that the required bond energy increases. This can be seen from the value of Gibbs free energy from the two bonds which concluded that catechin binds more easily to LDL receptors.18

Catechin has inhibition activity to the action of HMG-CoA reductase which results in reduced mevalonate synthesis from HMG-CoA. The results of research conducted by Ikeda stated that the decrease in total cholesterol levels in rat blood plasma was due to catechins being able to effectively inhibit cholesterol absorption in the intestine.38

The mechanism action of catechin in reducing triglyceride levels by inhibiting the accumulation of free fatty acids in the liver and stimulating thermogenesis, which is an increase in fat burning during resting conditions.39 In addition, research conducted by Mustofa explained that the decrease in triglycerides was caused by catechin being able to inhibit the absorption of exogenous fats from high cholesterol and fat diets.40

Synthesis of cholesterol is carried out in four stages and begins with acetyl-CoA. The first stage is the synthesis of mevalonate from acetyl-CoA; the second stage converts mevalonate to activated isoprenes; the third stage of squalene synthesis; the fourth stage of cholesterol synthesis. HMG-CoA plays a role to control the rate of cholesterol synthesis. By inhibiting HMG-CoA, it will reduce the synthesis of mevalonate so that the cholesterol will be reduced.24,41

Studies indicated that catechins in green tea contribute to cholesterol lowering effect by affecting lipids absorption in small intestine. Possible mechanisms are inhibiting lipids hydrolysis, inhibiting luminal emulsification, reducing micellar solubility and precipitating cholesterol from micelles.42 Catechin can reduce cholesterol content in micelles by forming insoluble precipitation with cholesterol, thereby reducing the absorption of cholesterol in the intestine. The adsorption energies between catechins and cholesterol are obviously stronger than that of cholesterol themselves, indicating that catechin has an advantage in reducing cholesterol micelle formation.43

CONCLUSION

Based on this review studies, showed a very strong potential of the gambir plant to be used in the treatment of hyperlipidemia with catechin as its bioactive compounds was shown. In silico, in vitro and in vivo studies on animal pharmacological showed mechanisms in reducing blood lipid levels through inhibition of the enzyme activity of HMG-CoA reductase and Lipase. Long-term use of gambir leaves is safe and does not cause abnormalities of the vital organs of the animal models. However, since there is no study on its clinical trial, clinical trial are needed. The potentials of gambir as antihyperlipidemia in this review should facilitate its commercialization for therapeutic applications.

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