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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Submitted: 16-02-2021, *Revised*: 27-18-2020, *Accepted*: 30-09-2021

DOI: https://doi.org/10.22435/mpk.v31i3.4472

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

Abstrak

Gambir (Uncaria gambir Roxb) adalah tumbuhan perdu dari suku Rubiaceae. Di Asia termasuk Indonesia, secara empiris ekstrak gambir digunakan sehari-hari untuk menyirih. Kandungan flavonoid katekin yang tinggi dalam gambir memiliki efek farmakologi dalam pengobatan hiperlipidemia yang berpotensi untuk dikembangkan menjadi obat tradisional. Kajian literatur ini bertujuan untuk mengkaji potensi farmakologi gambir sebagai terapi pengobatan hiperlipidemia berdasarkan hasil studi efek farmakologi in silico, in vitro, in vivo efek farmakologi dan keamanannya, sehingga memberikan bukti informasi ilmiah kepada masyakarat. Literatur yang digunakan dalam proses review meliputi literatur dengan berbasis bukti baik farmakologi maupun keamanan yang tersedia di Pubmed dan Google Scholar. Hasil

kajian menunjukkan potensi yang sangat kuat dari tanaman gambir dalam pengobatan hiperlipidemia dengan katekin sebagai senyawa bioaktif utama. Studi in silico menunjukkan mekanisme aksi katekin sebagai antihiperlipidemia menggunakan dua jalur yaitu penghambatan enzim HMG-CoA reduktase dan peningkatan reseptor LDL. Studi in vitro katekin mampu menghambat penyerapan lipid di usus melalui penghambatan aktivitas lipase pankreas, hidrolisis lipid dan emulsifikasi, serta pengendapan kolesterol misel. Studi in vivo menunjukkan fraksi etil asetat daun gambir mampu menurunkan kadar kolesterol total, trigliserida, LDL dan meningkatkan HDL plasma darah. Penggunaan daun gambir dalam jangka panjang terbukti aman, tidak mutagen, tidak ditemukan kelainan hematologi, biokimia klinis dan tidak menyebabkan kelainan organ vital hewan uji.

Kata kunci: Uncaria gambir Roxb.; pengobatan; hiperlipidemia

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.[2,3] Several recent studies reported that Gambir have potential effect as antioxidant, antibacterial and antihyperlipidemic.[4,5,6]

Hyperliperlidemia 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 antihiperlipidemic 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.[3] Kassim reported that the ethyl acetate extract has highest radical scavenging activity which is 88.63 % at 50 ppm followed by methanol extract.[3] 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.[12] Catechin has been reported as the main bioactive compounds and has been used as metabolite biomarker to determine the quality of gambir.[13] A comprehensive study by Nonaka found a new bioflavonoid compound, gambiriin. Gambir also contains the yellow flavonoid quercetin, as well as gambirdin and isogambirdin.[14] Andasuryani *et al.* also showed catechins as constituents of the most abundant content in gambir besides epicatechin and gambiriin.[15] A further study isolated (+) - catechin, (+) epicatechin, and seven dimer flavans known as gambiriins A1, A2, B1, B2, procyanidin B1, procyanidin B3, and gambiriin C from aqueous extracts.[4] Through rapid chromatography, four new indole alkaloids, namely gambirtannine, dihydrogambirtannine, oxogambirtannine, and neooxygambirtannine were identified from aqueous extracts of leaves and stems.[16] Table 1 shows the phytochemical content of the active compounds identified from the gambir plant.[8,17]

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 kacl/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.[18]

An in silico study conducted by Isnawati *et al.* showed that the Gibbs energy values of simvastatin, atorvastatin, catechin gallate, epicatechin gallate, gallocatechin gallate and epigallocatechin gallate of -6.4974; -8,543; -9,5736; -10.6395; -10,4765; and -10,598 kacl/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.[19]

Table 1. Chemical Compounds Isolated from Gambir (*Uncaria gambir* Roxb.)[8,17]

Compound	Group	Extract	Part of Plant	Method
(+)-Catechin	Flavonoid	Water	Leaves and young twigs	HPLC
		Water	Leaves and young twigs	Crystallization
		Water	Leaves	FT-NIR
		Water	Leaves	Spectroscopy
		Methanol	Leaves	Colorimetric
(-)-Epicatechin	Flavonoid	Water	Leaves and young twigs	Crystallization
		Water	Leaves and young twigs	HPLC
Gambiriin A1, A2, A3, B1, B2, B3	Flavonoid	Water	Leaves and young twigs	NMR
Gambiirin C	Flavonoid	Water	Leaves and young twigs	NMR
		Water	Leaves and young twigs	HPLC
Gambiriin A1, A2, B1, B2 Procyanidin B1, B3	Flavonoid	Water	Leaves and young twigs	HPLC
Gambirine	Flavonoid	Water	Leaves and young twigs	HPLC
		Water	Leaves	Spectroscopy
		Ethanol	Leaves	NMR
		Methanol	Leaves	NMR
Isogambirine	Flavonoid	Water	Leaves and young twigs	HPLC
		Water	Leaves	Spectroscopy
		Ethanol	Leaves	NMR
		Methanol	Leaves	NMR
		Methanol	Leaves	Colorimetric
Quercetin	Flavonoid	Water	Leaves	MS
		Water	Leaves	HPLC
Roxburghine A, B, C, D, E	Alkaloid	Water	Leaves	NMR
Gambirtannine Dihydrogambirtannine Oxogambirtannine Neoxygambitannine	Alkaloid	Methanol	Gambir powder	NMR, MS

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, 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 IC_{50} values ranging from (32.90±0.06) µg/mL to (166.35±4.10) µg/mL.

All the selected flavonoids contributed cholesterol esterase inhibitory activity.[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.[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.[23]

In Vivo Study

The catechin activity test of the gambir plant was conducted by Yunarto *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 ($p < 0.05$).[24]

Another study also strengthened the pharmacological effects of the gambir plant as an antihyperlipidemic. Experimental research by Sari *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 +), and 3 treatment groups (P1, P2, P3) were feeding with a high fat diet of beef brain for 14 days. 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 ($p<0.05$).[6]

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 ($p <0.05$).[25]

Cathechin also shows an effect on regulation of blood fat and prevention of atheroschlerosis in an experimental atheroschlerosis 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, $p<0.05$).[26]

Another study of catechin against atheroschlerosis in mice showed that the combined administration of catechins and caffeine has the inhibitory effect on the development of atherosclerosis in mice.[27]

In vivo study of catechin effect inhibit the increase of cholesterol level also reported by Mawarti *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 (+).[28]

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

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.[32] A mutagenicity test using mutant bacteria strains of *Salmonella typhimurium* TA 98, TA 100, *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).[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. While the results of sub-chronic toxicity test up to a dose of 985.6 mg / kgBW, based on clinical chemistry and microscopic observations on vital organs, revealed that it is safe for long-term consumption.[34]

A cytotoxicity test on HepG2 cells prior the in vitro anti-hyperlipidemic assay of catechin gallate showed no cytotoxic effects on HepG2 cells, thus might become an additional option for the treatment of hyperlipidemia.[35]

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.[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.[36] The ethyl acetate extract of gambir contain the highest catechin.[3] 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.[37] Solvent with higher molecular weight has lower polarity which allows other substances of about the same molecular weight to be easily extracted.[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.[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 promotor 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.

ACKNOWLEDGEMENT

The author would like to thank the Center for Research and Development of Biomedical and Basic Health Technology, Ministry of Health of the Republic of Indonesia for supported this study.

REFERENCES

1. Kasim A, Asben A, Mutiar S. Kajian kualitas gambir dan hubungannya dengan karakteristik kulit tersamak. Majalah Kulit, Karet, dan Plastik. 2015;31(1):55-64.

2. Rauf A, Siregar AZ. The condition of Uncaria gambir Roxb. as one of important medicinal plants in North Sumatra Indonesia. Procedia Chemistry. 2015;14:3-10

3. Kassim JN, Hussin MH, Achmad A, Dahon NH, Suan TK, Hamdan HS. Determination of Total Phenol, Condensed Tannin and Flavonoid Contents and Antioxidant Activity of Uncaria Gambir extracts. Indones J Pharm 2011; 22(1): 50–59.

4. Amir M, Mujeeb M, Khan A, Ashraf K, Sharma D, Aqil M. Phytochemical analysis and in vitro antioxidant activity of Uncaria gambir. International Journal of Green Pharmacy. 2012;6(1):67-72

5. Melia S, Novia D, Juliyarsi I. Antioxidant and antimicrobial activities of gambir (uncaria gambir roxb) extracts and their application in rendang. Pakistan Journal of Nutrition. 2015;14(12):938.

6. AlioesY, Sukma RR, Sekar SL.. Effect of Gambir Catechin Isolate (Uncaria Gambir Roxb.) Against Rat Triacylglycerol Level (Rattus novergicus). In IOP Conference Series: Earth and Environmental Science. 2019;217(1):1-6

7. Fadecko J, Singh RB, Chaithiraphan S, Vargova V, Tomlinson B, De Meester F, et al. Clinical manifestations of adverse effects of statins, oxidative stress and possible role of antioxidants in prevention. The Open Nutraceuticals Journal. 2010;3:154-65.

8. Zhang Q, Zhao JJ, Xu J, Feng F, Qu W. Medicinal uses, phytochemistry and pharmacology of the genus Uncaria. Journal of Ethnopharmacology. 2015;173:48-80.

9. Jiang Y, Ding S, Li F, Zhang C, Sun-Waterhouse D, Chen Y, Li D. 2019. Effects of (+)-catechin on the differentiation and lipid metabolism of 3T3-L1 adipocytes. Journal of Functional Foods. 2019;62:103558.

10. Suganya S, Nandagopal B, Anbarasu A. Natural Inhibitors of HMG□CoA Reductase—An Insilico Approach Through Molecular Docking and Simulation Studies. Journal of cellular biochemistry. 2017;118(1):52-7.

11. Ngamukote S, Mäkynen K, Thilawech T, Adisakwatanna. Cholesterol-lowering activity of the major polyphenols in grape seed. Molecules. 2011;16(6):5054-61.

12. Anggraini T, Tai A, Yoshino T, Itani T. Antioxidative activity and catechin content of four kinds of Uncaria gambir extracts from West Sumatra, Indonesia. African Journal of Biochemistry Research. 2011;5(1):33-8.

13. Ibrahim N, Yusoff NZM, Ahmad R. Quantification of Catechin in Leaves and Stems of Malaysian Uncaria Gambir (HUNTER) Roxb. by HPLC-DAD. Malaysian J Anal Sci. 2016;20(3):567–72.

14. Nordin NS, Thurairajah N, Rahim ZHA. Antioxidant and Cytoprotective Activities of Piper Betle, Areca Catechu, Uncaria Gambir and Betel Quid with and without Calcium Hydroxide. BMC Complement Altern Med. 2013;13(1):351.

15. Andasuryani, Purwanto YA, Budiastra IW, Syamsu K. Determination of Catechin Content in Gambir Powder from Dried Gambir Leaves Quickly Using FT NIR PLS Model. Int J Adv Sci Eng Inf Tech 2014; 4(5): 303-307.

16. Saad, MFM, Goh HH, Rajikan R, Yusof TRT, Baharum, SN, Bunawan H. Uncaria gambir (W. Hunter) Roxb: From phytochemical composition to pharmacological importance. Tropical Journal of Pharmaceutical Research. 2020;19(8):1767-73.

17. Musdja MY, Suryani N, Pitriyah. Anti-inlammatory activity of catechins isolate of Uncaria gambir Roxb on carrageenan induced paw edema in wistar male rats. International Journal of Recent Advances in Multidisciplinary Research. 2019;6(1):4518-21.

18. Adelina R, Kurniatri AA. Mekanisme Katekin sebagai Obat Antidislipidemia (Uji In Silico). Buletin Penelitian Kesehatan. 2018;46(3):147-54.

19. Isnawati A, Adelina R. Studi Docking Molekuler Catechin Gallate, Epicatechin Gallate, Gallocatechin Gallate, dan Epigallocatechin Gallate sebagai Obat Dislipidemia. Jurnal Kefarmasian Indonesia. 2015 Feb 28;5(1):25-32.

20. Sivashanmugam T, Muthukrishnan S, Umamaheswari M, Asokkumar K, Subhadradevi V, Jagannath P, et al. Cholesterol esterase inhibitory activity of flavonoids using in silico and in vitro studies. Asian Pac J Trop Dis. 2012;3(2):9.

21. Chen Y, She Y, Shi X, Zhang X, Wang R, Men K. Green tea catechin: does it lower blood cholesterol?. In IOP Conference Series: Earth and Environmental Science. 2020(August);559(1):012027.doi:10.1088/1755-1315/559/1/012027.

22. Jeon SY and Imm JY. Lipase Inhibition and Cholesterol-lowering Activities of Laccase-catalyzed Catechin Polymers. Food Sci. Biotechnol. 2014;23(5):1703-07.DOI 10.1007/s10068-014-0232-z.

23. Kim A, Chiu A, Barone MK, Avino D, Wang F, Coleman CI, et al. Green tea catechins decrease total and low-density lipoprotein cholesterol: a systematic review and meta-analysis. Journal of the American Dietic Association. 2011;111(11):1720-9.

24. Yunarto N, Elya B, Konadi L. Potensi Fraksi Etil Asetat Ekstrak Daun Gambir(Uncaria gambir Roxb.) sebagai Antihiperlipidemia. Jurnal Kefarmasian Indonesia. 2015;5(1):1-10.

25. Ar Birru AB. Pengaruh Isolat Katekin Gambir (Uncaria gambir Roxb.) terhadap Kadar Kolesterol Total Serum Tikus Rattus novergicus Galur Wistar Jantan dengan Diet Tinggi Lemak (Doctoral dissertation, UNIVERSITAS ANDALAS). 2018.

26. Yu CG, Li RY, Liu ZT, Gao H, Liang H, Liu K. Effects of catechin on blood fat level in experimental atherosclerosis model. Acta Acad. Med. 2012;48:324–26.

27. Liu L, Nagai I, Gao Y, Matsushima Y, Kawai Y, Sayama K. Effects of catechins and caffeine on the development of atherosclerosis in mice, Bioscience, Biotechnology, and Biochemistry. 2017;81:10. DOI: 10.1080/09168451.2017.1364618.

28. Mawarti H and Ratnawati R. Penghambatan peningkatan kadar kolesterol pada diet tinggi lemak oleh epigallocatechin gallate (EGCG) teh hijau klon Gmb4. Prosiding Seminas Competitive Advantage. 2021;1(2).

29. Suzuki T, Kumazoe M, Kim Y, Yamashita S, Nakahara K, Tsukamoto S. Green tea extract containing a highly absorbent catechin prevents diet-induced lipid metabolism disorder. Sci Rep. 2013;3:2749. doi: 10.1038/srep02749.

30. Labdia A, Amialib M, Bachirc YN, Merouaned A, Zouambie AD, Koceir EA, et al. Green tea extract attenuates non alcoholic fatty liver disease by decreasing hyperlipidemia and enhancing superoxide dismutase activity in cholesterol-fed rats. Mediterranean Journal of Nutrition and Metabolism. 2018;11(3):295-306.

31. Ahmad RS, Butt MS, Sultan MT, Mushtaq Z, Ahmad S, Dewanjee S, et al. Preventive role of green tea catechins from obesity and related disorders especially hypercholesterolemia and hyperglycemia. Journal of Translational Medicine. 2015;13:79. doi: 10.1186/s12967-015-0436-x.

32. Hasti S, Muchtar H, Bachtiar A, Uji Aktivitas Hepatoproteksi dan Toksisitas Akut dari ekstrak Gambir Terstandarisasi. Jurnal Penelitian Farmasi Indonesia. 2012;1(1):34-38.

33. Sulistyaningrum N. Rustanti L, Alegantina S. Uji Mutagenik Ames untuk Melengkapi Data Keamanan Ekstrak Gambir. Jurnal Kefarmasian Indonesia. 2013;2(1):36-45.

34. Yunarto N, Winarno M, Intan PR. Uji Toksisitas Akut dan Subkronis Fraksi Etil Asetat Ekstrak daun Gambir. Laporan Penelitian, Badan Litbang Kesehatan; 2016: Jakarta.

35. Zhang QW, Lin LG, Ye WC. Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review [Internet]. Chinese Medicine. 2018 [cited 2021 Aug 25];13:20. available from: https://doi.org/10.1186/s13020-018-0177-x.

36. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of Severinia buxifolia. Journal of Food Quality. 2019 [cited 2021 Aug 25];2019:8178294. Available from: https://doi.org/10.1155/2019/8178294.

37. Kah Hui C, Majid NI, Mohd Yusof H, Mohd Zainol K, Mohamad H, Mohd Zin Z. Catechin profile and hypolipidemic activity of *Morinda citrifolia* leaf water extract. Heliyon. 2020 Jun 28;6(6):e04337. doi: 10.1016/j.heliyon.2020.e04337.

38. Ikeda I. Multifunctional effects of green tea catechins on prevention of the metabolic syndrome. Asia Pacific Journal of Clinical Nutrition. 2018;17(1):273-4. 15.

39. Dulloo AG, Seydoux J, Girardier L. Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. International Journal Obesity Relation Metabolism Disorder. 2010;24:252-8.

40. Mustofa UE. Effect of green tea and green tea rich with catechin on blood glucose levels, serum lipid profile and liver and kidney functions in diabetic rats. Jordan Journal of Biological Sciences. 2014;7(1):7-12.

41. Chen ZY, Ma KY, Liang Y, Peng C, Zuo Y. Role and classification of cholesterol-lowering functional foods. Journal of Functional Foods. 2011 Apr 1;3(2):61-9.

42. Zheng XX, Xu YL, Li SH, Liu XX, Hui R, Huang XH. Green tea intake lowers fasting serum total and LDL cholesterol in adults: a meta-analysis of 14 randomized controlled trials. The American Journal of Clinical Nutrition. 2011;94(2):601-10.

43. Zheng K, Guo K, Xu J, Liu W, Chen J, Xu C, et al. Study on the interaction between catechin and cholesterol by the density functional theory. Open Chemistry. 2020;18(1):357-68.