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Evaluation Of Antilipid Peroxidation Activity Of Gambir Extract On Liver Homogenat In Vitro

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Abstract: Gambir [*Uncaria Gambir* (Hunter) Roxb] is a plant with specific location in Southeast Asia region proven widely used in medication. The activity of gambir as antioxidant had been studied with some type mechanisms but the activity as antilipid peroxidation had not been evaluated yet. Five sort of etanolic gambir extracts of gum gambir (gambir) and dried leaves were screened for lipid peroxidation inhibition on rat liver homogenate in vitro at the concentrations of 20, 50 and 100 ppm with malondialdehyde (MDA) level as measured parameter. Total phenolic content of all extracts were determined with Folin-Ciocalteu reagent and (+)catechin were by HPLC method. These results showed that the antioxidant activities were dose-dependent manner ag1000inst Fe^{2+}-induced lipid peroxidation. The 96% ethanolic extract of gambir (EtOH 96% gambir) exhibited the highest activity with IC50 value of 24.6 ppm that was equivalent to positive control Polyphenon60. Analysis of total phenolic content demonstrated that this extract contained 98% phenolic compounds in which 59% of this was (+)-catechine. All samples did not show pro-oxidant activity at the highest concentration. Therefore, this extract was potential to be developed as antilipid-peroxidation.
Keywords: Gambir [*Uncaria Gambir* (Hunter) Roxb], ethanolic extract, antilipid peroxidation, phenolic compound, (+)-catechin.

Introduction

Gambir known as *Uncaria Gambir* (Hunter) Roxb belonging to Rubiaceae family constituted a type of specific location plant in Southeast Asia, particularly in Indonesia and Malaysia[1,2]. Phytochemical study showed that the mayor compound contained in this plant were flavonoids in which flavon-3-ol derivative called (±)-catechin was around 40-80% of dried water extract besides (+)epicatechin 1,5% and gambiriin B1, B3, A1 about 1%, respectively[3,4]. The content of (+)-catechin dependeded on extraction methods and source of gambir come from. Hayani E.[3] proved that extraction with hot water from commercial gambir yielded the highest (+)-catechin content that reached around 7-88% depended on the source of gambir. Among the compounds, (+)-catechin and tannins (tannic acid) were the most useful of gambir constituents[5].

Pharmacological studies showed that gambir extract demonstrated some activities in preventing some damage caused of free radical-mediated process including antibacterial[6], inhibitor of alpha-glukosidase[7], anti-plaque of teeth[8], inflammation[9]. Most of the pharmacological effects of gambir flavonoid compounds seemed to be associated with the potencies as antioxidant[10].

Previous studies had elaborated antioxidant activity of gambir extract and (+)-catechin. Ethyl acetate extract of various commercial gambir from Indonesian market indicated antioxidant activity with IC50 value in range of 4.6 – 18.2 ug/mL by DPPH method[11]. Whilst claimed that IC50 values depended on the type of solvent. IC50 values of ethyl acetate and ethanolic extracts of Indonesian local gambir demonstrated different values even though not significant, namely, 20.70 and 16.88 ug/mL by DPPH method, repectively, and (+)-catechine standar was 15.88 ug/mL[7]. Amir *et.al* [12] studied the activity of methanolic gambir extract as antioxidant with some tests *in vitro* and their result showed that this extract demonstrated inhibition activity in range of 30% - 47% at 50 ppm of tested concentration. Whilst, Anggraini T.[13] claimed that inhibitoion value of commercial gambir using DPPH method was 40% at 250 ppm. However, the antioxidant activity in particular as an inhibitor of lipid peroxidation had not been studied yet. This paper was aimed to elaborate the efficacy of gambir to inhibited lipid peroxidation on liver homogenate in vitro. The protective effect of gambir extract in lipid peroxidation was evaluated by measuring the formation of the secondary component (malondialdehide) of the oxidative process on lipid matrix.

Method

Material

Materials and chemical reagents were obtained from following companies: 1-methyl-2-phenylindole, 1,1,3,3-tetramethoxypropane, trizma base, FTI-276 trifluoroacetate salt HPLC, folin–ciocalteu reagent, gallic acid, Polyphenon-60 (Sigma Aldrich), acetonitrile and methanol HPLC, HCl p.a., $NaH_2PO_4.H_2O$ p.a., $Na_2HPO_4.7H_2O$ p.a., trichloroacetic acid, $FeSO_4$ (Merck), butylated hydroxytoluene pharmaceutical grade, NaCl pharmaceutical grade, ether technical grade (local market), Na_2CO_3 (MP Biomedical). Gambir leaves and twigs were gained from Payah Kumbuh-West Sumatra.

Sample preparation

Gambir purposed here was prepared based on Indonesia Herbal Pharmacopeia[14] and stated as gambir (G). Gambir dried leaves were prepared by drying gambir leaves in oven at temperature under 50^{o}C for 24-48 hours and grinding them until obtained dried mass. Gambir ethanolic extracts were prepared as following method. Each fifty-five gram of gambir and gambir dried leaves powder were extracted with ethanol 96% and 50% at room temperature for 24 hour with 200 rpm agitation. Each filtrate was separated and evaporated at 45^{o}C with vacuum until semisolid mass was gained. To get the dried extract, all samples were kept at oven with temperature of 40^{o}C for 24 hour until dried mass was gained. All five samples obtained were coded as following G (gambir), EtOH 96% gambir (96% ethanolic extract of gambir), EtOH 50% gambir (50% ethanolic extract of gambir), EtOH 96% leave (96% ethanolic extract of gambir leaves), EtOH 50% leave (50% ethanolic extract of gambir leaves).

Determination of total phenolic content

Determination of total phenol was based on previous studied with slightly modification[15]. Briefly, into 2.0 mL disposable plastic microtube, 20 uL of each sample tested (20, 50, 100 ppm), 1.580 uL aquabidest, 100 uL the Folin-Ciocalteu reagent were put in order and mixed well. After 30 sec to 8 min left, 300 uL Na_2SO_4 20% was added to all tubes and shook to mix well for starting the reaction. The reaction was incubated at 40^{o}C for 30 minutes and read the absorbance of each reaction at 765 nm. Samples were dissolved in DMSO (40%). Blank solution was prepared by replacing the sample with aquabidest. Gallic acid stock was prepared by dissolving 0.5 g gallic acid with 10 mL ethanol and adding with aquabidest until 100 mL. The calibration curve was carried out by diluting gallic acid stock with aquabidest into a range of final concentration 1, 2, 3, 5, 8, 10 ppm and then plotting their absorbance vs. concentration to obtain standard curve equation. The concentration of total phenol was calculated from the equation and reported as percent of Gallic Acid Equivalent (GAE)/each gram of the extract as following equation, % total phenol content = (ug/mL GAE / ug/mL extract) x 100%.

Determination of catechin content

(+)Catechin content of samples was determined by HPLC method. Analysis was carried out on RP C18 column with gradient system. Acetonitrile and triflouro acetate 0.1% were used as eluent and set to a flow rate of 1 mL/min. The mobile phase was 20 minutes (1-54% gradient of acetonitrile:triflouro acetate 0.1% for 12 minutes first, followed by 1:99% for 8 minutes of the last). Wavelength for the maximum absorption of (+)-catechin was determined using the UV-visible spectrophotometer at 278 nm. Samples and (+)-catechin standard were dissolved in methanol to make a concentration stock of 20 mg/mL. For calibrating curve, the standard stock solution was diluted with methanol to final concentration at the range of 125-2000 ug/mL

Test animals

All animal testing procedures have to followed in accordance to The Center of Pharmaceutical and Medical Technology-BPPT Guide for the Care and Use of Laboratory Animals. Male rats (*Sprague dawley* strain) of 150-200 g body weight, at 2-3 month of age were gained from The National Agency of Drug and Food Control Republic of Indonesia for in vitro studies. The animals were kept in separate polycarbonate cages with free access to water and food *ad libitum*, in a room with controlled temperature (22˚C±3˚C) and in 12 h light/dark cycle before used.

Antilipid peroxidation test *in vitro*

Antilipid peroxidation tests were conducted on liver homogenate induced with Fe^{2+} ion *in vitro*. Preparation of liver homogenate was conducted based on previous research with slight modifications[16,17]. The livers from three healthy male rats sacrificed with ether anesthetion were quickly removed, perforated with cold saline and blotted until dry with clean tissue paper. Liver tissue was homogenized in cold 0.9% NaCl pH = 7 (1:5; w/v) solution using glass micropestle in ice bath and centrifuged at 4000 x g at 4^{o}C temperature for 10 minutes to obtain supernatant and its could be stored in -70^{o}C for further test.

Antilipid peroxidation reaction consisted of 400 uL of liver homogenate, 50 uL of Fe^{2+} 250 mM, and 50 uL of each gambir extract (the final concentration was 20, 50 and 100 ppm). Gambir extracts was dissolved in DMSO (40%) and added 0.9% NaCl pH = 7 solution to give a certain concentration. Fe^{2+} was an optimal value for lipid peroxidation test from a range concentration of 100-3,000 mM. As a control was designed by replacing gambir extract with 0.9% NaCl at pH = 7 solution. This reaction was carried out at 37^{o}C for 1 h with agitation of 200 rpm, stopped using 125 uL TCA 100% and centrifuged at 13.000xg at 4^{o}C temperature for 5 minutes to gain supernatant for MDA level determination. Experiments were performed in five replications.

MDA level was determinate by *N*-Methyl-2-phenylindole method as stated by Inoue T. and teams[18]. Briefly, into 2 mL disposable plastic tube, 200 uL of supernatant, 650 uL of 8.6 mM *N*-methyl-2-phenylindole in acetonitrile/methanol (3:1, v/v), 10 uL butylated hydroxytoluene (BHT) and 150 ul of concentrated HCl were placed in this order. This reaction was carried out in a thermoshaker at temperature of 45^{o}C for 60 minutes with agitation of 200 rpm. The color produced was measured by spectrophotometer at λ = 586 nm. The control, without extract, was prepared similar to that procedure above. As a blank, the supernatant was replaced with NaCl 0.9% pH 7.

To determine MDA level, the calibration curve of 1.1.3.3-tetrametoxipropane (TMOP) in 10 mM Tris-HCl was done at a range of final concentration of 0, 0.5, 1, 2, 3, 4 uM. Using the amount of MDA, all results were stated as % of antilipid peroxidation that was calculated using this equation[19]: % antilipid peroxidation = 1 –[MDA]sample/[MDA]control x 100%. Where [MDA]sample = MDA concentration of tested sample, [MDA]control = MDA concentration of control.

Results and discussions

Sample preparations and determination of total phenol and (+)-catechin contents

Five sort of gambir extracts reproduced in this experiment were gambir (G), 96% and 50% ethanolic extract of gambir leaves (EtOH 96% leave and EtOH 50% leave), 96% and 50% ethanolic extract of gambir (EtOH 96% gambir and EtOH 50% gambir) in which all of them were in the form of dried powder. All source materials were powdered and screened using mesh50 sieve. The extract rendement was presented in Table 1.

Table 1. The yield of gambir extract rendement

Type of samples	Source material	The rendement (%)
EtOH 96% leave	dried leaves powder	16%
EtOH 50% leave	dried leaves powder	13%
Gambir	fresh leaves and twigs	8%
EtOH 96% gambir	dried gambir powder	76%
EtOH 96% gambir	dried gambir powder	67%

The rendement of etahnolic extracts that were obtained from leaves were lower than gambir (Table 1). The results showed that the EtOH 96% gambir demonstrated higher of rendement compared to EtOH 50% gambir. The possible explanation for this result was the polarity of 96% ethanol as same as with polarity of some compounds in dried gambir powder.

Table 2 showed of total phenolic and (+)-catechin content of gambir extracts. The total amount of phenolic varied among the gambir extracts range from 53-98% in which EtOH 96% gambir exhibited the highest total phenolic content about 98%. Both of 96% and 50% ethanolic extracts of gambir leaves (EtOH 96% leave and EtOH 50% leave) yielded the same total phenolic content, around 52-53%.

(+)-Catechin content of samples that was stated as % of dried material weight widely demonstrated various values, range from n.d. (non-detected) to 59 %. Three samples (gambir, EtOH 96% gambir and EtOH 50% gambir) gave almost the same (+)-catechin level, around 50%. Whilst, two other extracts of dried leaves (EtOH 96% leave and EtOH 50% leaves) were lower than others, i.e. 13% and no-detected in 96% ethanolic extract and 50% ethanolic extract, respectively.

In this experiment, the content of (+)-catechin from gambir (gambir) was higher than that of previous one reported by Widiyarti G. *et.al*[20]. They claimed that (+)-catechin level of some commercial gambir was in range of 7-42%. The differences seemed to be due to the different sources of gambir, the axtraction process and the analyzing method. In addition to, the (+)-catechin level of etanolic extracst (EtOH 96% gambir and EtOH 50% gambir) in this experiment was lower than that of Widiyarti G.'s experiment using ethyl-acetate as eluen, i.e. 91-97%. It was due to the solubility of (+)-catechin in ethyl-acetate was higher than in alcohol 96%.

Table 2. Percentage of total phenolic and (+)-catechin content in gambir extracts

Type of samples	% Total phenol content	% (+)-Catechin content
EtOH 96% leave	53±0,33	13
EtOH 50% leave	52±0,33	n.d.
Gambir	87±0,24	59
EtOH 96% gambir	98±0,24	59
EtOH 96% gambir	82±0,06	51
Polyphenon-60	95±0,12	--

n.d. : not detected. % total phenol content = (ug/mL GAE / ug/mL extract) x 100%.
Values were expressed as mean (n=3).

Antilipid peroxidation test of gambir extracts *in vitro*

Lipid peroxidation test is a type of an antioxidant evaluation method by using the rich lipid media. The oxidation of lipid could be induced by some metals[10], or chemicals (tert-butylhydroperoxide (t-BuOOH)[21], 2,20-azobis (2-amidinopropane) dihydrochloride[22]. These tests will produce a secondary component of MDA in addition to the primary component like alcoxy compounds-LOOH. The ability in suppressing lipid peroxidation is characterized by decreasing of these compounds level.

The supernatant of liver homogenate was one of vulnerable media for evaluating the damaging effect of reactive species such as hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide substances (H_2O_2)**.** This media that was prepared with low rate centrifugation could serve the source of water, protein, lipids, ribonucleic acid (DNA and RNA) for lipid peroxidation test. This low rate media also gave other advantages, it's easier and cheaper in preparation compared to others[16,22].

The role of transition metals in the oxidation process has been intensely studied. These metal ions generate free radicals by decomposition of H_2O_2 or lipid hydroperoxide (LOOH) to produce hydroxyl and alcoxy radicals, respectively[10,23]. In this experiment, Fe^{2+} metal ion (FeSO4) was used as an oxidative induction. The concentrations of Fe^{2+} 100, 250, 500, 1000 and 3000 uM evaluated for determining the optimal concentration. The optimal concentration of Fe^{2+} required for induction of the highest level of MDA was at 250 uM (Figure 1). The higher concentration of MDA, above 250 uM, exhibited a saturated dose. This value would be used for further experiments.

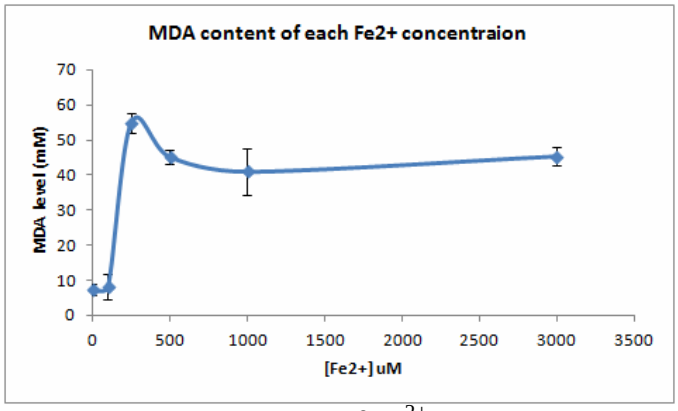

Figure 1. The optimation of Fe^{2+} concentration on lipid peroxidation.
Values stated mean values (n=5)

The oxidative mechanism of Fe^{2+} ion in lipid is claimed trough endogenous H_2O_2 to produce extremely reactive free hydroxyl radical, $OH^{\bullet}$ via the Venton reaction[16]. Furthermore, $OH^{\bullet}$ will steal hydrogen atom from bis-allyl position of lipid from neighboring molecule to generate lipid radical ($L^{\bullet}$). This reaction only took one million of a second. The presence of oxygen, $L^{\bullet}$ will become the $LOO^{\bullet}$ form. Lipid radicals will take other hydrogen atom of bis-allyl position of lipid source to be LOOH and $L^{\bullet}$. The reaction is taking place in cycle and will stop if the lipid source had been exhausted or the presence of antioxidants. If the lipid is from membrane of cell, oxidation process caused cell damage and apoptosis[16,24].

From five extracts that had already been tested, the effect of gambir in retaining MDA production presented by % antilipid peroxidation was conducted in three concentrations of 20, 50, and 100 ppm on liver homogenate induced by Fe^{2+} ion (Tabel 2). All tested concentrations demonstrated that the antioxidant activities were dose dependent manner against Fe^{2+}-induced lipid peroxidation. As a positive control was used the tea extract Polyphenon-60® with 95% total phenol content. Of the all concentrations, EtOH 96% gambir demonstrated the highest antioxidant activity with 24.6 ppm of IC50 value in which this value was equivalent to positive control. The potential antioxidant was indicated in the following decreasing order EtOH 96% gambir > gambir > EtOH 50% gambir > EtOH 96% leave > EtOH 50% leave.

Antioxidant strength of the samples was in line with the total phenolic content. The 96% ethanolic extract of gambir showed the highest both total phenol content and antioxidant activity (Figure 2). Although both ethanolic 96% gambir extract and gambir had the same (+)-chatecin content in which each of them was about 59%, but the IC50 values of both samples were different about 24.6 and 32.3 ppm, respectively. The possible reason was suggested that the antioxidant activity not only determined from (+)-chatecin content but also from other compound in gambir extract. This was consistent with previous studies that total phenol compounds were responsible for the antioxidant activity. Many studies indicate a linear relationship between total phenolics and antioxidant activity. Study antioxidant activity of commercial gambir from two regions of Sumatra-Indonesia (Payakumbuh and Padang Panjang) using DPPH method was consistent with these experiments in which IC50 of Payakumbuh's gambir with 41% of catechin content was higher than that of Padang Panjang's gambir (7% of catechin content), i.e. 10 vs 40 ug/mL[11]. These results were also supported by Oliveira and teams[25] in which there was a strong correlation between total polyphenol contents and the antioxidant activity (r^2 = 0.929) of *Sidastrum micranthum*; as well as *Wissadula periplocifolia* (r^2 = 0.814) extracts.

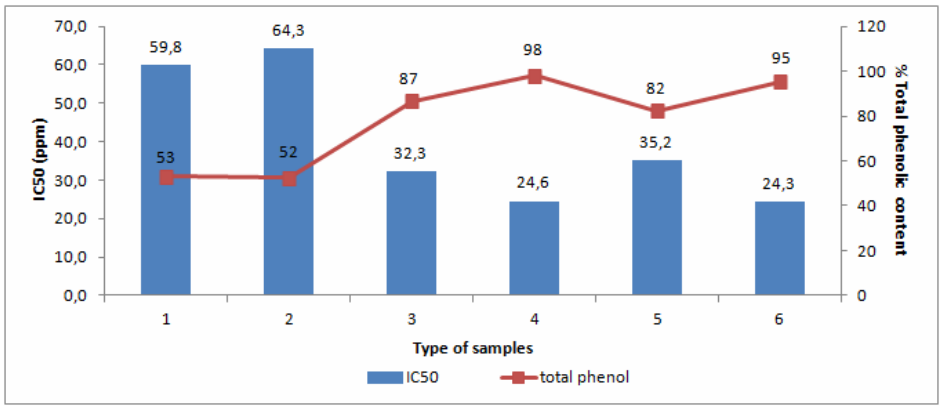

Figure 2. Antilipid peroxidation activity of gambir extracts at 50 ppm and total phenol content of gambir extract sample. (1) 96% ethanolic extract of gambir leaves. (2) 50% ethanolic extract of gambir leaves. (3) Gambir. (4) 96% ethanolic extract of gambir. (5) 50% ethanolic extract of gambir. (6) Polyphenon-60®. Data showed mean value of five independent replications. IC50 value were mean values of 5 independent experiments.

Another study showed that the activity of lipid peroxidation inhibitor from flavonoids had been deeply elaborated. Flavanoids were claimed having two mechanisms in suppressing lipid peroxidation, by scavenging for free radical and chelating metal ions[26]. Sugihara N. *et.al*.[26] stated that the main role of antilipid peroxidation of flavonoid is due to their ability to chelate metal ions rather than to scavenge them. The antilipid peroxidation effects of some flavonoids such as luteolin, taxifolin, apigenin, crysin and epigalocatechin declined with raising Fe^{2+} ion concentration on α-linolenic acid (LNA)-loaded cultured rat hepatocyte, indicating that the chelating ability of them was linked in their antioxidant activity. The complex bond between flavonoid and Fe^{2+} that was formed could be able to inhibit lipid peroxidation by restricting this ion toward LOOH in liposome, microsomes and cultured cells of hepatocyte[10]. These flavonoid compounds could become a metal ion complex because of the hydroxyl group in the ring of flavonoid structure. It had been proven that the three domain groups of ring structure contributed in chelating metal ion by hydrogen bonding i.e. 1) between the 3',4'-dihydroxy group on the B-ring, 2) between the 3-hydroxy and 4-carbonyl group on the C-ring, and 3) between the 5-hydroxy and 4-carbonyl group on the C-ring[26].

These studies were not in accordance with the previous paper that this experiment did not show no pro-oxidative activity of all gambir extract at high concentration of 100 ppm. Sugihara N. *et.al*.[26] demonstrated that (+)-catechin, the major compound of gambir, exhibited pro-oxidative effect at the concentration of 100 uM on $FeSO_4$-induced lipid peroxidation in LNA-loaded hepatocytes. The difference results were due to gambir contain other chemical compounds that be able to reduce the possibility of side effects caused by (+)-catechin. Biological activity of plant-derived extracts that commonly have a lot of compounds is a resultant from effect of each contained compound. Each compound is possible to prove the synergism effects and or lowering the risk effect so that the final results are getting well-balanced of the efficacy from total compound. This theory is known as SEES (side effect eliminating substance) that is commonly found in natural products[27]. There were many reports related to various oxidative properties of flavonoid compounds. They can alter their activities from antioxidant to pro-oxidant depend on some conditions, such as their physicochemical properties, their concentration, the concentration and sort of the metal ion used to induce lipid peroxidation, the molar ratio between flavonoid compound and metal ion, the substrate of lipid peroxidation reaction and the presence of chelating compounds[10,28].

Conclusions

From these experiments, it could be concluded that five types of gambir extracts at concentration of 20, 50, 100 ppm exhibited antilipid peroxidation against Fe^{2+}-induced lipid peroxidation at concentration of 250 uM. The activity was by means of the dose dependent manner without pro-oxidant effects at highest tested concentration. Ethanolic 96% extract of gambir demonstrated the highest antioxidant properties with IC50 value of 24.6 ppm in equivalent to Polyphenon-60 as positive control. The extract contained 98% total phenolic compound in which 59% of it was (+)-catechine. Therefore, this extract was potential to be developed as antilipid-peroxidation for properties and/medicinal or neutraceutical purposes.

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References

1. Sugiyama S., Recognize *Uncalia ganbir*, Yakushigaku Zasshi, 2004, 39(2), 355-62. (Abstract).
2. Hussin M.H., Kassim M.J., The corrosion inhibition and adsorption behavior of *Uncaria gambir* extract on mild steel in 1 M HCl. Materials Chemistry and Physics, 2011, 125 (3), 461–468.
3. Hayani E., Analisis kadar *catechin* dari gambir dengan berbagai metode [Analysis of catechin content of gambir by various methods]. Buletin Teknik Pertanian, 2003, 8(1), 31-33.
4. Taniguchi S., Kuroda K., Doi K., Inada K., Yoshikado N., Yoneda Y., Tanabe M., Shibata T., Yoshida T., Hatano T., Evaluation of gambir quality based on quantitative analysis of polyphenolic constituents. Yakugaku Zasshi, 2007, 127(8), 1291-300.
5. Bakhtiar A. Manfaat tanaman gambir [The benefit of Gambir]. [Azmi Dhalimi. Permasalahan gambir (*Uncaria gambir* L.) di Sumatera Barat dan alternatif pemecahannya. Perspektif, 2006, 5(1), 46 – 59]. Makalah Penataran Petani dan Pedagang Pengumpul Gambir di Kecamatan Pangkalan Kab. 50 Kota., 1991, Padang.
6. Pambayun R., Gardjito M., Sudarmadji S., Kuswanto K.R., Kandungan fenol dan sifat antibakteri dari berbagai jenis ekstrak produk gambir (*Uncaria gambir* Roxb) [Phenolic content and antibacterial properties of various extracts of gambir (*Uncaria gambir* Roxb)], Majalah Farmasi Indonesia, 2007, 18(3), 141 – 146.
7. Apea-Bah F. B., Hanafi M., Dewi R.T., Fajriah S., Darwaman A., Artanti N., Lotulung P., Ngadymang P., Minarti B., Assessment of the DPPH and α-glucosidase inhibitory potential of gambier and qualitative identification of major bioactive compound, Journal of Medicinal Plants Research 2009, 3(10), 736-757.
8. Lucida H., Rustini, Saufitri D., Dachriyanus, Formulation of anti-plaque toothpaste from standardized gambir extract and its antimicrobial activity, Jurnal Farmasi Indonesia 2010, 5(2), 70-77.
9. Jo T.H., Woo S.S., Cha J.M., Kim D.S., Lee Y.C., Do S.G., Kim J.H., Kim T.W., Sung H.S., Composition for Suppressing Cyclooxygenase and/or 5-Lypoxygenase. US 20070264361 A1, 2007.
10. Sugihara N.(1), Arakawa T., Ohnishi M., FurunoK., Anti- and pro-oxidative effects of flavonoids on metalinduced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with α-linolenic acid. Free Radical Biology & Medicine, 1999, 27(11/12), 1313–1323.
11. Widiyarti G., Sundowo A., Hanafi M., The free radical scavenging and anti-hyperglycemic activities of various gambiers available in indonesian market, Makara, 2011, 15(2), 129-134.
12. 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.
13. 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), 33-38.
14. Anonim, Farmakope Herbal Indonesia [Indonesan Herbal Pharmacopea], Depkes R.I., 2008, ed 1, 17.
15. Andrew Waterhouse, Folin-ciocalteau micro method for total phenol in wine, Waterhouse Lab., University of California. (no date). http://waterhouse.ucdavis.edu/faqs/davis-info (accessed 17 October 2013).
16. Kade I.G., Ibukun E. O., Nogueira C.W., Rocha J. B. T., Sun-drying diminishes the antioxidative potentials of leaves of *Eugenia uniflora* against formation of thiobarbituric acid reactive substances induced in homogenates of rat brain and liver, Experimental and Toxicologic Pathology, 2008, 60, 365–371.
17. Braughler J.M., Duncan L.A., Chase R.L., The involvement of iron in lipid peroxidation: Importance of ferric to ferrous ratios in initiation, The journal of biological and chemistry, 1986, 261(22), 10282-10289.
18. Inoue T., Ando K., Kikugawa K.. Specific Determination of Malonaldehyde by *N*-Methyl-2-phenylindole or Thiobarbituric Acid, JAOCS, 1998, 75(5), 597-600.
19. Singh R.P., Murthy K.N.C., Jayaprakasha G.K., Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models, J Agric Food Chem., 2002, 50(1), 81-6.

20. Widiyarti G., Sundowo A., Hanafi M., The free radical scavenging and anti-hyperglycemic activities of various gambiers available in Indonesian market, Makara sains, 2011, 15(2), 129-134.
21. Nuutila A. M., Pimia R.P., Aarni M., Caldentey K.M.O., Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity, Food Chemistry, 2003, 81, 485–493.
22. Kulisic T., Radonic A., Katalinic V., Milos M., Use of different methods for testing antioxidative activity of oregano essential oil, Food Chemistry, 2004, 85, 633–640.
23. Halliwell B., Gutteridge J.M.C., Oxygen toxicity, oxygen radicals, transition metals and disease: Review. Biochem. J., 1984, 219, 1-14.
24. Yoon J.H., Lee M.S., Kang J.H.,. Reaction of ferritin with hydrogen peroxide induces lipid peroxidation. Department of Genetic Engineering, Cheongju University, Cheongju, Korea (no date) [accessed from http://www.bmbreports.org/fulltext/bmbreports/view.php?vol=43&page=219], 13 Oktober 2013.
25. Oliveira A.M.F., Pinheiro L.S., Pereira C.K.S., Matias W.N., Gomes R.A., Chaves O.S., Souza M.F.V., Almeida R.N., Assis T.S., Total Phenolic Content and Antioxidant Activity of Some Malvaceae Family Species, *Antioxidants* 2012, *1*, 33-43.
26. Sugihara N.(2), Ohnishi M., Imamura M., Furuno K., Differences in Antioxidative Efficiency of Catechins in Various Metal-Induced Lipid Peroxidations in Cultured Hepatocytes, Journal of Health Science, 2001, 47(2), 99-106.
27. Christine T. Balancing power and weakness: social and cultural aspects of Malay Jamu in Singapore. Actes du 2e Colloque Europeen d'Ethnopharmacologie et de la 11e Coference international d'Ethnomedicine, Germany, Heidelberg, 1993, 34-42, [accessed from http://horizon.documentation.ird.fr/exl-doc/pleins_textes/pleins_textes_6/colloques2/010005517.pdf.], 3 March 2013.
28. Yoshida Y., Furuta S., Niki E., Effects of metal chelating agents on the oxidation of lipids induced by copper and iron, Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism, 1993, 1210(1), 81-88, (Abstract).

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