

A Review: Antioxidant Activity and Isolation of Compounds from Gambir (Uncaria Gambir(Hunter) Roxb.)

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ABSTRACT: Gambir (*Uncaria gambir* (Hunter) Roxb.) is certainly considered one among Indonesia's export commodities which in general grows in West Sumatra. Gambir is wealthy in flavonoids which include quersetin and catechins, that have antioxidant activity, anti-inflammatory, anti-bacterial, anti-most cancers and anti-diabetic properties. From the results of qualitative analysis, gambier contains quinones, terpenoids, alkaloids, tannins, flavonoids and saponins. Gambier extract contains several components, namely catechin, catechu tannic acid, quercetin, red catechu, flouresin gambier, ash, fat and wax. Phytochemical analysis shows that the main antioxidant compounds in *Uncaria gambir* are catechins, the dominant secondary metabolites. Catechins are able to block and clean up free radicals. Apart from catechins, other isolation results were obtained, namely (+) -epicatechin and several other flavan dimer such as gambiriin A1, A2, B1, B2, procyanidin B1, procyanidin B3, gambiriin C, gambirflavan D1 and gambirflavan D2 using HPLC (High Performance Liquid Chromatography), FT-NIR and Chromatography. This review has discussed the antioxidant activity and isolation of compounds from gambier (*Uncaria gambir* (Hunter) Roxb.). The most widely used method of determining antioxidants in vitro is 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is a strongloose radical, because of the delocalization of spare electrons for the duration of the molecule. But other than that, there are numerousdifferenttechniques that may be used to decide antioxidant activity, a number ofthat are 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferrous Reducing Antioxidant Power (FRAP), Total Phenolic Content (TPC) and the Response Surface Method (RSM).

Keywords: Antioxidants, Catechins, *Uncaria gambir* (Hunter) Roxb.

I. INTRODUCTION

Gambir (*Uncaria gambir*(Hunter) Roxb.) is certainly considered one among Indonesia's export commodities which in general grows in West Sumatra[1].This plant is very often used as traditional medicine and is prescribed by itself to relieve minor ailments such as fever, diarrhea, headaches, colds, coughs, and stomach aches. Herbal medicine is also used as a health supplement in promoting health and maintenance of physical health [2].This traditional management was carried out in the seventeenth century in Sumatra and the Malay Peninsula [3].

Uncaria gambir(Hunter) Roxbis a vine, especially native to Southeast Asia, Malaysia and Indonesia[4].This plant belongs to the Rubiaceae family, genus *Uncaria*. This plant is also known as the Sarawak gambier or gambier in Malaysia and Indonesia [5].Gambir is wealthy in flavonoids consisting of quercetin and catechins, that have antioxidant, anti-inflammatory, anti-bacterial, anti-most cancers and anti-diabetic activities[6][7][8][9][10].From the results of the qualitative analysis conducted by[11]that gambier contains quinones, terpenoids, alkaloids, tannins, flavonoids and saponins.The content of flavonoids and tannins from Gambier is reported to have antioxidant properties[12].The antioxidant content depends on the extraction conditions[13].Gambier extract contains several components, namely catechin, catechu tannic acid, quercetin, red catechu, flouresin gambier, ash, fat and wax. The main content is catechin (7-33%) and catechu tannic acid (20-55%)[14].Catechins are polyphenolic compounds that are reported to have antioxidant activity[15].

The most widely used method of determining antioxidants in vitro is 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is a solidloose radical, because of the delocalization of spare electrons during the molecule. Delocalization withinside the DPPH molecule determines the

prevalence of a crimson color, with an absorbance band with a most of approximately 520 nm. When DPPH reacts with a hydrogen donor, a reduced (molecular) form (DPPH) is produced, observed through a lack of crimson color. Therefore, the lower in absorbance relies upon linearly at the antioxidant concentration. But aside from that, there are numerous different techniques that may be used to decide antioxidant activity, a number of which might be 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferrous Reducing Antioxidant Power (FRAP), Total Phenolic Content (TPC) and the Response Surface Method (RSM) [16][17][18][19]. Based on the description above, this review will discuss the antioxidant activity and

isolation of compounds from gambier (*Uncaria gambir* (Hunter) Roxb.)

II. METHOD

Data Collection

Literature search for information on antioxidant activity and isolation of compounds from *Uncaria gambir* using literature study techniques by searching for sources or literature in the form of primary data or official books and national and international journals for the last 20 years (2000-2020). The main references used in this review article were searched through trusted websites such as ScienceDirect, ResearchGate, Google Scholar, as well as other published and trusted journals.

ANTIOXIDANT ACTIVITY

No	Method	Solvent	Results	Reference
1	DPPH	Hot water	92-93%	[6]
2	DPPH	Ethyl acetate	88.63%	[20]
		Methanol	85.98%	
		Hot water	82.23%	
3	DPPH	Methanol	18.27 µg/ml	[21]
4	DPPH	Aquades: Ethanol (1:1)	49.70 ± 0.60%	[22]
		Ethanol:Ethyl Acetate (1:1)	49.52 ± 0.68%	
		Ethyl Acetate	50.13 ± 0.74%	
5	DPPH	Ethyl Acetate	31.35% 51.37% 92.24%	[23]
6	FRAP	Aquades	5717.8 ± 537.6 µmol Fe(II)/mg	[18]
	DPPH		6.4 ± 0.8 µg/mL	
	TPC (Total Phenolic Content)		1142.5 ± 106.8 µg TAE/mg	
7	DPPH	Ethyl Acetate	13.8 µg/ml	[24]
		Residue after the Ethanol extract	16.2 µg/ml	
		Aquades	27.4 µg/ml	
8	Metode Permukaan Respon (RSM)	Ethyl Acetate ; Ethanol (0.37:0.55)	82.735 ± 0.362 mg/ml	[19]
9		Ethyl Acetate	Dose 20 mg/kg BB	[25]
10	DPPH	Ethanol	92.54%	[26]
11	DPPH + Nanogold	Aquades	89.89%	[27]
12	TPC	Methanol	197.35 and 182.00 mg/g GAE	[28]
	DPPH		91.33 dan 91.55%	

Phytochemical analysis shows that the main antioxidant compounds in *Uncaria gambir* are catechins, the dominant secondary metabolites. Catechins are able to block and clean up free radicals. To prove the antioxidant properties of *Uncaria gambir*, an extract of gambier was made using a traditional method, namely by boiling the leaves and stems of gambier for 1.5 hours then squeezed to get the extract. Then the thick extract was put into 'paraku' (a special container for thick gambier extract from wood measuring 3 mx 30 cm × 10 cm (L×W×H)) for 24 hours. The extract is then printed and dried in the sun for approximately 3 days. Then the radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was carried out on four extracts of the popular gambier cultivar. Four cultivars of gambier showed the same antioxidant activity with a very significant ability to scavenge free radicals (92-93%)[6].

Gambier extract turned into made through grinding it right into a first-class powder and filtered thru a 250 µm sieve. Part of this gambier (5.0 g) turned into dissolved in 80°C distilled water (100 mL). The aggregate turned into shaken at 200 rpm (IKA® KS 260, Sweden) for 1 hour. Then it turned into transferred to a centrifugation tube and centrifuged for 5 minutes till a clean answer turned into obtained. The unsolved gambier turned into eliminated through filtering it thru a vacuum clear out out and the mom liquor turned into handled with n-hexane (50 mL, QR&C) 3 instances to do away with fats and oil from the extract. Purified water turned into freeze-dried (Labconco, USA). The ensuing dilute extract powder (1.0 g) turned into then dissolved in 3 distinct solvents, particularly ethyl acetate, methanol and warm water (50 mL, QR&C). Then the extract turned into focused at 50°C below low strain in a rotary evaporator (Heidolph, Germany). The focused extract is then dried in a single day in an oven (50 °C) and subsequently the dry extract is floor right into a best powder. *Uncaria gambir* which has been efficaciously extracted with 3 distinct solvents indicates that ethyl acetate extract has the best fee of radical scavenging interest of 88.63% at a attention of 50 ppm observed through methanol extract of 85.98% at a attention of fifty ppm. And minimum antioxidant interest became proven through warm water extract (82.23%) on the equal attention. It may be concluded that gambier extract the use of ethyl acetate solvent gives the best antioxidant interest as compared to different solvent extracts[20].

The consequences of the initial phytochemical screening confirmed that the

Uncaria gambir extract contained flavonoids, alkaloids and phenolics. In this take a look at, quantitative estimation of flavonoid and phenolic content

material become additionally executed through colorimetric approach, the usage of the reagent approach of aluminum chloride and Folin-Ciocalteu, respectively, to set up the connection among antioxidant interest, general phenolics and flavonoid content material. The general phenolic and flavonoid contents have been found, respectively, 18.37 ± 2.79 mg gallic acid equivalent (GAE)/g dry weight and 5.82 ± 2.23 mg habitual equivalent (RE)/g dry weight. Then the DPPH radical scavenging interest of *Uncaria gambir* (Hunter) Roxb extract become executed, in comparison with ascorbic acid as the same old which had the IC₅₀ cost of the extract and the same old become $18.27 \mu\text{g/ml}$ and $79 \mu\text{g/ml}$. The consequences received on this take a look at suggest that *Uncaria gambir* extract may be a capacity supply of herbal antioxidants[21].

The gambier is overwhelmed after which sifted (± 60 mesh), the sieve is within the shape of gambier powder. Gambier powder as a lot as 10 g dissolved in 100 ml of solvent aquadest, ethanol, ethyl acetate, a mixture of aquadest:ethanol (1:1), and a mixture of ethanol: ethyl acetate (1:1), stirred the use of a shaker water tub at temperature of 30°C, for one hour. Then it become filtered with Whatman No. 42. The residue is extracted once more 2 instances with 100 mL of solvent every. The extracted answer is then evaporated the use of a rotary vacuum evaporator, till the gambier extract is received in dry shape. In general, gambier extract has a DPPH radical scavenging interest this is better than routine ($32.07\% \pm 0.75\%$ and BHT ($22.04\% \pm 0.80\%$). Gambier extract which has the very best antioxidant interest is distilled water extract:ethanol (1:1), ethanol extract: ethyl acetate (1: 1), and ethyl acetate extract. The 3 sorts of extracts had DPPH radical scavenging sports that had been now no longer notably different, every of $49.70\% \pm 0.60\%$, $49.52\% \pm 0.68\%$, and $50.13\% \pm 0.74\%$. Meanwhile, distilled water extract ($42.45\% \pm 0.31\%$) and ethanol extract ($42.62\% \pm 0.55\%$) has a decrease DPPH radical scavenging interest than different gambier extracts. From those consequences it may be concluded that ethyl acetate extract has a better DPPH radical scavenging interest as compared to different solvents. DPPH radical, the extra the antioxidant interest[22].

In the studies of Melia et al, 2015, 1000 gr gambier pollen became located in a darkish bottle

(2.5L) which became then delivered with one thousand ml of ethyl acetate; the aggregate is stirred via way of means of swinging for 10 minutes. It became macerated for 36 hours (swung 3 instances a day) after which filtered with clear out paper; rubbish became re-macerated 3 instances. Filtration is smoothed with a rotary evaporator. Then the antioxidant interest take a look at became completed the usage of the DPPH radical scavenging interest technique with ascorbic acid as a comparison. Then the effects of % inhibition of gambier extract with concentrations of 15.625, 31.25, and 62.50 µg/ml had been 31.35, 51.37 and 92.24%, respectively. Then the ascorbic acid widespread curve became in comparison sequentially to attain the DPPH cost with an IC_{50} of 25.55 µg/ml. The effects confirmed that the cost of the DPPH radical scavenging interest of gambier extract became better than that of ascorbic acid. This approach that gambier extract has proper interest as an antioxidant [23].

A look at via way of means of Nur Sazwi et al., 2013, 5 samples (betel leaf, areca nut, gambier, betel and betel containing calcium hydroxide) had been extracted in deionized distilled water for 12 hours at 37°C. Antioxidant pastime changed into evaluated for the presence of radical scavenging pastime the usage of the DPPH take a look at, iron discount pastime the usage of the FRAP take a look at and overall phenolic content (TPC) changed into decided the usage of the Folin-Ciocalteu procedure. Among betel components, gambier indicates the best antioxidant (DPPH - $IC_{50} = 6.4 \pm 0.8$ µg/mL, FRAP - 5717.8 ± 537.6 µmol Fe (II)/mg) and overall phenolic content (TPC - 1142.5 ± 106.8 µg TAE/mg). In the DPPH and FRAP tests, it changed into confirmed that gambier changed into one of the betel components which confirmed barely higher antioxidant pastime than areca nut. The addition of DPPH gambier scavenger pastime is sort of similar to ascorbic acid, indicating a robust loose radical scavenger. The DPPH take a look at measures the capacity of the extract to donate hydrogen to the DPPH radical produced via way of means of bleaching the DPPH solution. The extra the bleaching action, the better the antioxidant pastime. DPPH and FRAP take a look at have a comparable response mechanism, specifically to lessen positive radicals (eg, iron ions and DPPH radicals). Thus the better the DPPH radical scavenging pastime, the better the iron-decreasing capacity [18].

The outcomes of studies carried out with the aid of using Apea-Bah et al., 2009 ethanol extract of industrial gambier have been extracted

with ethyl acetate. Ethanol and ethyl acetate extracts and water extracts after ethyl acetate extraction and residues from ethanol extraction have been examined totally free radical scavenging interest. the usage of 2,2-diphenyl-1-picrylhydrazyl (DPPH). It became located that everyone extracts had excessive interest for DPPH inhibition. Apart from the aqueous extract, 92% DPPH inhibited with the aid of using the extract became performed at 30 µg/ml. The ethanol and ethyl acetate extract had extensively higher ($p < 0.01$) DPPH inhibitory activity than the aqueous extract. The IC_{50} of the natural extracts and residues ranged from 13.8 to 16.2 µg/ml for DPPH inhibition while the aqueous extract became 27.4 µg/ml. From those research it may be concluded that the ethanol and ethyl acetate extracts of industrial gambier, in addition to their residues on extraction have the capacity to scavenge excessive reactive unfastened radicals inclusive of the ones produced with the aid of using DPPH and therefore, have excessive antioxidant pastime in vitro [24].

To get the best extraction conditions for phenolic compounds from gambier with high antioxidant activity, an optimization process was carried out using the response surface method (RSM) on three variables, namely the ratio of the solvent, the ratio of the sample to the solvent, and the extraction time with the response of antioxidant activity to anti-radical DPPH (IC_{50}). The extraction process was carried out using the maceration method with stirring at room temperature. The optimization result of 50 shows the quadratic regression polynomial equation, namely $Y = 137.25 + 5.18 (R \text{ solvent}) + 24.75 (R \text{ sample}) + 11.53 (\text{old ex}) + 6.17 (R \text{ solvent} \times \text{sample}) + 3.14 (R \text{ sample} \times \text{old ex}) + 13.79 (R \text{ sample} \times \text{old ex}) - 5.44 (R \text{ solvent}^2) - 15.33 (R \text{ sample}^2) - 5.42 (\text{Old ex}^2)$. The optimum conditions were achieved at a ratio of ethyl acetate to ethanol 0.37: 0.55, the ratio of the sample to the solvent 1: 2, and the extraction time of 20 hours. The validation results show the predictive value ($IC_{50} = 82.89$ mg/ml) of model 50 is not significantly different from the validation result value ($IC_{50} = 82.735 \pm 0.362$ mg/ml) [19].

The catechins from Gambier had been removed via way of means of partitioning approach with ethyl acetate as solvent. A overall of 25 male rats had been divided into five groups, every organization such as five mice. Catechins had been stopped in 0.5% Na CMC with a catechin dose of five mg/kg, 10 mg/kg and 20 mg/kg, for tremendous control, nutrition E suspension changed into used at a dose of 20 mg/kg in Na-CMC 0.5%

and for poor control, 0.7% Na-CMC suspension changed into used. The checkpractisechanged into administered orally as soon asin line with day for 7 days. The statistical checkoutcomes of catechin gambir isolates confirmedthat everyonecheck doses of catechin gambir isolates had antioxidant consequences and had been substantiallyunique from poor controls ($p < 0.05$). Catechin doses of 5, and 10 mg/kg BW did now no longer display great variations with nutrition E as a fine control, whilst the catechin dose of 20 mg/kg b.w gave the most powerful antioxidant impact and had a great distinction with the positive control ($p < 0.05$). It can be concluded that the catechin gambier has a stronger potential as an antioxidant than vitamin E [25].

From testing the antioxidant activity of the research of Irma and Ahmad [26], it was found that isolate 1, which contained more -OH groups, had a much higher antioxidant activity than isolate 2, which was indicated by the percent inhibition value of isolate 1 of 92.54%, while for isolate 2 only 3.27% at the same concentration. This significant difference supports the theory that antioxidant activity depends on the number of -OH groups [26].

In Sari & Taufikurohmah's research, 2019 [27] antioxidant activity was determined by the percentage of free radical scavenging. The results showed that the addition of nanogold to gambier extract had an effect on increasing antioxidant activity. Nanogold testing of the antioxidant activity of gambier extract was carried out by preparing each sample and DPPH with a 1:1 ratio,

namely 10 mL of sample from 5 mL of nanogold at a concentration of 5-25 ppm with 5 mL of 100 ppm gambir extract solution and 10 mL of 0.003% DPPH solution. Put in 5 different dark bottles. The answer combination changed into shaken till homogeneous and allowed to face for 30 minutes in order that the DPPH interacted with the samples, specifically nanogold and gambir. The best nanogold concentration that supports the antioxidant activity of gambier extract is 25 ppm and has the best free radical scavenging results, which is 89.89%. The results showed that the greater the nanogold concentration, the greater the percentage of attenuation.

Gambir is the dried extract of Uncaria gambier leaves and branches. Pesisir Selatan and Fifty Cities are production centers for gambier in West Sumatra, Indonesia. This study identifies gambier from Pesisir Selatan and Lima Puluh Kota of West Sumatra Indonesia for its quality. Gambier analysis includes antioxidant activity, total polyphenol content and catechin content. Catechin content was determined by High Performance Liquid Chromatography (HPLC). The results showed that the antioxidant activity, total polyphenol and catechin content of gambier from the Pesisir Selatan and Fifty Cities areas were 91.33 and 91.55%, 197.35 and 182.00 mg/g GAE and 50.87, respectively. 55.40%. From these results it can be judged that the quality of gambier from the cities of Pesisir Selatan and Lima Puluh Kota is influenced by the raw material [28].

CHEMICAL COMPOSITION

Compound	Class	Extract	Parts of Plants	Identification Method	Reference
(+)-Catechin	Flavonoid	Aqueous	Young leaves and twigs	HPLC	[29]
		Aqueous	Leaf	FT-NIR	[30]
(+)-Epicatechin		Aqueous	Young leaves and twigs	HPLC	[29]
Gambirin A1 Procyanidin B1, B3		Aqueous	Young leaves and twigs	HPLC	[29]
Gambirin A1, A2, B1, B2 Procyanidin B1, B3 Gambirin C	Flavonoid	Aqueous	Young leaves and twigs	Chromatography	[31]
Catechin-(4 α -8)-ent-epicatekin, Gambirflavan D1 dan Gambirflavan D2		Aqueous	Young leaves and twigs	Chromatography	[32]

Table 1. Isolation of compounds from gambier

In the study of Taniguchi et al., 2007 [29] Polyphenol constituents were analyzed in 31 gambier and related products to determine the evaluation method, because gambier contained a large number of polyphenol constituents. The

general flavan content material within the samples found out the use of the vanillin-HCl estimation technique ranged from 24-79 %. The opposite segment excessive overall performance

liquid chromatography (RP-HPLC) evaluation confirmed that catechins had been the maximum plentiful parts in every sample, with contents within the variety 7-76 %. The content material of catechins in gambier merchandise among the primary and 0.33 quartiles is 28-54%. Thus, the decrease restriction of catechin content material in gambier merchandise may be set at round 20 for great management. Fifteen samples examined have been subjected to HPLC evaluation to expose the presence of epicatechin (mean 1.5%) and the dimer compounds procyanidin B1, procyanidin B3, and gambirinin A1 (about 1% each).

Conventional gambier manufacturing method regularly produces gambier with low catechin content material. The engineering of the gambier leaf manufacturing method to provide gambier powder has been advanced via way of means of preceding researchers. The motive of this examine is to expand a calibration version to expect catechin content material in dry gambier leaf powder quick the usage of the PLS FT-NIR version. The reflectance spectrum of the dried gambier leaf powder became acquired at a wavelength of one thousand to 2500 nm. The pre-processing spectra processing approach used is a mixture of the normalization approach among zero and one the primary approach of 9-factor Savitzky-Golay derivative (dg1). The consequences confirmed that the correlation coefficient and preferred blunders of prediction (SEP) had been 0.99, and 2.10%, respectively acquired while 6 partial least square (PLS) elements had been used. The advanced calibration version has proven better accuracy and precision to expect the catechin content material of gambier powder from dried gambier leaves [30].

The gambier manufacturing product (2 kg) became homogenized in MeOH (22 l) at room temperature to supply an extract (1.3 kg). Part of the extract (309 g) became given a Dia-ion HP-20 column (10x60 cm; solvent, H₂O - MeOH in incremental gradient mode). (+) -catechin (1) became crystallized from eluate (61 g) with 20% MeOH, and mom liquor (14 g) became chromatographed on Toyopearl HW-40 (3.0x40 cm; solvent, 70% EtOH) to present a fragment containing monomers and flavan dimers (2 g). The fractions have been then chromatographed at the ODS column (solvent, 10-30% MeOH), and the mixed fractions (frs) 21-37 (409 mg), 38-47 (167 mg), and 51-78 (849 mg) of the column have been similarly chromatographed. within the ODS and Sephadex LH-20 columns to supply

procyanidin B3 (3) (58 mg) (from frs 21-37), procyanidin B1 (4) (26 mg) (from frs 38-47), and gambirinin A1 (6) (90 mg), and Gambirinin C (5) (17 mg) (from frs 51-78). The mixture of frs 79-99 (139 mg) became similarly chromatographed on silica gel-ODS and MCI-gel CHP-20P to yield (+) -epicatechin (2) (12 mg). Eluate with 40% MeOH (52 g) from a Dia-ion column HP-20 became chromatographed on a Toyopearl HW-40 column (3.040 cm; solvent, 70% EtOH) to supply a dimer-containing fraction (4.5 g). The dichromatographic fraction at the MCI-gel CHP-20P column (solvent, 30-50% MeOH), and the mixed frs 33-103 (1.6 g) became similarly chromatographed at the ODS, and the Toyopearl HW-40 and Sephadex LH-20 columns for gave gambirinin A2 (7) (57 mg). Combined 133-186 (544 mg) of MCI gel column chromatographed in Toyopearl Column HW-40, Sephadex LH-20, and MCI-gel CHP-20P, and similarly purified via way of means of HPLC on YMC Pack ODS A-324 column (10x300 mm) with solvent extension H₂O - HCOOH - CH₃CN (81.95: 0.05: 18) and H₂O - HCOOH - MeOH (74.95:0.05:25) gave gambirinin B1 (8) (8.8 mg), and gambirinin B2 (9) (9.8mg). The outcomes of this examine received the outcomes of isolation (+) -catechin, (+) epicatechin, and 7 dimer flavans called gambirinin A1, A2, B1, B2, procyanidin B1, procyanidin B3, and gambirinin C from water extract [31].

A further study was carried out by Taniguchi et al., 2008 [32] That the gambier became extracted with MeOH, and the extract became given Dia-ion HP-20 CC.2 20% MeOH eluate (14.07 g) from the Dia-ion HP-20 column chromatographed on a Toyopearl HW-40 column, after elimination of one through crystallization. The fractions acquired have been similarly purified on silica gel-ODS, Toyopearl HW-forty, and MCI-gel CHP-20P columns, and through preparative HPLC, to present catechins- (4 α → 8) -ent-epicatechin (7) (4.5 mg). Eluate with 40% MeOH (51.89 g) of ion Dia Column HP-20 became chromatographed on a Toyopearl HW-40 column, and a CHP-20P gel MCI column. Two fractions acquired have been purified through CC on silica gel-ODS, Sephadex LH-20, Toyopearl HW-40, and or MCI-gel CHP-20P, in addition to through preparative HPLC, to present flavan D1 images (8) (11.1 mg) and gambirflavan D2 (9) (5.8 mg). The consequences of this examine observed three new dimer flavan, namely "catechin- (4 α -8) -ent-epicatechin, gambirflavan D1 and gambirflavan D2, which have been removed from gambier *Uncaria gambir* (leaves and younger twigs).

III. CONCLUSION

Phytochemical analysis shows that the main antioxidant compounds in *Uncaria gambir* are catechins, the dominant secondary metabolites. Catechins are able to block and clean up free radicals. Apart from catechins, other isolated results were obtained, namely (+) - epicatechin and several other flavan dimer such as gambiriin A1, A2, B1, B2, procyanidin B1, procyanidin B3, gambiriin C, gambirflavan D1 and gambirflavan D2 using HPLC (High Performance Liquid Chromatography), FT-NIR and Chromatography. The most widely used method of determining antioxidants in vitro is 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is a stable free radical, due to the delocalization of spare electrons throughout the molecule. But apart from that, there are several other methods that can be used to determine antioxidant activity, some of which are 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferrous Reducing Antioxidant Power (FRAP), Total Phenolic Content (TPC) and the Response Surface Method (RSM).

REFERENCE

- [1]. A. Rauf, Rahmawaty, and A. Z. Siregar, "The Condition of *Uncaria gambir* Roxb. as One of Important Medicinal Plants in North Sumatra Indonesia," *Procedia Chem.*, vol. 14, pp. 3–10, 2015, doi: 10.1016/j.proche.2015.03.002.
- [2]. S. U. Ahmad, A. Azam, A. N. Shuid, and I. N. Mohamed, "Antioxidant and anti-inflammatory activities of *Marantodes pumilum* (Blume) Kuntze and their relationship with the phytochemical content," *Rec. Nat. Prod.*, vol. 12, no. 6, pp. 518–534, 2018, doi: 10.25135/rnp.58.17.11.188.
- [3]. N. Ferdinal, "A Simple Purification Method of Catechin from Gambier," *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 4, no. 6, p. 441, 2014, doi: 10.18517/ijaseit.4.6.460.
- [4]. H. Fauza, "Gambier: Indonesia Leading Commodities in The Past," *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 4, no. 6, p. 455, 2014, doi: 10.18517/ijaseit.4.6.463.
- [5]. M. Hazwan Hussin and M. Jain Kassim, "Thermodynamic characterisation of mild steel corrosion and inhibition adsorption of *Uncaria gambir* and catechin in 1 M HCL," *J. Corros. Sci. Eng.*, vol. 13, no. June 2014, 2010.
- [6]. T. Anggraini, A. Tai, T. Yoshino, and T. Itani, "Antioxidative activity and catechin content of four kinds of *Uncaria gambir* extracts from West Sumatra, Indonesia," *African J. Biochem. Res.*, vol. 5, no. 1, pp. 33–38, 2011.
- [7]. M. Y. Musdja, N. Suryani, and P. Pitriyah, "Anti-inflammatory activity of catechins isolate of *Uncaria gambir* Roxb on carrageenan induced paw edema in wistar male rats," *Int. J. Recent Adv. Multidiscip. Res.*, vol. 6, no. 1, pp. 4518–4521, 2019.
- [8]. N. V. Magdalena and J. Kusnadi, "Antibacterial from Gambier Leaves Crude Extract (*Uncaria gambir* var *Cubadak*) Microwave-Assisted Extraction Method against Bacterial Pathogens," *J. Pangan dan Agroindustri*, vol. 3, no. 1, pp. 124–135, 2015.
- [9]. S. Syarifah, T. Widyawati, D. Rita Anggraini, A. Sari Wahyuni, and M. Indah Sari, "Anticancer activity of *Uncaria gambir* roxb on T47D breast cancer cells," *J. Phys. Conf. Ser.*, vol. 1317, no. 1, 2019, doi: 10.1088/1742-6596/1317/1/012106.
- [10]. E. Yanti, H. Diana Morika, Harmawati, and S. Aisyah Nur, "J. Kesehatan. Sainatika Meditory J. Kesehatan. Sainatika Meditory, vol. 2, no. II, pp. 79–88, 2018, [Online]. Available: <http://jurnal.syedzasaintika.ac.id/index.php/meditory/article/view/244>.
- [11]. N. Ferdinal, J. Sulistyono, and N. Nazir, "Enzymatic synthesis of flavonoid-glycosides from Gambier (*Uncaria gambir*) using CGT-ase enzymes from *Bacillus licheniformis*," *Pros. Semirata FMIPA Univ. Lampung*, pp. 289–296, 2013.
- [12]. N. Ibrahim, N. Z. Mohd Yusoff, and R. Ahmad, "Quantification of Catechin in Leaves and Stems of Malaysian *Uncaria gambir* (Hunter) Roxb. By HPLC-DAD," *Malaysian J. Anal. Sci.*, vol. 20, no. 3, pp. 567–572, 2016, doi: 10.17576/mjas-2016-2003-15.
- [13]. G. Yeni, K. Syamsu, O. Suparno, E. Mardiyati, and H. Muchtar, "Repeated extraction process of raw gambiers (*Uncaria gambir* Roxb.) for the catechin production as an antioxidant," *Int. J. Appl. Eng. Res.*, vol. 9, no. 24, pp. 24565–24578, 2014.
- [14]. Amos, I. Zainuddin, A. Triputranto, B. Rusmandana, and S. Ngudiwaluyo, "Gambi Post Harvest Technology," p. 65, 2004.
- [15]. Ediningsih and S. Rahayuningsih, "Extraction, Isolation, Characterization and

- Antioxidant Activity Test of Gambir Catechin Compounds (*Uncaria gambir* (Hunter). Roxb,," *Alkimia*, vol. 7, no. 2, pp. 177–188, 2019, [Online]. Available: <http://journal.uin-alauddin.ac.id/index.php/>.
- [16]. A. M. Pisoschi and G. P. Negulescu, "Methods for Total Antioxidant Activity Determination: A Review," *Biochem. Anal. Biochem.*, vol. 01, no. 01, pp. 1–10, 2011, doi: 10.4172/2161-1009.1000106.
- [17]. D. Gupta, "Methods for determination of antioxidant capacity: A review," *Int. J. Pharm. Sci. Res.*, vol. 6, no. 2, pp. 546–566, 2015, doi: 10.13040/IJPSR.0975-8232.6(2).546-66.
- [18]. N. Nur Sazwi, T. Nalina, and Z. H. A. Rahim, "Antioxidant and cytoprotective activities of Piper betle, Areca catechu, *Uncaria gambir* and betel quid with and without calcium hydroxide," *BMC Complement. Altern. Med.*, vol. 13, 2013, doi: 10.1186/1472-6882-13-351.
- [19]. G. Yeni, E. G. Sa'id, K. Syamsu, and E. Mardiyati, "Determination of the Best Conditions for Extraction of Antioxidants from Gambir Using the Response Surface Method," *J. Litbang Ind.*, vol. 4, no. 1, p. 39, 2014, doi: 10.24960/jli.v4i1.637.39-48.
- [20]. T. K. S. and H. S. H. M. Jain Kassim, M. Hazwan Hussin, A. Achmad, N. Hazwani Dahon, "Determination of total phenol, condensed tannin and flavonoid contents and antioxidant activity of *Uncaria gambir* extracts," *J. Chem. Inf. Model.*, vol. 22, no. 1, pp. 50–59, 2011.
- [21]. M. Amir, M. Mujeeb, A. Khan, K. Ashraf, D. Sharma, and M. Aqil, "Phytochemical analysis and in vitro antioxidant activity of *Uncaria gambir*," *Int. J. Green Pharm.*, vol. 6, no. 1, pp. 67–72, 2012, doi: 10.4103/0973-8258.97136.
- [22]. R. Rauf et al., "DPPH Radical Capture Activity of Gambir Extract (*Uncaria gambir* Roxb.)," vol. 30, no. 1, pp. 1–5, 2010.
- [23]. S. Melia, D. Novia, and I. Juliyarsi, "Antioxidant and antimicrobial activities of gambir (*Uncaria gambir* Roxb) extracts and their application in rendang," *Pakistan J. Nutr.*, vol. 14, no. 12, pp. 938–941, 2015, doi: 10.3923/pjn.2015.938.941.
- [24]. F. B. Apea-Bah et al., "Assessment of the DPPH and α -glucosidase inhibitory potential of gambier and qualitative identification of major bioactive compound," *J. Med. Plants Res.*, vol. 3, no. 10, pp. 736–757, 2009.
- [25]. M. Y. Musdja, H. A. Rahman, and D. Hasan, "Antioxidant Activity of Catechins Isolate of *Uncaria Gambier* Roxb in Male Rats," *LIFE Int. J. Heal. Life-Sciences*, vol. 4, no. 2, pp. 34–46, 2018, doi: 10.20319/ijhls.2018.42.3446.
- [26]. I. Kresnawaty and A. Zainuddin, "Antioxidant and Antibacterial Activity of Methyl Derivative Ethanol Extract of Gambir Leaves (*Uncaria gambir*)," *J. Penelit. Tanam. Ind.*, vol. 15, no. 4, pp. 145–151, 2009, doi: 10.21082/jlittri.v15n4.2009.145-151.
- [27]. D. N. Sari and T. Taufikurohmah, "Effect of Addition of Nanogold on Antioxidant Activity of Gambir Extract (*Uncaria gambir* Roxb.)," *UNESA J. Chem.*, vol. 8, no. 1, pp. 20–26, 2019.
- [28]. T. Anggraini, Neswati, and A. Asben, "Gambir Quality from West Sumatra Indonesia Processed with Traditional Extraction," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 347, no. 1, 2019, doi: 10.1088/1755-1315/347/1/012066.
- [29]. S. Taniguchi et al., "Evaluation of gambir quality based on quantitative analysis of polyphenolic constituents," *Yakugaku Zasshi*, vol. 127, no. 8, pp. 1291–1300, 2007, doi: 10.1248/yakushi.127.1291.
- [30]. A. Andasuryani, Y. A. Purwanto, I. W. Budiastara, and K. Syamsu, "Determination of Catechin Content in Gambir Powder from Dried Gambir Leaves Quickly using FT NIR PLS Model," *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 4, no. 5, p. 303, 2014, doi: 10.18517/ijaseit.4.5.423.
- [31]. S. Taniguchi et al., "Revised structures of gambirins A1, A2, B1, and B2, chalcane-flavan dimers from gambir (*Uncaria gambir* extract)," *Chem. Pharm. Bull.*, vol. 55, no. 2, pp. 268–272, 2007, doi: 10.1248/cpb.55.268.
- [32]. S. Taniguchi et al., "New dimeric flavans from gambir, an extract of *Uncaria gambir*," *Heterocycles*, vol. 74, no. C, pp. 595–605, 2008, doi: 10.3987/com-07-s(w)37.