Trop J Nat Prod Res, August 2022; 6(8):1215-1218
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antioxidant Activity of *Uncaria Gambir* (Hunter) Roxb Extracts

Mutia D. Hidayati* and Arief Rahmatulloh

Department of Chemical Engineering, Politeknik Negeri Malang, Malang, Indonesia

ARTICLE INFO

Article history:
Received 27 May 2022
Revised 27 July 2022
Accepted 29 August 2022
Published online 02 September

ABSTRACT

Uncaria Gambir is a popular plant in Southeast Asia, used traditionally for healing various diseases. Pharmacologically, *U. Gambir* has many potentials. This study aimed to investigate the antioxidant activities of *U. Gambir* stem extracts (methanol, ethyl acetate and n-hexane) using DPPH (2,2-diphenyl-2-2-picrylhydrazyl) and ABTS (2,2'-azinobis (3-ethylbenzothiazole-6-sulfonic acid) methods. The results of this study show that methanol extract has the highest antioxidant activity in the DPPH assay with IC_{50} value of 9.71 µg/mL. In addition, it has the highest ABTS activity with the IC_{50} value of 6.63 µg/mL. This study showed that the methanol extract of *U. Gambir* can potentially be used as an antioxidant.

Keywords: Antioxidant, ABTS, DPPH, *Uncaria gambir* (Hunter) Roxb, Stem Extracts.

Introduction

Free radical formation occurs continuously as a consequence of oxidation reaction of stable compounds in the body's metabolism. Free radicals are unstable and highly reactive due to the unpaired electron in their atomic orbital.[1] In addition, it can either donate an electron or accept an electron from other molecules. Furthermore, free radicals such as hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical and peroxinitrite radical cause many diseases.[2] They can attack important macromolecules such as DNA, protein, carbohydrate, and lipids. This condition leads to the damage of macromolecules, and this can induces human diseases like Alzheimer's disease and cancer.[3] Hence, chemical substances are needed for the prevention of free radicals.

One of the chemical substances that play a role in inhibiting free radicals is antioxidant. This compound works by neutralizing free radicals through electron donors. The neutralization process forms more stable compound, thereby inhibiting the mechanism of oxidative reaction.[4] Antioxidant compounds can be obtained from natural or synthetic sources. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), propyl gallate (PG) and octyl gallate (OG) are popular synthetic antioxidant compounds which are commonly used as antioxidants in food, cosmetic and industrial products.[5,6] However, synthetic antioxidants pose high risks on human body since they are potentially carcinogenic and chemically unstable. This justifies the need for natural antioxidant sources for humans. The sole alternative to synthetic antioxidant is natural antioxidant.[4,7] One of the natural antioxidant sources is *Uncaria gambir* (Hunter) Roxb, which is ubiquitous in Southeast Asia. *Uncaria gambir* (Hunter) Roxb, which is locally known as Gambir, is a climbing shrub native to Southest Asia, particularly in Sumatra and Borneo. This plant is traditionally used for treating various illnesses like diarrhea, dysentery, and sore throat.[8] *U. gambir* also can be used as mouthwash[9] and wound dressing.[10]

*Corresponding author. E mail: mutiadevi@polinema.ac.id
Tel: +62-341-404424

Citation: Hidayati MD and Rahmatulloh A. Antioxidant Activity of *Uncaria Gambir* (Hunter) Roxb Extracts. Trop J Nat Prod Res. 2022; 6(8):1215-1218. doi.org/10.26538/tjnpr/v6i8.9

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Furthermore, this plant is utilized as an astringent and antiseptic as well.[11] This plant has been known to possess pharmacological properties: it can be used as anti-inflammation,[12] antiseptic,[13] anticancer,[14] and anticholesterol.[15] Previous researches reported that phenolics in *U.* gambir extract such as catechin, quercetin, quinic acid have contributed to antioxidant activities.[16] Gambirin A1, A2, B1, B2 have also been isolated from methanol extract of *U.gambir*.[11]

Free radical scavenging activity of *U gambir* using DPPH method has been reported. Ethyl acetate extract of *U. gambir* pollen has DPPH radical scavenging activity with IC_{50} value of 25.55 µg/mL.[17] In addition, methanol extract of *U. gambir* leaves and shoots has DPPH radical scavenging activity with IC_{50} value of 18.27 µg/mL.[18] Aqueous extract of *U gambir* leaves and stems has DPPH radical scavenging activity which ranged from 92 to 93.1%.[19] Recently, numerous researchers reported about the antioxidant which was derived from leaves, roots and shoots of this plant. However, reports about stem extract of *U. gambir* are limited in number. Additionally, there are many reports on free radical scavenging activity of *U. gambir* using DPPH method, whereas reports on free radical scavenging acvitity using ABTS methods is nonexistent so far.

The study aims mainly to find out the antioxidant activity of three solvents extracts of *U. gambir* stem: ethyl acetate, hexane and methanol extracts.

Materials and Methods

Plant material

Uncaria gambir stems were obtained from West Kalimantan, Indonesia, in May 2021. They were identified at Balai Materia Medika in Batu, Malang and were authenticated with voucher specimen number 074/495/102.20-A/2021.

Materials and instrumentation

High grade organic solvents including methanol, ethanol, ethyl acetate, n-hexane and dimethylsulfoxide (DMSO) were purchased from Merck. DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), and $K_2S_2O_8$ (potassium peroxydisulfate) were purchased from the same source. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma Aldrich) was used as antioxidant standard. Incubator EYELA SLI-400 was used to incubate the samples. The reaction was monitored by spectrophotometer UV (HITACHI U-2900, Japan). Rotary Evaporator (IKA RV 8 Basic, Germany) was used to obtain crude extracts.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Preparation of extracts
Dried gambir stem (25 g each) was extracted separately with methanol, ethyl acetate, and hexane (250 mL for 24 h) at room temperature. The extracts were filtered using filter paper then concentrated with a rotary evaporator under reduced pressure. *U. gambir* crude extracts were obtained from this treatment.

DPPH assay
DPPH activity of the crude extracts were assayed using a method described by Brand Williams,[20] modified by Dudonne *et al.*[21] Each of the crude extracts (10 mg) was dissolved in 1 mL methanol. The mixture consisted of 1 mL DPPH solution 6 x 10^{-5} M and 33 µL of methanol solution of crude extract. After 20 minutes of incubation at 37°C, the absorbance of the reaction mixture was measured at 515 nm by UV-VIS spectrophotometer to get the Absorbance value (As). Methanol was used as a blank and measured at same wavelength (Ab). The experiment was carried out in triplicate and DPPH activity was calculated using the following formula: [21]

$$\text{Antioxidant activity (\%)} = \frac{A_b - A_s}{A_b} \times 100\ \% \qquad (1)$$

Note: Ab = absorbance of solution without sample
As = absorbance of sample solution

Furthermore, the extract which have a good DPPH inhibition (%) was measured for 50% inhibitory concentration (IC_{50}) value (µg/mL). The value of IC_{50} was represented as the concentration of the extracts which is needed to reduce the intensity of 50% DPPH concentration.[22]

ABTS assay
ABTS activity of crude extracts were assayed using the method described by Pellegrini *et al.*[23] ABTS was dissolved in water at 7 mM concentration. Then, the ABTS radical cation was generated by reacting 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium peroxydisulfate ($K_2S_2O_8$). The mixture was allowed to stand at room temperature for 12-16 h to yield a dark blue solution. The ABTS solution was diluted with 99.5% ethanol to give an absorbance of 0.7 ± 0.02 at 734 nm, before starting the assay. 1 mL DMSO was utilized to dissolve each of crude extracts (10 mg). The reaction mixture consisted of 1 mL ABTS solution and 10 µL of crude extract were shaken for 10 sec. After 4 min incubation at 30°C, the absorbance of the reaction mixture was measured at 734 nm by spectrophotometer UV-Vis to provide the A_s value. Blank sample with 10 µL of DMSO in ABTS solution was prepared and measured at same wavelength to find out the A_b value. Trolox was used as a positive control. The ABTS activity was calculated by formula 1 with triplicate experiment. Further, the extract which has a good ABTS activity (%) was measured for its IC_{50} value (µg/mL).

Statistical analysis
The antioxidant activity analyses were performed on three varieties of stem extracts: methanol, ethyl acetate, and n-hexane solvent using independent t-test at an error rate of 5%. The tests were conducted to compare the above extracts' antioxidant activity against that of Trolox's as the positive control.

Results and Discussion

The extraction of *U. gambir* was performed by using methanol, ethyl acetate, and n-hexane which provided varying extraction yield; 1.59, 0.92, and 0.92 g, respectively. The highest yield extraction for various stem extracts was obtained with methanol solvent with the value of 0.0636 % w/w. The result showed different yield extraction for each solvent, depending on the sample's polarity. Then, the stem extract showed the tendency to be polar when extracted with methanol that has the highest polarity. This indicated that the main compounds of *U.gambir* stems are polar solvent soluble. The determination of antioxidant activity of *U. gambir* extracts was carried out using DPPH and ABTS methods. The antioxidant activity of *U. gambir* extracts which is based on DPPH assay at concentration of 39.93 µg/mL can be seen in Figure 1 which presents the percentages of antioxidant activity of stem extracts; methanol, ethyl acetate, and n-hexane were 90.31 ± 0.93%, 86.23 ± 0.146% and 27.5 ± 0.31%, respectively. Trolox as a positive control has antioxidants activity of 95.11 ± 0.086%. Figure 1 shows that the methanol extract of the stem of *U. gambir* has the highest activity in comparison with the other extracts, because these extracts may contain phenolic compounds that contributed to the antioxidant activity.[16] The previous study by Phongpaichit *et al*,[24] showed the strength of antioxidant activity based on DPPH assay: IC_{50} > 250 µg/mL, inactive; >100-250 µg/mL, weakly active; >50-100 µg/mL, moderately active; 10-50 µg/mL, strongly active; < 10 µg/mL, very strongly active. Figure 2 shows that, the IC_{50} value of *U. gambir* methanol extract is 9.71 µg/mL, while Trolox as positive control has IC_{50} value of 4.12 µg/mL. This result indicates that *U. gambir* methanol extract is a strongly active antioxidant (IC_{50} < 10 µg/mL).

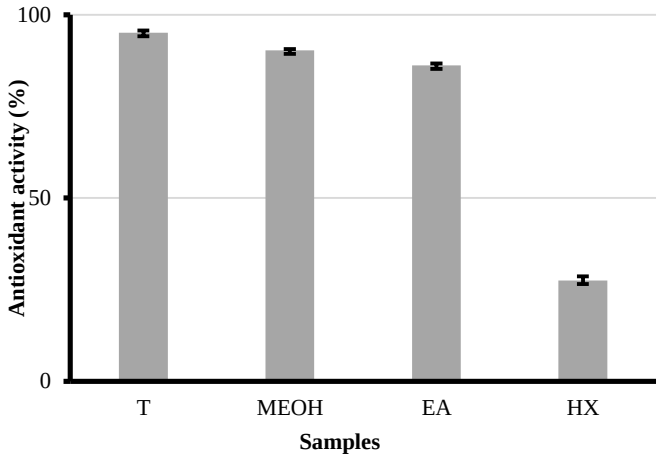

Figure 1: DPPH Scavenging activity of *U. gambir* extracts at a concentration of 39.93 µg/mL, MeOH, methanol extract; EA, ethyl acetate extract; HX, hexane extract and T, Trolox (positive control). Each column represents the mean±SD, n = 3

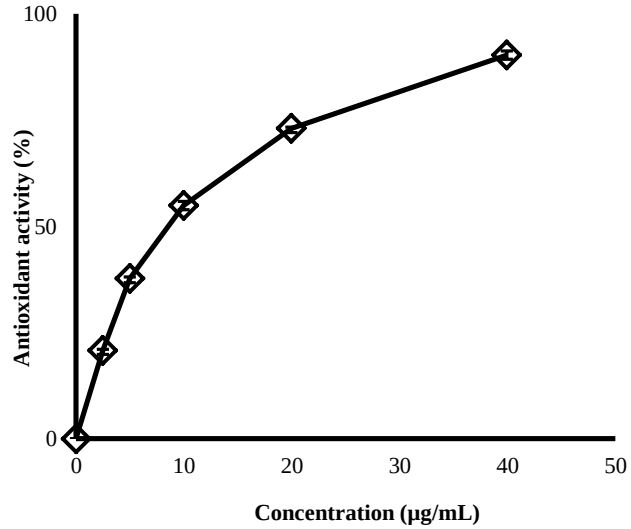

Figure 2: DPPH Scavenging activity of *U. gambir* methanol extract

Antioxidant activity of *U. gambir* extracts was also investigated using the ABTS method. The antioxidant activity of three extracts (methanol, ethyl acetate, and n-hexane) at a concentration of 49.5 µg/mL are presented in Figure 3. The antioxidant activity percentage of *U. gambir* stem extracts were 94.88 ± 0.405, 75.47 ± 0.234, and 44.42 ± 0.219%, respectively, while Trolox as a standard has

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

antioxidant activity of 98.04 ± 0.673%. Figure 3 illustrates the ABTS scavenging activity of *U.gambir* stem extract on several solvents: ethyl acetate, hexane and methanol with Trolox. ABTS scavenging activity of methanol extract of *U. gambir* is shown in Figure 4. The IC_{50} value of methanol extracts was 6.63 µg/mL, while, Trolox which functions as positive control has IC_{50} value of 2.25 µg/mL. The IC_{50} value obtained from the methanol extract of *U. gambir*, the antioxidant activity of *U. gambir* is very strong. The result shows the correlation between IC_{50} value and antioxidant activity: the lower the IC_{50} value, the higher the antioxidant activity of the compound. The IC_{50} value of Trolox indicated that it had a higher activity than the methanol extract (the same as scavenging activity with DPPH method). Such result was made possible by the fact that Trolox is a derivative of vitamin E, which consist of aromatic ring with substitution of hydroxy and carboxylate group. The presence of distinct substituents in the backbone structure of phenol compound gives its antioxidant property. Further, it is also based on its structure, especially on several properties: the position, number of hydroxyl groups and the characteristic of the aromatic ring substituents.[7]

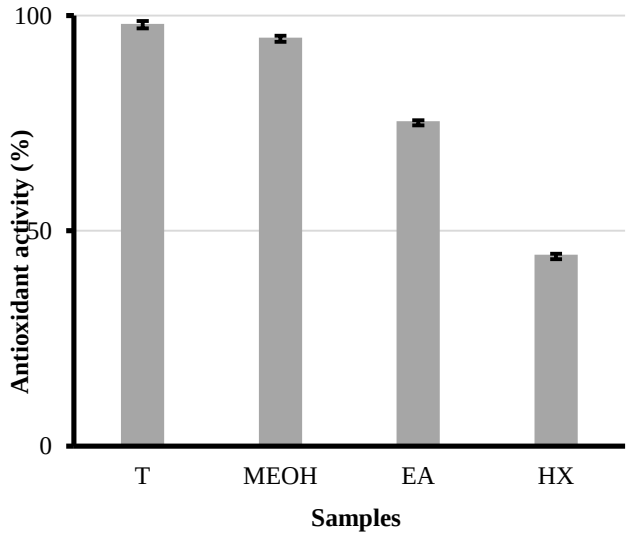

Figure 3: ABTS Scavenging activity of *U. gambir* extracts at a concentration of 49.5 µg/mL, MeOH, methanol extract; EA, ethyl acetate extract; HX, hexane extract and T, Trolox (positive control). Each column represents the mean±SD, n = 3

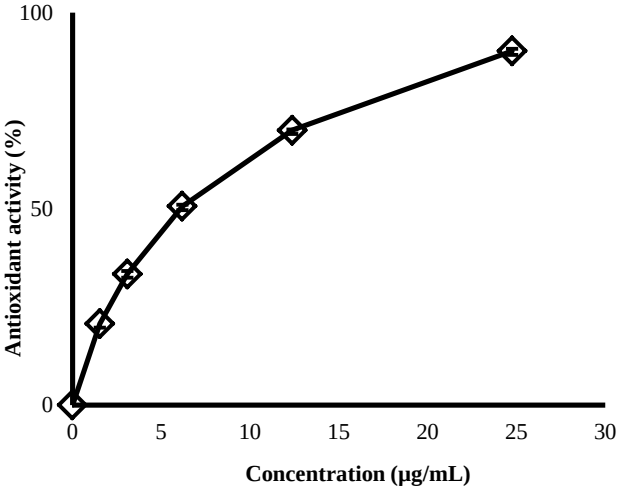

Figure 4: ABTS Scavenging activity of *U. gambir* methanol extract

Table 1: DPPH and ABTS radical scavenging activity of *U. gambir* extracts

Extraction solvent	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
Hexane	107.11 ± 0.58	73.07 ± 0.54
Ethyl acetate	23.39 ± 0.32	22.45 ± 0.18
Methanol	9.71 ± 0.45	6.63 ± 0.36
Trolox	4.12 ± 1.13	2.25 ± 0.77

Value are the Mean ± SD for three parallel assessment (n=3)

Table 1 shows various extracts of *U. gambir* that were tested for antioxidant activity using DPPH and ABTS methods. The table also indicates that methanol extract of *U. gambir* has the highest antioxidant activity on both methods. The extract has an IC_{50} value of 9.71 µg/mL, whereas Trolox has IC_{50} value of 4.12 µg/mL using DPPH method. Furthermore, the methanol extract has IC_{50} value of 6.63 µg/mL, while Trolox has IC_{50} value of 2.25 µg/mL using ABTS method. There was no meaningful difference of the results based on t-test between the antioxidant activity of the *U. gambir* extracts and Trolox as a positive control since the t-tailed value was > 0.05. Furthermore, the result shows that the scavenging activity with ABTS method provides higher antioxidant activity than DPPH method. Such result was made possible by the fact that the ABTS method test was performed with the absorbance reading of the ABTS•+ measured at 734 nm. The wavelength was chosen to give the minimal interference from the other absorbing components and turbidity of sample. Another reason for this condition is the presence of phenolic which is contained in the extract of *U. gambir*. The phenolic interferences resulted in the near visible region rather than the far ones. DPPH posed serious problem of interference since it was analyzed at 515 nm, while ABTS scavenging method give less serious problem since the chromogen provides the absorbance peaks at 734 nm.[25]

Conclusion

The antioxidant activity of *U.gambir* stems extract were confirmed by DPPH and ABTS methods on several solvent namely, methanol, ethyl acetate, and n-hexane. The methanol extract showed the highest antioxidant activity on both methods, DPPH with IC_{50} of 9.71 µg/mL and ABTS with IC_{50} of 6.63 µg/mL. Based on this result, methanol extract of *U. gambir* could be utilized as a potential source of antioxidant.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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