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Toxicity Test, Antioxidant Activity, and Determination of Total Flavonoid Content (TFC) of *N*-Hexane and Ethyl Acetate Fraction from *Uncaria Nervosa* Elmer (Bajakah) Root Wood

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ABSTRACT

Uncaria nervosa Elmer (local name: Bajakah) is one of the medicinal plants used as a cancer drug by the people of Muara Badak, East Kalimantan. The purpose of this study was to determine the total flavonoid content (TFC), toxicity, and antioxidant activity of the n-hexane (NH) and ethyl acetate (EA) fractions of *Uncaria nervosa* root wood. Determination of total flavonoids was carried out using a UV-Vis spectrophotometer with quercetin as a standard, toxicity test was carried out using the brine shrimp lethality test (BSLT) method, and antioxidant test using the DPPH radical reduction method. Based on the results of this study, the total flavonoid content (TFC) of NH and EA fractions were 192 and 425 mg QE/g extract, respectively. Based on the results of the toxicity test on *Artemia salina* L. shrimp, the LC_{50} values were 11.91 ppm for NH and 33.19 ppm for EA. These LC_{50} values indicate that NH is very toxic and EA is toxic against *Artemia salina* L. While the antioxidant test results showed that EA was very significant as an antioxidant and NH was moderate with IC_{50} values of 17.54 ppm and 75.93, respectively.

KEYWORDS: *Uncaria nervosa*, bajakah, toxicity, antioxidant activity, BSLT, quercetin

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INTRODUCTION

Efforts to discover new drugs continue to grow by looking for natural compounds that can be used as guidelines in the search for new drugs. The slogan of returning to nature and the belief that traditional medicines have relatively small negative effects make it the right moment for research on traditional medicines, which mainly come from medicinal plants [1].

Bajakah is a climbing stem that is known to be used to treat cancer by the Dayak people in Kalimantan. Several types of plants known as bajakah are known as cancer drugs, namely *Jasminum elongatum* (PJ Bergius) Wild., *Merremia peltata* (L.) Merr, *Fibraurea tinctoria* Laur. *Uncaria acida* (Hunter) Roxb[2], and *Spatholobus littoralis* Hassk[3]. In Muara Badak Kutai Kertanegara, *Uncaria nervosa* is also known as Bajakah which is believed to be used as a cancer drug[4]. In addition, the leaves of *U. nervosa* are used to treat headaches and the roots are used as a remedy for fever[2].

The previous study's results showed that the crude extract of bajakah root wood contained alkaloids, flavonoids, terpenoids, and phenolics. Toxicity test also showed the crude extract of root bark and root wood were very toxic against *Artemia salina* with LC_{50} values of 1.76 and 2.66 ppm, respectively[4]. Although several biological activities of the genus of *Uncaria* have been studied, published studies on the biological activity and content of active compounds are limited for *U. nervosa*[5]. As a continuation of the research on *U. nervosa*, in this paper, we will report on the toxicity properties of *Artemia salina* L., DPPH radical scavenging, and the total flavonoid content of the *n*-hexane and ethyl acetate fractions of *U. nervosa* root wood

MATERIAL AND METHODS

Extraction and Partition

The sample in this study was dry root wood of *U. nervosa* Elmer extracted by maceration using methanol. The crude extract obtained was then partitioned using *n*-hexane solvent and then continued with ethyl solvent. The filtrate obtained from the maceration of 1.01 kg of the *U. nervosa* root wood, then separated at low temperature using a rotary evaporator, obtained 80 grams of crude extract. 40 grams of crude extract was dissolved in methanol then partitioned using n-hexane and then partitioned using ethyl acetate. The fractions of *n*-hexane (NH) and ethyl acetate (EA) obtained were 5 and 12 grams, respectively.

Brine Shrimp Lethality Test

A toxicity test was carried out using *Artemia salina* L larvae. This method refers to previous studies [4],[6],[7]. The n-hexane and ethyl acetate fractions of bajakah wood root were first dissolved to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 ppm. For each concentration of the sample added 10 shrimp larvae. After 24 hours, the dead shrimp larvae were counted and the probit value was determined. Determination of LC_{50} was carried out using profit analysis.

Antioxidant Activity Test

Antioxidant test with DPPH radical scavenging method was carried out according to previous research methods [8], [9]. The sample was put in a test tube and made in concentrations of 20, 40, 60, and 80 ppm. Each sample was taken 2 mL and then added 2 mL of DPPH solution (0.024 mg/mL). Absorbance measurements of samples, blanks, and positive control (vitamin C) were measured using a UV-Vis spectrometer (at max 517 nm). Determination of the IC value is carried out using the formula:

(%) Inhibition = $\frac{A\ blan}{k\ sample}$ $100\%\ A\ blank$

Data analysis

Determination of LC_{50} for the BSLT test and IC_{50} for the DPPH radical scavenging test was carried out using linear regression. Analysis of the total flavonoid content in the sample was expressed as mg quercetin equivalent per gram of extract weight (w/w QE) with the formula: C=(Cq.V)/m .FP where C = Total flavonoids (mg QE/g extract, Cq = Sample concentration (mg/L), V = Extract volume (L), m = Extract Weight, and FP = Dilution Factor Then IC50 to DPPH reduction was determined based on the linear regression equation, y = a + bx, where y = 50 and x is IC_{50}, which is obtained from the graph of % Inhibition vs. concentration [8], [9], [10].

Determination of Total Flavonoids Content (TFC)

Based on previous research, obtained a standard quarcetine calibration curve with a linear regression equation is y = 0.0091x + 0.0366, R^2 = 0.9951[11].

Table 1. Total Flavonoids Content (TFC) of NH and EA

Sample	Absorbance				TFC (mg QE/g extract)
	I	II	III	Average	
NH	0.391	0.415	0.353	0.386	192
EA	0.825	0.799	0.805	0.810	425

The calculation of the total flavonoid content was carried out using the linear regression formula y = 0.0091x + 0.0366, where y = absorbance and x = flavonoid content after 5 times dilution, where the total flavonoid content of NH and EA was 192 and 425 mg QE/g extract, respectively. The total flavonoid content of EA is higher than that of NH, flavonoid is a type of natural phenolic which is somewhat polar so it is more likely to dissolve in ethyl acetate compared to n-hexane. The presence of flavonoid content in these fractions is supported by data from previous research, the color test (qualitative) of the crude extract of bajakah wood showed the presence of flavonoid [4].

Toxicity Test

From the graph of the relationship between the log concentration of the NH and the probit value, the linear regression equation obtained is y = 0.4105x + 4.5585, where R^2 = 0.6412, while the graph of the relationship between the log concentration of the EH and the probit value obtained by the linear regression equation is y. = 0.3089x + 4.5301, where R^2 = 0.7405. Based on these two linear regression equations, the LC_{50} values for the n-hexane and ethyl acetate fractions were 11.91 and 33.19 ppm, respectively.

Table 2. The LC_{50} value of NH and EA

Sample	Concentration (ppm)	Log Concentration	Number of larvae	Number of ***Mortality***	% Mortality	Probit	LC_{50} (ppm)
NH	1000	3.0000	10	7.67	76.70	5.71	11.91
	500	2.6989	10	7.67	76.70	5.71	
	250	2.3979	10	6.67	66.70	5.41	

	125	2.0969	10	6.33	63.30	5.33	
	62.5	1.7959	10	7.00	70.00	5.52	
	31.25	1.4948	10	7.33	73.30	5.61	
	15.625	1.1938	10	4.33	43.30	4.82	
	7.815	0.8928	10	4.00	40.00	4.75	
EA	1000	3.0000	10	6.30	63%	5.33	33.19
	500	2.6989	10	7.00	70%	5.52	
	250	2.3979	10	6.30	63%	5.33	
	125	2.0969	10	5.00	50%	5.00	
	62.5	1.7959	10	5.60	56%	5.15	
	31.25	1.4948	10	5.60	56%	5.15	
	15.625	1.1938	10	4.00	40%	4.75	
	7.815	0.8928	10	4.30	43%	4.82	

The results of the LC_{50} value showed that NH was very active (<31 ppm) while EA was in the active category (<1000 ppm). Although there is no direct relationship with anticancer properties, the brine shrimp lethality test (BSLT) is a bioactivity screening test carried out to predict the presence of a fraction or extract that has the potential to anticancer. BSLT testing is a simple, easy, and inexpensive method to determine the potential for bioactivity so that it can be used as a guide in the search for anticancer compounds from active fractions [7]. The lower LC_{50} value in the BSLT test indicates a very large potential of an extract to find compounds that can be developed as cancer drugs.

From the two fractions obtained from the extract of the root wood of Bajakah, it was shown that both were toxic to *Artemia salina* L, although the NH was categorized as very toxic, while the EA was toxic, the LC_{50} EA value was also low even though it was not included in the very active category. Meanwhile, the flavonoid content of the EA is much higher than NH so the potential to find active compounds as anticancer can be obtained from both these fractions. Various studies have shown that flavonoid compounds have anticancer properties. The reaction mechanism of flavonoid compounds shows the ability to inactivate carcinogens, antiproliferation, cell cycle arrest, induction of apoptosis, inhibition of angiogenesis, antioxidants, and reversal of multidrug resistance or a combination of these mechanisms[12]. Other types of secondary metabolites that can be used as anticancer drugs are alkaloids. Crude extract of *Uncaria nervosa* stem wood also contains alkaloids, so in this plant, it is also possible to have alkaloid compounds that can be developed into anticancer drugs[4]. As taxol and camptothecins are two types of anticancer drugs, alkaloid compounds. These two compounds were isolated from plants [13].

The genus *Uncaria* is an important source of natural medicinal products, one of which is a type of alkaloid [14]. From several plant species belonging to the genus *Uncaria*, alkaloid compounds with anticancer properties have been isolated such as activities of *Uncaria* alkaloids, including rhynchophylline, isorhynchophylline, corynoxeine, isocorynoxeine, hirsutine and hirsutein [15]. *Uncaria nervosa* which has been used as a traditional medicine as an anticancer drug is also likely to be found with alkaloid compounds that have anticancer properties. This may be the reason why the n-hexane fraction was more toxic to *Artemia salina* shrimp than the ethyl acetate fraction even though the flavonoid content of the n-hexane fraction was smaller than the ethyl acetate fraction.

Antioxidant Test

The antioxidant test was carried out by DPPH radical scavenging method. Measurement of the absorbance of the sample solution and vitamin C as a positive control with UV-Vis spectrophotometer (see table 3). IC_{50} calculation results for NH, EA, and Vitamin C obtained 75.93, 17.54, and 2.74 ppm, respectively.

Table 3. The IC_{50} value of NH and EA

Sample	Concentration (ppm)	Absorbance				% Inhibition	IC_{50} (ppm)
		1	2	3	Average		
NH	20	0.049	0.049	0.049	0.0490	26.86	75.93
	40	0.049	0.048	0.049	0.0486	27.46	
	60	0.047	0.047	0.047	0.0470	29.85	
	80	0.026	0.026	0.026	0.0260	61.19	
EA	5	0.045	0.045	0.044	0.0447	33.42	17.54
	10	0.036	0.036	0.036	0.0360	46.26	
	20	0.028	0.028	0.028	0.0280	58.20	

	40	0.023	0.023	0.023	0.0230	65.67	
Vit. C	2	0.036	0.036	0.036	0.0360	46.26	2.74
	4	0.028	0.028	0.028	0.0280	58.21	
	6	0.019	0.019	0.019	0.0190	71.64	
	8	0.005	0.005	0.005	0.0050	92.53	

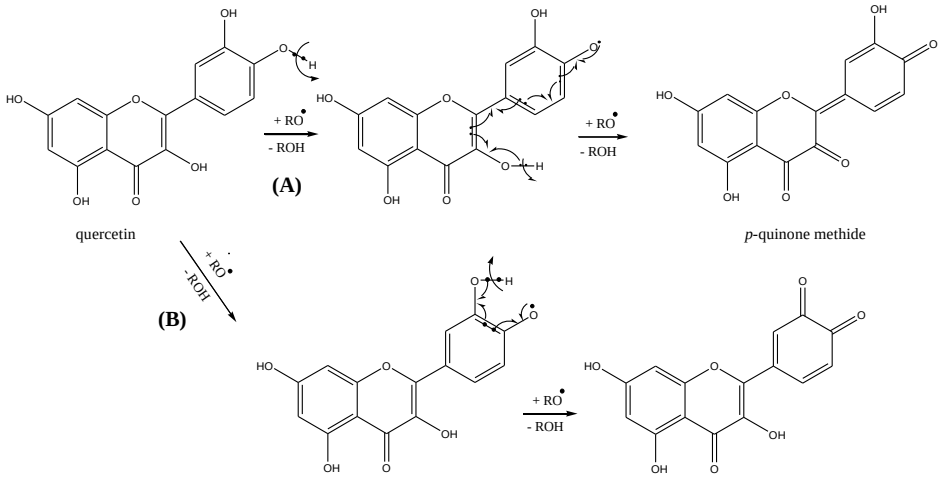
Based on the IC_{50} value, both samples showed that the NH was active, while the EA was very active [16]. Total flavonoid content and DPPH radical reduction showed a positive correlation. The flavonoid content of EA is greater than NH as well as the radical scavenging activity of DPPH where EA is higher than NH. One of the main pharmacological effects of flavonoids is antioxidant activity [17]. Flavonoids are found in many types of plants. Flavonoids are strong antioxidants due to their ability to scavenge reactive oxygen species (ROS). ROS are classified as superoxide anion radicals ($O_2^{\cdot-}$), singlet oxygen (O_2), hydrogen peroxide (H_2O_2), the highly reactive hydroxyl radical (OH), and lipid peroxy radicals, perhaps the most important function of flavonoids [18], [19]. When reactive oxygen species (ROS) are formed, flavonoids have the ability to control their accumulation via scavenger ROS [20].

Flavonoids that have an OH group that is directly attached to the main framework of the flavonoid are able to donate H from the OH group to radicals to become stable compounds. The part of the flavonoid, which loses $H^{\cdot}$, will become a radical (Fl-O•) which has a lower energy because it can be self-stable by the conjugation of a radical that enters the benzene core before reacting with other radicals which will cause the formation of stable compounds. In general, the process of adsorption of radicals by flavonoids occurs by releasing H atoms or releasing electrons through two pathways: (1) transfer of H atoms, and (2) transfer of electrons/proton transfers [17].

R-O• + Fl-OH → R-OH + Fl-O• (1)

R-O• + Fl-OH → $R\text{-}O^-$ +Fl-OH•+ →R-OH + Fl-O• (2)

Fl-O• is then stabilized by termination reactions that occur intermolecularly or intramolecularly. One type of flavonoid that has very high activity, even higher than vitamin C, is quercetin. The two proposed pathways (A and B) to predict the mechanism of the quercetin antioxidant reaction occur intramolecularly [17], [21].



Pigure.1. Two pathways (A and B) to predict the mechanism of action of the antioxidant quercetin

CONCLUSIONS

The results of determining the total flavonoid content (TFC) showed that the flavonoid content of EA was higher than that of NH with concentrations of 425 and 192 QE/g of extract, respectively. While the toxicity properties showed that both of them were significantly toxic against *Artemia salina* shrimp, NH was more toxic than EA with LC_{50} values of 11.91 and 33.19 ppm respectively. The antioxidant test on DPPH radical scavenging showed that EA was highly significant as an antioxidant while NH was moderate with IC_{50} values of 75.93 and 17.54 ppm, respectively.

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