

Compound Characterization and Evaluation of Antioxidant Potential of Ethanol Extract *Spatholobus littoralis* Hassk in Kolaka, Southeast Sulawesi, Indonesia

ABSTRACT

The bajakah tampala plant (*Spatholobus littoralis* Hassk) has been traditionally believed by the public to cure cancer, tumors, heart disease, stroke, and diabetes. However, empirically the public does not yet know the chemical compound content and there have been no studies on biological activity tests, especially the antioxidants of bajakah tampala from Kolaka, Southeast Sulawesi. The aim of this research is a qualitative analysis of the chemical compounds of the ethanol extract of bajakah tampala roots and testing of antioxidant activity. Analysis of the chemical compounds of the ethanol extract of bajakah tampala roots using an FT-IR spectrophotometer and LC-MS/MS. The antioxidant activity of the bajakah tampala root ethanol extract was tested using the DPPH immersion method and the absorption was measured using a UV-Vis spectrophotometer and using the positive control quercetin. The results of the research showed that the ethanol extract of bajakah tampala roots had 8 chemical components that were detected, namely compound 1 apocynoside I, compound 2 quercetin, compound 3 hexosylphingosine, compound 4 3-hydroxy-7-methoxybaicalein, compound 5 momor-cerebroside I, compound 6 ambronal, compound 7 stigmastan-3,6-dione, and compound 8 trilaurin. The ethanol extract of bajakah tampala roots has an antioxidant activity of 21.75 µg/mL which is lower, compared to the antioxidant activity of quercetin of 2.91 µg/mL as a positive control, however the antioxidant activity of the ethanol extract of bajakah tampala roots is still included in the very strong category.

Keywords: Characterization, bajakah tampala, Spatholobus littoralis Hassk, antioxidant

1. INTRODUCTION

Intake of foods high in antioxidants containing flavonoids can prevent heart disease, cancer, stroke, and diabetes. Antioxidants have many health benefits around the world. Antioxidants protect the body from damage caused by free radicals, molecules that can damage cells and tissues in the body. Antioxidants are substances that can ward off and inhibit the formation of free radicals which are harmful to the body [1]. Indonesia is a country with high biodiversity which is rich in natural antioxidant compounds. One of the plants that contains high antioxidant natural compounds is the bajakah Tampala plant (*Spatholobus littoralis* Hassk) [2-4]. Based on previous research, the bajakah tampala plant (*Spatholobus littoralis*

Hassk) apart from having antioxidant activity, also has anticancer or antitumor activity [5-7], anti-inflammatory [7-9], and antibacterial activity [10-12].

Red bajakah root plants and white bajakah root plants, both in the skin and roots, contain secondary metabolites including phenolic compounds [13,14], alkaloids [13,14], saponins [14], terpenoids [14], tannins [13,14], flavonoids [13, 15], carotenoids [14], and steroids [14] which have antioxidant activity [13], and antibiofilm activity [16]. The antioxidant activity of bajakah root plants is in the very strong category, even higher than the antioxidant activity of vitamin C and vitamin E comparable to the antioxidant activity of secang wood [13]. The levels of flavonoids in red and white bajakah roots, both the bark and wood, are higher, when compared to secang wood [13]. Based on the content of secondary metabolites and antioxidant activity in bajakah roots, it can be used as a pharmaceutical preparation [13]. Natural chemical compounds in bajakah stems have also been modeled in silico for their inhibitory activity on the protease enzyme of the SARS-CoV-2 virus [18].

Free radicals are reactive compounds towards atoms that have unpaired electrons in their outer shell. Free radicals can be found in the environment, such as cigarette smoke, medicine, packaged food, additives, etc. [19]. Free radicals in normal amounts are beneficial for health in fighting inflammation, killing bacteria, and controlling smooth muscle tone in blood vessels, as well as organs in the body. Excessive amounts result in oxidative stress [20]. Excessive free radicals in the human body play a role in the pathology of various degenerative diseases such as cancer, atherosclerosis, rheumatism, coronary heart disease, cataracts, and Parkinson's neurodegeneration disease. An increase in free radicals can cause oxidative damage to cells, tissues, and body organs, so the body needs antioxidant compounds to stabilize free radicals [21].

Antioxidant compounds can neutralize free radicals in the human body so that cell damage can be prevented [22]. Natural antioxidants are the body's defense system to ward off cell damage found in food in small quantities and are needed in large quantities to maintain normal metabolic processes [23]. The physiological role of antioxidants is to prevent damage to cellular components resulting from chemical reactions involving free radicals. One of the plants that has antioxidant activity is bajakah tampala [21].

Bajakah tampala (*Spatholobus littoralis* Hassk) is one of the traditional medicinal plants from Central Kalimantan but has not been widely used [24]. Bajakah tampala also grows in Kolaka and Konawe, Southeast Sulawesi Province. Bajakah tampala Kolaka is believed by the public to be able to cure cancer, but empirically the public does not yet know the chemical compound content and biological activity, especially antioxidant activity. This is due to a lack of public and government knowledge about the potential of bajakah tampala root plant extracts cultivated outside their natural habitat [24].

Based on the results of previous research, bajakah has the potential to have antioxidant activity because it contains alkaloids, phenols, hydroquinone/tannins, and flavonoids. Triterpenoid and saponin compounds have the activity of counteracting free radicals and act as scavengers [25]. Flavonoid compounds have potential as antioxidants because they have hydroxyl groups attached to aromatic rings so they can capture free radicals produced by fat peroxidation reactions. Flavonoid compounds will donate one hydrogen atom to stabilize fat peroxidation radicals [23]. Research on tampala bajakah (*Spatholobus littoralis* Hassk) continues to be of interest to researchers, this can be seen in silico research on the inhibitory activity of the SARS-CoV-2 virus protease enzyme [18]. Based on this, research on many bajakah species in various regions becomes more important, especially the characterization of the chemical components and biological activities of many bajakah species in Kolaka, Southeast Sulawesi Province.

2. MATERIAL AND METHODS

2.1 Subjects

Bajakah tampala stems (*Spatholobus littoralis* Hassk) that have been pruned from the planting area are immediately cut into smaller sizes and dried using room temperature for 3-6 days, when the bajakah stems have a minimum water content, then proceed with the smoothing process by blending them so that during the maceration process it is more optimal and more extract is produced.

2.2 Isolation of Active Compounds from Bajakah of Kolaka with Maceration Method

Maceration extraction of samples of tampala bajakah root powder (*Spatholobus littoralis* Hassk) using ethanol solvent was carried out by weighing 100 grams of powder, and then placing it in a dark or brown bottle. The bottle containing the bajakah tampala stem powder sample was added with 250 mL of ethanol solvent. The bajakah tampala trunk powder sample was macerated using an orbital shaker for ± 6 days or until the sample was maximally extracted by the solvent. Next, the bajakah tampala root extract is filtered using filter paper and then evaporated which produces a brown solid. The chemical components of the brown bajakah tampala (*Spatholobus littoralis* Hassk) root extract were identified using an FT-IR and LC-MS/MS spectrophotometer.

2.3 Antioxidant Activity Test on Bajakah Root Extract

The antioxidant activity test of the ethanol extract of bajakah tampala roots was carried out at the Advanced Characterization Laboratory, Serpong-Indonesian Institute of Sciences (LIPI). The antioxidant activity of the ethanol extract of bajakah tampala against DPPH free radicals was measured according to the Yen and Chen method [26] and the method of Prasetyo *et al.* [27]. The extract solution in 2 mL of methanol was added to a solution of 0.5 mL of 1,1-Diphenyl-2-Picrylhydrazyl or DPPH (1 mM in methanol). The mixture was shaken and left at room temperature for 30 minutes. Absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 515 nm. The percent inhibition of the sample is calculated based on the difference in absorbance between the blank solution and the sample solution, using the following formula.

$$\% \text{Inhibition} = \left(1 - \frac{\text{The absorbance of blank solution}}{\text{The absorbance of the sample solution}}\right) \times 100\%$$

The percentage of DPPH-reducing activity was plotted against the sample concentration. The 50% concentration inhibition value (IC_{50}) is calculated from the linear regression graph of the percentage of inhibition against the sample concentration. The antioxidant activity test was carried out 2 times using quercetin as a comparison or positive control. A stock solution with a concentration of 1000 ppm was obtained by weighing 10 mg of sample dissolved in 10 mL of methanol. Antioxidant activity tests were carried out at various sample concentrations: 10; 50; 100; and 200 ppm which is made by dilution from a 1000 ppm stock solution. Measurement of the antioxidant power of quercetin was carried out at various concentrations: 1 ppm, 5 ppm, 10 ppm, and 20 ppm. Then 3.5 ml DPPH was added to each, vortexed, and incubated at 37^o C in a dark room. If all the electrons in DPPH are paired, the color of the solution changes from dark purple to bright yellow, so the color change can be measured with a UV-visible spectrophotometer. The ability of antioxidant compounds is inversely proportional to the antioxidant activity value (IC_{50}). The smaller the IC_{50} value, the greater the antioxidant compounds the ethanol extract has. Antioxidant compounds that have an $IC_{50} < 50 \mu\text{g/mL}$ are classified as very strong, $50 \mu\text{g/mL} < IC_{50} < 100 \mu\text{g/mL}$ are considered strong, $101 \mu\text{g/mL} < IC_{50} < 250 \mu\text{g/mL}$ are moderate, $251 \mu\text{g/mL} < IC_{50} < 500 \mu\text{g/mL}$ is classified as weak, and if the IC_{50} value $> 500 \mu\text{g/mL}$ is classified as very weak [28].

3. RESULTS AND DISCUSSION

3.1 Preparation of Bajakah Tampala Root Samples (*Spatholobus littoralis* Hassk)

Samples of bajakah tampala roots (*Spatholobus littoralis* Hassk) from Sembilanbelas November Village, Wundulako District, Kolaka Regency, Southeast Sulawesi. Tampala bajakah root samples (*Spatholobus littoralis* Hassk) were dried at room temperature for 3-6 days. Dried bajakah root samples are blended so that the maceration process is more optimal and to prevent enzymatic reactions or microbial activity, as well as preventing the growth of fungus so that it is not damaged and the chemical composition does not change [29]. Sample refinement aims to expand the surface of the sample so that the solvent can easily penetrate the sample, where the smaller the sample size, the larger the outer surface, making it easier to extract the sample to obtain maximum results [30].

The finely powdered samples of bajakah roots were extracted using the maceration method using ethanol solvent, because this method uses simple, easy, cheap equipment, and does not go through a heating process which can damage the composition of chemical compounds in the tampala bajakah root samples (*Spatholobus littoralis* Hassk). The principle of the maceration method is like dissolves like, meaning that the polar solvent process will dissolve the polar compounds contained in simplicia [31]. The maceration method has advantages, including that easily damaged compounds are well preserved because high temperatures are not used during the maceration process [32].

The extraction process uses a 96% ethanol solvent gradient under research conducted by Aminah [34] stating that ethanol solvent has advantages compared to distilled water and methanol solvents. Chemical compounds can be extracted with more ethanol than methanol and distilled water. This is following the research results of Rizqiana and Sudarmin [32] that ethanol can attract active substances flavonoids, interquinones, glycosides, basic alkaloids, coumarins, tannins, and saponins compared to the solvents methanol and distilled water.

The initial stage of the maceration process is where the bajakah tampala root powder is soaked using 96% ethanol solvent in a dark bottle. The soaking process is accompanied by stirring using an orbital shaker for 4-7 days until all the ingredients are mixed evenly. The ethanol extract solution was evaporated using a rotary vacuum evaporator. The evaporation process aims to remove the solvent used during the maceration extraction process to obtain a brown solid with a yield of 2.01%. The brown solid ethanol extract of bajakah tampala roots (*Spatholobus littoralis* Hassk) dissolves well in n-hexane and acetonitrile solvents but does not dissolve in distilled water.

3.2 Characterization of Chemical Components of Bajakah Tampala Root Ethanol Extract (*Spatholobus littoralis* Hassk)

Characterization of the compound content of the ethanol extract of bajakah tampala roots (*Spatholobus littoralis* Hassk) was identified using an FT-IR spectrophotometer and LC-MS/MS. The results of the FT-IR spectrophotometer analysis can be stated that the functional groups of the chemical component of the ethanol extract of bajakah tampala roots (*Spatholobus littoralis* Hassk) have the hydroxyl (-OH) functional group which is a wide spectrum at the wave number 3437.30 cm^{-1} , the functional group -C-Hsp² aromatic at wave number 3074.89 cm^{-1} which is supported by spectra at wave numbers 1553.84 cm^{-1} and 1511.90 cm^{-1} , aliphatic -C-Hsp³ functional group at wave numbers 2916.56 cm^{-1} and 2849.06 cm^{-1} which is supported by methylene spectra (-CH₂-) at a wave number of 1458.89 cm^{-1} with cutout bending vibrations, and the spectrum of the methylene functional group (-CH₃) at a wave number of 1373.89 cm^{-1} with symmetric bending vibrations. The sharp spectra at wave numbers 1728.43 and 1689.02 cm^{-1} are thought to be the carbonyl

functional group spectra of the chemical components of the ethanol extract of bajakah tampala roots (*Spatholobus littoralis* Hassk). Another clue that can support this reason is the appearance of bands in the wavelength area $980.90\text{--}536.99\text{ cm}^{-1}$, namely the bands 884.05 cm^{-1} , 828.51 cm^{-1} , and 791.41 cm^{-1} , and 677.08 cm^{-1} which indicates that the alkyl group probably contains three or more adjacent methylene groups ($-\text{CH}_2\text{-CH}_2-$). The band 1728.43 cm^{-1} is thought to come from the carbonyl group ($\text{C}=\text{O}$). Ester has strong absorption bands from $\text{C}=\text{O}$ stretching and $\text{C}-\text{O}$ stretching vibrations. This can be shown by the $\text{C}=\text{O}$ absorption band occurring at a frequency near 1728.43 cm^{-1} , while the $\text{C}-\text{O}$ absorption band is found in the fingerprint area, namely in the 1263.84 cm^{-1} , 1233.23 cm^{-1} and 1164.91 cm^{-1} bands. These bands are sometimes difficult to mark, but the $\text{C}-\text{O}$ bands are strong and can be used to differentiate between esters and ketones.

The results of LC-MS/MS analysis on the ethanol extract of tampala bajakah roots (*Spatholobus littoralis* Hassk) contain chemical components including: compound 1 apocynoside I ($\text{C}_{19}\text{H}_{30}\text{O}_8$; m/z 409.1826), compound 2 quercetin ($\text{C}_{15}\text{H}_{10}\text{O}_7$; m/z 303.0500), compound 3 hexosylphingosine ($\text{C}_{24}\text{H}_{47}\text{NO}_8$; m/z 478.3378), compound 4 3-hydroxy-7-methoxy-baicalein ($\text{C}_{16}\text{H}_{12}\text{O}_6$; m/z 301.0713), compound 5 momor-cerebroside I ($\text{C}_{48}\text{H}_{93}\text{NO}_{10}$; m/z 844.6878), compound 6 ambronal ($\text{C}_{30}\text{H}_{46}\text{O}_2$; m/z 439.3571), compound 7 stigmastan-3,6-dione ($\text{C}_{29}\text{H}_{48}\text{O}_2$; m/z 429.3726), and compound 8 trilaurin ($\text{C}_{39}\text{H}_{74}\text{O}_6$; m/z 661.5360) whose complete compound structures are presented in Figure 1.

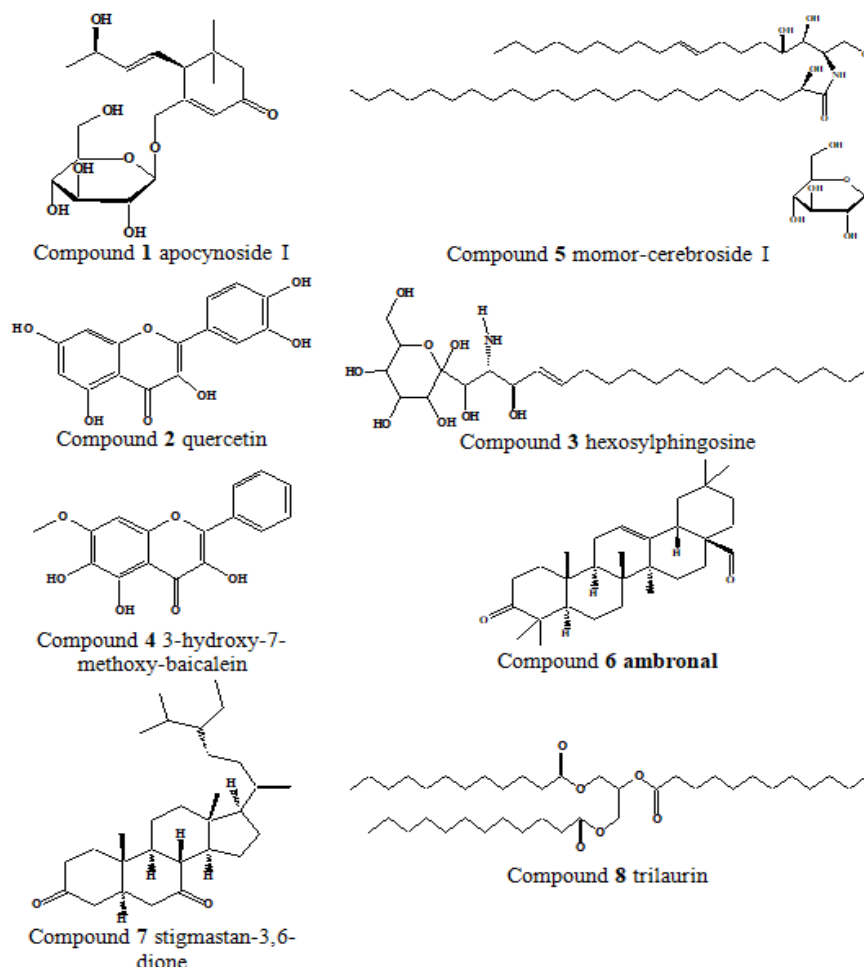


Fig. 1. Components of chemical compounds of bajakah tampala root extract (*Spatholobus littoralis* Hassk)

A total of 8 chemical components could be identified from the roots of bajakah tampala Kolaka which were isolated by the maceration method using ethanol solvent. The compounds in the ethanol extract of bajakah tampala roots have polar functional groups, including the hydroxyl (-OH) functional group found in compounds 1-6; the ester functional group (RCOOR) is found in compound 8, the ketone functional group (-C=O) is found in compounds 1, 2, 4, 6, and 7, the ether functional group (R-O-R) is found in compounds 1-5, the amine functional group and amino are found in compounds 3 and 5, the phenol functional group (PhOH) is found in compounds 2 and 4, the phenoxy functional group is found in compound 4, and the aldehyde functional group (RCOH) is found in compound 6. Two of the 8 compounds are the flavonoid group has a C₆A-C₃-C₆B structural framework which has a phenol (PhOH) functional group, namely compound 2 (quercetin) and compound 4 (3-hydroxy-7-methoxy-baicalein) which have antioxidant activity [33-34].

According to Fitriani et al. [13] the main components of bajakah tampala roots are phenolics, tannins, and flavonoids which have a very strong category, even higher than vitamin C and vitamin E, and comparable to the antioxidant activity of secang wood. Based on the results of this research, strengthen the reasons for isolating the components of chemical compounds and testing biological activity, especially antioxidant activity on the roots of bajakah tampala (*Spatholobus littoralis* Hassk) originating from Kolaka Regency, Southeast Sulawesi Province.

3.3 Antioxidant Activity Test of Bajakah Tampala Extract (*Spatholobus littoralis* Hassk)

Based on literature searches, many antioxidant molecules are circulating on the market, including vitamin E [35-36], Vitamin A [36], vitamin C [36], quercetin [34], catechin [37], andrographolide [37], curcuminoid [37], and baicalein [34]. Generally, these antioxidant compounds have a phenol (PhOH) functional group such as quercetin [34], baicalein [34], vitamin E [35-36], andrographolide [37], curcuminoid [37], and catechin [37], hydroxyl functional groups aliphatic (-OH) compounds such as Vitamin A [36], and vitamin C [36], and conjugated double carbon bond functional groups such as Vitamin A [36] and curcuminoids [37]. This functional group is analogous to the 8 chemical components found in the roots of bajakah tampala (*Spatholobus littoralis* Hassk). Among these antioxidant compounds are the flavonoid compounds quercetin [34], catechin [37], andrographolide [37], and baicalein [34] analogs with components of compound 2 quercetin (C₁₅H₁₀O₇; m/z 303.0500) and compound 4 3-hydroxy-7-methoxybaicalein (C₁₆H₁₂O₆; m/z 301.0713) which are owned by the roots of bajakah tampala (*Spatholobus littoralis* Hassk). Meanwhile, antioxidant compounds that have aliphatic hydroxyl (-OH) functional groups such as vitamin A [36] and vitamin C [36] are analogous to the chemical component of compound 8 trilaurin (C₃₉H₇₄O₆; m/z 661.5360) which is also found in roots bajakah tampala (*Spatholobus littoralis* Hassk), while antioxidant compounds that have aliphatic hydroxyl (-OH) functional groups such as vitamin A [36] and vitamin C [36] are analogous to the chemical component of compound 1 apocynoside I (C₁₉H₃₀O₈; m/z 409.1826), compound 3 hexosylphingosine (C₂₄H₄₇NO₈; m/z 478.3378), and compound 5 momor-cerebroside I (C₄₈H₉₃NO₁₀; m/z 844.6878) which are components of chemical compounds contained in the roots of bajakah tampala (*Spatholobus littoralis* Hassk). Based on the structure of the chemical components of bajakah tampala roots (*Spatholobus littoralis* Hassk) which are similar to antioxidant compounds that are already circulating on the market, this strengthens the urgency of research into antioxidant tests and the importance of publications that are beneficial for people who use bajakah tampala roots (*Spatholobus littoralis* Hassk) as traditional medicine.

The percent inhibition of the antioxidant activity of bajakah tampala (*Spatholobus littoralis* Hassk) roots in this study are presented in Tables 1 and 2. Based on Tables 1 and 2, the

results show that the higher the concentration of the bajakah tampala (*Spatholobus littoralis* Hassk) extract solution and the quercetin solution, the higher the absorbance.

Table 1. Antioxidant test results on Bajakah Tampala (*Spatholobus littoralis* Hassk)

Replication	Concentration (ppm)	Percent Inhibition (%)	IC ₅₀ (µg/mL)	Average IC ₅₀ (µg/mL)
1	10	34.48	21.84	21.75
	50	65.24		
	100	82.87		
	200	87.91		
2	10	34.57	21.65	21.75
	50	65.70		
	100	82.78		
	200	87.42		

Table 2. Antioxidant test results against the quercetin standard

Replication	Concentration (ppm)	Percent Inhibition (%)	IC ₅₀ (µg/mL)	Average IC ₅₀ (µg/mL)
1	1	20.25	2.96	2.94
	5	64.04		
	10	88.64		
	20	92.56		
2	1	19.81	2.91	2.94
	5	66.53		
	10	88.72		
	20	92.04		

The same thing as the percent inhibition value shows that the higher the concentration of the solution, the higher the percentage inhibition value. This happens because the higher the concentration of the solution, the higher the antioxidant content, thereby increasing the extract's ability to reduce free radicals. This is in line with the research results of Ikhlar *et al.* [37] that the concentration of the added extract affects the ability of the extract to reduce the increase in free radicals.

The linear regression curve of the relationship between the concentration of bajakah tampala extract (*Spatholobus littoralis* Hassk) and the percent inhibition for replications 1 and 2 can be seen in Figures 2 and 3. Figure 2 which is replication one, obtained the results of the linear regression equation, namely $y=18.6416 \ln(x)-7.4909$ with a correlation coefficient (R) of 0.9813. This value shows that between concentration and percent inhibition there is a correlation of 98.13%. In Figure 3, which is replication 2, the linear regression results obtained are $y=18.4612 \ln(x)-6.7711$ with a correlation coefficient (R) of 0.9796. This value shows that concentration and percent inhibition correlate with 97.96%. Based on the results of the linear regression equation of the antioxidant activity test of bajakah tampala extract, it was found that the concentration was directly proportional to the percent inhibition value. The greater the concentration of the standard solution, the greater the percent inhibition. This is under Lindawati's research [38] stating that if the R value is close to 1 it can be said that the relationship between percent inhibition and concentration has a very strong correlation.

Based on antioxidant activity test data, the results showed that bajakah tampala Kolaka had an IC₅₀ value of 21.75 µg/mL and the quercetin control solution had an IC₅₀ value of 2.9 µg/mL. Based on the IC₅₀ value of Bajakah Tampala (*Spatholobus littoralis* Hassk) and the quercetin control solution, it can be stated that bajakah tampala Kolaka has very strong antioxidant activity. This is under research by Ibroham *et al.* [39] stated that if the IC₅₀ value

<50 µg/mL is included in the category of very strong antioxidant activity. The IC₅₀ value of the quercetin control solution has a higher value, compared to the IC₅₀ value of tampala bajakah (*Spatholobus littoralis* Hassk) but is still in the very strong category. The quercetin standard solution has very strong antioxidant activity because it is a pure compound (single compound) that has a C₆A-C₃-C₆B flavonoid framework structure that has 2,3 double bonds and 4-oxo bonds in the quercetin skeleton ring, allowing electron delocalization to occur. from the C₆A ring to the C₃ framework and further delocalizes to the C₆B ring which shows broad resonance. This results in significant efficiency for radical destruction. Therefore, quercetin has a structure that is responsible for the higher effectiveness of antioxidant activity [40].

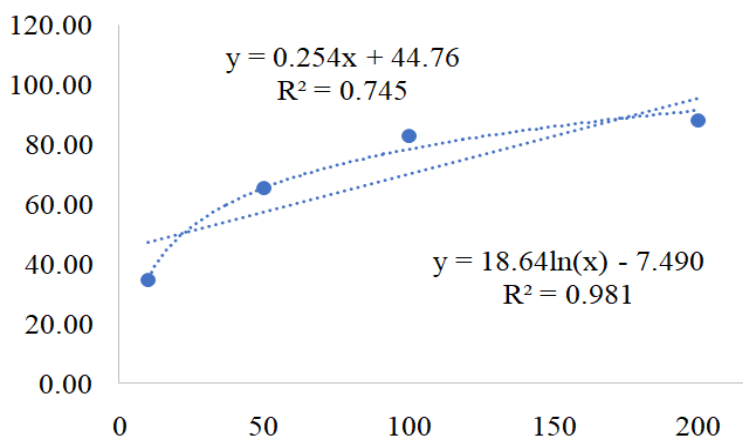


Fig. 2. Linear regression of the relationship between concentration and percent inhibition of replication 1

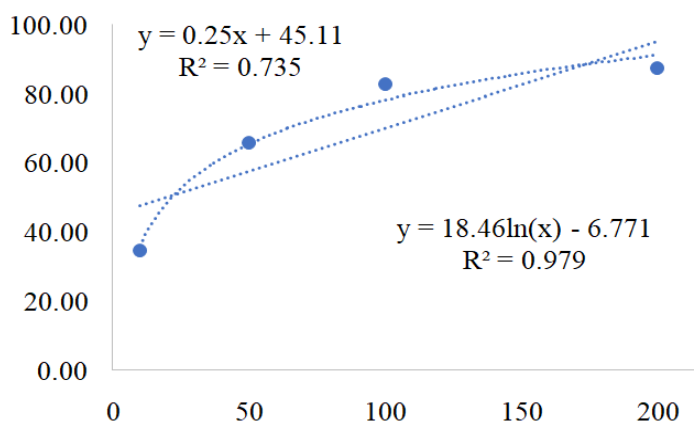


Fig. 3. Linear regression of the relationship between concentration and percent inhibition of replication 2

Quercetin was used as a control solution because it is a flavonol from the group of polyphenolic flavonoid compounds found in almost every type of plant and quercetin is a natural antioxidant that has very strong antioxidant activity. This is under research by Nastati [41], quercetin is known to have very strong antioxidant activity because it has a phenolic group (PhOH) as a functional group which is responsible for antioxidant activity because it is capable of being a hydrogen or electron donor. Quercetin was chosen as a positive control solution because quercetin is a natural antioxidant that has very strong activity so it is very suitable as a comparison to determine the antioxidant activity of bajakah tampala extract (*Spatholobus littoralis* Hassk) [42].

The results of the antioxidant activity test for bajakah tampala (*Spatholobus littoralis* Hassk) obtained an IC₅₀ value of 21.75 µg/mL (very strong), this is in line with the research results of Amiani et al. [21] that the antioxidant compounds in bajakah tampala root extract have an antioxidant IC₅₀ value of 13.2535 µg/mL (very strong). However, different from the results of research by Salsabilla et al. [22] the IC₅₀ value of bajakah tampala infusion extract originating from Kalimantan has an antioxidant activity value of 70.81 µg/mL (strong). This is caused by differences in the extraction methods used in the research. The research of Salsabilla et al. [22] used the infusion method, namely hot extraction, while in this study the maceration method was used because the maceration method is easier and there is no heating process, thus allowing no reduction in the chemical compound content of the extraction results. This is by Lindawati's research [39] that the maceration method has a simple process and does not involve heating so it can prevent damage to chemical compounds that are not resistant to heating, especially flavonoids. Apart from this, it is caused by different extraction methods and types of solvents. The aim of using 96% ethanol solvent is to adjust the polarity of the solvent to the polarity of the compound to be isolated. Flavonoid compounds have a hydroxy group (-OH) which is the same as ethanol solvent which has a -OH functional group that functions to bind compounds in the ethanol extract of bajakah tampala. This is the opinion of Patria and Soegihardjo [43] that ethanol was chosen as a solvent because it presents a better amount of polyphenols compared to methanol and water extracts, is more efficient in penetrating cell walls to attract polyphenols in cells, and is preservative for microorganisms. Meanwhile, research by Salsabilla et al. [22] using distilled water as a solvent showed that the use of ethanol was better than distilled water. This is the opinion of Azizah and Salamah [44] that in the extraction process, ethanol has advantages compared to the solvents distilled water and methanol. The number of chemical compounds extracted with ethanol is greater than using the solvents methanol and water, in other words, ethanol can dissolve more chemical compounds than methanol and distilled water. This is under Aliviyanti's research results [45] that 96% ethanol is the best solvent, compared to water, methanol, and *n*-hexane solvents.

Based on research results, the bajakah tampala (*Spatholobus littoralis* Hassk) has antioxidant activity of 21.75 µg/mL, which is included in the very strong category. The results of this research are under the research results of Amiani et al. [21] that bajakah tampala has antioxidant activity that is classified as strong to very strong. If the antioxidant activity of bajakah is associated with secondary metabolites such as flavonoids, phenolics, tannins, polyphenols, alkaloids, and terpenoids, then flavonoids are the main content of bajakah which is largely responsible for causing high antioxidant activity. Flavonoids are compounds in the phenolic group that have various bioactivities such as enzyme inhibitors, protection from ultraviolet, pigmentation, and defense against various diseases. Flavonoids can act as anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, antithrombotic, antiviral, and anti-carcinogenic. As antioxidant agents, flavonoids can stabilize free radicals through their reaction with free radical reactive compounds so that the compounds become more stable and less reactive.

4. CONCLUSION

Based on the results of the research above, it can be concluded that the ethanol extract of Bajakah tampala roots (*Spatholobus littoralis* Hassk) from Kolaka Regency, Southeast Sulawesi Province has 8 chemical compounds that were detected, namely: compound 1 apocynoside I, compound 2 quercetin, compound 3 hexosylphingosine, compound 4 3-hydroxy-7-methoxybaicalein, compound 5 momor-cerebroside I, compound 6 ambronal, compound 7 stigmastan-3,6-dione, and compound 8 trilaurin. The ethanol extract of bajakah tampala roots (*Spatholobus littoralis* Hassk) using the maceration method had an antioxidant activity of 21.75 µg/mL which was lower, when compared to the antioxidant activity of quercetin of 2.94 µg/mL as a positive control, but the antioxidant activity of bajakah tampala

root ethanol extract tampala (*Spatholobus littoralis* Hassk) is still included in the very strong category.

CONSENT

We all authors declare that this research did not involve patients, but involved samples from the bajakah tampala plant (*Spatholobus littoralis* Hassk) and chemicals.

ETHICAL APPROVAL

All authors hereby declare that all stages of the experiment did not involve humans and humans as research objects.

REFERENCES

1. Aryanti R, Perdana F, and Rizkio RAM. Study of Antioxidant Activity Testing Methods of Green Tea (*Camellia sinensis* (L.) Kuntze). *Journal of Surya Medica*. 2021; 7(1):15-24.
2. Ulfa SR and Susanto BNA. Effect of Decoction of Bajakah Stems (*Spatholobus littoralis* Hassk) with Cigarette Smoke Exposure to Ovarian Morphometry of Mice (*Mus musculus*). *Nusantara Hasana Journal*. 2022; 1(9): 65-69.
3. Hasna LZ, Sehkaemi P, and Aviciena MA. Review: Bajakah Wood Roots and their Health Benefits. *FoodTech: Jurnal Teknologi Pangan*. 2022; 4(1): 32-39. DOI: <http://dx.doi.org/10.26418/jft.v4i1.56637>.
4. Yudiane IPG, Gama SI, and Ahmad I. Secondary Metabolite Profile and Antioxidant Activity Tests from the Ethanol Fraction of Bajakah Root (*Uncaria nervosa* Elmer). *Proc. Mul. Pharm. Conf*. 2023; Samarinda, 15-17 Maret 2023.
5. Supriatno, Yasmin T, Rizkiyani A, and Akmalia MF. Ethanol Extract of Borneo's Bajakah Tampala Root (*Spatholobus littoralis* hassk) Suppresses the Cell Proliferation and Chemotactic Migration and Induces Apoptosis on a Human Oral Tongue Squamous Cell Carcinoma Cell. *Journal of International Dental and Medical Research*. 2023; 16(2): 607-612.
6. Abdulrahman, Utami SR, Widia, dan Roanisca O. Study of Secondary Metabolites of Flower Stem *Spatholobus Littoralis* Hassk. in Development as an Anticancer Herbal Medicine Breast and Antioxidants. Conferences. *National Seminar on Research and Community Service*. 2021; Faculty of Engineering-University of Bangka Belitung.
7. Nastati K, Nugraha DF. Anti-inflammatory Activity of Bajakah Wood Extract (*Spatholobus Littoralis* Hassk). *Journal of Surya Medica*. 2022; 7(2): 45-50.
8. Rousdy DW, Wardoyo ERP, and Ifadatin S. Anti-inflammatory Activity of Bajakah Stem (*Spatholobus littoralis* Hassk.) Ethanolic Extract in Carrageenan-Induced Paw Edema Mice. *Journal of Biodjati*. 2022; 7(1): 66-74.
9. Rousdy DW and Wardoyo ERP. In Vitro Antiinflammatory Activity of Bajakah (*Spatholobus littoralis*) Stem Extract. *Biosaintifika*. 2023; 15(2): 150-157.
10. Nastiti K, Nugraha DF, and Kurniawati D. Identification of Active Antibacterial Compounds from *Spatholobus Littoralis* Hassk Extract With GCMS (Gas Chromatography-Mass Spectroscopy). *Journal of Surya Medica*. 2023; 9(1): 277-282.
11. Ikwanti D, Perawati S, and Andriani L. Test of Antibacterial Activity of the Yellow Bajakah Plant Fraction (*Arcangelisia Flava* (L.) Merr.) Against *Propionibacterium Acnes*. *Syntax Literate: Jurnal Ilmiah Indonesia*. 2023; 8(11): 1-17.

12. Saparuddin L, Suleman AW, and Mansur. Antibacterial Activity Test of Bajakah Wood (*Spatholobus littoralis* Hassk.) Against the Growth of *Staphylococcus aureus*. *Journal of Pharmaceutical Sciences*. 2023; 4(1): 108-114.
13. Fitriani F, Sampepana E, and Saputra SH. Characterization of Bajakah Root Plants (*Spatholobus littoralis* Hassk) from Loa Kulu Kutai Kartanegara Regency. *Indonesian Journal of Industrial Research*. 2020; 12(2): 365-376.
14. Bandy NA, Erniwati, Muthmainnah, Ariyanti, Hapid A, and Asniati. Phytochemical Analysis of Bajakah Leaf Extract (*Poikilospermum suaveolens* (Blume) Merr) From Kapiroe Village Palolo District, Sigi District, Central Sulawesi Province. *Journal of Warta Rimba*. 2021; 9(1): 31-42.
15. Octaviana YN, Febriyanti R, and Kusnadi. Determination of Total Phenol Content in Bajakah Root Extract (*Spatholobus littoralis* Hassk) Results of the Inflation Process of Several Brands Circulating on the Market. *Pharmacy Scientific journal*. 2023; 10(1): 1-10.
16. Hafifah DK and Suparno. Effect of Red Bajakah Tampala Flavonoid Concentration as Antibacterial against *Bacillus subtilis*. *Scientific Journal of Science*. 2022; 23(2): 99-107. DOI: <https://doi.org/10.35799/jis.v23i2.49392>.
17. Hamzah H, Pratiwi SUT, Jabbar A, Hafifah AS, Al-Fajri BA, Nurhalisah N. Bioactivity Tracing of the Ethanol Extract of Bajakah Tampala (*Spatholobus littoralis* Hassk.) Typical Plant of Kalimantan Island as Antibiofilm of *Staphylococcus aureus*. *Macedonian Journal of Medical Sciences*. 2023; 11(A): 8-14. <https://doi.org/10.3889/oamjms.2023.10676>.
18. Norhayati, Andika, and Purwanto A. In Silico Study of Bajakah Compounds (*Spatholobus suberectus*) to Protease SARS-CoV-2 Inhibitor. *Journal of Science and Health*. 2023; 5(2): 78-89. DOI: <https://doi.org/10.25026/jsk.v5i2.950>.
19. Ridho AE. 2013. Test the antioxidant activity of the methanol extract of lacum fruit (*Cayratia trifolia*) using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) method. *Journal of Pharmacy Students*, Faculty of Medicine, UNTAN, 1(1).
20. Yuslianti ER. Introduction to free radicals and antioxidants. Publisher Deepublish. Yogyakarta. Indonesian. 2018.
21. Amiani W, Fahrizal MR, and Aprelea RN. Secondary Metabolite Content and Activity of Bajakah Plants as Antioxidant Agents. *Jurnal Health Science*. 2022; 3(4): 516-522.
22. Salsabila H, Febriyanti R, and Amananti W. Determination of Antioxidant Activity of Bajakah Tampala (*Spatholobus Littoralis* Hassk) and Kalalawit (*Uncaria Gambir* Roxb) Root Infusion Using the DPPH Method. *Jurnal Crystal: Chemical Research Publications and Applications*. 2023; 5(1): 22-29.
23. Martingsih NW, Widana GAB and Kristiyanti P. Phytochemical Screening and Antioxidant Activity Test of Ethanol Extract of Matoa (*Pometia pinnata*) Leaves using the DPPH Method. *FMIPA Undiksha*. 2016; 332-339.
24. Ayuchecaria N, Saputera MMA, and Niah R. Determination of Total Phenolic Content of Bajakah Tampala (*Spatholobus littoralis* Hassk.) Stem Extract Using UV-Visible Spectrophotometry. *Journal of Indonesian Pharmaceutical People*. 2020; 3(1): 132-141.
25. Adhityasmara D and Ramonah D. Hepatoprotective Effect of Ethanol Extract of Bajakah Tampala Stems (*Spatholobus Littoralis* Hassk) on Isoniazid-Induced Rats. *Scientific Journal of Science*. 2022; 40-48.

26. Yen GC and Chen HY. Antioxidant Activity of Various Tea Extracts with their Antimutagenicity. *Journal of Agricultural and Food Chemistry*. 1995; 43(1): 27-32. <http://dx.doi.org/10.1021/jf00049a007>.
27. Prasetyo E, Kharomah NZW, and Rahayu TP. Antioxidant Activity Test Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) Method on Ethanol Extract of Durian Fruit Peel (*Durio zibethinnus* L.) from Alasmalang Village, Banyumas Regency. *Jurnal Pharmascience*. 2021; 8(1): 75-82.
28. Ibroham MH, Jamilatun S, and Kumalasari ID. The Potential of Plants in Indonesia as Natural Antioxidants. *LPPM UMJ National Research Seminar*. E-ISSN:2745-6080. 2022.
29. Handayani V, Ahmad AR, and Sudir M. Test of Antioxidant Activity of Methanol Extracts of Flowers and Leaves Patikala (*Etingera elatior* (Jack) R.M.Sm) Uses DPPH Method. *Pharm Sci. Res*. 2014; 1(2): 86-93.
30. Abdulrahman A, Utami SR, Widia W, and Roanisca O. Study of Secondary Metabolites of Bajakah Stem (*Spatholobus littoralis* Hassk.) in Development as an Antioxidant and Anti-Breast Cancer Herbal Medicine. *In Proceedings of National Colloquium Research and Community Service*. 2021; 5: 46-49.
31. Dewatisari WF. Comparison of Chloroform and Ethanol Solvents on the Yield of Mother-in-law's Tongue (*Sansevieria trisfasciata* Prain) Leaf Extract using the Maceration Method. *Journal UIN Alauddin*. 2020; 127-132.
32. Rizkiana A and Sudarmin. Analysis of Antioxidant Activity on the Ethanol of Indonesian Tropical Forest Plants. *Indonesian Journal of Chemical Science*. 2023; 12(1): 47-57.
33. Ozgen S, Kilinc OK, and Selamoglu Z. Antioxidant Activity of Quercetin: A Mechanistic Review. *Turkish Journal of Agriculture-Food Science and Technology*. 2016; 4(12): 1134-1138.
34. Peng-fei L, Fu-gen H, Bin-bin D, Tian-sheng D, Xiang-lin H, and Ming-qin Z. 2013. Purification and antioxidant activities of baicalin isolated from the root of huangqin (*Scutellaria baicalensis* gcorsi). *J Food Sci Technol*. 50(3): 615-619.
35. Pekiner BD. Vitamin E As An Antioxidant. *J. Fac. Pharm Ankara*. 2023; 32(4): 243-267. DOI: 10.1501/Eczfak_0000000393.
36. Didier AJ, Stiene J, Fang L, Watkins D, Dworkin LD, and Creeden JF. Antioxidant and Anti-Tumor Effects of Dietary Vitamins A, C, and E. *Antioxidants*. 2023; 12 (632): 1-26. <https://doi.org/10.3390/antiox12030632>.
37. Ikharrar MS, Yudistira A, and Wewengkang DS. Antioxidant Activity Test on *Stylissa* Sp. using the DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Method. *Pharmacon*. 2019; 8(4): 961-967. <https://doi.org/10.35799/pha.8.2019.29376>.
38. Lindawati NY. Ethanol Extract of Red Beans (*Phaseolus Vulgaris* L.) using Complex Colorimetric Methods using Visible Spectrophotometry. *Manuntung Scientific Journal*. 2020; 6(1): 83-91.
39. Ibroham MH, Jamilatun S, and Kumalasari ID. The Potential of Plants in Indonesia as Natural Antioxidants. *LPPM UMJ National Research Seminar*. E-ISSN:2745-6080. 2022.
40. Mlcek J, Jurikova T, Skrovankova S, and Sochor J. Quercetin and Its Anti-Allergic Immune Response. *Molecules*. 2016; 21(623): 1-15. doi:10.3390/molecules21050623.

41. Nastiti K, Noval, and Kurniawati D. Antioxidant Activity Combination of *Piper Betle* Leaf Infusion, Ethanolic Extract of Bundung (*Actinuscirpus Grossus*) And Citrus Fruit Peel of Citrus Aurantifolia. *Journal of Surya Medica*. 2021; 7(1): 115-122.
42. Jatmiko MP and Mursiti S. Isolation, Identification, and Activity Test of Flavonoid Compounds in Jamblang Leaves (*Syzygium cumini* L.) Skeel as Antioxidants. *Indonesian Journal of Chemical science*. 2021; 10(2): 129-138.
43. Patria WD and Soegihardjo C.J. Antioxidant Activity Test using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical and Determination of Total Phenolic Content of Ethyl Acetate Fraction of Ethanolic Extract of Mistletoe Leaves (*Dendrophthoe Pentandra* L. Miq.) Growing on Kepel Trees (*Stelechocarpus Burahol* (Bl.) Hook F.). *Journal of Science and Community Pharmacy*. 2013; 10: 51-60.
44. Azizah B And Salamah N. Standardization of Non-Specific Parameter and Comparative Levels of Curcumin Extract Ethanol and Extract of Purified Turmeric Rhizome. *Pharmaceutical Scientific Journal*. 2013; 3(1): 21-30.
45. Aliviyanti RUY, Sudiby RS, and Murwanti. Cytotoxic Effect of Kalimantan Bajakah Roots on T47D Breast Cancer Cells. *Scientific Research Journal*. 2021; 26(2): 131-140. <https://journal.uny.ac.id/index.php/saintek>.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

Bajakah tampala kolaka: a traditional medicinal plant of the species *Spahtolobus littoralis* Hassk which grows in Kolaka district, Southeast Sulawesi Province, which is used as a tradisional medicine for the community of Kolaka district, Southeast Sulawesi Province.

APPENDIX

IC₅₀ : Inhibition Concentration 50%

FTIR : Fourier Transform Infra Red

LC-MS/MS: Liquid Chromatography-Mass Spectrometry/Mass Spectrometry

DPPH : 1,1-Diphenyl-2-Picrylhydrazyl

UV-Viss : Ultra Violet-Vissible