ORIGINAL ARTICLE

Antioxidant activity of roots, stems, and leaves *Spatholobus littoralis* Hassk.: an experimental study

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ABSTRACT

Introduction: Tooth extraction is an action that will leave scars where the procedure is conducted. Post-extraction wounds will heal after going through 4 complex healing phases. *Reactive Oxygen Species* (ROS) play a role in wound healing, where antioxidants become substances that can control ROS levels in the body. *Spatholobus littoralis* Hassk., which comes from Central Kalimantan, has benefits in the wound healing phase. The aim of study was to analyze the effectiveness of *Spatholobus littoralis* Hassk., extracts on antioxidant activity. **Methods:** The type of research was an in vitro laboratory experimental study. Extracts of roots, stems, and leaves of *Spatholobus littoralis* Hassk. DPPH 2, 2-Diphenyl-1-picrylhydrazylradical as negative control, and Vitamin C as positive control were tested for antioxidants by using DPPH solution. Tests using a spectrophotometer with a wavelength of 517 nm were conducted after each sample was incubated for 30 minutes. Then, It would be calculated to determine the percentage of inhibition and IC50 of each sample. Data analysis used in this research was One-way ANOVA. **Results:** One-way ANOVA test showed no significant differences in the root extract, DPPH, and vitamin C groups; besides, there were significant differences in the stem and leaf extract groups. In the post hoc Tukey test, a concentration of 2500 ppm in stem extract was the most effective concentration, and a concentration of 2000 ppm in leaf extract was the most effective, with IC50 values from lowest to highest: stem extract (9.46), vitamin C (11.52), root extract (23.86), leaf extract (47.71), and DPPH (1660710) **Conclusion:** Extract of *Spatholobus littoralis* Hassk. has antioxidant activity, with the highest antioxidant activity in *Spatholobus littoralis Hassk*. stem extract, the most effective concentration is at 2500 ppm.

KEYWORDS

DPPH, *reactive oxygen species*, *spatholobus littoralis* Hassk.

INTRODUCTION

Teeth extraction is done by separating the tooth that can no longer be maintained from the surrounding soft tissue.[1] Tooth extraction is an act of removing teeth from the alveolar and carried out if there is an indication that the tooth cannot be restored or maintained.[2,3] After the extraction action, it will leave a wound where the action was carried out and around it.[4] Wounds in tooth extraction have an opportunity for infection due to bacteria in the mouth.[5]

The infection also triggers complications after tooth extraction.[6] Derivatives of radical compounds from oxygen, commonly known as Reactive Oxygen Species (ROS), also play a role in the healing process of wounds that act as secondary messengers to the injured area.The Controlled ROS can help speed up the wound healing process.[7]

Antioxidants have a role in controlling ROS levels.[7] Antioxidants act by preventing the formation of excess ROS by binding ions.[8] Antioxidants will defend cells from oxidative damage and accelerate the wound healing process.[9] Antioxidants can come from natural ingredients. One of the natural ingredients that contain antioxidants is *Spatholobus littoralis* Hassk. Extract from *Spatholobus littoralis* Hassk. contains alkaloids, saponins, tannins, phenols, flavonoids, and triterpenoids.[10]

One of the methods used to determine antioxidant activity qualitatively is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.[11] This method is often used because it is easier and cheaper than the other methods. DPPH method is usually used to find out the antioxidant in qualitative way.[11]

Based on previous research, it was revealed that *Spatholobus littoralis* Hassk. stem extract has effectiveness as an anti-inflammatory and accelerates fibroblast migration in the wound healing process in vitro.[12] Not only used for the stem, roots, and leaves, the *Spatholobus littoralis* Hassk. plant is also rich in flavonoids which have a role as active compounds in antioxidants.[13]

Wound healing will be completed after passing through 4 phases: hemostasis, inflammatory, proliferation, and remodeling. Antioxidants have a role in every healing process. In the hemostasis phase, antioxidants control ROS levels, which help in the blood clotting process.[14] Later on, at the inflammatory phase, antioxidants inhibit ROS, resulting in excess inflammation.[15]

In the proliferative phase, antioxidants will regulate ROS levels so that further damage does not occur.[7] In the proliferative phase, antioxidants will regulate ROS levels so that further damage does not occur. While in the remodeling phase, antioxidants will regulate ROS levels so that collagen increases quicker.[7]

There still needs to be more research conducted on the effectiveness of the active compounds in *Spatholobus littoralis* Hassk. extract in the process of wound healing. It is necessary to carry out further research to determine the antioxidant activity contained in the extracts of the roots, stems, and leaves of *Spatholobus littoralis* Hassk. so that it can be known as the most exciting part of a controller of ROS in the wound healing process. The aim of the study was to analyze the effectiveness of *Spatholobus littoralis* Hassk. extracts on antioxidant activity.

METHODS

This research was an in vitro laboratory experimental research using the DPPH method to determine antioxidants qualitatively. The research group was *Spatholobus littoralis* Hassk. obtained from Muara Teweh, Central Kalimantan, with root, stem, and leaf extracts concentrations of 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm, DPPH as negative control and Vitamin C positive control.

Phytochemical testing to determine the active compounds contained in the extracts of the roots, stems, and leaves of *Spatholobus littoralis* Hassk. was carried out at Balai Penelitian Tanaman Rempah dan Obat (Balitro).

The preparation of the roots, stems, and leaves extract of *Spatholobus littoralis* Hassk was done through the maceration method at Balitro. Roots, stems, and leaves of *Spatholobus littoralis* Hassk. were first dried in indirect sunlight, then crushed into powder using a 60-mesh filter blender (Test Mesh Shiver, Guangzhou, China). Powdered roots, stems, and leaves of *Spatholobus littoralis* Hassk. were extracted using the maceration method with 70% ethanol solution for three days and evaporated with an evaporated machine (New Style RE201D Rotary Evaporator, China). The extract was stored at -20°.

Results of extracts of roots, stems, and leaves of *Spatholobus littoralis* Hassk. were divided into 4 concentration groups of 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm. Extracts that had been made, negative control (DPPH) and positive control (vitamin C), were placed in a pyrex glass (Pyrex, Indonesia). DPPH reagent was prepared by mixing 4 mg of DPPH powder which had been weighed with 100 ml of 96% ethanol. Each group was mixed with 1 ml of DPPH reagent, which was measured using a micropipette.

All groups added with the reagent were mixed to become homogeneous using a centrifugal apparatus (TOMY MDX-310, Tokyo, Japan.). Then it was covered using aluminum foil, and incubated for 30 minutes. After 30 minutes, measurements were made using a spectrophotometer (Biorad, Berkeley, USA.) with a wavelength of 517 nm. The DPPH test was done at Laboratorium BIOCORE Fakultas Kedokteran Gigi, Universitas Trisakti. The score obtained from the spectrophotometer measurement was calculated using the following formula Percentage of Inhibition from Abrosbansi Blanko minus Absorbansi Sample divided by? Absorbansi Blanko and times 100.The scores obtained were then applied to a linear diagram.

The Y value obtained calculated the percentage formula for antioxidant activity. A material is said to contain antioxidant activity if the percentage of its antioxidant activity is more than or equal to 50%, or it is called the concentration of inhibitor 50 (IC 50). IC50 is the standard for determining antioxidant activity.[16]

The data obtained were tested statistically using the Statistical Package for the Social Sciences (SPSS) version 25. All groups were tested for normality, then a one-way ANOVA test was carried out and followed by Tukey's Post Hoc test to determine significant differences in each concentration.

RESULTS

The results of the phytochemical test found that the root and leaf extracts of *Spatholobus littoralis* Hassk. contained the same compounds, but the stem extracts of *Spatholobus littoralis* Hassk. found different compounds, namely triterpenoids. (Table 1). The calculation of percentage of inhibition resulted in each extract group were obtained from the spectrophotometric readings, which can be seen in Table 2.

Table 1. Phytochemical test

Compound	Roots	Stems	Leaves	Methods
Alkaloid	-	-	-	Qualitative
Saponins	+	+	+	
Tannins	+	+	+	
Phenolics	+	+	+	
Flavonoids	+	+	+	
Steroids	-	-	-	
Glycosides	+	+	+	
Triterpenoids	-	+	-	

Table 2. percentage of inhibition

No	Group	Concentration in ppm	% Inhibition
1.	Roots	1000	28.633
		1500	30.709
		2000	28.826
		2500	31.772
2.	Stems	1000	26.943
		1500	24.287
		2000	22.018
		2500	16.127
3.	Leaves	1000	31.772
		1500	25.591
		2000	23.804
		2500	24.915
4.	Vitamin C	1000	6.035
		1500	6.373
		2000	5.939
		2500	3.283
5.	DPPH	1000	0.096
		1500	0.434
		2000	0.144
		2500	0.241

Table 2 shows that the percentage of inhibition of each extract group had a high percentage of inhibition. Furthermore, the highest percentage of inhibition value in the root group was obtained at a concentration of 2500 ppm (31.7721). Meanwhile, in the stem extract group, the highest % inhibition was at a concentration of 1000 ppm (29.9435). The leaf group got the highest % inhibition at a concentration of 1000 ppm (31.7721). In the positive control group used in this study, the highest inhibition was obtained at a concentration of 1500 ppm (6.3737), and the negative control group at a concentration of 1500 ppm (0.4346).

The application of the results of percentage of inhibition to the linear diagram produced the Y value of root extract (7.533x + 26.102), stem extract (6.900x + 34.495), leaf extract (4.0500x + 34.346), DPPH (3E-05x + 0.1787) and vitamin c (17.00x + 8.450). The Y value would then be applied to the IC50 calculation to produce an X value.[16] Based on the IC50 calculation, it shows that the resulting antioxidant activity varied. (Table 3). IC50 values were in sequence from the highest to the lowest: the negative control group (1660710) and the *Spatholobus littoralis* Hassk. leaf extract group. (47.71), *Spatholobus littoralis* Hassk. root extract group. (23.86), positive control group (11.52) and *Spatholobus littoralis* Hassk. stem extract. (9.46). IC50 calculation results in the extract group can be seen in Table 3.

Table 3. IC50 Calculations

No.	Group	IC50
1.	Roots	23.86
2.	Stems*	9.46
3.	Leaves*	47.71
4.	Vitamin C	11.52
5.	DPPH	1660710

*shows a significant difference ($p<0.05$)

The results of statistical tests were carried out on the SPSS version 25 application using one-way ANOVA. Based on the results of statistical tests, there was no significant difference ($p>0.005$) in

Spatholobus littoralis Hassk. root extract (p=0.018), DPPH negative control (p=0.099), and vitamin C positive control (p=0.083). There was a significant difference (p<0.005) in *Spatholobus littoralis* Hassk. stem extract (p=0.001) and *Spatholobus littoralis* Hassk. leaf extract. (p=0.000).

Post Hoc Tukey test results on *Spatholobus littoralis* Hassk. stem extract. 2500 ppm concentration was obtained as the most effective concentration. Whereas in *Spatholobus littoralis* Hassk. leaf extract. 2000 ppm concentration was obtained as the most effective concentration (p<0.05). (Table 4)

Table 4. SD Mean Value. P-value one-way ANOVA and post hoc Tukey test stem and leaf extract group of *Spatholobus littoralis* Hassk.

Group	Concentration	MD ± SD	p-value
Stems	1000 vs. 1500	-0.014 ± 0.010 [abc]	0.001*
	1000 vs. 2000	-0.026 ± 0.010 [abc]	
	1000 v 2500	-0.056 ± 0.010 [ac]	
	1500 vs 1000	0.014 ± 0.010 [abc]	
	1500 vs 2000	-0.012 ± 0.010 [abc]	
	1500 vs. 2500	-0.043 ± 0.010 [ac]	
	2000 vs 1000	0.026 ± 0.010 [abc]	
	2000 vs 1500	0.012 ± 0.010 [abc]	
	2000 vs. 2500	-0.031 ± 0.010 [ac]	
	2500 vs. 1000	0.056 ± 0.010 [ac]	
	2500 vs. 1500	0.042 ± 0.010 [ac]	
	2500 vs. 2000	0.031 ± 0.010 [ac]	
Leaves	1000 vs. 1500	-0.032 ± 0.007 [abc]	0.000*
	1000 vs. 2000	-0.041 ± 0.007 [ac]	
	1000 v 2500	-0.036 ± 0.007 [abc]	
	1500 vs 1000	0.032 ± 0.007 [abc]	
	1500 vs. 2000	-0.009 ± 0.007 [ac]	
	1500 vs 2500	-0.004 ± 0.007 [abc]	
	2000 vs. 1000	0.041 ± 0.007 [ac]	
	2000 vs. 1500	0.009 ± 0.007 [ac]	
	2000 vs. 2500	0.006 ± 0.007 [ac]	
	2500 vs. 1000	0.036 ± 0.007 [ac]	
	2500 vs 1500	0.004 ± 0.007 [abc]	
	2500 vs 2000	-0.006 ± 0.007 [abc]	

a-c in different columns shows a significant difference (p<0.05);
*shows a significant difference (p<0.05)

DISCUSSION

Results of phytochemical tests that have been carried out on *Spatholobus littoralis* Hassk. extract showed that the root and leaf extracts of *Spatholobus littoralis* Hassk. contains saponins, tannins, phenolics, flavonoids, and glycosides. *Spatholobus littoralis* Hassk. stem extract contains one different compound namely triterpenoids (Table 1). These results are in accordance with research that has been done before.[17] Triterpenoids which are known to have a role as high antioxidant active compounds are aligned with research on *Spatholobus littoralis* Hassk. stem extract is proven to accelerate fibroblast migration and as an anti-inflammatory agent in the wound healing process.[12]

Tannins, flavonoids, and saponins are contained in *Spatholobus littoralis* Hassk. The extract also has a role in active antioxidant compounds. This was proven in research conducted on *Sprague Dawley* rats, that *Persea ameracana* Mill seed extract, which contains these active compounds, can increase the formation of fibroblasts and act as an anti-inflammatory in wound healing.[10]

The active compounds contained in the extracts of the roots, stems, and leaves of *Spatholobus littoralis* Hassk. plays a significant role in the presence of antioxidant activity which acts as a controller of ROS in the process of wound healing.[13,18] It is known that the highest antioxidant content is in *Spatholobus littoralis* Hassk. stem extract (Table 3). This is in line with studies conducted on lindur (*Bruguiera gymnorrhiza*) plants to compare antioxidant activity in roots, stems, and leaves and found that lindur (*Bruguiera gymnorrhiza*) has the highest antioxidant activity in lindur (*Bruguiera gymnorrhiza*) stem parts.[19]

Spatholobus littoralis Hassk. stem extract has the most effective concentration at 2500 ppm. Antioxidant activity of *Spatholobus littoralis* Hassk. stem extract has a lower IC50 value (9.46) (Table

3) compared to the highest antioxidant activity in the lindur (*Bruguiera gymnorrhiza*) plant, which is found in the stem of the lindur plant (*Bruguiera gymnorrhiza*) (14.21). This shows the antioxidant activity of *Spatholobus littoralis* Hassk. stem extract is more effective than lindur (*Bruguiera gymnorrhiza*) stem. So, in the research conducted on *Spatholobus littoralis* Hassk. stem extract, it is proven that the stem extract of *Spatholobus littoralis* Hassk. can accelerate the migration of fibroblasts and as an anti-inflammatory in the wound healing process.[12,20]

CONCLUSION

Extracts of roots, stems, and leaves of *Spatholobus littoralis* Hassk. has antioxidant activity. The best antioxidant activity was found in Spatholobus littoralis Hassk. stem extract with an IC50 value of 9.46 and a concentration of 2500 ppm as the most effective concentration.

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