SANITAS: JURNAL TEKNOLOGI DAN SENI KESEHATAN
ISSN : 1978-8843 (*PRINT*) / 2615-8647 (*ONLINE*) **Vol. 14 No. 2: 95 - 105**

COMPARISON OF ANTIOXIDANT ACTIVITY OF CRUDE EXTRACT AND GEL PREPARATION FROM Alstonia scholaris L. LEAF EXTRACT

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Submitted: 11th November 2023; Accepted: 31th December 2023

https://doi.org/10.36525/sanitas.2023.9

ABSTRACT

Alstonia scholaris L. contains several compounds, which has been proven to be potential as skin protection by formulating in gel preparation. The aims of this study were to obtained the antioxidant activity of crude extract from leaf part of *Alstonia scholaris* L. and gel preparation contain *Alstonia scholaris* L. leaf extract. Extract was prepared using destilled methanol by maceration method. The gel preparation was developed with extract (1% w/v) in carbomer base (1,67% w/v). The chemical compound was evaluated qualitatively used some reagent. The antioxidant assays performed by DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The result shown that methanolic extract of *Alstonia scholaris* leaf was found had flavonoids, tannins, phenols, alkaloids, saponins, and steroids compound. The methanolic crude extract and gel preparation had strong antioxidant activity with IC_{50} value was found to be 70,588±0,135 ppm and 87,153±0,432 ppm, respectively. From the result we conclude that the methanolic crude extract of Alstonia scholaris leaf sole has strong scavenging free radical activity even formulated in gel preparation but has decreased IC_{50} value.

Keywords: *Alstonia scholaris; antioxidant; DPPH; gel; carbomer*

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INTRODUCTION

Alstonia scholaris L. is one of the plants that is widely used by the community as a medicinal plant (1). The most frequently isolated active compounds from this genus are Monoterpenoid Indole Alkaloids (MIAs) (2). Apart from that, the phenolic compounds, flavonoids and proanthocyanidins were proven to be found in the leaf, follicles and latex part of this plant with the highest content in the leaf with phenol levels of 49.66 ± 1.52 mg GAE/g, flavonoid levels of 97.33 ±1.52 mg QE/g and proanthocyanidin levels 99.33±1.52 mg CE/g (3).

Research was proven that *Alstonia scholaris* L. has antioxidant, antibacterial, antihyperuricemia and anticancer activity (3–5). Other research also showed that *Alstonia scholaris* L. has antitussive, expectorant, anti-asthmatic, anti-inflammatory and analgesic activity (6,7). However, several studies show that there are differences of chemical active compounds in *Alstonia scholaris* L. in several places where they grow, causing differences in the pharmacological activity of these plants, including activity as antioxidants (8–11).

From previous study, antioxidant profiles of methanol extracts of leaves displayed most potent antioxidant activity (70-80%) followed by follicle part (50-80%) and latex (40-80%) (3). The scavenging activity of methanolic extract of *Alstonia scholaris.* L from previous study has the lowest IC50 value (84,48 ppm which is good chategory of antioxidant activity) follow ethyl acetate and n-hexana, 237,29 ppm and 295,40 ppm, respectively (12).

On the other hand, the formulation can cause some differences in pharmacological activity from before it was formulated due to the presence of excipients (13). Therefore, in this research was carried out the differences of antioxidant activity of *Alstonia scholaris* L. methanolic extract and after formulation in gel preparations using native Indonesian plants.

MATERIAL & METHODS

Collecting samples and simplicia process

Alstonia scholaris L. leaf were collected from the ex-MTQ area of Jambi City, Jambi Province and determined at the Plant Taxonomy Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Padjadjaran University. Fresh leaf were taken, wet sorted then dried using oven at 45℃. The simplicia was powdered with a grinder and the

percentage of yield was calculated by comparing the weight of simplicia to the initial weight of the sample.

Preparation of Extract

The extract was made using methanol distillate as solvent with maceration method for 3x24 hours with occasional stirring. the ratio of simplicia and solvent were 1:10. The maserate was collected and the solvent was evaporated using a rotary evaporator at a temperature of 50℃ until a thick extract was obtained. the percent of yield was calculated by comparing the weight of the extract obtained to the weight of the initial simplicia powder used.

Non-Specific and Specific Parameters, and Screening of Phytochemical

The methanolic extract of *Alstonia scholaris* L was determined non-specific parameters (water content and ash content) and specific parameters (identity and organoleptics). The extracts were identified chemical compounds of flavonoids, alkaloids, saponins, steroids, tannins and phenolics.

Gel Preparation

Formula of gel preparation was an optimal gel formula with extract concentration of 1%; carbopol 1.67%; glycerin 14.83%; methyl paraben 0.18%; propyl paraben 0.02%; NaOH 0.7%; and distilled water ad 100%. Gel preparation was expanded with carbopol in distilled water for 24 hours. Next, in a separate container, mix methyl paraben, propyl paraben, and glycerin while heating. The extract was added to the mixture and homogenized using homogenizer for 20 minutes. The expanded carbopol was mixed into the solution with stirred. Add NaOH and add distilled water, stir until homogeneous (14).

Antioxidant Activity

The antioxidant test was carried out using the DPPH (1,1-diphyenyl-2-picrylhydrazyl) method based on research by Zaky, et al (2021) with slight modifications. A series of antioxidant tests are carried out as follows (15):

a. Preparation of blank solution

   10 mL of 0,05 mM DPPH solution in methanol pro analysis was made and incubated in dark room. The absorbance of blank solution was measured by spectrophotometry method at 517 nm.

b. Preparation of ascorbic acid solution

   Ascorbic acid solution was made at a concentration of 1000 ppm in methanol pro analysis and diluted to 10; 20; 30; 40 and 50 ppm as positive control. Then, 3.8 mL of DPPH blank solution was homogenized with 0.2 mL various concentration of ascorbic acid solution, incubated in the dark room for 30 minutes then measured at a wavelength of 517 nm using a UV-Vis spectrophotometer.

c. Preparation of sample solution

   Sample solution was made contained 10,000 ppm extract and diluted to a concentration of 400; 600; 800; 1000; and 2000 ppm. 3.8 mL of sample solution was added to 0.2 mL of DPPH blank solution. The solution was homogenized, stored in a dark place and the absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer.

d. Determination of percent of inhibition and IC_{50} value

   Percent of inhibition was calculated using this following formula:

   Percent of inhibition=(blank absorbance-sample absorbance)/blank absorbancex100%. Then, linear regression equation was created between concentration (x) and percent inhibition (y). The IC_{50} value can be find as the x value from the regression equation with y=50.

RESULT & DISCUSSION

Plant determination was carried out with the result that was Alstonia scholaris (L.) R. Br from the Apocynaceae family. The results of simplicia was 4.388 kg of from 5,3 kg of sample. The results of the percent of simplicial and extract yield are presented in table 1.

Tablel 1. Percent of yield

Sample	Percent of yield (%)
Simplicia	82,79
Crude extract	5,7

Determination of plants is the initial stage of research which aims to determine and ensure the viability of the plants in this research. The samples used were fresh pulai leaf which is hoped increase the economic value of these plant. Pulai leaf simplicia was extracted by maceration method, therefore components that cannot tolerate heating are still present in the extract (16). Crude extract was determined the specific, non-specific parameters and phytochemical screening, shown in table 2 and table 3.

Table 2. Nonspecific and specific parameter of crude extract

Parameters	Result
Nonspecific parameters	
Water content	1,8%
ash content	1,87%
Specific parameters	
Identity	
Extract	*Alstonia scholaris* L. methanolic Leaf extract
Botanical Name	*Alstonia scholaris* L.
Part of plant	Folium (leaf)
Indonesian Name	Pulai, Pule
Organoleptics	
Smell	Typical Pulai Extract Smell
Type of extract	Dry extract
Flavor	Bitter
Color	Blackish Green

Based on the screening results, pulai leaf contain secondary metabolites, such as flavonoids, phenolics, tannins, alkaloids, saponins and steroids. The orange color from the identification of flavonoids was due to magnesium powder can reduction reaction with flavonoid compounds resulting in a change in the color of the extract. Because of reaction with FeCl3, crude extract contains phenolic and tannins compound become blue or blackish green.

The identification reaction of alkaloids produced precipitate because the nitrogen atom in alkaloid structure with a lone pair of electrons substitutes the iodine ion in Dragendorf reagent covalently. Furthermore, because of saponin has hydrophilic and hydrophobic groups, the extract containing saponin compounds generates foam and stable when 2 N HCl was added. HCl can increase the polarity of saponin caused by the polar (hydrophilic) groups to face outward and the non-polar (hydrophobic) groups to face inward, resulting in the

formation of a micelle. *Alstonia scholaris* L. leaf extract also contains steroid compounds which are characterized by the formation of a green or blue color due to oxidation through the formation of conjugated double bonds. These results were accordance with several studies, one was conducted by Kalaria, et al, 2012 shown that *Alstonia scholaris* L. leaf extract has flavonoids, phenolics, tannins, alkaloids, saponins and steroids (17,18).

Table 3. Phytochemical screening of crude extract

Phytochemical compounds	Reagents	Result
Flavonoids	Mg powder+HCl	positive (orange color was formed)
Tannins	FeCl3	Positive (a blackish green/blackish blue color was formed)
Alkaloids	Dragendorf	positive (precipitated)
Saponins	Aquadest, HCl	positive (foams was formed)
Steroids	Anhydrate acetic acid+H2SO4	positive (blue color was formed)

Flavonoid molecules are the most compound that function as natural antioxidants. As antioxidants, flavonoids can provide radical molecules with hydrogen atoms. Nonetheless, the quantity and orientation of the OH groups determine how potently flavonoids exhibit antioxidant activity. The antioxidant activity of flavonoids increases with their hydroxyl group count.

The DPPH method was selected for the antioxidant activity test because simple, fast, and does not require a lot of reagents. With one unpaired of electron, DPPH is a radical molecule which gives it a purple color. According to Zaky (2021), discoloration from deep purple to clearer liquid indicates the formation of an electron pair (15).



Figure 1. *Alstonia scholaris* L. Gel Preparation

Table 4. Antioxidant Activity of Crude Extract

Concentration (ppm)	Replication 1		Replication 2		Replication 3	
	Abs.	Inhibition (%)	Abs.	Inhibition (%)	Abs.	Inhibition (%)
20	0,523	19,290	0,521	19,599	0,523	19,290
30	0,461	28,858	0,461	28,858	0,461	28,858
40	0,429	33,796	0,425	34,414	0,426	34,259
50	0,408	37,037	0,409	36,883	0,41	36,728
100	0,215	66,821	0,217	66,512	0,217	66,512
Blank Abs.			0,648			
Linear Regression	y=0,5719x+9,7111		y=0,5641x+10,174		y=0,5666x+9,9322	
IC_{50}			70,588±0,135			

The results of determining the antioxidant activity of crude extracts (table 4) and gel preparation (table 5) shown that both have strong antioxidant activity with IC_{50} value 70,588±0,135 ppm and 87,153±0,432 ppm, respectively. However, it was seen that the extract in gel preparation decrease in antioxidant activity as indicated by an increase in the IC_{50} value. The IC_{50} value is the sample concentration that can ward off 50% of free radicals. A smaller IC_{50} value indicates better antioxidant activity. The IC_{50} value of the sample (*Alstonia scholaris* L crude extract and *Alstonia scholaris* L extract in gel preparation) was strong category but still far from the IC_{50} value of the positive control (ascorbic acid) with 19,769±0,659 ppm which has very strong category (table 6). According to previous study, if IC_{50} value is <50 ppm, a compound is classified as very strong antioxidant activity, strong when the IC_{50} value is 50-100 ppm, moderate when the IC_{50} value is 101-150 ppm, and weak antioxidants when the IC_{50} value is >150 ppm (19).

Table 5. Antioxidant Activity of Gel Preparation

Concentration (ppm)	Replication 1		Replication 2		Replication 3	
	Abs.	Inhibition (%)	Abs.	Inhibition (%)	Abs.	Inhibition (%)
20	0,336	36,364	0,338	35,985	0,334	36,742
30	0,321	39,205	0,321	39,205	0,32	39,394
40	0,314	40,530	0,315	40,341	0,314	40,530
50	0,3	43,182	0,3	43,182	0,299	43,371
100	0,252	52,273	0,252	52,273	0,251	52,462
Blank Abs.			0,528			

Linear Regression	y=0,195x+32,953	y=0,1981x+32,689	y=0,194x+33,189
IC_{50}		87,153±0,432	

Table 6. Antioxidant Activity of Vitamin C

Concentration (ppm)	Replication 1		Replication 2		Replication 3	
	Abs.	Inhibition (%)	Abs.	Inhibition (%)	Abs.	Inhibition (%)
10	0,396	38,889	0,396	38,889	0,403	37,809
20	0,338	47,840	0,328	49,383	0,288	55,556
30	0,227	64,969	0,26	59,877	0,258	60,185
40	0,178	72,531	0,18	72,222	0,18	72,222
50	0,093	85,648	0,093	85,648	0,093	85,648
blank absorbance			0,53			
linear regression	y=1,1821x+26,512		y=1,1636x+26,296		y=1,1235x+28,58	
IC50			19,769±0,659			

The difference of antioxidant activity of crude extracts and its gel preparation was contributed of excipient. Carbomer as gelling agent can increase viscosity value of gel preparation. The higher value of viscosity is, the denser of particles in gel base. It will affect the diffusion process of crude extract out from gel base (20). The other previous study shown that difference in the drug release could be attributed to the difference in the basic properties of polymers (among these was carbomer) due to functional group substitution (21).

CONCLUSION

The antioxidant activity of methanol extract of *Alstonia scholaris* L leaf extract and gel preparation containing this extract was strong category of antioxidant activity. However, there was a decrease in the value of its antioxidant activity after it was formulated in gel preparation.

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