

Phytochemical Analysis And Antioxidant Activity Of N-Hexane And Ethyl Acetate In Flower *Sphagneticola trilobata* (L.) J.F Pruski

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Abstract : The *S. trilobata* flowers grow throughout the year, so they are rarely used and eventually wasted. *S. trilobata* flowers contain metabolite compounds that have potential as antioxidants. Antioxidants are useful for reducing free radical activity which can cause degenerative diseases. The purpose of the study was to determine the antioxidant activity of *S. trilobata* flower extract in n-hexane and ethyl acetate solvents and to measure the value of antioxidant activity of n-hexane extract and ethyl acetate extract of *S. trilobata* flowers expressed by IC_{50} . Extraction of *S. trilobata* flowers used the maceration method and measurement their antioxidant activities were carried out by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with a spectrophotometer UV-Vis at a wavelength of 517. The results exhibited that the n-hexane extract had weak antioxidant activity with an IC_{50} value of 410.18 $\mu\text{g} / \text{mL}$, while the ethyl acetate extract had strong antioxidant activity with an IC_{50} value of 249.50 $\mu\text{g} / \text{mL}$.

Index Terms: Flowers, *Wedelia trilobata*, Antioxidants, DPPH, n-hexane, ethyl acetate.

1 INTRODUCTION

Most degenerative diseases appear to be initiated by an oxidation reaction in the body. This reaction results in continuous oxidative damage between molecules and eventually, if it accumulates, it causes important macro molecules in cells, especially DNA to be damaged [1]. Khaira (2016) states that cells in our bodies begin to experience damage due to reactive oxygen species (ROS) (such as radicals, hydroxyl and superoxide). Cells also experience oxidative stress, most likely due to the increasing number of free radicals in the body. When the exposure to free radicals is more than natural antioxidants as an effort to defend the body, natural antioxidants will be directly disrupted and cause cell and tissue damage. To anticipate the negative effects of free radicals, it requires the intake of antioxidants from outside the body to help reduce free radical activity [3]. According to Gutteridge and Halliwell (2010), antioxidants can be complex molecules produced by the body such as superoxide dismutase, catalase and peroxiredoxins. Antioxidants can also be simple compounds derived from plants (exogenous antioxidants) such as vitamins (vitamins E, C, A, and β -carotene), and secondary metabolites [5]. Therefore, it is highly recommended to consume foods with high antioxidant content to counteract the effects of free radicals. One of the plants that has antioxidants and is used as a medicinal plant is *Sphagneticola trilobata* (L.) J. F. Pruski (previously *Wedelia trilobata*) [6]; [7]. Some literature states that *S. trilobata* has biological activity [8]; [9]; [10]; [11]. *S. trilobata* flowers are reported to have antioxidant activity [12], antibacterial [13]; [12], anti-inflammatory [14], hypoglycemic and hypolipidemic [15]. Other parts of *S. trilobata* such as leaves, stems and roots are reported to have potential as analgesic, antiparasitic, antipyretic, chemopreventive / anticancer [16]; [17]; [18]; [19]; [20]; [21]; [22]; [23]; [24]. Research on antioxidants from *S. trilobata* flower extract using a polar solvent, namely methanol has been reported [6].

However, antioxidant research of *S. trilobata* flower extract using other non-polar solvents has never been reported, even though Kumar et al. [25] and Senbagalakshmi et al. [26] stated that n-hexane and ethyl acetate solvents were also able to attract secondary metabolites in plant materials such as compounds flavonoids, triterpenoids, alkaloids, phenolics, and tannins. Based on the description above, it is important that this study be conducted to report the antioxidant activity of *S. trilobata* flower extract using n-hexane and ethyl acetate as a first step in developing a source of antioxidant-rich herbal medicine using various types of solvents.

2 MATERIAL AND METHODE

2.1 Material

The sample used in this study was the flower of *S. trilobata* (L.) J. F. Pruski which was collected from Langsa City, Aceh-Indonesia. The chemicals used in this study were methanol p.a, ethyl acetate, n-hexane, aquades, 2,2 - diphenyl - 1 - picrylhydrazil (DPPH), vitamin C and filter paper (Whatman No. 1).

2.2 Methode

2.2.1 Extraction Process

The flowers of *S. trilobata* were extracted using the maceration method as suggested by Mardina et al. [27] with n-hexane and ethyl acetate solvents. As much as 300 g of *S. trilobata* flowers were put into the container, n-hexane solvent was added until the sample was completely immersed in the macerated sample placed in a place protected from direct sunlight. After 3x24 hours of maceration I using n-hexane, followed by treatment using ethyl acetate solvent for 3x24 hours as well. Stirring is done regularly every day [28]. After that, it was filtered using Whatman Paper No. 1 filter paper was used to filter / separate impurities. The viscous extract was obtained by concentrating the solution using a rotary evaporator [27].

2.2.2 Phytochemical Screening

Alkaloids. Alkaloid was identified by Wagner's and Mayer's reagents. 5mg of flowers extracts were dissolved in the Wagner's reagent (1mL) and added few drops Mayer's reagent. Positive alkaloid was marked by appearance reddish

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brown and yellow colours precipitates for the Wagner's reagent and Mayer's reagent respectively [6]. Flavonoids. Flavonoid test were established by dissolving 5 mg of flower extract to few drop of diluted NaOH. This would result the yellow colour. Positive flavonoid in the extract was confirmed by adding few drops of diluted H₂SO₄ and would disappear or become colourless [11], [27]. Fenol. The phenol test was carried out by dissolving 5 mg of flower extract then adding 3-4 drops of FeCl₃. The formation of a bluish black color indicates a phenol compound in the material [29]. Saponins. Saponin was investigated by mixing 1g of the flower extract to 5ml of distilled water and shaken vigorously (±10 min) for a stable persistent froth. Formation of froth approved the presence of saponins in the extract [30]. Steroids. Steroid was examined by adding 10mg of the extract to 1mL of concentrated H, boiled and filtered before adding anhydrous acetic acid (1mL). When the dark reddish green colour appears, it confirmed the steroids [6]. Tannins. Tannin was detected by dissolving 5mg of the extract in 40% of the ethanol, then boiled for 5 min, and allowed for 3 min in room temperature before adding few drops of 15% FeCl. The greenish to black colour would be formed when tannins available in the extract [6].

2.2.3 Antioxidant Evaluation

The test for the antioxidant activity of the n-hexane, ethyl acetate extracts of *S. trilobata* flowers, and vitamin C (positive control) were carried out by immersing free radicals (DPPH) using UV-Vis spectrophotometry. DPPH was prepared in fresh form with a concentration of 0.4 mM. Flower extracts of n-hexane and ethyl acetate were dissolved in methanol p.a, adjusted in a concentration range of 25 - 400 µg/mL while Vitamin C was used as a comparison solution made in a concentration range of 3 - 15 µg/mL. Next, 1 mL of DPPH solution was mixed with the extract at various concentrations then adjusted using methanol p.a to a total volume of 5 mL. Then homogenized and incubated for 30 minutes at room temperature, and the absorbance was measured at a wavelength of 517 nm [31].

2.2.4 Data Analysis Technique

The data obtained in the form of the absorbance value of the sample at a predetermined concentration were analyzed using the formula below:

$$\text{Antioxidant Activity\%} = \frac{\Delta \text{blanko} - \Delta \text{sampel}}{\Delta \text{blanko}} \times 100\%$$

Data in the form of the percentage of antioxidants were then analyzed using Microsoft Excel to produce a linear regression equation.

3 RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The results of phytochemical screening were carried out using n-hexane and ethyl acetate solutions. Crude n-hexane extract only showed positive results from alkaloids and flavonoids, while ethyl acetate extract showed positive results from alkaloids, flavonoids, phenols, steroids and tannins (Table 1).

Table 1. Phytochemical screening of flower extracts of *n-hexane and ethyl acetate of Sphagneticola trilobata*

Compound Chemicals	Flower Extract	
	N-Hexane	Ethyl Acetate
Alkaloids	+	+
Flavonoids	+	+
Phenol	-	+
Saponins	-	-
Steroids	-	+
Tannins	-	+

Ket: (+) Available and (-) Not Available

3.2 Antioxidant Activity of *S. trilobata* Flowers

The results of the free radical scavenging of n-hexane and ethyl acetate extracts of *S. trilobata* flowers are presented in Figure 1 and Figure 2. The IC₅₀ value was obtained from a linear regression equation constructed by plotting the scavenging percentage curve against the concentration of flower samples. Thus the IC₅₀ values of the n-hexane extract and the ethyl extract of *S. trilobata* flowers were 410.18 µg/ml and 249.50 µg/ml, respectively, indicating the extract concentration needed to clean 50% of DPPH free radicals. According to Mangkasa et al., [32], antioxidant activity is categorized as very strong with IC < 50 ppm, strong in the 50-100 ppm range, moderate at 101-250 ppm, weak at 250-500 ppm, and classified as inactive in IC > 500 ppm. The antioxidant activity of n-hexane extract was in the weak category, while the ethyl acetate extract of *S. trilobata* flowers was in the strong category. The comparison used as a positive control is vitamin C (ascorbic acid) because it functions as a synthetic (standard) antioxidant by capturing free radicals, the IC₅₀ value is 9.15 µg/mL which is classified as a very strong antioxidant activity.

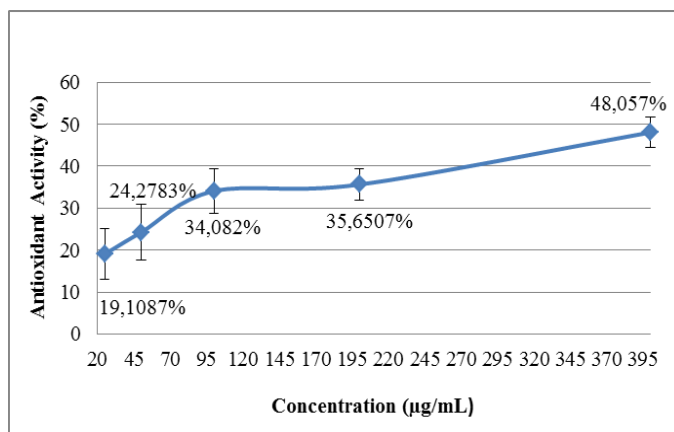


Figure 1. Average of antioxidant activity of *S. trilobata* flower n-hexane extract

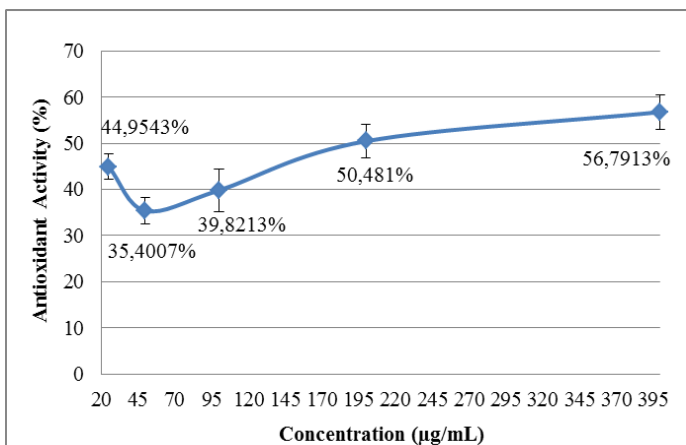


Figure 2. Average of antioxidant activity of S. trilobata flower ethyl acetate extract

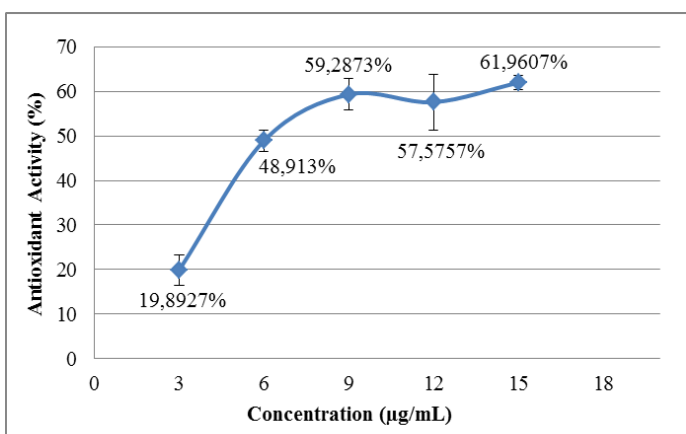


Figure 3. Average antioxidant activity of vitamin C as standard antioxidant.

Table 2. Comparison of IC50 values between antioxidant standards and samples

Sample	Equation	R ²	Value IC50 (µg/mL)
Vitamin C	y = 3,0933x + 21,686	0,7217	9,15
N-hexsan	y = 0,0696x + 21,451	0,8985	410,18
Etil aasetat	y = 0,0477x + 38,099	0,7371	249,50

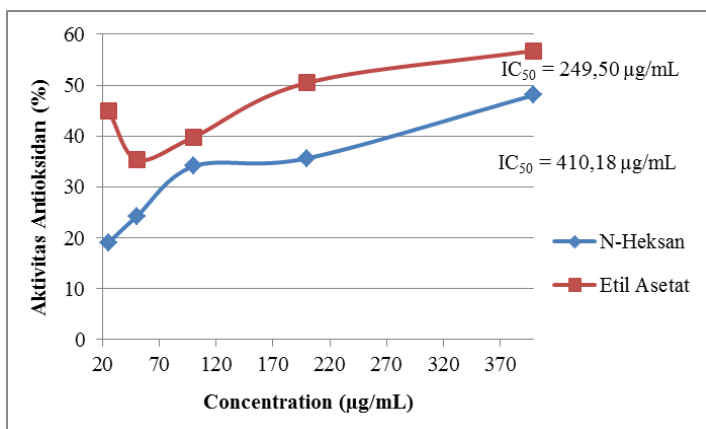


Figure 4. Comparison chart of IC50 values of N-hexane and ethyl acetate extract

4 CONCLUSION

The results of the study, it can be concluded that the antioxidant activity in the n-hexane extract of S. trilobata flowers resulted in an antioxidant activity value of 48.057% while the ethyl acetate extract of S. trilobata flowers had an antioxidant activity value of 56.7913%. The antioxidant activity of S. trilobata flower extract in n-hexane solvent had weak antioxidant activity as indicated by IC50 of 410.18 µg/mL while ethyl acetate solvent had strong antioxidant activity with an IC50 value of 249.50 µg/mL.

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