Flower of *Sphagneticola trilobata* (L.) J.F Pruski from Aceh, Indonesia: Antioxidant and Cytotoxic Activity on HeLa Cells

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**Abstract.** This study evaluated antioxidant and cytotoxicity of methanol extract from flowers of *Sphagneticola trilobata* (L.) J.F. Pruski. Phytochemical Screening of the flowers exhibited the presence of alkaloid, flavonoid, phenol, saponin, steroid and absent of tannin. The flowers extract also showed good source of antioxidant with an IC₅₀ value of 19.072 µg/ml which was categorized in the range of strong antioxidant activity (<150 ppm). Cytotoxic activity of the flowers against HeLa cell line was found to 331.287 (LC₅₀) or 83% inhibition. The results might suggest/support the flower of *S. trilobata* as source of natural antioxidant. However, further study about in vivo antioxidant and lethal dose of this sample are recommended.

1. Introduction

Flower of *Sphagaticola trilobata* (L.) J.F Pruski (Figure 1) are very attractive flowers due to prolific and constant blooming. Interesting yellow flower colors like small sunflowers. They often found on the side of the road, along streams, house buildings, unused gardens or in oil palm plantations. They are salt resistant. Some people considered weed or invasive plant due to they spreads very easily, forms a thick carpet in soil creeper. This plant can also be used as an ornamental plant. This plant has a scientific name, *Sphagaticola trilobata* (L.) Pruski. On the other hand, the familiar name is *Wedelia trilobata* (L.) Hitch.) which is widely used common name in the Pacific. Australia calls as “Singapore daisy” [1, 2, 3, 4].

Literatures reported that the *S. trilobata* has been practiced for treatments of various diseases [5, 6, 7, 4]. It is ordo Asterales with family Asteraceae which record long history in medicinal practice for treatment of various diseases such as skin diseases, snakebite, wounds, sore throat, headache, cold, fever, ulcer, varicose, dysfunction of kidney and amenorrhea [3]. Asian countries also utilized them as antibacterial, antifungal, anti-plasmodium, antidiabetic, hepatoprotective, antipyretic-thenalgestic and antitumor [6, 7, 8, 9, 10, 11]. Other people employ Asteraceae against bronchitis, and enhance fertility [12].

Many literatures reported the biological activities of *S. trilobata* [5, 13]. However, no report about the cytotoxic activity of methanolic extract of flowers of *S. trilobata* against HeLa cell line. Therefore, in this study, assessment cytotoxic activities of flowers of *S. trilobata* toward HeLa cells...
were evaluated. Besides, pharmalogical activities of the sample including phytochemical screening, and antioxidant were also examined.

![Figure 1. Flower of *Sphagneticola trilobata* (L.) J.F. Pruski](image)

2. Materials and Methods

2.1. Materials

The flowers of *S. trilobata* were accumulated from Langsa-Aceh. The study used chemicals that were analytical grade and procured from C.V. Multikreasi Bersama and CV Rudang Jaya, Medan Indonesia including methanol p.a, 1,1-difenil-2-pikrilhidrazil (DPPH) (Sigma Aldrich), aquadest, and Whatman filter paper No.1. Hela cells recognized as ATCC CCl 2 that cultured in Primate Research Center of Bogor Agricultural University, Indonesia. Others chemicals used were RPMI1640, Dulbecco's Modified Eagle's Medium (D-MEM), Fetal Bovine Serum (FBS) 5% dan Penicillin 100 U/mL, Streptomycin 100 ug/mL.

2.2. Methods

2.2.1. Extraction Process

The flowers of *S. trilobata* dried at room temperature for 10 days and extracted with methanol for 3x24 h. The maserasi extracts were subjected to a rotary vacuum evaporator (Heidolph, Germany), after filtered by Whatman filter paper No.1. Subsequently, they stored at 4°C for further experiment [14].

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 brown and yellow colours precipitates for the Wagner’s reagent and Mayer’s reagent respectively [3].

**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 [15].

**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 [16].

**Steroids.** Steroid was examined by adding 10mg of the extract to 1mL of concentrated H₂SO₄, boiled and filtered before adding anhydrous acetic acid (1mL). When the dark reddish green colour appears, it confirmed the steroids [3].
**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% FeCl₃. The greenish to black colour would be formed when tannins available in the extract [3].

### 2.2.3. Antioxidant Evaluation

Antioxidant property of the extract was evaluated by the DPPH method. The DPPH solution was prepared in the fresh one at the concentration of 0.4 mM. The flowers extract that was dissolved in the methanol p.a, was arranged in the range concentration of 25 – 200 µg/mL. An aliquot of 1mL of the DPPH solution was mixed with the extract at the various concentrations then adjusted to 5mL total volume solution using methanol buffer. They were homogenized and incubated in the dark at 30°C for 37 min. The triplicate optical density (OD) value was documented at 517 nm before analysed by the formula: [17].

\[
DPPH \text{ scavenging activity (\%)} = \left( \frac{(\text{Reference OD} - \text{Sample OD})}{\text{Reference OD}} \right) \times 100
\]  

### 2.2.4. Cytotoxic Evaluation Using MTT Assay

Cytotoxic activity of the flowers extract was evaluated using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay and carried out in triplicate. HeLa cells (5000 cells/100µL) were seed separately into plates and exposed to different concentration of the extracts (1, 5, 10, 25, 50, 100, 200, and 500µg/mL). After overnight incubation, 10µL of MTT reagents (5mg/mL) were transferred into each well for 4h re-incubation. The reaction was discontinued by adding 10% SDS in 0.1N HCL. The absorbance was read at 595nm wavelengths. The inhibitory rate against viability HeLa was calculated based on this formula:

\[
% \text{ cell inhibition} = \left( \frac{A_{570 \text{ of control cells}} - A_{570 \text{ treated cells}}}{A_{570 \text{ of control cells}}} \right) \times 100
\]

The cytotoxic activity calculated from interpolation the plot of concentration and percentage of mortality cell line and stated as LC₅₀ [5, 14].

### 3. Results and discussion

#### 3.1. Phytochemical Screening

Phytochemical screening was performed using methanol and aqueous (distilled water) solutions. These crude extract exhibited positive results of alkaloids, flavonoids, phenol, saponin, steroid and tannin (Table 1).

| Chemicals compounds | Methanol extract of *S. trilobata* (L.) J.F Pruski flowers extracts |
|---------------------|---------------------------------------------------------------|
| Alkaloid            | +                                                             |
| Flavonoid           | +                                                             |
| Phenol              | +                                                             |
| Saponin             | +                                                             |
| Steroid             | +                                                             |
| Tannin              | -                                                             |

* Available

* Not available.

#### 3.2. Antioxidant Activity of *S. trilobata* Flowers
The result for the free radical scavenging of the methanol extract from *S. trilobata* flowers were presented in Figure 2. The IC$_{50}$ value were obtained from the linear regression equation that were constructed by the plotting a curve of percentage scavenging against the concentration of the flowers sample. Thus the IC$_{50}$ value of the flowers of *S.trilobata* was 19.072 µg/ml, indicating the concentration of extract required scavenging 50% of the DPPH free radicals. According to Mustarichie *et al.* [18], antioxidant activity is categorized as a very powerful with IC$_{50}$ <50 ppm, strong in the range of 50-100 ppm, moderate at 101-250 ppm, weak at 250-500 ppm, and classified as inactive at the IC$_{50}$ >500 ppm. Antioxidant activity of the *S. trilobata* leaves was strong category.

![Figure 2. Antioxidant activity of the methanol extract of *S. trilobata* flowers following DPPH radical scavenging assay.](image)

### 3.3. Cytotoxic Activity of *S. trilobata* Flowers

Furthermore, the study conducted the cytotoxic activity of methanol extracts from flowers of *S. trilobata* against HeLa cells line for 24 h incubation. The method used MTT assay at the concentration extract of 1 – 500 µg/mL and LC$_{50}$ values were determined. Cell survival analyse indicated that *S. trilobata* extract caused growth inhibition of HeLa cells in dose dependedet manner. After 24 h incubation, methanol extract of flowers from *S. trilobata* exhibited the most cytotoxic activity at a concentration of 500µg/mL with 83% of mortality (Figure 3).

![Figure 3. Toxicity of flowers extract of *S. trilobata* against HeLa cells lines for 24 h observation](image)
4. Conclusion
The study concluded that the flowers of *Sphagneticola trilobata* might be a source of antioxidant as it exhibited the IC$_{50}$ value of 19.072 µg/ml and classified as strong category. Besides, it showed toxicity capability against HeLa cell line with LC$_{50}$ values of 331.287. This is might be due to the presence of bioactive compound as displayed in phytochemical screening viz. alkaloids, flavonoids, fenol, saponin, steroids.

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