Antioxidant and cytotoxic activities of the ethyl acetate extract of *Sphagneticola trilobata* (L.) J.F. Pruski on MCF-7 breast cancer cell

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**Abstract**

*Sphagneticola trilobata* (L.) J.F. Pruski is the perennial herb distributed at tropical temperature. In this study, the antioxidant and anticancer properties of the ethyl acetate extract from *S. trilobata* leaves were investigated against MCF-7 breast cancer cells. The antioxidant and anticancer activities were assessed by 1,1-diphenyl-2-picrylhydrazyl free radical scavenging and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay methods, respectively. The extract demonstrated DPPH free radical scavenging effect with IC₅₀ value equaling to 127.43 μg/mL. The cytotoxic study was conducted on the concentration range of 1–200 μg/mL, and the results exhibited the strongest inhibitory activity against MCF-7 with the LC₅₀ value of 58.143 μg/mL. The cytotoxic activity of the extract was supported by the induction of apoptosis cell which possessed the apoptosis percentage of 78.80%. Thus, the cheap herbal drug treatment might highly be recommended to treat effectively breast cancers as an ideal choice or combinational therapy.

**Key words:** Antioxidant, cytotoxicity, MCF-7, *Sphagneticola trilobata*

**INTRODUCTION**

Free radicals and antioxidants are two opposite components in the body that play a vital role in the development of human illnesses.¹ Free radicals may be derived from endogenous processes and/or other external sources. If the body exposed to free radicals for a long time, it will increase the progression of degenerative diseases including aging, cancer, arteriosclerosis, diabetes, Alzheimer’s and Parkinson’s diseases, and immune deficiency diseases.²,³

In contrast, the body also produced antioxidant to protect and/or clean up the effect of free radicals routinely.¹ However, if free radicals have accumulated in excessive amounts, additional intake of antioxidants from outside the body is necessary.¹ Currently, natural antioxidant from the plant extract as a potential substitute for the synthetic ones has been increased demand in the therapeutic treatment due to the safety of side effect.¹,⁴ In fact, synthetic antioxidants donate their electrons to quench free radical once, without being recycled by the organisms; as a result, they could develop harmful metabolic byproducts that rise, rather than minimize the total load of oxidative stress.⁶ Thus, discovery and development of natural antioxidants should

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S. trilobata is a member of family Asteraceae that has been utilized as old traditional medicine in India, Chinese, Caribbean, and Central and South America. It is known as “Wedelia” in the Pacific, Singapore daisy in Australia, creeping daisy in English, Seruni/Sernai in Indonesia, and Wedelia kuning in Malaysia. For six decades ago, some species of this family (Wedelia chinensis, Wedelia paludosa, Wedelia calendulaceae, Wedelia prostrata, Wedelia spp., and Wedelia trifoliosa) have been great interest in the pharmaceutical therapy since they exhibited biological activities including antibacterial, antifungal, anti-plasmodium, antidiabetic, hepatoprotective, antipyretic- –analgesic, and anticancer activities. Nevertheless, the available current literature has not documented yet about the evaluation of antioxidant and cytotoxic properties of the ethyl acetate extract from S. trilobata leaves against MCF-7 cell line, especially the sample from Aceh-Indonesia. Thus, this study would be examined the antioxidant and cytotoxic activities of S. trilobata against human breast cancer. The finding of the study is expected to contribute in the phytopharmacy to develop S. trilobata as a new anticancer drug.

**SUBJECTS AND METHODS**

**Plant material and chemicals**

The leaves of S. trilobata was accumulated from Aceh, Indonesia, in March–April 2019 and identified at the Herbarium Medanense, Universitas Sumatera Utara (Indonesia), with the voucher specimen number of 4542/ MEDA/2019. All materials (analytical grade) were provided by C. V. Multi Kreasi Bersama Medan, Indonesia, that consist of methanol p. a, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich), aquadest, ascorbic acid, Whatman filter paper No. 1, and 96-well flat-bottomed microtiter plates (Nunclon, USA). The MCF-7 (ATCC® HTB-22TM) was cultured in DMEM with addition of FBS, penicillin and streptomycin which were 5%, 10% and 100 U/mL respectively. In addition, rotary vacuum evaporator (Heidolph, Germany), UV-Vis spectrophotometer (722N, China), and ELISA reader (Infinite M200, Tecan, Switzerland) were used in this study. For apoptosis detection, Annexin V- FITC/PI kit (BD Biosciences, USA) was adopted.

**Extraction procedure**

The samples were cut into thick slices (±0.3 cm) and dried at room temperature for 7 days. Subsequently, they were subjected to ethyl acetate extraction at room temperature for 3 × 24 h and changed three times. The extracts were filtered by Whatman filter paper No. 1 and then concentrated in a rotary vacuum evaporator before stored at 4°C for further experiment.

**Phytochemical screening**

**Alkaloids**

Alkaloid was detected by Wagner’s and Mayer’s tests. The extracts (± 5 mg) were dissolved in 1 ml of the Wagner’s reagent and few drops of Mayer’s reagent. Alkaloid was confirmed by appearance reddish brown and yellow color precipitates for the Wagner’s reagent and Mayer’s test reagent, respectively.

**Flavonoids**

Flavonoids were confirmed by dissolving 5 mg extract to few drops of diluted NaOH. This would result the yellow color. Adding few drops of diluted H2SO4 would disappear or become colorless that confirmed the presence of flavonoid in the extract.

**Saponins**

Saponins were detected by mixing the extract (1 g) with 5 ml of distilled water and shaken vigorously (± 10 min) for a stable persistent froth. Formation of froth showed the presence of saponins in the extract.

**Steroids**

Steroids were examined by adding the extract (10 mg) to 1 mL of concentrated H2SO4, boiled, and filtered before adding anhydrous acetic acid (1 mL). When the dark reddish-green color appears, it confirmed the steroids.

**Tannins**

Tannins were detected by dissolving 5 mg of the extract in 40% of the ethanol, boiled for 5 min, and allowed for 3 min in room temperature before adding few drops of 15% FeCl3. The greenish to black color would be formed when tannins or become colorless that confirmed the presence of flavonoid in the extract.

**Antioxidant evaluation**

Antioxidant capability of the extract was assayed by the DPPH method. First, the extract and the DPPH were prepared in methanol solution at a concentration of 500 μg/mL and 5.53 mg/mL, respectively. An aliquot of 1 mL of the DPPH solution was mixed with the sample at various concentrations (250–1000 μL) and complemented to 5 mL total volume of mixture solution by adding methanol buffer solution. Subsequently, the mixture was homogenized and allowed to incubate in the dark at 30°C for 37 min. The average of triplicate absorbance result was recorded at 517 nm and then was analyzed by the following formula:

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

The study used ascorbic acid as a standard antioxidant and antioxidant capacity was expressed as IC50.
Cytotoxic evaluation using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay
Cytotoxic evaluation by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was performed in triplicate. MCF-7 cancer cells (5000 cells/100 μL) were seeded separately into plates and exposed to different concentrations of the extracts (1, 5, 10, 25, 50, 100, and 200 μg/mL) and then allowed for overnight incubation. Ten microliters MTT reagent (5 mg/mL) was transferred into each well for 4 h re-incubation. The reaction was blocked by adding 10% SDS in 0.1N HCl. The OD of assay was read at 595 nm wavelengths. The inhibitory rate against viability MCF-7 was calculated based on this formula: [19]

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

The cytotoxic of the extract was stated as LC50 which was calculated from interpolation the plot of concentration against percentage of cell line mortality.

Cell apoptosis assay
Annexin V-FITC/PI was adopted to detect cell apoptosis that exposure to the extract. 5 × 10^5 cells/mL of MCF-7 was incubated overnight and treated the following day with the extract at a concentration of LC50 value. The cell was rinsed twice using 1 mL PBS, trypsinized, and suspended by 100 μL of ×1 binding buffer. Moreover, Annexin V-FITC and PI staining solution was transferred into each tube and set for 30 min in the dark room before adding 400 μL of ×1 binding buffer which was place on ice. Flow cytometry was run to detect cell apoptosis within 1 h and distribution of cell cycle was recorded as data. [19]

RESULTS AND DISCUSSIONS
Phytochemical screening
The phytochemical analysis was performed to evaluate the presence bioactive compound in the sample and support for qualitative separation. [27] The results revealed that the extract contained flavonoids, alkaloid, and steroid and absence of phenol, tannin, and saponin (Table 1).

| Chemicals compounds | Ethyl acetate extract of Sphagneticola trilobata |
|---------------------|--------------------------------------------------|
| Flavonoids          | +                                                |
| Alkaloids           | +                                                |
| Phenol              | -                                                |
| Tanin               | -                                                |
| Steroid             | +                                                |
| Saponin             | -                                                |

+: Presence, -: Absent

Chemical compounds contained in the S. trilobata might be used for formulating new drugs, particularly for cancer treatment. [8,20] Alkaloid, flavonoids, and steroid can act as antioxidant that led to the invention of antianalgesic, anti-inflammatory, and cardio- tonic activities, respectively. [9,10,21]

Antioxidant activity
Free radical can cause degenerative diseases including cancer. Currently, researches have increased interest to discovery therapeutic medicinal plants that have high antioxidant activity to reduce oxidative stress tissue injury. [22,23] One of them was S. trilobata. In this study, antioxidative activity of ethyl acetate S. trilobata leaves was measured by DPPH method (Figure 1), and its scavenging activity was compared with the synthetic antioxidant [Figure 2]. The selection of DPPH free radical scavenging method is due to simple and inexpensive methods. [24] The results revealed that the extract possesses an IC50 value of 127.43 μg/mL. This value was lower than the commercial antioxidant (the IC50 of ascorbic acid was 7.106 μg/mL) [Table 2]. However, the result could be classified into moderate antioxidant activity ranging 101–250 ppm. [25] Chethan et al. [26] reported that the methanol extract of S. trilobata flower exhibited antioxidant activity with an IC50 value of 9 μg/mL. An experiment was conducted to evaluate the antioxidant capacity of different parts of W. trilobata that were extracted using methanol solvents. The results exhibited that the flower part had the highest antioxidant activity when compared to other plant parts with an IC50 value of 90 μg/mL. [27]

Cytotoxic activity
The result of anticancerous activity by MTT assay is presented in Figures 3 which displays mortality values of the MCF-7 cells incubated for 24 h with the ethyl acetate extract of S. trilobata leaves. The mortality values of cells were found to raise with upgrade the extract concentration. The maximum inhibition was found around...
90% at a concentration of 100 μg/mL with the average LC$_{50}$ of 58.143 μg/mL. This result was almost accordance by Venkatesh et al.,[28] who studied the cytotoxic activity of methanol extract of W. trilobata on MEG-01 cells and found that the IC$_{50}$ value was 80 μg/ml after 48 h incubation. Moreover, other study examined the cytotoxicity of the essential oil of W. chinensis against B16 F-10 cancer cell with the LC$_{50}$ value of 50 μg.[13] This cytotoxic effect might be caused the presence of secondary metabolites contained in the extract. This is accordance with Nithin et al.[29] and Ahmed et al.[11] that reported S. trilobata (the previous name is W. trilobata) consists of 3α-tigloyloxypterokaurene L3, wedelobatins A and wedelobatins B, sesquiterpene lactones, trilobed-6-O-isobutyrate, kaurenoic acid, eudesmanolidelactones, and luteolin.

**Inducing apoptosis cell**
To determine the programmed breast cancer cell death due to exposure of the extract, further evaluation of apoptosis using Annexin V method was carried out. In this study, the apoptotic test was performed with the concentration of LC$_{50}$ where the flow cytometry exhibited that the percentage of apoptotic and necrotic was 78.80% and 1.42%, respectively [Figure 4]. The percentage of cell death due to apoptosis was greater than necrosis; hence, it was concluded that the extract was potential to induce apoptosis in MCF-7 cells.

**CONCLUSIONS**
The ethyl acetate extract of S. trilobata possessed antioxidant and anticancer properties. It had moderate antioxidant activity with an IC$_{50}$ value of 96.272 μg/mL. The cytotoxic properties showed that more than 90% mortality of MCF-7 occurred with a LC$_{50}$ of 58.143 μg/mL. The cytotoxic properties of S. trilobata against breast cancer cells were also confirmed by the induction of apoptosis that revealed a percentage of apoptosis cells of 78.80%. Overall, the study suggested for further purification, structural analysis, and in vivo studies of breast cancer activity.

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**Conflicts of interest**
There are no conflicts of interest.

| Samples | IC$_{50}$ (μg/mL) |
|---------|-----------------|
| Extract ethyl acetate of Sphagneticola trilobata leaves | 58.143 |
| Ascorbic acid | 7.10645 |

Table 2: Comparison the IC$_{50}$ value between the sample and the reference standard
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