ORIGINAL ARTICLE

# Anticancer, Antioxidant, and Antibacterial Activities of the Methanolic Extract from *Sphagneticola trilobata* (L.) J. F Pruski Leaves

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*J. Adv. Pharm. Technol. Res.*

## ABSTRACT

This study aims to investigate the potential of bioactive secondary metabolites contained in *Sphagneticola trilobata* (L.) J.F Pruski leaves as novel plant-derived anticancer agent. Qualitative bioactive compound contents in the methanolic extract of *S. trilobata* leaves were screened using phytochemical method. Antioxidant evaluation was carried out using 2,2-diphenyl-1-picrylhydrazyl assay; antibacterial – using well diffusion method on *Escherichia coli* and *Salmonella typhi*; and cytotoxicity – using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay on MCF-7 cell line and Vero Cell. It was found that the methanolic extract exhibited antioxidant activity with an $IC_{50}$ value of 124.34 µg/mL. The inhibition zone values against *E. coli* and *S. thypi* (at extract concentration of 100 mg/mL) were 34.33 and 36 mm, respectively. *In vitro* MTT assay showed that our extract successfully reached 96% mortality with $LC_{50}$ = 189.287 µg/mL, where the selective index of 2.5 suggest its selectivity against MCF-7 breast cancer cell line. In conclusion, the data of biological activities suggest the potential development of methanolic extract from *S. trilobata* leaves as a phytomedicine for breast cancer treatment.

**Key words:** Antibacterial, antioxidant, cytotoxicity, MCF-7, *Wedelia trilobata*

## INTRODUCTION

Bioactive compounds derived from natural product have been massively researched for their utilization in medicinal practices[1] on the basis that they are safer than synthetic drugs.[2-4] These medicinal plants have been suggested to promise a wide spectrum of therapeutic effects, including anticancer activities.[5] In this study, we aim to preliminarily investigate the potential of developing a new drug from a natural resource, namely *Sphagticola trilobata* (L.) J.F Pruski.[6-8]

*S. trilobata* (L.) or known as *Wedelia trilobata* is a medicinal plant known for its therapeutic effects for ulcer, sore throat, varicose, headache, fever, epilepsy, amenorrhea, snakebite, wounds, kidney dysfunction, hepatitis, cold, and indigestion.[9,10] Some literatures have reported the plant's bioactivities such as antioxidant, antibacterial, anti-inflames, and antimalarial, antifungals, hepatoprotective, antidiabetic,

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**Submitted:** 01-Feb-2021 **Revised:** 10-Mar-2021
**Accepted:** 12-Apr-2021 **Published**: 16-Jul-2021

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.japtr.org |
| | DOI: 10.4103/japtr.JAPTR_131_21 |



**How to cite this article:** Mardina V, Ilyas S, Halimatussakdiah H, Harmawan T, Tanjung M, Yusof F. Anticancer, antioxidant, and antibacterial activities of the methanolic extract from *Sphagneticola trilobata* (L.) J. F pruski leaves. J Adv Pharm Technol Res 2021;12:222-6.

and antitumor.[11-14] Previously, we have investigated the ethyl acetate extract from *S. trilobata* leaves yielding 78.80% apoptosis percentage against MCF-7 breast cancer cell.[7] Herein, we used methanolic extract, a more polar solvent, which an expectation of obtaining wider ranges of bioactive secondary metabolites.

## SUBJECTS AND METHODS

### Plant material and identification

The fresh leave samples were collected from Langsa, Aceh, Indonesia within March till May 2019. The taxonomic identification of plant was confirmed at the Herbarium Laboratorium, Universitas Sumatera Utara, Indonesia by Dr. Nursahara Pasaribu, M.Sc (voucher No. 4542/MEDA/2019). The plant was classified as a part of Spermatophyta (division), Angiospermae (sub-division, Dicotyledone (class), Asterales (ordo), Asteraceae (family), and Sphagneticola (genus) and identified as *S. trilobata* (L.) J.F Pruski (species).

### Extraction and phytochemical studies

The extract was obtained by chopping (±3 mm) and soaking the leaves of *S. trilobata* in the methanol solvent for 3 days maceration. Then, *Whatman paper* (No. 1) was to filter the filtrate, and concentrated using a rotary flash evaporator (Heidolph, Germany). The methanolic extract was then phytochemically screened for the presence of flavonoids, alkaloids, saponins, steroids, tannins, and phenols employing the procedures used in our previous report.[6]

### Antioxidant evaluation

The antioxidant activity was quantitatively analysis *in vitro* carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (in triplicate) as previously described.[6,15] The extract solution with different concentrations (25–200 µg/mL) was prepared by dissolving the extract using mL methanol p.a. As much as 4 mL dissolved extract was then mixed with 1 mL (0.4 mM), followed by 30 min incubation in a dark condition at 37°C and measured using ultraviolet-visible spectrophotometer (Infinite M200, Tecan, Switzerland) at`. A negative blank was prepared by adding 1 ml DPPH (0.4 mM) into 4 mL methanol buffer. The calculation of antioxidant activity was based on: Antioxidant activity (%) = 100% × (blank absorbance–sample absorbance)/blank absorbance).

### Antibacterial evaluation

The antimicrobial activity of the extract was determined by agar well diffusion method as used previously.[16] The microorganisms used were *Eschericia coli* and *Salmonella typhi* (obtained from The Gadjah Mada University Indonesia). A volume of 100 µL bacterial inoculum ($10^8$ CFU/mL) were prepared on Nutrient Broth, followed by the introduction of serial dilutions of the extract and positive standard (5–100 mg/mL) into the well. The inhibition zone was measured after 24 h incubation. For the positive controls, we used tetracycline, clindamycin, ciprofloxacin, ofloxacin, chloramphenicol, and ampicillin. Meanwhile, ddimethyl sulfoxide (DMSO) was use as a negative control. These antibacterial evaluations were performed in triplicate.

### *In vitro* cytotoxic evaluation using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide Assay

*Cell line culture*

Cytotoxic activity of the methanolic extract from *S. trilobata* leaves was tested against the positive standards; breast cancer cell line (MCF-7) and Vero cell labeled as ATCC HTB 22 and ATCC CCl 81, respectively. Cells were grown at a concentration of 5000 cell/100 µL in Dulbecco's modification of Eagle medium, fetal bovine serum (5%), penicillin (100 U/mL), and streptomycin 100 µg/mL at 37°C and 5% $CO_2$ saturation.[6,7]

### 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide Assay and Selectivity Index

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was carried out in triplicate using 96-well plate according to the published work.[6,17] MCF-7 and Vero cell lines at a concentration of $1 \times 10^5$ cells/mL were seeded separately into 96-well flat-bottomed microliter plates (Nunclon, US.), followed by the exposure of the methanolic extract (1–1000 µg/mL) untreated cells were served as controls. After 1 day incubation, a volume of 100 µL of MTT reagent (5 mg/mL in DMSO) was put into each well and re-incubated for 4 h before added with 10% SDS (prepared in 0.1N HCl solution). The triplicate absorbances were read at 595 nm (Infinite M200, Tecan, Switzerland) to obtain percentage of mortality. The cytotoxicity was stated as $LC_{50}$ obtained from the interpolation of the plot of log concentration (the dose that inhibits 50% of the cells population) and mortality percentage of the cell line. The selective cytotoxicity against cancer cells was calculated using the formula below.[18]

$$\text{Selectivity Index } (\text{SI}) = \frac{\text{LC50 of vero cell}}{\text{LC50 of cancer cell}} \quad (3)$$

## RESULTS AND DISCUSSIONS

### Phytochemical properties

The screening test results of the secondary compounds contained in the extract of *S. trilobata* leaves using methanol and water solvent have been presented [Table 1].

### Antioxidant activity

The results of antioxidant evaluation using DPPH methods of the methanolic extract from *S. trilobata* leaves have been presented. The $IC_{50}$ value was calculated based on the linear regression equation from the plot in Figure 1. The

model yields correlation value ($R^2$) of 0.9384 with IC50 of 124.34 µg/ml.

### Antibacterial activity

Based on the evaluation of the antibacterial activity, shown in Figures 2 and 3, the inhibition zone was obtained within the range of 3–34.5 mm at the concentration of 5–100 mg/mL. The Antibacterial activity of the extract was compared with several antibiotics commercial, namely, tetracycline, clindamycin, ciprofloxacin, ofloxacin, chloramphenicol, and ampicillin.

### Cytotoxic activity

The results of *in vitro* cytotoxic activity against MCF-7 cell line are shown in Figure 4. Vero cell line as a normal cell was used to compare the effect of cytotoxic. The specific levels of toxicity were stated as $LC_{50}$ and calculated using probit analysis; relationship of data between log concentration curves against the probit value of the mortality percentage [Table 2]. The average score of $LC_{50}$ of MCF7 and Vero cell lines were 189.287 µg/mL and 465.357 µg/mL, respectively. The visualization analysis of selected cytotoxic activity against MCF-7 and Vero cell line are described in Table 3.

**Table 1: Screening of phytochemical compounds of the methanolic *Sphagneticola trilobata* leaves**

| Constituents | Solvent | |
|---|---|---|
| | Methanol | Distilled water |
| Flavonoids | + | + |
| Alkaloids | − | + |
| Phenol | + | + |
| Tannin | + | + |
| Steroid | − | − |
| Saponin | + | − |

+: Presence, −: Absent

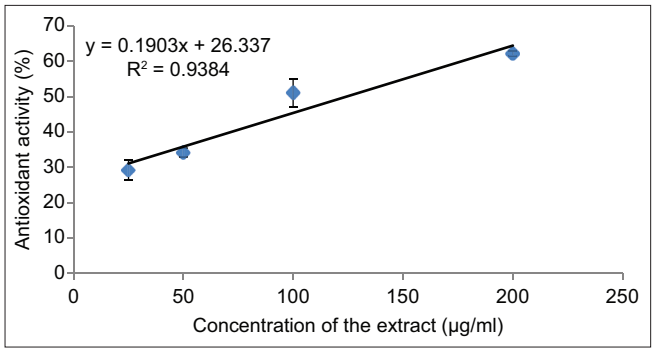



**Figure 1:** Percentage of antioxidant activity of the sample scavenge 2,2-diphenyl-1-picrylhydrazyl

## DISCUSSIONS

A well-researched medicinal *S. trilobata* (L.) J.F Pruski[6-8] had been qualitatively and phytochemically screened showed that the methanolic extract from its leaves contain flavonoids, alkaloids, phenols, saponins, and tannins. These compounds possess antioxidant activities, which were analysed using DPPH assay. The $IC_{50}$ value produced by the methanolic extract from *S. trilobata* (L.) leaves was 124.34 µg/mL. Based on literature,[19] our extract can be considered to have moderate activities ($IC_{50}$ = 101–250 µg/mL). Therefore, the leaves extract in our study is categorized as moderate antioxidant. Antioxidant activities are important for anticancer mechanism, as cancer

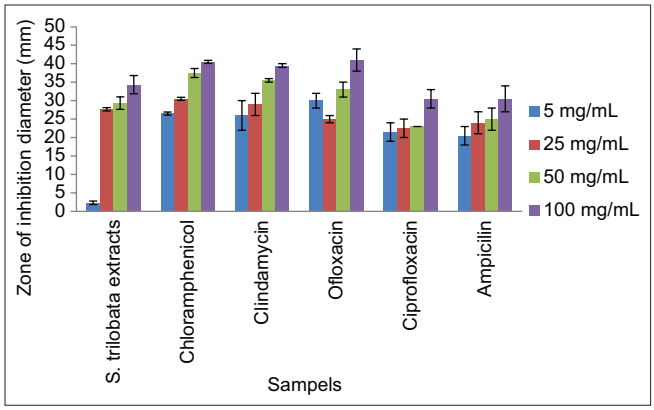


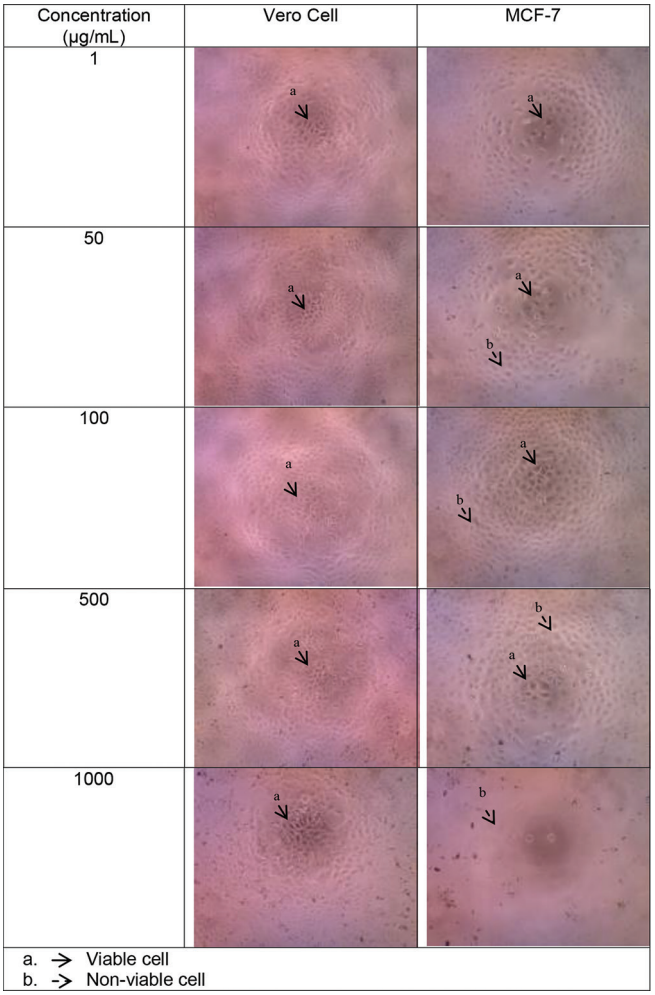

**Figure 2:** Antibacterial activity (zone of inhibition) of methanol extract of *Sphagneticola trilobata* (L.) J.F Pruski leaves against *Escherichia coli* and comparison among the several antibiotics

**Table 2: The Relationship of data between log concentration and probit percentage of mortality cell line**

| Concentration Log (µg/mL) | Probit of percentage of mortality cell line | | | |
|---|---|---|---|---|
| | MCF-7 | LC50 of MCF-7 | Vero | LC50 of vero cell |
| 0 | 1.0098±0.9906 | 189.287 µg/mL | 3.2134±0.6801 | 465.357 µg/mL |
| 0.699 | 3.6213±0.8334 | $R^2$=0.8074 | 3.6016±0.7244 | $R^2$=0.9277 |
| 1 | 3.8448±0.063 | | 4.338±0.2007 | |
| 1.398 | 3.9015±0.4093 | | 4.277±0.2506 | |
| 1.699 | 4.1184±0.2592 | | 4.411±0.2015 | |
| 2 | 3.9593±0.1485 | | 4.461±0.1822 | |
| 2.699 | 4.8032±0.0215 | | 4.93 ± 0.2192 | |
| 3 | 6.7302±0.0944 | | 5.24 ± 0.1213 | |
| SI | 2.458>2 | | | |

SI: Selectivity index

**Table 3: Morphology description of cytotoxic activity of methanol extract on MCF 7 and Vero cells with respect to the different concentration**



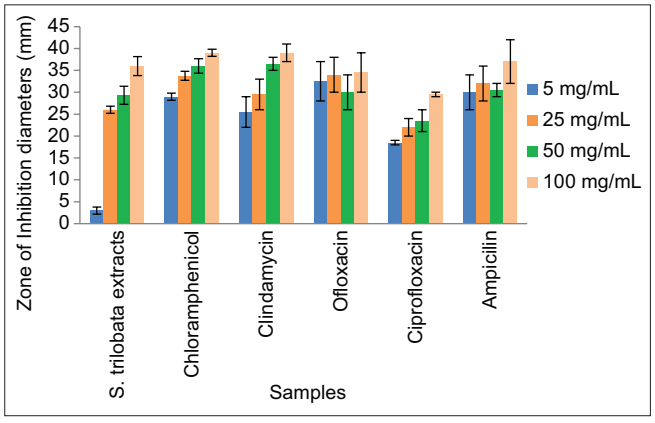




**Figure 3:** Antibacterial activity (zone of inhibition) of methanol extract of *Sphagneticola trilobata* (L.) J.F Pruski leaves against *Salmonella typhi* and comparison among the several antibiotics

initiation and development are strongly correlated with reactive oxygen species.[20,21]

The methanolic extract from *S. trilobata* leaves with the concentrations of 5, 25, 50, and 100 mg/mL were assessed for their potentiating affects against *E. coli* and *S. typhi* [Figures 2 and 3]. The leaves extracts depicted the best potentiating effect (at 100 mg/mL) with inhibitory zones of 34.33 and 36 mm for *E. coli* and *S. typhi*, respectively. These results were close to inhibition zones of commercial antibiotics (chloramphenicol, clindamycin, ofloxacin, ciprofloxacin, and ampicillin) at the concentration of 100 mg/mL. The activity against *E. coli* and *S. typhi* may be caused by the presence of bioactive compounds, which can be enhanced through purification. Combination with antimicrobial releasing agents, such as polyurethane,[22,23] can also be the enhancement strategy.

Based on the cytotoxicity studies, the LC50 value for MCF-7 was lower (189. 287 µg/mL than the Vero cells 465.357 µg/mL). The selective cytotoxicity against MCF-7 breast cancer cell line was expressed as SI, which we had achieved SI = 0.5. Hence, our extract can be classified as selectively for MCF-7 breast cancer cell lines (SI ≥ 2).[18] These anticancer activities are corroborated with the morphological description contrast to experience morphological description, contrast cell deformation was observed in MCF-7-treated cells. The cells were observed to experience a shrinkage and lysis; indicating the inhibited cellular growth. This appearance can be associated with the characteristics of cell death, where nuclear condensation occurs resulting in the formation of apoptotic bodies.[17]

## CONCLUSIONS

Our studies selective anticancer properties of the methanolic extract from *S. trilobata* leaves against MCF-7 breast

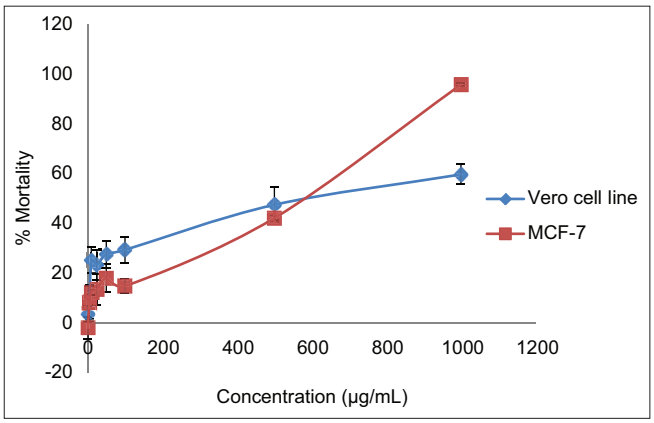



**Figure 4:** Toxicity of crude extract of *Sphagneticola trilobata* leaves against MCF-7 and Vero cells lines for 24 h observation

cancer cell lines, attributed to its moderate antioxidant activity. In addition, it also has inhibitory activities against Gram-negative *E. coli* and *S. typhi* with similar efficacy compared with commercial drugs, namely tetracycline, clindamycin, ciprofloxacin, ofloxacin, chloramphenicol and ampicillin. The bioactivate activities of the methanolic extract can be associated with the presence of flavonoids, alkaloids, phenols, saponin, and tannin. Future researches strategy can include the investigation of the anticancer mechanisms, purification, and isolation of the bioactive compounds, as well as *in vivo* study to evaluate the acute/chronic cytotoxicity of *S. trilobata* extract.

## Acknowledgment

This study was fully supported by the Ministry of Research Technology and Higher Education through the PKPT research grant of 207/SP2H/AMD/LT/DRPM/2020 (219/UN54.6/PG/2020).

## Financial support and sponsorship

The Ministry of Research, Technology and Higher Education of Republic of Indonesia (KEMENRISTEKDIKTI RI) with Grant No. of 207/SP2H/AMD/LT/DRPM/2020 (219/UN54.6/PG/2020).

## Conflicts of interest

There are no conflicts of interest.

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