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Original Research Article

In Vitro Antioxidant Activity and Cytotoxic Effect of Ethanol Extract of *Vernonia elaeagnifolia* Leaves against Breast Cancer Cell Lines

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ABSTRACT

Vernonia elaeagnifolia is an evergreen creeping plant belonging to the sunflower family. It has been investigated for various medicinal properties, but there is limited information on its antioxidant and anticancer properties. This study therefore aims to examine the antioxidant and anticancer potentials of the ethanol extract of *Vernonia elaeagnifolia* leaves (VEE). VEE was obtained by maceration in 96% ethanol. Preliminary phytochemical screening was done according to standard method. Antioxidant activity of VEE was assessed using the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) radical scavenging assay. Cytotoxic effect against 4T1 and MCF7 breast cancer cells, as well as Vero non-cancerous cells was examined by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Phytochemical screening of VEE reveals the presence of alkaloids, flavonoids, saponins, phenolics, and tannins. VEE exhibited modest antioxidant activity with IC_{50} of 147.28 µg/mL, compared to ascorbic acid which had an IC_{50} of 5.43 µg/mL. VEE possessed moderate cytotoxic effect against 4T1, and MCF7, and a mild cytotoxic effect against Vero cells with IC_{50} of 165.50, 185.68, and 299.66 µg/mL, respectively. VEE was less potent than Doxorubicin which exhibited IC_{50} values of 0.44, 2.09, and 4.51 µg/mL, against 4T1, MCF7, and Vero cells, respectively. The selectivity index of VEE was 1.6 - 1.8, indicating limited selectivity for cancer cells. The findings from this study have shown the potentials of *Vernonia elaeagnifolia* leaves as an antioxidant and a mild cytotoxic agent against breast cancer cells.

Keywords: *Vernonia elaeagnifolia*, Antioxidant, Cytotoxic, Breast cancer, Selectivity index.

Introduction

Vernonia elaeagnifolia also known as *Vernonia elliptica* or *Tarlmounia eliptica* is commonly called curtain creeper. The plant belongs to the Asteraceae family and is usually used in vertical gardens to increase density and decrease air pollution.[1] Asteraceae family have been used for medicinal purposes for a long time, spanning several millennia.[2,3] In the past, *Vernonia elaeagnifolia* was mostly used for warding off leeches and as a natural fiber for textiles. As the plant became increasingly popular, it was later used for it medicinal properties, including anti-hyperlipidemia, anticancer, antibacterial, antioxidant, and antifungal properties.[4–7] The medicinal value of *Vernonia elaeagnifolia* can be attributed to its secondary metabolites, such as alkaloids, flavonoids, tannins, phenolics and flavonoids.[6] Several phytochemicals including flavonoids, phenolic compounds, alkaloids, and terpenes have been shown to possess cytotoxic effect.[8]

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However, the relationship between cytotoxic effect of plant extracts and their pharmacological properties is complex and multifaceted, and understanding this relationship is crucial for the development of effective and safe anticancer remedies from plant-derived compounds. *Vernonia elaeagnifolia* has gained a lot of interests among scientists due to its pharmacological properties, and its potential as a promising source of novel therapeutic agents for the prevention and treatment of cancer.[9,10] In recent times, considerable research has been conducted to examine the cytoprotective properties of *Vernonia amygdalina*, specifically its capacity to protect cells from the harmful effect of a variety of stressors.[11,12] The cytoprotective effect of *Vernonia amygdalina* is believed to be a result of its antioxidant and anti-inflammatory activities, which help to mitigate the harmful effects of inflammation and free radicals.[13] The *in vitro* and *in vivo* cytoprotective properties of *Vernonia amygdalina* can be harnessed for the prevention and treatment of numerous diseases, including cancer. *Vernonia amygdalina* has demonstrated *in vitro* cytotoxic properties against several types of cancer cells, including those of the breast, colon, cervix, lungs, and prostate.[14] In addition to its antioxidant properties, *Vernonia amygdalina* exerts cardioprotective effect by ameliorating the damage caused by high levels of reactive oxygen species (ROS) associated with the use of doxorubicin.[15] *Vernonia elaeagnifolia* and *Vernonia amygdalina* belong to the same genus, *Vernonia*, however, the anticancer and antioxidant properties of *Vernonia elaeagnifolia* have not been thoroughly explored, therefore, additional investigations of this genus may yield novel therapeutic agents.

The present study investigated the potential antioxidant and cytotoxic properties of *Vernonia elaeagnifolia* leaves ethanol extract (VEE).

Three distinct cell types; MCF7, which represents estrogen-positive luminal breast cancer cell, 4T1, which is a triple-negative breast cancer cell, and Vero, which represents a non-cancerous kidney epithelial cell, were used to assess the cytotoxic effect of VEE.[16] Vero cells were used to compare the effect of the extract on normal cells. Doxorubicin, a conventional anticancer drug known to cause toxicity and cellular senescence was used as the positive control.[17] This preliminary investigation of the antioxidant and cytotoxic activities of *Vernonia elaeagnifolia* was conducted to explore the potential of the plant as a source of novel chemotherapeutic agent.

Materials and Methods

Plant collection and identification

The leaves of *Vernonia elaeagnifolia* were collected from the main building of Siti Walidah, Universitas Muhammadiyah Surakarta in December, 2023. The plant material was identified at the taxonomist named Siti Kartika Sari, Biology Laboratory, Universitas Muhammadiyah Surakarta with voucher number 036/A-E-I/LAB.BIO/II/2024.

Preparation of extract

Vernonia elaeagnifolia leaves were washed and dried in an oven at 40°C. The dried leaves were pulverized using a mechanical grinder. The powdered leaves were macerated in ethanol (96%) at room temperature for 48 h. After filtration, the marc was remacerated in ethanol (96%) at room temperature for 24 h. the combined ethanol extracted was concentrated to dryness, and the percentage yield was calculated. The extract obtained was referred to as VEE.

Phytochemical screening

VEE was screened for the presence or absence of phytochemicals such as polyphenols, alkaloids, flavonoids, anthraquinones, tannins, and saponins according to the method previously reported by Sultana *et al*. (2017).[5]

Antioxidant assay

The antioxidant activity of VEE was assessed using the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay according to the methods previously described by Kusumorini *et al*. (2022)[18] and Dong *et al*. (2015).[19] Briefly, ABTS cation radical ($ABTS^{\bullet+}$) solution was prepared by mixing 5 mL of 7 mM aqueous ABTS solution with 88 µL of 140 mM $K_2S_2O_8$ solution. The mixture was then kept in the dark at room temperature (25°C) for 12–16 h. The absorbance of the dark blue coloured $ABTS^{\bullet+}$ solution formed was adjusted to 0.7 ± 0.02 at a wavelength of 734 nm by dilution in water. One milliliter (1 mL) of various concentrations (25, 50, 100, 200, and 400 ppm) of VEE in ethanol or ascorbic acid (5, 10, and 15 ppm) in methanol was added to 2 mL of the $ABTS^{\bullet+}$ solution. The reaction mixture was incubated in the dark at room temperature for 6 min, and the absorbance was measured at 734 nm. The ABTS free radical-scavenging activity (RSA) was calculated using the formula below:

$$ABTS\ RSA = (1 - \frac{A_{sample}}{A_{blank}}) \times 100\%$$

Where; A_{sample} is the absorbance of $ABTS^{\bullet+}$ solution mixed with sample and A_{blank} is the absorbance of $ABTS^{\bullet+}$ solution mixed with solvent. Subsequently, the IC_{50} value was calculated using a linear regression equation.

Determination of cytotoxic activity

Cell lines

The MCF7 (ATCC: CRL-2539), 4T1 (ATCC: HTB-22), and Vero (ATCC: CCL81.2) cells were obtained from the Molecular Biology Laboratory of the Research Centre for Medicinal Raw Materials and Traditional Medicine in Tawangmangu, Indonesia. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% penicillin-streptomycin (Gibco, New Zealand), 10% fetal bovine serum (Sigma, United States), sodium bicarbonate (Sigma, United States), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer.

MTT assay

The cell lines (MCF7, 4T1, and Vero) were seeded in a 96-well plate at a density of 7×10^3 cells per well, they were treated with VEE at different concentrations ranging from 1 to 200 µg/mL [in Dimethyl sulfoxide (DMSO)]. The cells were incubated at 37°C in a 5% CO_2 atmosphere for 24 h. Cell viability was assessed by treatment with 0.5 mg/mL MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent. Following the formation of formazan crystals, a stopper reagent (10% Sodium dodecyl sulfate in 0.01N HCl) was added. The absorbance of the resulting solution was measured at 595 nm using a spectrophotometer. Doxorubicin was used as the positive control. The absorbance data from each well were converted to percentage of viable cells. The concentration that will cause 50% inhibition of cell viability (IC_{50}) was determined from a regression analysis. The selectivity index (SI) of the extract was calculated by the ratio of the IC_{50} value in cancer cells and the IC_{50} value in normal cells.[20,21]

Statistical analysis

Data were presented as mean ± standard error of mean (SEM) of triplicates determination. The data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using GraphPad Prism v.9 software.

Results and Discussion

In recent times, there has been an increasing interest in the use of herbal remedies for the treatmet of a number of diseases. This increasing surge in the use of medicinal plants is because these plants are perceived to be safe, and do not result in serious side effects.[22] This study was conducted on the basis of the potential therapeutic benefits that have been linked to the numerous bioactive constituents of the tropical plant *Vernonia amygdalina* a close related species to *Vernonia elaeagnifolia*. In addition, *Vernonia amygdalina* has been shown to have cytotoxic effect against breast, colorectal, cervical, lung, and prostate cancer cells.[9-11,14] However, the study on the antioxidant and anticancer effects of *Vernonia elaeagnifolia* is limited, but it is believed that it would have similar pharmacological properties as *Vernonia amygdalina* because of the close botanical relationship. Therefore, the present study investigated the antioxidant and cytotoxic effects of *Vernonia elaeagnifolia*.

Phytochemical constituents of VEE

The qualitative phytochemical screening of VEE revealed the presence of alkaloids, flavonoids, phenolic compounds, and saponins (Table 1). These results corroborated the phytochemical profile of the ethanol extract of *Vernonia amygdalina*.[9,14] These findings provides the basic data for future investigations of the bioactive constituents of *Vernonia elaeagnifolia*.

Table 1: Phytochemical Constituents of VEE

Phytochemical	Inference
Flavonoids	+
Polyphenols	+
Alkaloids	+
Anthraquinones	-
Tannins	-

Saponins	+

+: Present, -: Absent, VEE: *Vernonia elaeagnofolia* leaves ethanol extract.

Antioxidant effect of VEE

Currently, *Vernonia elaeagnifolia* has been used extensively for a variety of purposes. Despite the absence of substantial evidence, the antioxidant properties of this plant is often associated with its use as an anti-pollution and sun protection agent.[23] The antioxidant assay of VEE was performed using the ABTS method, in which the antioxidant compounds in VEE is reacted with the ABTS radical cation ($ABTS^{\cdot+}$) which was produced from the reaction of potassium persulfate with ABTS reagent. The solution containing the cation radical is dark blue in colour and absorbs light at a maximum wavelength of 743 nm. When antioxidant compounds donate electrons or hydrogen atoms, the cation radical solution becomes colourless.[24] In the present study, the antioxidant activity of VEE was assessed on the basis of the IC_{50} value of ABTS radical scavenging activity. A lower IC_{50} value indicates a higher antioxidant activity. VEE exhibited an IC_{50} value of 147.28 µg/mL (Figure 1), which suggests that the plant extract has moderate antioxidant activity.[25] The plant extract was about 30 times less potent than ascorbic acid, which showed an IC_{50} value of 5.43 µg /mL.

Diverse methodologies including radical-scavenging activity and the quantification of total phenols and flavonoids have been employed to assess the antioxidant capacity of *Vernonia amygdalina*.[9] Antioxidants, due to their ability to scavenge free radicals, can either prevent or slow down cellular damage.[13,27] A previous study evaluated the antioxidant properties of *Vernonia elaeagnifolia* under glycophytic and halophytic conditions, and the extract was found to cause significant changes in the levels of enzymatic antioxidants such as superoxide dismutase, catalase, guaiacol peroxidase, ascorbate peroxidase, total polyphenols, proline, and ascorbic acid.[6] These findings indicate that *Vernonia elaeagnifolia* show promise as natural therapeutic agent for combating oxidative stress and potentially combating cancer.

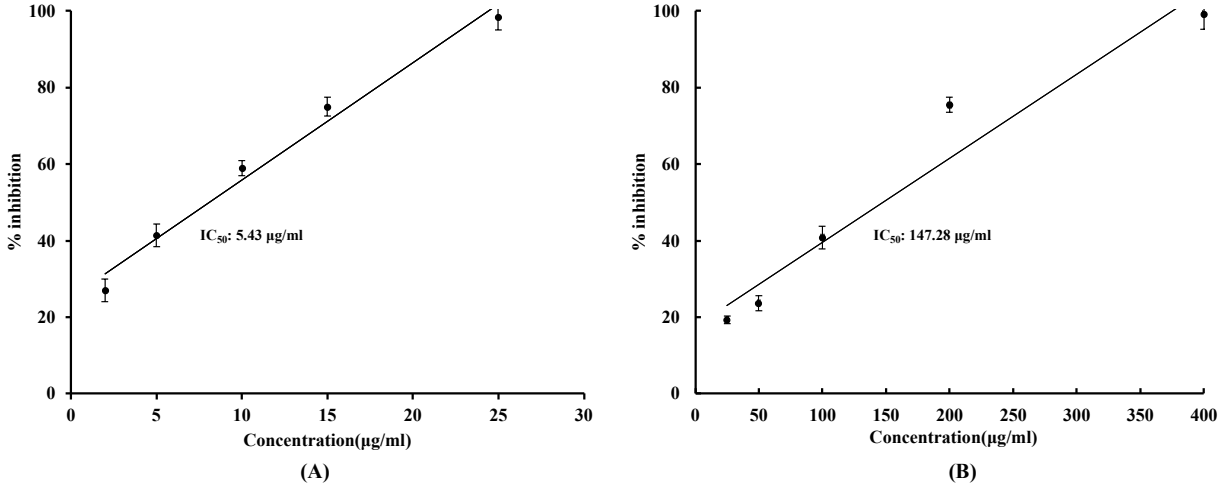

Figure 1: Antioxidant activity of ascorbic acid (A) and VEE (B). The antioxidant activity was determined using ABTS Radical Scavenging Assay. VEE: *Vernonia elaeagnofolia* leaves ethanol extract.

Cytotoxic effect of VEE

The cytotoxic effect of VEE was evaluated against two breast cancer cell lines; 4T1 and MCF7 cells. VEE inhibited the proliferation of the two cell lines in a dose-dependent manner. The IC_{50} values of VEE were 165.5 and 185.68 µg/mL for 4T1 and MCF7, respectively (Figure 2A-B). To determine the selectivity against cancer cells, the cytotoxic activity of VEE was assessed using Vero cells. The IC_{50} value of VEE against Vero cells was 299.66 µg/mL (Figure 2C). For the positive control (doxorubicin), the IC_{50} was found to be 0.44, 2.09, and 4.51 µg/mL against 4T1, MCF7, and Vero cells, respectively (Figure 2D). According to the 2017 World Health Organization (WHO) classification of cytotoxic agents, VEE exhibited a moderate cytotoxic activity against 4T1 and MCF-7 breast cancer cells, and a mild cytotoxicity against Vero cells. The selectivity index (SI) was calculated from the ratio of the IC_{50} value obtained for normal cells and that obtained for cancer cells, and was found to be 1.81 and 1.61 for 4T1 and MCF-7, respectively (Table 2). A substance is said to exhibit selectivity toward cancer cells when the SI value is ≥ 3.[4] From the results obtained in this study, VEE does not demonstrate selective toxicity for malignant cells.

This study proposed a hypothesis that *Vernonia elaeagnifolia* and *Vernonia amygdalina*, being members of the same genus, would exhibit similar antioxidant and anticancer effects. The alkaloids, flavonoids, phenolics, and terpenoids present in *Vernonia amygdalina* are also present in VEE, potentially contributing to their therapeutic effects, and offering a natural and sustainable health benefits.[26] As the hunt for effective and sustainable therapies continues, further research on the bioactive compounds from *Vernonia elaeagnifolia* is expected to play a significant role in the development of novel therapeutic agents with unique chemical moieties. It is important to note that this low cytotoxicity is observed exclusively for Vero cells, indicating that VEE might possess lower cytotoxic activity against normal cells compared to conventional drugs such as doxorubicin. However, this is subject to further investigations.

Table 2: Cytotoxic activity (IC_{50}) and Selectivity Index (SI) of VEE against 4T1, MCF7, and Vero cells

Cell line	IC_{50} (µg/mL)	Selectivity Index (SI)
4T1	165.50	1.81
MCF7	185.68	1.61
Vero	299.66	

VEE: *Vernonia elaeagnofolia* leaves ethanol extract

The findings from this study, though premature, might be regarded as significant, as *Vernonia elaeagnifolia* exhibits considerable

therapeutic potential due to its antioxidant and cytoprotective activities.

This study reveals an intriguing discovery, *Vernonia amygdalina* extract is effective in inhibiting 4T1 cancer cells, however, *Vernonia elaeagnifolia* extract demonstrates a relatively reduced ability to do so. Furthermore, the ethanol fraction of the leaves of *Vernonia amygdalina* has demonstrated a notable cytoprotective effect on Vero cells. Whereas *Vernonia elaeagnifolia* extract exerted a weak cytotoxic effect against Vero cells, which suggest that it has a potential of exerting minimum damage to normal cells. However, further investigation on the potential cytotoxic effect of *Vernonia elaeagnifolia* extract against different cancer cells is necessary. The results obtained in this study suggest that exploring the effect of the combination of VEE with a known chemotherapeutic agent could offer better outcome.[28,29] This is particularly important, as chemotherapy can often exert negative effects on normal cells.[17,30,31] This study provides valuable information regarding the use of VEE with respect to its antioxidant and cytotoxic effects, and has shown the potential for the development of VEE as a natural chemotherapeutic agent. Hence, conducting a thorough study on the mechanisms of action of VEE can help gain a deeper understanding of the molecular targets of the plant extract, and its practical applications.

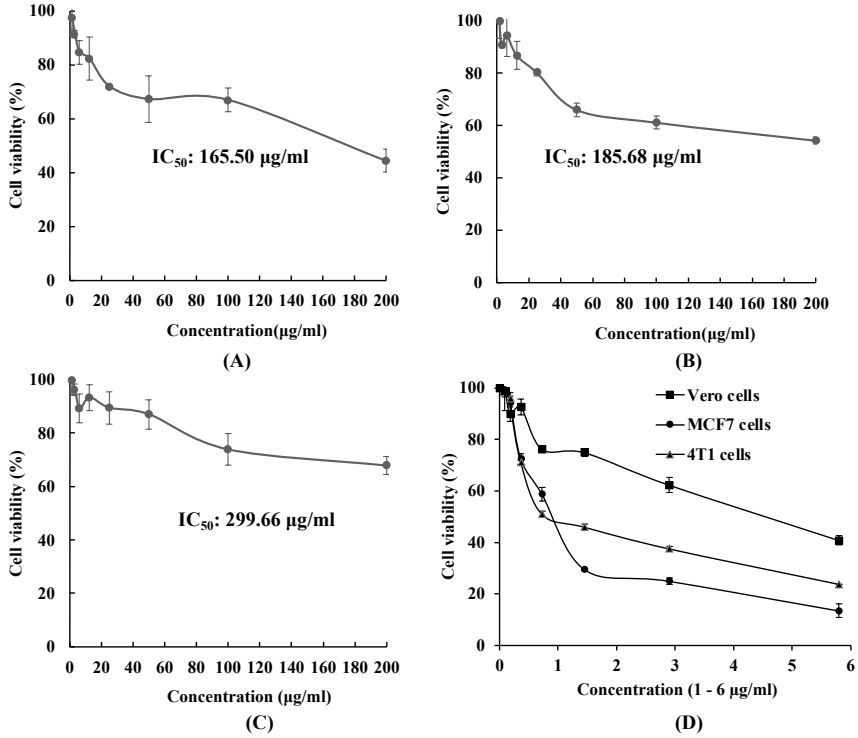

Figure 2: Cytotoxic activity of VEE against 4T1 cells **(A)**, MCF7 cells **(B)**, Vero cells **(C)**, and doxorubicin against three different cells **(D)**. The cytotoxic effect was determined using MTT assay. The data are mean ± standard error of mean (SEM) (n=3). VEE: *Vernonia elaeagnofolia* leaves ethanol extract.

Conclusion

Phytochemical screening of VEE has shown that the plant contains potentially bioactive compounds like alkaloids, flavonoids, phenolics, and saponins. VEE demonstrated moderate antioxidant activity, moderate cytotoxic activity against MCF7 and 4T1 breast cancer cells, and a weak cytotoxic effect against Vero (a non-cancerous) cell. The selectivity index shows that VEE is not selectively toxic to malignant cells.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be answered by them.

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