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ETHYL ACETATE FRACTION OF *SPHAGNETICOLA TRILOBATA* AS A POTENTIAL SOURCE OF ANTIOXIDANT COMPOUNDS

Runing title: *S. trilobata* antioxidative activity was identified in the ethyl acetate fractions

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ABSTRACTS
As demonstrated in our previous paper, the crude extract of *S. trilobata* has antioxidant activity. The current investigation aims to identify the bioactive compound responsible for *S. trilobata*'s antioxidant effect. The results shown that the ethyl acetate extract has the lowest EC_{50} values, indicating the highest DPPH scavenging (EC_{50} values for F- hexane, F- chloroform, F- ethyl acetate, and F-aqueous were > 800.00 µg/ml, 625.64 ± 43.05 µg/ml, 19.67 ± 0.98 µg/ml, 478.17 ± 34.67 µg/ml, respectively). Semi-polar compounds can be antioxidant bioactive compounds. Furthermore, there appeared to be a good correlation between phenolic content and antioxidant activity of the extracts, with higher phenolic content ethyl acetate fractions demonstrating higher antioxidant activity.
Keywords*: Sphagneticola trilobata*, antioxidant activity, fraction, polyphenol, ethyl acetate fraction

INTRODUCTION

Sphagneticola trilobata (L.) Pruski, or its former name, *Wedelia trilobata*, has been used as traditional medicines from many countries worldwide to treat various human ailments. It could be thought to be due to its naturally abundant phytochemical compounds, which result in pharmacological properties. According to Coe & Anderson (1996), fruits, leaves, and stem of *S. trilobata* were used to treat childbirth care and the treatment of bites and stings, fever, and infection, specifically in Eastern Nicaragua [1]. Remarkably, leaves were commonly used to treat kidney dysfunction, colds, wounds and amenorrhea, and dysmenorrhea [1; 2]. The crude extract of *S. trilobata* has antioxidant activity, as demonstrated in our previous paper [3]. The current study aims to identify the bioactive compound responsible for *S. trilobata*'s antioxidant effect.

MATERIALS AND METHODS

Sampling, extraction and fractions

The crude methanol extract of *S. trilobata* [4] was fractioned with water and hexane in the ratio of 1: 6, respectively. The aqueous extract was further extracted with chloroform and then with ethyl acetate.

The hexane, chloroform, ethyl acetate and aqueous extract were concentrated using a rotary evaporator giving fractions [5].

Antioxidant Properties

The 1'-1' diphenylpicryl-hydrazyl radical scavenging assay (DPPH) was carried out with minor modifications using the method described by Ghatak *et al* [6]. A DPPH (0.3 mM) solution was prepared to react with the plant extract in a 1 : 1 ratio. After 30 minutes of incubation at 37°C, the absorbance at 517 nm was measured. Vitamin C was used as a positive control in this test. The following formula is used to calculate the ability to scavenge DPPH radicals:

% DPPH radical scavenging = [(sample – negative control)/negative control] x 100

Determination of total content polyphenols

The total polyphenol content of *S. trilobata* extracts was determined using the Folin-Ciocalteu reagent [7] and a slightly modified Nunzia *et al* [8]. A volume of 200 µL extract at a concentration of 1000 µg/mL was mixed with 200 µL of Folin-Ciocalteu (100%) reagent. The mixture was incubated for 5 minutes at room temperature, after which 1600 µL of 5% sodium carbonate solution (w/v) was added. The reaction mixture was incubated at 40°C for 20 minutes before being placed in 96 plates, and the absorbance at 765 nm was measured. Gallic acid (range from 0 to 500 µg/mL) was used for the linear equation of a standard curve. The total polyphenol content was expressed as mg/g Gallic acid equivalent (GAE) of dry extract.

Statistical analysis

Each experiment was carried out three times. Graphpad Prism 7.04 was used to calculate the EC50 value. p-values less than 0.001 (****), 0.01 (***), 0.1 (**), and 0.5 (*) were used to determine significant differences. The data were presented as the mean standard error of the mean.

RESULTS

Antioxidant Properties of S.trilobata fractions by DPPH

S.trilobata ethyl acetate fractions demonstrated potent antioxidant activity in the DPPH assay. As illustrated in Figure 1, Among the aqueous, n-hexane, and chloroform fractions, the ethyl acetate fraction had the highest effect (8.88±1.65 %, 25.32±4.49 %, 13.07±4.59 % and 86.51±1.23 %, respectively at 100µg/ml concentration).

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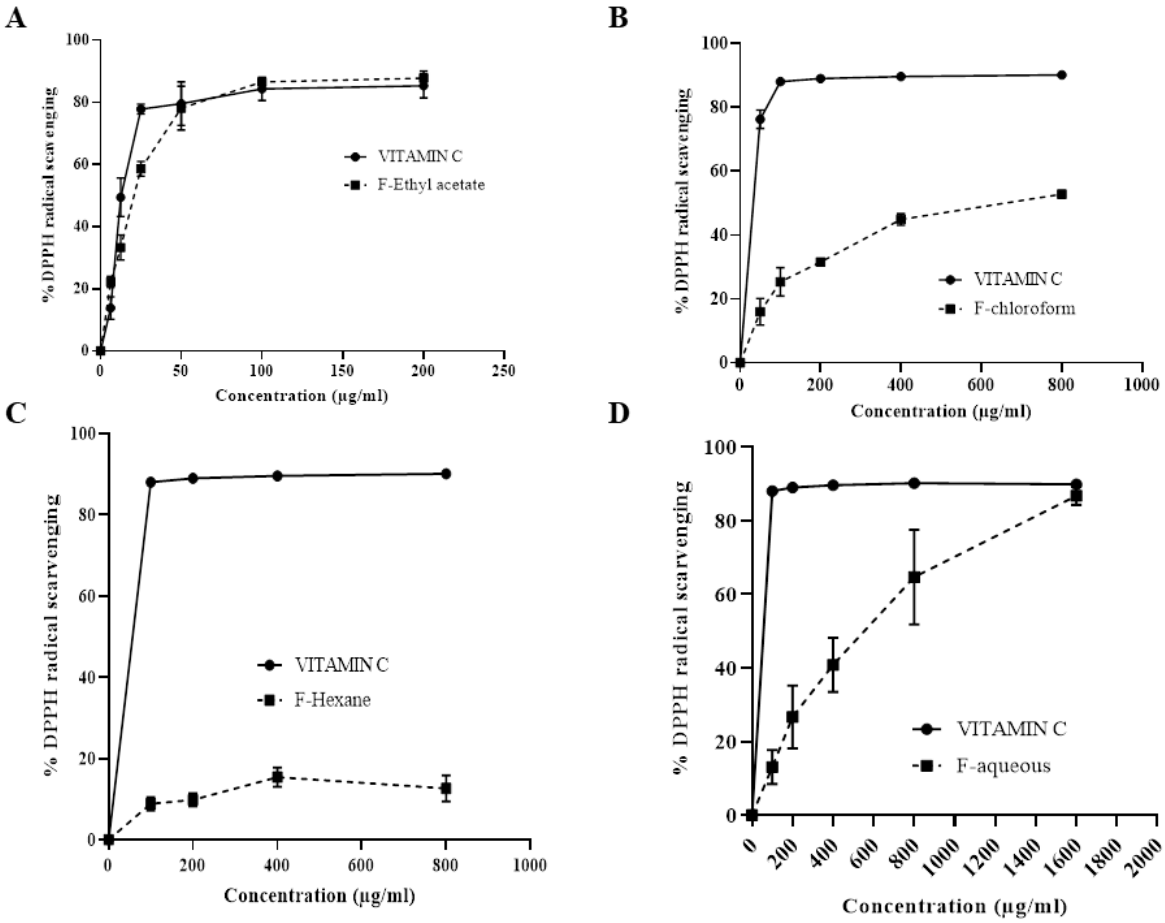

Figure 1: Antioxidant activity of the *S. trilobata* fractions extract. Determination of antioxidant activity of the *S. trilobata* fractions extracts by the DPPH method, using different concentrations, expressed as a percentage of antioxidant activity. Average of analysis obtained in triplicate (n = 3).

The results in table 1 show that the ethyl acetate extract has the lowest EC_{50} values, indicating the highest DPPH scavenging (EC_{50} values for F- hexane, F- chloroform, F- ethyl acetate, and F-aqueous were > 800.00 µg/ml, 625.64 ± 43.05 µg/ml, 19.67 ± 0.98 µg/ml, 478.17 ± 34.67 µg/ml, respectively). Antioxidant bioactive compounds may be semi-polar compounds.

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Table 1: The EC_{50} DPPH value of *S. trilobata* fractions

Compound	EC_{50} (μg/ml)
F-Hexane	> 800.00
F-Chloroform	625.64 ± 43.05
F-Ethyl acetate	19.67 ± 0.98
F-Aqueous	478.17 ± 34.67
Vitamin C	14.57 ± 1.47

Total polyphenol content of S.trilobata fractions

In general, the fraction may obtain a number of compounds belonging to groups such as sesquiterpen, diterpen, coumarin, quinon, aglycon, ect. due to the specific chemical properties of the solvent ethyl acetate. Compounds from *S. trilobata* in these groups have also been discovered in previous studies and are likely to provide the majority of the extract's biological activity [4]. The ethyl acetate solvent could extract polyphenols, which could be the key activity of the fraction. Furthermore, the antioxidant activity of plant extracts is frequently associated with the phenolic compounds found in them.

As expected, the contents of phenolic compounds and flavonoids were significantly higher in the fractions obtained in ethyl acetate than in the other fractions. Figure 2 depicts the total phenolic content of all *S.trilobata* fractions. The phenolic content of the ethyl acetate fractions was found to be higher than that of the other fractions in mg/g as gallic acid equivalent, i.e. at the same concentration. This is due to the higher solubility of such compounds in ethyl acetate, which is more polar than other solvents [9].

DPPH, a protonated radical, has a characteristic absorbance maxima at 517 nm that decreases with proton radical scavenging and has been widely used to assess the free radical scavenging effect of natural antioxidants [10]. Many studies have found a link between free radical scavenging activity and total phenolic compound levels. Oki *et al.* [11] discovered that the phenolic compound content increased the radical scavenging activity. Both the Lu and Foo [12] and Siriwardhana *et al.* [13] studies found a strong relationship between DPPH radical scavenging potential and total phenolic content.

In our study, there appeared to be a good correlation between phenolic content and antioxidant activity of the extracts, as ethyl acetate fractions with higher phenolic content demonstrated higher antioxidant activity.

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Finally, our findings suggest that phenolic acids are to blame for the higher antioxidant activity of the *S.trilobata* ethyl acetate fraction.

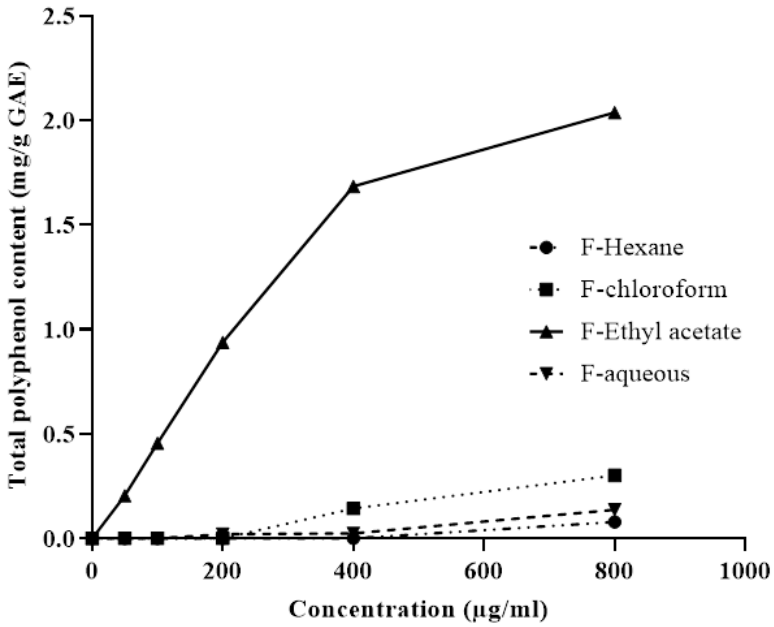

Figure 2: Total polyphenol content in *S.trilobata* fractions

CONCLUSION

When compared to other fractions, the ethyl acetate fraction was found to be the most effective, while the hexane fraction was found to be the least effective. The study also reveals that *S.trilobata* can be an interesting source of antioxidants, with potential applications in food and pharmaceuticals. Our research is focused on isolating and identifying antioxidant molecules in *S.trilobata* ethyl acetate fractions, as well as studying their health-promoting potential and mammalian safety.

LIST OF ABBREVIATIONS

F.: Fraction

S. Sphagneticola trilobata

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AUTHORS' CONTRIBUTIONS

Dr. Hoang Thanh Chi and Dr. Bui Thi Kim Ly were in charge of the experimental design, as well as conducting the experiments and writing, revising, and correcting the MS. The MS was revised and corrected by Dr. Nguyen Thi Lien Thuong. The submitted final MS was read and corrected by all of the authors.

COMPETING INTEREST

The authors declare that they have no competing interests

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