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COMPARATIVE FREE-RADICAL SCAVENGING OF CRUDE EXTRACTS AND VARIOUS FRACTIONS OF *ERUCA SATIVA*

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Abstract

The present study investigates the antioxidant potential of crude extracts and solvent fractions of Eruca sativa (rocket or arugula), a leafy vegetable belonging to the family Brassicaceae. The plant was successively extracted with solvents of increasing polarity, hexane, chloroform, ethyl acetate, methanol, and butanol to determine their comparative antioxidant activity using the DPPH free radical scavenging assay. The findings indicated that there was a proportional increase in antioxidant activity with the solvent polarity with the best effects of butanol and methanol fraction. The butanol fraction had 85.66 + -1.31% and the methanol fraction had 79.66 + -1.73% scavenging activity in 100 -mL of solution as compared to the standard ascorbic acid (95.24 + -0.64%). Polar fractions showed high activity indicating that the phenolic and flavonoid compounds are the chief contributors. The results also identify E. sativa as a useful source of natural antioxidant that can be utilized in nutraceutical and pharmaceutical products to prevent the occurrence of oxidative stress-related conditions.

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are by-products of normal cellular metabolism. Under physiological conditions, a balance exists between their generation and the body's antioxidant defense systems. However, when this balance is disturbed, oxidative stress occurs, leading to damage of lipids, proteins, and DNA (Ozcan & Ogun, 2015). This oxidative stress is implicated in the development of chronic and degenerative diseases, including cancer, atherosclerosis, neurodegenerative disorders such as Alzheimer's and Parkinson's disease, diabetes mellitus, and the aging process itself (Behl et al., 2021). In recent decades, there has been growing interest in identifying safe and effective natural antioxidants that can mitigate oxidative damage (Carocho & Ferreira, 2013; Li et al., 2014). The synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been popularly used as food preservatives, but their possible toxicity and carcinogenicity have raised concerns. This has led to shift in the world towards a more natural and plant-based antioxidants as safe substitutes (Felter, Zhang, & Thompson, 2021; Zhang, Diao, & Zhang, 2023).

A variety of secondary metabolites have been produced by plants, including polyphenols, flavonoids, tannins, alkaloids, and terpenoids, many of which possess very good antioxidant activities (Khan et al., 2024; Rahman et al., 2023). These compounds act by scavenging the free radicals, chelating transition metals, and modulating the antioxidant enzymes. Their protective effects are often

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associated with the prevention of cellular damage and inflammation (Flora, 2009; Mucha, Skoczyńska, Małecka, Hikisz, & Budzisz, 2021). Therefore, evaluating the antioxidant activity of plants not only provides scientific validation for traditional medicinal uses but also helps identify new sources for natural antioxidant agents (López-Alarcón & Denicola, 2013; Nićiforović et al., 2010).

Eruca sativa Mill., commonly known as rocket, arugula, or "taramira," is a leafy vegetable of the Brassicaceae family. It is commonly grown in the Mediterranean area, South Asia and the Middle East (Pignone & Gómez-Campo, 2010; Rana & Kumar, 2017). The seeds and leaves of E. sativa have long been used in the treatment of liver diseases, asthma and cough, skin irritation and aphrodisiac (Jilani, Ali, Rehman, & Nisar, 2015). The plant contains a high number of bioactive compounds including glucosinolates, isothiocyanates, flavonoids (e.g., quercetin and kaempferol), carotenoids and phenolic acids all of which have been shown to have antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Marwat, ur Rehman, & Khan, 2016). Phytochemical research has indicated that E. sativa has sulfur-based compounds which have contributed to its distinctive pungent smell, and the biological effects. It is also known that Glucosinolates and the products of their hydrolysis (Miękus et al., 2020; Zhu, Yang, Sanchez, Hamilton, & Fonseca, 2022). In addition, it has vitamin and such minerals as zinc, which improves the antioxidant profile (Mahmoud & Taha, 2018; Matev, Dimitrova, Petkova, Ivanov, & Mihaylova, 2018).

Although the phytochemical composition and part of the antioxidant activity of E. sativa have been analyzed in earlier reports, not much is known about the antioxidant capacity of the solvent fractions of E. sativa compared to each other. As antioxidant compounds have different polarity, application of increasing polarity solvents will give a complete picture of which category of compounds is most contributing to the activity. This gap will be addressed by the current study, which would provide a systematic assessment of the DPPH radical scavenging activity of various solvent fractions of E. sativa, therefore, identifying the strongest antioxidant extract and supporting its possible use in nutraceutical activity.

2.0 Materials and Methods

2.1 Plant Collection

Fresh aerial parts of *Eruca sativa* were collected from District Swabi during the flowering season. The plant was authenticated by a qualified taxonomist, and voucher specimens were (Uos/BOt 145) deposited in the herbarium of the Department of Botany University of Swabi for reference. The collected material was thoroughly washed for the removal of the dust. After that the plant material was shade-dried at room temperature, and ground into a fine powder using a grinder machine. The grounded powdered plant material was kept in the airtight containers for the further use.

2.2 Extraction and Fractionation

The powdered plant material (approximately 500 g) was extracted using Methanol by means of maceration and 72 hours at room temperature shaking intermittently. To produce a dark green residue, the methanolic extract was filtered on Whatman No. 1 filter paper and concentrated by use of rotary evaporator under reduced pressure. The crude extract was dissolved in distilled water and further partitioned with increasing polarity solvents; hexane, chloroform, ethyl acetate and methanol butanol. Each fraction was evaporated to dryness, weighed, and stored in labeled glass vials at 4°C.

2.3 Antioxidant Activity

The antioxidant potential of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, a widely used and reliable method to measure free radical–scavenging capacity (Uddin & Rauf, 2012). The stock solution of DPPH (0.1 mM) was made in methanol. Supersamples were made in methanol with various concentrations (10 100 µg/mL). A sample (1 mL) was combined with 1 mL of DPPH solution and left at room temperature, in the dark, incubating them during 30 minutes. The absorbance was measured at 517 nm with a UV- Vis spectrophotometer. Ascorbic acid was considered as a reference standard. The equation used to calculate the percentage of inhibition of DPPH radicals was as given below:

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$$\%\text{Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

2.4 Statical Analysis

Each experiment was done thrice. The data were presented in the form of the standard deviation (SD) and mean. One-way ANOVA was used to conduct statistical analysis.

3.0 Results

The antioxidant potential of the crude extract and various solvent fractions of *Eruca sativa* was evaluated using the DPPH radical scavenging assay. The findings, as stated in **Table 1**, show that the radical scavenging activity significantly and significantly increases with the concentration in all fractions. The higher the concentration of the extracts in 10 to 100 µg/mL, the higher the percentage inhibition of DPPH radicals and this indicated that the antioxidant constituents worked more efficiently with the increase in dose. At the lowest concentration (10 µg/mL), the butanol fraction was found to have 45.49 +/- 1.34% scavenging activity, then the methanol(40.77 +/- 1.91%), ethyl acetate(33.88 +/- 2.00%), chloroform(26.33 +/- 1.04%), and the hexane fraction had the least activity (12.66 +/- 1.8 Ascorbic acid was the standard antioxidant and exhibited 91.50 + -0.93 percent inhibition at the same level, which depicts that the E. sativa extracts are also not potent as compared to the pure standard; nevertheless, they carry a significant level of free radical neutralizing potential. With increased concentration (40 and 60 µg/mL) all the fractions had a significant increase in activity. The 55.05 ± 1.61% and 75.90 ± 1.45% inhibition of the methanol and butanol fractions, respectively, at 60 µg/mL were significantly higher compared to 29.02 ± 1.66 and 43.45 ± 1.39 per cent of the hexane and chloroform fractions, respectively. The ethyl acetate fraction was intermediate (50.74 ± 2.31%), which is the contribution of moderately polar phenolic compounds. The butanol fraction had the greatest DPPH scavenging potential at its highest concentration (100 µg/mL) (85.66 ± 1.31%), and was nearly as active as ascorbic acid (95.24 ± 0.64%). The methanol fraction followed closely with 79.66 ± 1.73%, while the ethyl acetate fraction reached 62.76 ± 2.31%. In contrast, the hexane fraction showed only 38.00 ± 1.40%, indicating limited antioxidant potential due to the extraction of non-polar constituents such as lipids and sterols.

Table 1: DPPH radical scavenging activity of crude extracts and various fractions of Eruca sativa

Concentration	Hexane	Chloroform	Ethyl Acetate	Methanol	Butanol	Standard
10 µg/mL	12.66±1.87	26.33±1.04	33.88±2.00	40.77±1.91	45.49±1.34	91.50±0.93
20 µg/mL	18.98±1.90	31.08±1.05	39.45±2.03	49.09±1.50	53.08±1.55	92.80±0.90
40 µg/mL	24.67±1.49	37.49±1.56	45.06±2.06	55.05±1.61	60.44±1.60	93.09±0.87
60 µg/mL	29.02±1.66	43.45±1.39	50.74±2.31	69.87±1.65	75.90±1.45	94.00±0.92
80 µg/mL	33.55±1.50	48.45±1.55	56.12±2.11	73.04±1.70	80.37±1.33	94.98±0.85
100 µg/mL	38.00±1.40	54.76±1.09	62.76±2.31	79.66±1.73	85.66±1.31	95.24±0.64

4.0 Discussion

When comparing E. sativa extracts, a distinct solvent-specific differences in antioxidant activity were observed. Methanol and butanol were polar solvents that extracted the greatest amounts of active compounds which are usually phenolics and flavonoids. These substances have been known to provide hydrogen atoms to free radicals to stabilize them and interrupt chain reactions leading to oxidative stress. It has been previously demonstrated

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that E. sativa possesses chemicals such as quercetin, kaempferol, sinapic acid, and glucosinolates, which have great antioxidant properties (Kumar et al., 2022). The performance improvement of the butanol fraction can be explained by the fact that it is intermediate polarized and, therefore, glycosides and phenolic substances with high radical scavenging potential can be extracted. The trend that is observed (Butanol > Methanol > Ethyl Acetate > Chloroform > Hexane) indicates the hypothesis that the antioxidant activity is proportional to solvent polarity. The weak activity was observed with nonpolar fractions like hexane which presumably had little extraction of phenolic constituents.

These findings are in line with reports by Nawaz, Haq, et al. (2020) , which emphasized that polar solvent extracts generally show stronger antioxidant potential (Nawaz, Shad, Rehman, Andaleeb, & Ullah, 2020). The high DPPH inhibition also suggests potential for *E. sativa* extracts in preventing lipid peroxidation and cellular oxidative damage. Given its edible nature and safety profile, *E. sativa* could serve as a functional food ingredient or a natural antioxidant additive in pharmaceuticals and cosmetics.

5.0 Conclusion

This paper shows that the Eruca sativa has exceptional antioxidant properties which are localized in the polar portions. DPPH radical scavenging capacity of the butanol and methanol extracts was found to be high and close to the ascorbic acid. These results confirm the conventional application of E. sativa as an ingredient of health-promoting preparations and justify the additional research to isolate and characterize the active antioxidant compounds.. Future research should include assays targeting other radicals (ABTS, FRAP, hydroxyl radical) and in vivo studies to confirm the biological relevance of these findings.

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Competing of Interests

All the authors declare that they have no conflicts of interest.

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Data availability statement

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