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ANTIMICROBIAL POTENTIAL OF CRUDE EXTRACTS AND ISOLATED FRACTIONS OF *ERUCA SATIVA*

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Abstract

This study aimed to investigate the antimicrobial potential of crude extracts and solvent fractions of Eruca sativa (rocket), a member of the Brassicaceae family widely known for its nutritional and medicinal properties. The plant was extracted using solvents of increasing polarity hexane, chloroform, ethyl acetate, methanol, and butanol to isolate bioactive fractions. The antibacterial activity was found using the agar well diffusion method against Escherichia coli, Klebsiella pneumoniae, Staphylococcus epidermidis, Staphylococcus aureus, and Bacillus subtilis. The results obtained show a wide variation in antibacterial efficacy among the different fractions. Methanol and butanol fractions showed the strongest inhibition of bacteria, with maximum zones of inhibition recorded against B. subtilis (28.98 ± 2.20 mm) and S. aureus (25.76 ± 1.33 mm), comparable to the standard reference antibiotic streptomycin (35.11 ± 1.32 mm). Moderate antibacterial activity was observed in ethyl acetate and chloroform fractions, while hexane showed weak inhibition. E. coli and K. pneumoniae displayed the least susceptibility, highlighting the resistance of Gram-negative bacteria. These findings suggest that E. sativa contains polar bioactive compounds, possibly phenolics and glucosinolates, with significant antibacterial efficacy. The results support the potential of E. sativa as a natural source for developing plant-based antimicrobial agents.

INTRODUCTION

Antimicrobial resistance (AMR) has become a critical global health concern, threatening the effectiveness of modern medicine (Salam et al., 2023). The overuse and misuse of antibiotics have led to the emergence of resistant bacterial strains that no longer respond to conventional treatment (Saleem, Deters, de la Bastide, & Korzen, 2019). As a result, the discovery of new and effective antimicrobial agents from natural sources has gained renewed attention (Abdallah, Alhatlani, de Paula Menezes, & Martins, 2023). Plants represent one of the most promising sources of bioactive molecules, offering a vast reservoir of secondary metabolites such as flavonoids, alkaloids, terpenoids, and phenolic acids that possess antimicrobial, antioxidant, and anti-inflammatory properties (Bhatti, Ismail, & Kayani, 2022; Rauf et al., 2023). *Eruca sativa* (Mill.), commonly known as rocket or arugula, belongs to the family Brassicaceae. It is cultivated widely across Europe, the Middle East, and South Asia as a leafy vegetable (Pignone & Gómez-Campo, 2010; Rana & Kumar, 2017). Beyond its dietary value, *E. sativa* has been used in traditional medicine to treat varieties of diseases (Jilani, Ali, Rehman, & Nisar, 2015). Several studies have demonstrated that members of the Brassicaceae family are rich in sulfur-

containing compounds, especially glucosinolates and their hydrolysis products such as isothiocyanates, which are known to exhibit antimicrobial and anticancer activities (Miękus et al., 2020).

The phytochemical composition of *E. sativa* includes flavonoids (quercetin, kaempferol), phenolic acids and other secondary metabolites (Jaafar & Jaafar, 2019; Kumar et al., 2022). These compounds act synergistically to provide antioxidant and antibacterial properties (Formagio et al., 2014). Methanolic and aqueous extracts of *E. sativa* have been reported to inhibit the growth of both Gram-positive and Gram-negative bacteria, as well as exhibit strong free radical scavenging activity (A. Ahmad & Shehta, 2020; Bassyouni, Kamel, Algameel, Ismail, & Gaber, 2022). Despite these promising biological properties, systematic evaluation of antibacterial activity across different solvent fractions of *E. sativa* remains limited. The extraction solvent plays a crucial role in determining the yield and type of phytochemicals extracted (Ingle et al., 2017). Non-polar solvents (like hexane) extract fats and sterols, while polar solvents (like methanol and butanol) extract phenolics, flavonoids, and glycosides, which are often responsible for antimicrobial activity (Mukherjee et al., 2024). Hence, studying multiple solvent fractions provides insight into the polarity and solubility of bioactive compounds.

The present research was therefore designed to evaluate and compare the antibacterial potential of crude extracts and various solvent fractions of *E. sativa* against a panel of pathogenic bacterial strains. The findings from this study could contribute to the development of new natural antimicrobial formulations that are safer, effective, and eco-friendly.

2.0 Material and Methods

2.1 Plant Collection and Identification

Fresh plant material of *Eruca sativa* was collected from agricultural fields during the early flowering season to ensure maximum phytochemical content. The plant was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the departmental herbarium. The plant material was washed to remove dust and debris, shade-dried at room temperature for two weeks, and ground into fine powder using a mechanical grinder. The powdered material was stored in airtight containers away from light and moisture until extraction.

2.2 Extraction and Fractionation

About 1 kg of dried plant powder was macerated in 3 L of methanol for seven days with intermittent shaking. After extraction, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure to yield a thick methanolic crude extract. The crude extract was suspended in distilled water and successively partitioned with solvents of increasing polarity: hexane, chloroform, ethyl acetate, methanol, and butanol. Each solvent fraction was evaporated to dryness, weighed to calculate yield percentage, and stored at 4°C for further analysis.

2.3 Antibacterial Activity

The antibacterial activity was assessed by the agar well diffusion method (Z. Ahmad et al., 2024). Nutrient agar plates were inoculated with freshly prepared bacterial suspensions equivalent to 0.5 McFarland standard (approximately 1×10^8 CFU/mL). Using a sterile cork borer, 6 mm wells were made in the agar, and 100 µL of each extract (1 mg/mL) was added to the wells. Streptomycin (30 µg/mL) was used as the positive control, while the corresponding solvent served as a negative control. The plates were incubated at 37°C for 24 hours, and the diameters of inhibition zones were measured in millimeters (mm) using a digital caliper. All tests were performed in triplicate.

2.4 Statistical Analysis

Results were expressed as mean ± standard deviation (SD). One-way analysis (ANOVA) was performed.

3.0 Results

The results of antibacterial assays are summarized in **Table 1**. Distinct variations in inhibitory activity were observed across different extracts and bacterial species.

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

Bacterial strain	Hexane	Chloroform	Ethyl Acetate	Methanol	Butanol	streptomycin
E. coli	-	-	-	16.09±1.00	22.65±1.65	26.22±1.04
K. pneumonia	-	18.44±1.54	20.65±1.43	15.09±1.09	13.09±1.87	30.54±1.82
S. epidermis	12.87±1.00	16.87±1.66	19.66±1.86	14.98±2.00	18.76±1.65	34.02±1.12
S. aureus	10.04±1.04	14.98±1.87	25.98±1.09	25.76±1.33	23.87±2.01	28.55±1.23
B. subtilis	8.23±1.54	20.87±1.85	17.87±1.33	26.87±1.54	28.98±2.2	35.11±1.32

Among all fractions, the methanol and butanol extracts exhibited the highest inhibitory effects. Methanol fraction showed notable inhibition against *B. subtilis* (26.87 ± 1.54 mm) and *S. aureus* (25.76 ± 1.33 mm), while butanol fraction displayed maximum inhibition against *B. subtilis* (28.98 ± 2.20 mm). Hexane extract showed weak or no inhibition, whereas chloroform and ethyl acetate fractions demonstrated moderate activity.

Gram-positive bacteria (*S. aureus*, *B. subtilis*, *S. epidermidis*) were more sensitive to the extracts than Gram-negative strains (*E. coli*, *K. pneumoniae*).

4.0 Discussion

The findings from this study confirm the antibacterial potential of *Eruca sativa* extracts and fractions, with marked activity in the methanol and butanol fractions. This suggests that the antibacterial compounds in *E. sativa* are predominantly polar and likely include flavonoids, phenolic acids, and glucosinolates. The degree of extraction depends heavily on solvent polarity (Nawaz, Shad, Rehman, Andaleeb, & Ullah, 2020). Polar solvents such as methanol and butanol are more efficient in extracting compounds like phenolics, tannins, and glycosides, whereas non-polar solvents such as hexane extract fats and waxes with little antimicrobial activity (Borges, José, Homem, & Simões, 2020; Mamoona, Nosheen, Riaz, Shah, & Shahid, 2025). The superior performance of methanol and butanol fractions in this study supports the hypothesis that the bioactive constituents of *E. sativa* are polar in nature (Pagnotta, Ugolini, Matteo, & Righetti, 2022). Chloroform and ethyl acetate fractions showed intermediate activity, suggesting they contain semi-polar bioactive metabolites such as terpenoids or alkaloids. The weak inhibition by hexane extract indicates that non-polar compounds are not major contributors to antibacterial activity in this plant. The bacterial response varied notably between Gram-positive and Gram-negative strains. Gram-positive bacteria such as *S. aureus* and *B. subtilis* were more susceptible, likely due to their simpler cell wall structure. In contrast, Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides that limits the penetration of antibacterial compounds, resulting in reduced susceptibility. The strong inhibition of *S. aureus* and *B. subtilis* by methanol and butanol extracts aligns with earlier reports where *E. sativa* essential oils and extracts inhibited similar Gram-positive bacteria. The consistent activity across these strains highlights the broad-spectrum nature of *E. sativa* bioactives. Previous studies on *E. sativa* have identified numerous phytoconstituents responsible for antimicrobial activity (Gulfraz et al., 2011). Glucosinolates and their breakdown products, particularly isothiocyanates, are known to disrupt bacterial enzyme systems and compromise membrane integrity (Borges et al., 2015). Additionally, flavonoids such as quercetin and kaempferol exhibit bactericidal effects by inhibiting nucleic acid synthesis and energy metabolism (Li, He, Zhang, & Shi, 2022).

Phenolic compounds can bind to bacterial cell walls, leading to increased membrane permeability and leakage of vital cellular contents. The presence of multiple phytochemicals in *E. sativa* may act synergistically, enhancing antibacterial efficacy beyond what is achievable by individual compounds. Although streptomycin exhibited the largest inhibition zones, some *E. sativa* fractions produced

comparable effects (up to 28.98 mm). This suggests that the plant extracts possess substantial antibacterial potential. The difference in activity between natural extracts and synthetic antibiotics may also be due to concentration differences and the presence of crude mixtures in the extracts rather than isolated compounds.

The growing threat of antibiotic resistance underscores the need for alternative therapeutic sources. Natural products derived from plants such as *E. sativa* provide a sustainable and accessible means of combating resistant pathogens. Since the plant is already widely consumed as food, its bioactive fractions could be developed into nutraceuticals or topical formulations for skin infections and wound healing.

Further work is needed to isolate and purify the specific antibacterial constituents using chromatographic techniques. Structural elucidation via NMR and mass spectrometry could provide insights into their mechanisms of action. Additionally, in vivo testing and cytotoxicity assessments are essential before clinical application. Combining plant-derived compounds with conventional antibiotics could also enhance efficacy and reduce the development of resistance.

5.0 Conclusion

The present study provides clear evidence that *Eruca sativa* exhibits significant antibacterial activity, particularly in methanol and butanol fractions. The highest inhibition zones were observed against *Bacillus subtilis* (28.98 ± 2.20 mm) and *Staphylococcus aureus* (25.76 ± 1.33 mm), indicating strong activity against Gram-positive bacteria. The results demonstrate that the antibacterial potential of *E. sativa* is associated with polar phytochemicals such as flavonoids, phenolic acids, and glucosinolates. Given its broad spectrum of biological activity and widespread availability, *E. sativa* represents a promising candidate for the development of natural antimicrobial agents. Future research should focus on the isolation of pure compounds, assessment of synergistic effects, and evaluation of potential applications in pharmaceutical formulations.

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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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