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Antibacterial Activity of *Eruca sativa* Mill Leaves Against Some Pathogenic Bacteria Isolated from Prostatitis Samples

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Abstract:

This study was conducted in Hillah city, Babil province in Iraq, to know the extent of the effects of the secondary metabolites, such as flavonoids and terpenoids extract from *Eruca sativa* Mill leaves, against some bacterial species isolated Prostatitis Samples represented by *Escherichia coli*, *Staphylococcus aureus*, *streptococcus pyogenes, Pseudomonas aeruginosa, and Klebsiella pneumonieae*. An ability of antibacterial was totally completed by utilizing the method of agar well diffusion by preparing three concentrations 25, 50 &100mg/ml. sterile distal water was utilized as a negative control. Flavonoid extract at 50 and 100 mg/ml exhibited significant supremacy at Probability ≤ 0.05 over the negative control when applied to *Escherichia coli*. *Staphylococcus aureus* was sensitive to flavonoids and terpenoid compounds at all concentrations under study P≤ 0.05 in comparison with the negative control. Whereas *streptococcus pyogenes, Pseudomonas aeruginosa, and Klebsiella pneumonieae are* totally resistant to all concentrations of flavonoid compounds and *Escherichia coli*, *Klebsiella pneumonieae,* and *Pseudomonas aeruginosa* fully resistance to all concentration of terpenoid compounds. Lastly, flavonoids and terpenoid compounds in leaves of *Eruca sativa* respected a good source for controlling some bacterial species isolated from Prostatitis samples, especially against *Staphylococcus aureus*.

Keywords: *Eruca sativa, Antibacterial activity, Secondary metabolites.*

Introduction

Eruca sativa, often known as jarjeer, is a yearly plant belonging to the *Brassicaceae* family. It is rich in various compounds and minerals that possess nutraceutical and organoleptic properties. Jarjeer was primarily utilized as a dietary source and traditionally taken mostly for its aphrodisiac qualities. This crop is rich in

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phytochemicals, including flavonoids, phenolic acids, terpenes, carotenoids, tannins, glycosides, saponins, sterols, alkaloids, and other secondary metabolites. The primary flavonoids found in leaves are kaempferol and its derivatives, specifically glucosativin. Additionally, the main fatty acid present is erucic acid, while the primary glucosinolate is glucoerucin. The plant possesses medicinal properties such as antibacterial, antidiabetic, antihypertensive, antiplatelet, and antioxidant activity. Additionally, it promotes hair development and exhibits various other effects (Jaafar, & Jaafar, 2019). *E. sativa* is a plant-based medication that mostly consists of Erucic acid, along with oleic acid, linoleic acid, saturated fatty acids, flavonoids, phenolics, glucosinolate, vitamin C, and carotenoids (Qaiyyum, & Nergis, 2022). *E. sativa* is a diploid herb with 22 chromosomes, it has 350 genera and about 3,500 species having medicinal values. It is about 1 to 1.5 feet long and is widely grown all over the world. It mainly originated from the Mediterranean region, Middle-East, South Asia, North Africa, Iran, and Pakistan, and in India, it is mostly cultivated in Haryana, Punjab, and around Delhi (Tarique, 2010). The seeds have long been used in folk medicine as a lactagogue, aphrodisiac, diuretic, antis -corbutic, antimicrobial, to disintegrate renal calculi and induce vomiting; This used seeds were taken a long time in traditional medicine as an aphrodisiac, diuretic, lacagog, antimicrobial, and anti-bacterial, to induce vomiting and destroy kidney stones (Boulos, 1983). Its seed is commonly yellow but sometimes is reddish yellow or spotted with brown-green spots (Koocheki, Razavi, & Hesarinejad, 2011). Antiulcer effect of *E. sativa* is known in traditional medicine. *Helicobacter pylori* which are involved in the pathogenesis of ulcer have a high urease activity, and urease enzyme is essential to *H. pylori* metabolism and required for its colonization in gastric mucosa. *E. sativa* extract produces a marked reduction of urease activity and thus provides scientific confirmation for its use as antiulcer agent (Guenane, et al., 2017). Lipid autoxidation is initiated by a chain of lipophilic radicals. *In vivo,* hydrogen peroxide (H_2O_2) is generated by several oxidase enzymes. H_2O_2, through hydroxyl free radical serves as a messenger molecule in the inflammatory mediators' synthesis and activation; these mediators are involved in tissue damage and pathogenesis of various diseases such as diabetes (Alqasoumi, et al., 2009). Rocket leaf oil extracted by steam distillation has a significant antifungal effect assessed by the well-diffusion method. The extracted oil has a high rate of inhibition (60–67%) against *Dreschlera halodes*, *Cola clavata*, *Rhizopus oryzae,* and *Aspergillus nidulans*. While the oil moderately suppress *Alternaria kiliense* (49%), *Alternaria alternata* (38%) and exhibited minimum inhibition against *F. oxysporum* with (13%) (Perez, Pauli, & Bazerque, 1990). Powdered seeds of *E. sativa* demonstrate antifungal effect. Crude aqueous seeds exhibited a strong, powerful antifungal effect against the fungus *Spadicoides stoveri* and *Paecilomyces variotii* while insignificant inhibition against other fungal strains (Ansari, 2014). Medicinal plant secondary metabolites are thought to be a promising starting point for the synthesis of novel antibiotics and medications for the treatment of infectious disorders because they have the ability to function as both bactericidal and bacteriostatic against "multidrug resistance" pathogens. However, the objective of this investigation was to assess the efficaciousness of *E. sativa* leaves' secondary antibacterial compounds against specific bacterial species that were isolated from samples of prostatitis.

Materials and Methods

Plant Material

Leaves of *Eruca sativa* Mill, had been purchased from local markets and identified based on the taxonomic features of Iraqi Flora (Ghazanfar, Edmondson, & Nicholas Hind, 2019). (Table 1). Leaves of these plant was cleaned, dried, and kept according to (Harborne, Mabray, & Marby, 1975), Table: 1.

Table 1. Scientific, Local, English Name, Family, and Active Parts

Scientific name	Local name	English name	Family	Active part used
Eruca sativa Mill	jarjeer	Rocket	Brassicaceae	Leaves

Extraction of the Crude Flavonoid Compounds

Crude Flavonoid compounds were extracted according to (Bohm, & Koupai-Abyazani, 1993).

Extraction of the Crude Terpenoid Compounds

Crude terpenoid compounds were extracted according to Bohm, & Koupai-Abyazani, (1993). The stock solution of 100 mg/ml for Flavonoids and Terpenoids was prepared in 10% Dimethyl Sulfoxide (DMSO), then sterilized by a Millipore filter (0.22µm) and stored at (-20C°) until use (Al-Jassani, 2018).

Antibacterial Activity

The agar well diffusion method was utilized to test the ability of antibacterial *Eruca sativa* leaves against some bacterial species isolated from Prostatitis Samples (Perez, Pauli, & Bazerque, P990). Cork porer with a size of 6mm in diameter are used to make wells in agars. Control negative was made by adding sterile distal water in wells.

Pathogenic Bacteria Isolates

Isolates of some bacterial species isolated from Prostatitis Samples obtained from Microbiology laboratories in different hospitals within the boundaries of the municipality of Hillah-Iraq (Table 2).

Table 2. Pathogenic Isolates and Sources of isolates

No	Isolates	Source
1	*Escherichia coli*	Prostatitis Samples
2	*Staphylococcus aureus*	
3	*streptococcus pyogenes*	
4	*Pseudomonas aeruginosa*	
5	*Klebsiella pneumonieae*	

Statistical Analysis

All data of treatments were dictated by three replicates. Data were subjected to an analysis of variance by using SPSS 20.0 program, a completely randomized design was used and least significant difference (L.S.D) was performed at P≤ 0.05.

Results

The results of antibacterial activity of the crude flavonoid compounds extracted from the leaves of *Eruca sativa* against pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, streptococcus pyogenes, Pseudomonas aeruginosa,* and *Klebsiella pneumonieae* isolated from Prostatitis samples are presented in (table 3). The antibacterial activity of the crude flavonoid secondary metabolites with three concentrations (25, 50, and 100 mg/ml) was screened by the agar well diffusion method. The results revealed that the crude flavonoid compounds extracted from the leaves of *Eruca sativa* showed a significant reduction at P≤ 0.05 in the growth of *Escherichia coli* and *Staphylococcus aureus*. The antibacterial activity was applied at (25, 50, and 100) mg/ml. The inhibitory zone of flavonoid ranged from 0± 0 in 25 mg/ml, 12± 1 in 50 mg/ ml, and 15± 1 in 100 mg/ml when applied to *Escherichia coli* and 15±1, 18± 1, and 20± 1 when applied to *Staphylococcus aureus*. Other organisms like *streptococcus pyogenes, Pseudomonas aeruginosa,* and *Klebsiella pneumonieae* were revealed to be completely resistant to the flavonoid compounds (Figure: 1, 2).

Table 3. An Antibacterial Efficacy of Flavonoid Compounds Extract from *Eruca sativa L* Leaves Against Some Bacterial Species Isolated from Prostatitis Samples. LSD= 1.54

Bacteria	Flavonoid compounds Concentration mg /ml Inhibition zone/mm			
Concentration	Negative control	25 mg/ml	50 mg/ml	100 mg/ml
Escherichia coli	0± 0	0± 0	12± 1	15± 1
Staphylococcus aureus	0± 0	15±1	18± 1	20± 1
streptococcus pyogenes	0± 0	0± 0	0± 0	0± 0
Pseudomonas aeruginosa	0± 0	0± 0	0± 0	0± 0
Klebsiella pneumonieae	0± 0	0± 0	0± 0	0± 0

In the same context, the crude Terpenoid compounds extracted from the leaves of *Eruca sativa* showed a significant reduction at P≤ 0.05 in the growth of *Staphylococcus aureus* and *streptococcus pyogenes*. The inhibitory zone of Terpenoid ranged from 0± 0 in 25 mg/ml, 15± 1 in 50 mg/ ml, and 18± 1 in 100 mg/ml when applied *Staphylococcus aureus* and 0± 0, 12± 1, and 17± 1 when applied *streptococcus pyogenes*. Other organisms like *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumonieae* were revealed to be completely resistant to the flavonoid compounds (Figure 2).

Table 4. An Antibacterial Efficacy of Terpenoid Compounds Extract from *Eruca sativa L* Leaves Against Some Bacterial Species Isolated from Prostatitis Samples. LSD= 1.48

Bacteria	Terpenoid compounds Concentration mg /ml Inhibition zone/mm			
Concentration	Negative control	25 mg/ml	50 mg/ml	100 mg/ml
Escherichia coli	0± 0	0± 0	0± 0	0± 0
Staphylococcus aureus	0± 0	0± 0	15± 1	18± 1
streptococcus pyogenes	0± 0	0± 0	12± 1	17± 1
Pseudomonas aeruginosa	0± 0	0± 0	0± 0	0± 0
Klebsiella pneumonieae	0± 0	0± 0	0± 0	0± 0

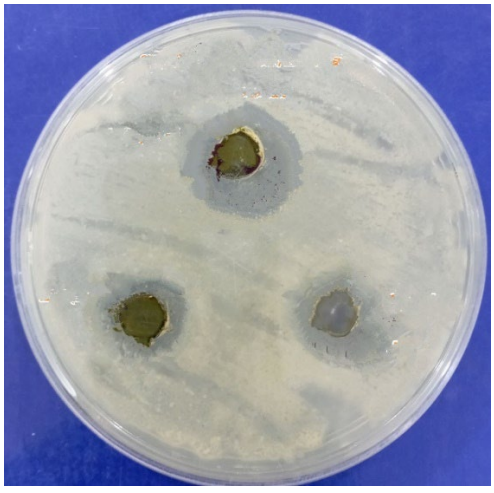


Figure 1. Antibacterial activity of the crude Flavonoid compounds at (25, 50, 100 mg/ml against S. aureus

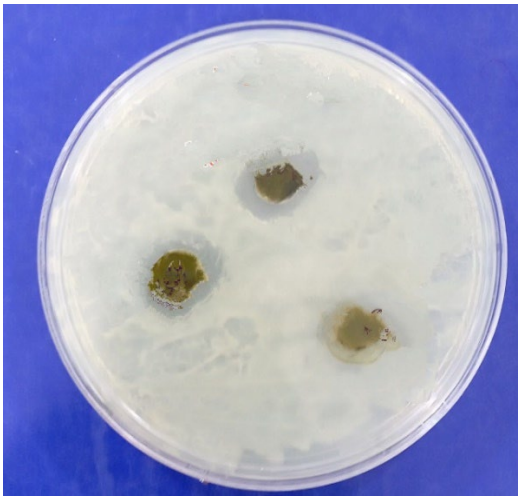


Figure 2.. Antibacterial activity of the crude Flavonoid compounds at (25, 50, 100 mg/ml against *E. coli*

Disscution

There is no doubt that the effective compounds extracted from medicinal plants remain one of the most important, if not the most important, sources in the fight against diseases, especially in light of the aggravation of the problem of microorganism's resistance to antibiotics, Medicinal plants are also less harmful in terms of side effects compared to chemical drugs. Constituents separated from different active parts of numerous medicinal plants such as (*Lactuca serriola* leaves; *Lepidium sativum* leaves; *Myrtus Communis* leaves; *Cassia senna* leaves; *Ricinus communis* leaves; *Cassia didymobotrya* leaves; *Melia azedarach* leaves; *Dianthus caryophyllus* flowers bud; and *Salvia hispanica* seeds), possess the ability of antibacterials for controlling several pathogenic microorganisms isolated from different clinical samples (Al-Marzoqi, Hussein, & Al-Khafaji, 2015; Al-Marzoqi, Sahi, & Hussein, 2016; Hussein, et al., 2017; Hussein, et al., 2018a; Hussein, et al., 2018b; Hussein, et al., 2019; Hussein, & Al-Marzoqim, 2020; Kamil, Hussein, & Al-Marzoqi, 2020; Hussein, Kamal, & Sahi, 2020). Hussein, Naji, & Al-Khafaji, (2018) reported that constituents separated from the unicellular primitive plant like *Chlorella vulgaris* possess the ability of antibacterial counter to pathogenic bacteria. Kamal, Hussein, & Tolaifeh, (2019) Used phytochemical compounds separated from *Hibiscus sabdarifa* for controlling *E. coli* and *Proteus* sp. Kamal, Al-Kaim, & Hussein, (2020) Used constituents extracted from *Ficus carica* L. for controlling *E. coli* and *Pseudomonas aeruginosa*. AL-Masoodi, Hussein, & Al-Rubaye, 2020) Used phytochemical compounds extracted from *Boswellia carteri* and *Curcuma longa* for controlling *Fusarium* spp. isolated from seeds of corn. Hussain, Hussein, & Al-Rubaye, (2021) used terpenoid compounds extracted from *Carthamus tinctorius* against *Aspergillus* species isolated from stored medicinal plant seeds. Secondary metabolites represented by Alkaloids and flavonoid compounds separated from *M. Communis* leaves are respected as a worthy source for controlling pathogenic microorganisms segregated from hemodialysis fluid specimens (Sharara, Al-Marzoqi, & Hussein, 2021). Hasan Radhi, et.al. (2022) used *Callistemon viminalis* leaves extracts for controlling isolates of Urinary Tract Infections. Alkaloids and Terpenoids extracted from the roots of *Saussurea costus* have powerful antifungal activity against *Candida* species (Karim, Hussein, & Al-Rubaye, 2022), *S. costus* roots also have powerful antifungal activity against Aspergillus species isolated from rice seeds (Al-Nafie, Hussein, & Al-Rubaye, 2024). *Allium sativum* is considered a reliable means of suppressing some bacterial species that have been isolated from people infected with the Corona Virus (Hussein, et al., 2023). Secondary metabolite compounds extracted from the *D. caryophyllus* L. flower buds such as terpenoid and flavonoid have powerful antifungal activity against *Candida* species (Karim, Hussein, & Al-Rubaye, 2023). Ethyl acetate extract of seeds of *E. sativa* was highly efficient in controlling the growth of *Staphylococcus aureus, Bacillus subtilus, Methicillin resistant Staphylococcus aureus, Streptococcus pyogenes,* and *Escherichia coli* (Rizwana, et al., 2016). *Eruca sativa* seed, as well as seed oil, have antibacterial activity against gram-positive and gram-negative bacteria, but *Klebsiella pneumoniae* and *Staphyllococcus epidermidis* were found to be less susceptible as compared to other clinical isolates (Gulfraz, et al., 2011). Seeds aqueous extract inhibits gram-positive bacteria, and the mean inhibitory zone for *streptococcus faecalis* and *Staphylococcus aureus* was 10.4 mm and 14.0 mm, respectively (Hussain, et al., 2020). Flowers of *E. sativa* showed a good growth inhibition zone compared to positive controls when tested against Salmonella *typhimurium* (Koubaa, et al., 2015). The crude extract of *E. sativa* was active against all tested food-borne bacteria. Furthermore, inhibition of developed biofilm and also reduced the viability of bacterial cells within biofilms (Awadelkareem, et al., 2022). In general, the inhibitory effect of plant extracts against microorganisms can be explained as follows: i) inhibit the formation of the cell wall of the organism or inhibit the synthesis of some essential proteins, ii) Damage in DNA synthesis iii) disruption in membranes permeability (Tyler, Brady, & Robbert, 2019). In the same context,

Terpenoids and flavonoids make their effects by disruption of microbial membranes and Polypeptides embarrassment of linkage of bacterial proteins to host polysaccharide receptors (Okusa, Stévigny, & Duez, 2009). On the other hand, some types of bacteria have the ability to resist the effect of bioactive compounds extracted from medicinal plants, and this may depend on the nature of the components of the bacterial cell wall in addition to its ability to get rid of these compounds. Finally, antibacterial efficacy of *E. sativa* might be belonging to phytochemical compounds such as flavonoids and terpenoids and their effect in proteins and polysaccharides and disruption in membranes permeability or inhibiting of efflux pump.

Conclusion

Flavonoids and terpenoid compounds in leaves of *Eruca sativa* respected a good source for controlling some bacterial species isolated from Prostatitis samples, especially against Staphylococcus aureus.

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