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Antibacterial activity of Secondary Metabolites Extracted from *Eruca sativa* L. Leaves against some pathogenic bacteria isolated from Clinical Samples

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Antibacterial activity of Secondary Metabolites Extracted from *Eruca sativa* L. Leaves against some pathogenic bacteria isolated from Clinical Samples

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Abstract:

A study was conducted in Hillah city, Babil province in Iraq, for the purpose of knowing the extent of the effects of the secondary metabolites such as flavonoids and terpenoids extract from *Eruca sativa* L. leaves, against some bacterial species isolated clinical samples represented by *Escherichia coli, Staphylococcus aureus, streptococcus pyogenes, Pseudomonas aeruginosa, and Klebsiella pneumonieae*, and, the ability of antibacterial was 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 terpenoids compounds at all concentration under study at (P≤ 0.05) in compared with the negative control. Whereas *streptococcus pyogenes, Pseudomonas aeruginosa, and Klebsiella pneumonieae* fully resistance to all concentration of flavonoid compounds and *Escherichia coli, Klebsiella pneumonieae,* and *Pseudomonas aeruginosa* fully resistance to all concentration of terpenoid compounds. Lastly, flavonoids and terpenoids compounds leaves of *Eruca sativa* respected a good source for controlling some bacterial species isolated from clinical samples especially against *Staphylococcus aureus*.

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

INTRODUCTION

Eruca sativa (jarjeer) is an annual herb (family Brassicaceae), which contains a wide range of chemicals and minerals with nutraceutical and organoleptic characteristics. Jarjeer was generally used as a food and traditionally mainly consumed due to its aphrodisiac properties. This crop known to contain various phytochemicals such as flavonoids, phenolic acids, terpens, carotenoids, tannins, glycosides, saponins, sterols, alkaloids, and other secondary metabolites. In leaves, kaempferol and its derivatives, glucosativin, are the main flavonoids and glucosinolate, respectively, while erucic acid and glucoerucin are the main fatty acid and glucosinolate, respectively. Medicinally, the plant has antibacterial, antidiabetic, antihypertensive, antiplatelet, and antioxidant activity and stimulates hair growth and other effects. Trails on topical pharmaceutical preparations involve the use of *E. sativa* which had been done. These preparations include creams and waxs which are intended to be used for potentiating hair growth and skin fungal and bacterial infection [1]. *Eruca sativa* is one of the plant origin drugs, It contains Erucic acid (major contain), oleic acid, linoleic acid, saturated Fatty acids, Flavonoids, Phenolics, Glucosinolate, Vitamin C and Carotenoids [2]. *Eruca sativa* is an annual herb diploid (2n = 22) in nature belongs to Brassicaceae family, 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 world. It mainly originated from Mediterranean region, Middle-East, South Asia, North Africa, Iran and Pakistan and in India it is mostly cultivated in Haryana, Punjab and around Delhi [3]. 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 long time in traditional medicine as an aphrodisiac,

diuretic, lacagog, antimicrobial, anti-bacterial, to induce vomiting and destroy kidney stones[4]. Its seed is commonly yellow, but sometimes is reddish yellow or spotted with brown green spots [5]. 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 [6]. Lipid autoxidation is initiated by a chain of lipophilic radicals. *In vivo* hydrogen peroxide (H2O2) is generated by several oxidase enzymes. H2O2 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 [7]. Rocket leaf oil that extracted by steam distillation has significant antifungal effect assessed by 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%) [14]. Powdered seeds of *E. sativa* demonstrate antifungal effect. Crude aqueous seeds exhibited strong powerful antifungal effect against the fungus *Spadicoides stoveri* and *Paecilomyces variotii* while insignificant inhibition against other fungal strains [8]. Secondary metabolites produced by medicinal plants have ability to act as bacteriostatic and bactericidal against "multidrug resistance" microorganisms and regarded a good precursor for synthesis new antibiotics and drugs for controlling infectious diseases. However, this study was aimed to examine the ability of antibacterial of secondary

metabolites of *Eruca sativa* L. leaves against some bacterial species isolated from clinical samples.

MATERIALS&METHODS

Plant material: Leaves of (*Eruca sativa* L), had been purchased from local markets, identified based on the taxonomic features in Iraqi Flora [9]. (Table: 1). Leaves of these plant were cleaned, dried, and kept according to [10], Table: 1.

Table 1: Scientific, Local, English name, Family, and active parts

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

Extraction of the Crude Flavonoid Compounds: Crude Flavonoid compounds were extracted according to [11].

Extraction of the Crude Terpenoid Compounds: Crude terpenoids compounds were extracted according to [12]. Stock solution of 100 mg/ml for Flavonoids, and Terpenoids were prepared in 10% Dimethyl Sulfoxide (DMSO) then sterilized by Millipore filter (0.22µm) and stored at (-20C˚) until use [13].

Antibacterial Activity: Agar well diffusion method was utilized to test the ability of antibacterial of *Eruca sativa* leaves against some bacterial species isolated from clinical samples [14]. Cork porer with size 6mm in diameter 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 clinical samples obtained from Microbiology laboratories in different

hospitals within the boundaries of the municipality of Hillah-Iraq (Table:-1).

Table:-1: Pathogenic Isolates and sources of isolates

N0	Isolates	Source
1	***Escherichia coli***	**Different sources of clinical 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 16.0 program, a completely randomized design was used and least significant difference (L.S.D) was performed at $P \leq 0.05$.

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 clinical samples are presented in (table 2). The antibacterial activity of the crude flavonoid secondary metabolites with three concentrations (25, 50, and 100 mg/ml) was screened by agar well diffusion method. The results revealed that, the crude flavonoid compounds extracted from the leaves of *Eruca sativa* showed significant reduction at $P \leq 0.05$ in the growth of *Escherichia coli* and *Staphylococcus aureus*. The antibacterial activity was

applied at (25, 50, and 100) mg/ml. Inhibitory zone of flavonoid ranging from 0± 0 in 25 mg/ml, 12± 1 in 50 mg/ ml, and 15± 1 in 100 mg/ml when applied of *Escherichia coli* and 15±1, 18± 1, and 20± 1 when applied of *Staphylococcus aureus*. Other organisms like *streptococcus pyogenes, Pseudomonas aeruginosa,* and *Klebsiella pneumonieae* revealed completely resistant to the flavonoid compounds, (Figure: 1, 2).

Table:-2: An antibacterial efficacy of Flavonoid compounds extract from *Eruca sativa* L leaves against some bacterial species isolated from clinical 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 significant reduction at P≤ 0.05 in the growth of *Staphylococcus aureus* and *streptococcus pyogenes*. Inhibitory zone of Terpenoid ranging from 0± 0 in 25 mg/ml, 15± 1 in 50 mg/ ml, and 18± 1 in 100 mg/ml when applied of *Staphylococcus aureus* and 0± 0, 12± 1, and 17± 1 when applied of *streptococcus pyogenes*. Other organisms like *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella*

pneumonieae revealed completely resistant to the flavonoid compounds, (Figure: 2).

Table:-3: An antibacterial efficacy of Terpenoid compounds extract from *Eruca sativa* L leaves against some bacterial species isolated from clinical 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**

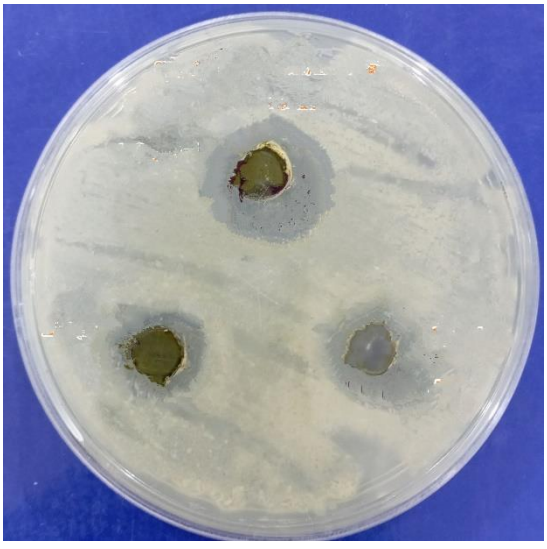

Figure: 1. Antibacterial activity of the crude Flavonoid compounds at (25, 50, 100 mg/ml against ***S. aureus***

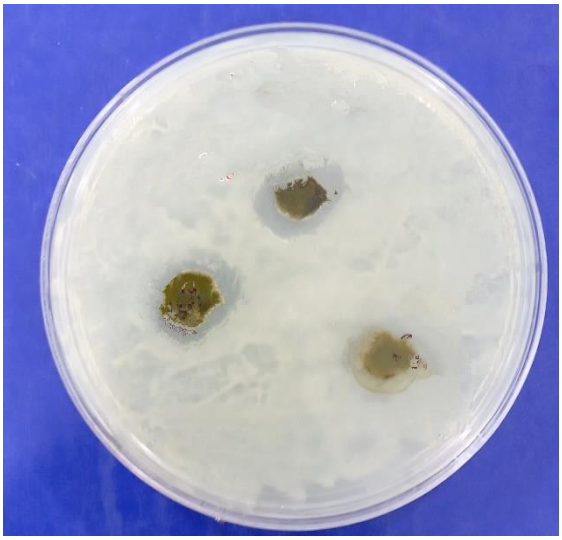

Figure: 1. Antibacterial activity of the crude Flavonoid compounds at (25, 50, 100 mg/ml against ***E. coli***

DISSCUTION

There is no doubt that the effective compounds extracted from medicinal plants remain one of the 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 ability of antibacterials for controlling several pathogenic microorganisms isolated from different clinical samples [15, 16, 17, 18, 19, 20, 21, 22, 23]. [24] Reported that, constituents separated from the unicellular primitive plant like *Chlorella vulgaris* possess ability of antibacterial counter to pathogenic bacteria. [25] Used phytochemical compounds separated from *Hibiscus sabdarifa* for controlling *E. coli* and *Proteus* sp. [26] Used constituents extracted from of *Ficus carica* L. for controlling *E. coli* and *Pseudomonas aeruginosa*. [27] Used phytochemical compounds extracted from *Boswellia carteri* and *Curcuma longa* for controlling *Fusarium* sp. isolated from seeds of corn. [28] Used terpenoids compounds extracted from *Carthamus tinctorius* L. against *Aspergillus* species isolated from stored medicinal plant seeds. Secondary metabolites represented by Alkaloids and Flavonoids compounds separated from *M. Communis* leaves respected a worthy source for controlling pathogenic microorganisms segregated from hemodialysis fluid specimens [29]. [30] 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 [31], and also Secondary metabolite

compounds extracted from the *D. caryophyllus* L. flower buds such as terpenoid and flavonoid have powerful antifungal activity against *Candida* species [32]. 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* [33]. *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 [34]. 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 [35]. Flowers of *E. sativa* showed good growth inhibition zone compared to positive controls when tested against Salmonella *typhimurium* [36]. 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 [37]. 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 [38]. 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 [39]. 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.

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الفعالية التضادية للمركبات الثانوية المستخلصة من اوراق نبات الجرجير ضد بعض الاحياء المجهرية المعزولية من عينات سريرية

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الخلاصة:

اجريت الدراسة الحالية في مدينة الحلة مركز محافظة بابل. وكان هدف الدراسة هو معرفة مدى تاثير المركبات الثانوية مثل الفلافونويدات والتربينات المستخلصة من اوراق نبات الجرجير ضد بعض انواع الاحياء المجهرية المرضية المعزولة من عينات سريرية متمثلة بـــ:

Escherichia coli, Staphylococcus aureus, streptococcus pyogenes, Pseudomonas aeruginosa, and Klebsiella pneumonieae

تم تقييم الفعالية التضادية ضد الاحياء المجهرية بطريقة الانتشار في الحفر داخل الاكار وبتحضير ثلاثة تراكيز مختلفة هي (25و 50 و 100) وقد استخدم الماء المقطر المعقم كمعاملة سيطرة سالبة لغرض المقارنة. اضهر مستخلص الفلافونويدات بالتراكيز (50 و 100) تفوقا على معاملة السيطرة السالبة المتمثلة بالماء المقطر عند تطبيقها على تحت مستوى احتمال 0.05 . كما واضهرت حساسية تجاة كل التراكيز المستخدمة قيد الدراسة للمركبات الفلافونويدة والتربينة. كما واضهرت كل من مقاومة تجاة كافة التراكيز المستخدمة قيد الدراسة. وفي الختام فأن المركبات الفعالة المستخلة من نبات الجرجير تعد مصدرا جيدا للسيطرة على الاحياء المجهرية قيد الدراسة.