

# Phytochemical Investigation and Antibacterial Assessment of Iraqi *Tropaeolum majus* L. Extracts (Ethyl Acetate Fraction)

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## ABSTRACT

*Tropaeolum majus* has a long history of use as a medicinal plant with a wide range of therapeutic applications. The purpose of this study was to evaluate the antibacterial effect of ethyl acetate fraction of *Tropaeolum majus* plant against four genera of bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) obtained from nawah scientific center. The antibacterial activity of this plant (0.0003, 0.0007, 0.0015, 0.003, 0.006, 0.012, 0.025, 0.05, 0.1 and 0.2 mg/ml) were evaluated using broth macro dilution method (methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically) and the Minimum Inhibitory Concentration (MIC) values of tested ethyl acetate extract were found from 0.025 to 0.05 mg/ml. Preliminary phytochemical investigation of *Tropaeolum majus* leaves extract revealed the presence of different chemical constituents like alkaloids, glycosides, tannins, terpenoids, phenols and flavonoids, while for ethyl acetate fraction indicated the presence of glycosides, tannins, phenols and flavonoids. Analysis of ethyl acetate fraction by High Performance Liquid Chromatography (HPLC) showed the presence of four phenolic compounds in the ethyl acetate fraction using (gallic acid, p-coumaric, chlorogenic acid and rutin) as standards. In conclusion, the presence and diversity of secondary metabolites may be responsible for the observed antibacterial activity.

**Key words:** Phytochemicals, Antibacterial activity, *Tropaeolum majus*, Alkaloids, Glycosides

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## INTRODUCTION

For thousands of years, humans have known about the benefits of using plants to treat or alleviate illness. Novel chemical compounds can be found in the plant kingdom which may be significant due to their potential use in medicine (e.g. food and forage conservation) [1].

Plant extracts were regarded as important for the treatment of different ailments by ancient civilizations and natural products account for approximately 30% of global drug sales. It is estimated that there are approximately 2500000 species of higher plants in the world and the majority of them have not been thoroughly investigated for their pharmacological activities. However, there has been a growing interest in plant uses and the identification of plant constituents with antibacterial activity for several decades. The most important reason for the latter was that infections are one of the leading causes of illness and mortality worldwide, particularly

infections caused by *Enterococcus* and *Staphylococcus* species, which are agents of many intra hospital infections [2]. Furthermore, the emergence of drug resistance and the emergence of undesirable side effects of various antibiotics has encouraged the quest for new antimicrobial agents, especially among plant extracts, to identify new chemical structures to avoid the a fore mentioned disadvantages [3,4].

Despite the fact that pharmaceutical industries have developed several new antibiotics over the last three decades, microorganism resistance to these drugs has increased. Bacteria, in general, have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents. This is cause for concern, given the number of patients in hospitals who have suppressed immunity, as well as new multi resistant bacterial strains. As a result, new infections can occur in hospitals, resulting in a high mortality rate [5,6].

The problem of microbial resistance is worsening and the future use of antimicrobial drugs remains uncertain. As a result, steps must be taken to address this issue, such as limiting antibiotic use, conducting research to better understand the genetic mechanisms of resistance and continuing studies to develop new drugs, both synthetic

and natural. The ultimate goal is to provide patients with appropriate and efficient antimicrobial drugs.

*Tropaeolum majus* is a member of the tropaeolaceae family and is commonly known as garden nasturtium, Indian cress, or monk's cress. It's a cultigen that originated in the South American Andes, where it has been grown as a crop plant since ancient times [7]. It had been brought to Europe in 1684 from Peru by a Dutch monk, Pater Beverning [8]. It is cultivated currently in most countries of the world, including Iraq, as an ornamental plant. It's widely cultivated as a decorative as well as a therapeutic plant [9]. It is a bushy (about 30 cm tall) or vining (may stretch out up to 90 cm) plant that grows rapidly. It has tender, rounded, watercress flavored leaves (5 to 15 cm across), held by long fleshy stalks and showy trumpet shaped yellow or orange flowers with reddish patches. The plant as a whole has a spicy peppery flavor. Fresh leaves, flowers and stems are used in salads and green pods can be pickled and used as a caper alternative [10-12].

*Tropaeolum majus* is prevalently utilized as an antiseptic, diuretic, laxative, hair tonic, antiscorbutic, anti-inflammatory, antihypertensive and antidepressant. Likewise, it is applied in the cleaning of skin, eyes and the treatment of skin disorders, furunculosis, acne, pulmonary disorders, amyotrophic lateral sclerosis, psoriasis, eczema and scrofula [13,14].

The few studies that are available indicate the existence of certain chemical components in specific parts of the plant, but no complete characterization has been done. These chemical constituents can be assigned to various classes of polyphenols, flavonoids, alkaloids, saponins, tannins, anthocyanins, carotenoids and terpenoids [15,16].

The purpose of our study was to determine the antibacterial activity of *Tropaeolum majus* extracts (ethyl acetate fraction). The antibacterial activity from *Tropaeolum majus* was tested against four genera of bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*).

## MATERIALS AND METHODS

### Plant material collection

The leaves of *Tropaeolum majus* of the family (tropaeolaceae) were collected from Babil in January and February 2021. The plant was identified and authenticated by Prof Dr. Sukaena Abass/Department of Biology/College of Sciences/University of Baghdad. The leaves were thoroughly washed, dried in the shade and ground to a fine powder in a mechanical grinder.

### Method of work

The leaves' air dried powder is weighed, then defatted with n-hexane to remove chlorophyll and waxy material, then extracted in soxhlet with 80% ethanol for 18 hours, then mixed and dried using a rotary evaporator, the dry extract is weighted and the yield of extraction is

calculated. The dry extract is dissolved in water and treated with various reagents to screen for the type of phytochemicals present and then partitioned 2-3 times with various solvents of different polarities such as petroleum ether, chloroform, ethyl acetate and n-butanol then each fraction is dried and weighted.

### Preliminary phytochemical examination of plant extract

Phytochemical analysis for the detection and identification of bioactive chemical components in the medicinal plants studied was carried out using the standard methods described by Harborne [17].

The preliminary phytochemical screening tests is also helpful within the detection of the bioactive principles and after might result in the drug discovery and development. In addition, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

In this study we used ethyl acetate fraction to evaluate the antibacterial activity of *Tropaeolum majus*, since contains several active constituents like flavonoids, phenols, tannins and other bioactive compounds.

### HPLC analysis

HPLC was utilized to assess the flavonoids and phenolic acids contained in the ethyl acetate fraction of *Tropaeolum majus*. The phenol standards used included (gallic acid, p-coumaric acid, chlorogenic acid and rutin). The standards were dissolved in a mobile phase at concentrations of 1 mg/mL and then filtered through a 0.22 µm syringe filter then 10 µl were injected into the HPLC column. The measurements were done using a Waters 2690 Alliance HPLC system equipped with a Waters 996 photodiode array detector. The column type used was column C18 Inertsil: 4.6 x 250 mm, 5 µm and the mobile phase consist of buffer (0.1% acetic acid in water) and methanol. The phenols were identified by comparing retention times of standards and calculating absorbance ratios following co-injection of samples and standards.

### Test organisms

The test organisms used in this study were obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt), which include (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 25668 and *Staphylococcus aureus* ATCC 6538).

### Preparing inoculum (colony suspension method)

A disc of *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 25668 and *Staphylococcus aureus* ATCC 6538, were inoculated separately into 100 ml of tryptic Soy broth medium and incubated at 37.0°C ± 1.0°C for 24.0 ± 2.0 hr. For the preparation of fresh (18-24 h) culture agar plate, a loopful from the broth was streaked onto tryptic soy agar medium, incubated at 37.0°C ± 1.0°C for 21.0 ± 3.0

hr. Inoculating 3-4 colonies (from each organism plate) yielded a straight sterile saline solution, which was then adjusted to reach turbidity corresponding to a 0.5 McFarland standard. A 0.5 McFarland standard and the DENSICHEK optical instrument were used to standardize inoculum density. That adjustment results in a suspension containing approximately  $1.0 \times 10^8$  CFU/mL for each test organism. The suspension was diluted to  $1.0 \times 10^7$  CFU/mL by taking 1.0 ml and diluting it in 9.0 ml broth. Then, 5.0 ml was added to 5.0 ml sterile broth to yield approximately  $5.0 \times 10^6$  CFU/ml [18].

### Broth macro dilution method

First, 5.0 ml of each sample was added to 5.0 ml tube broth (1:2 dilution), mixed well and then 5.0 ml of the 1:2 dilution was aspirated using a fresh tip and added to the next 5.0 mL broth (1:4). This procedure was repeated for each antibacterial sample to produce at least (9) dilutions. In the first well of the 24 well plates, 1.0 mL of the sample was directly inoculated and 1.0 mL of each dilution was inoculated in the subsequent wells. Each well received 100  $\mu$ l of prepared inoculum, resulting in a final concentration of  $5.0 \times 10^5$  CFU/mL (the optimum concentration is  $2-8 \times 10^5$  CFU/mL). To validate inoculum density, another 100  $\mu$ l of each organism solution was diluted and cultivated (externally). Each organism/sample plate received a growth control well containing inoculation broth but no sample. To each

organism/sample plate, a negative control well containing only broth and no bacteria was introduced. All plates were incubated for  $24.0 \pm 2.0$  hours at  $37.0^\circ\text{C} \pm 1.0^\circ\text{C}$ . Plates were taken from the incubator and put on a dark surface to monitor growth after incubation [18].

### Determination of MIC values

The broth macro dilution technique was used to determine the Minimum Inhibitory Concentrations (MIC) of plant crude extracts [19]. The sample concentration range was prepared by diluting the stock solutions twice in sterile broth. Ten dilutions of the samples were examined, ranging from 0.0003 to 0.2 mg/ml.

## RESULTS

### Phytochemical screening

Preliminary phytochemical examination of crude leaves extracts of *Tropaeolum majus* revealed the presence of flavonoids, phenols, tannins, alkaloids and terpenoids, while the preliminary phytochemical examination of ethyl acetate fraction revealed the presence of flavonoids, phenols and tannins. The results of these tests are summarized in Tables 1 and 2.

**Table 1: Phytochemical screening results of *Tropaeolum majus* crude extract.**

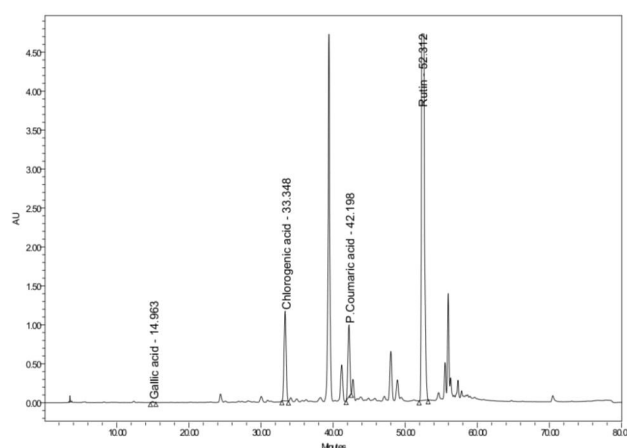
Crude extract	Alkaloids	Flavonoids	Glycosides	Phenols	Tannins	Terpenoids
	+	+	+	+	+	+

**Table 2: Phytochemical screening results of ethyl acetate fraction.**

Ethyl acetate fraction	Alkaloids	Flavonoids	Glycosides	Phenols	Tannins	Terpenoids
	-	+	+	+	+	-

### HPLC analysis

Four phenolic compounds (gallic acid, p-coumaric, chlorogenic acid and rutin) were detected in the ethyl acetate fraction of *Tropaeolum majus* leaves extract by comparison of their retention time with the retention time of the standards as shown in Figure 1.



**Figure 1: HPLC chromatogram of the *Tropaeolum majus* extract (ethyl acetate fraction).**

**Antibacterial activity**

The results obtained for *Tropaeolum majus* leaves extract exhibits a valuable antibacterial activity against the tested microorganisms at a concentration from 0.025 to 0.05 mg/mL as shown in Table 3 and MIC values of the

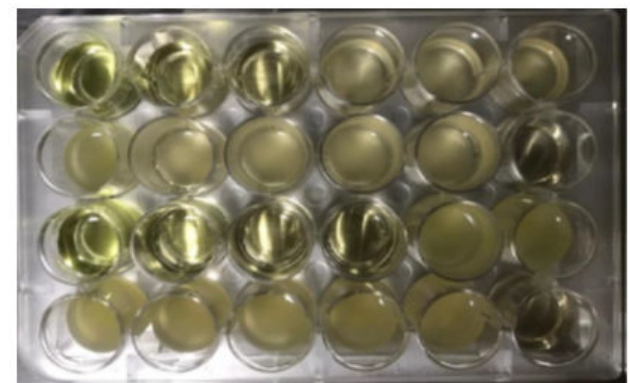
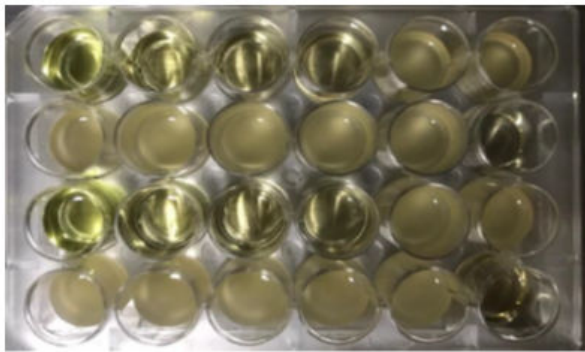
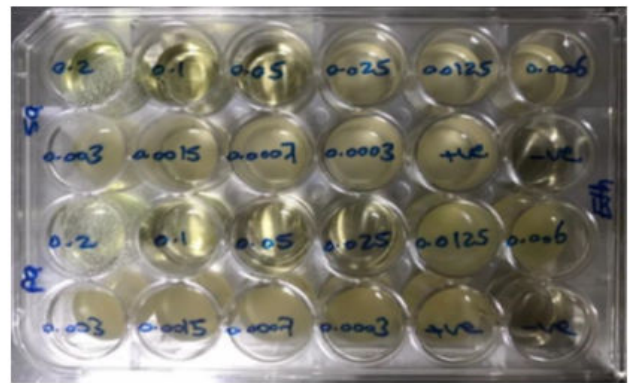
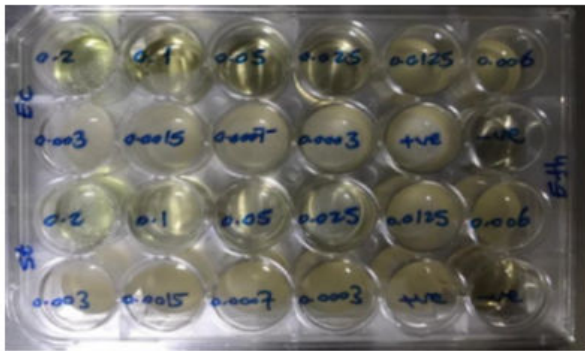
plant extract against these bacterial species are presented in Table 4 and shown in Figures 2 and 3.

**Table 3: Minimal Inhibitory Concentrations (MIC) reading results**

Dilution (mg/mL)	0.2	0.1	0.05	0.025	0.012	0.006	0.003	0.0015	0.0007	0.0003
<i>E. coli</i> ATCC 25922	C	C	C	C	T	T	T	T	T	T
<i>S. typhimurium</i> ATCC 14028	C	C	C	C	T	T	T	T	T	T
<i>P. aeruginosa</i> ATCC 25668	C	C	C	C	T	T	T	T	T	T
<i>S. aureus</i> ATCC 6538	C	C	C	T	T	T	T	T	T	T

**Table 4: Minimal Inhibitory Concentrations (MICs) of *Tropaeolum majus* extract determined by the agar macro dilution method**

Test organism	MIC value
<i>Escherichia coli</i> ATCC 25922	0.025 mg/ml
<i>Salmonella typhimurium</i> ATCC 14028	0.025 mg/ml
<i>Pseudomonas aeruginosa</i> ATCC 25668	0.025 mg/ml
<i>Staphylococcus aureus</i> ATCC 6538	0.05 mg/ml



**Figure 2: The agar macro dilution test of *Tropaeolum majus* extract against *E. coli* and *S. typhimurium*.**

**Figure 3: The agar macro dilution test of *Tropaeolum majus* extract against *S. aureus* and *P. aeruginosa*.**

## DISCUSSION

Natural plant substances may successfully serve as an alternative to synthetic compounds commonly used in medicine. The plant assayed in this study is commonly used as medicinal plants in different parts of the world. The previous studies showed that there is no or weak antibacterial activity of *Tropaeolum majus* due to the low amount of phenols in the plant extract as a result of the type of methods used for plant extraction (decoctions and sonication), but our study showed that the Iraqi *Tropaeolum majus* leaves extract (extracted in soxhlet) possesses good antibacterial activity against selected pathogens due to the presence of the phenolic acids and flavonoid mentioned above. Many studies showed that phenolic compounds that have been identified in *Tropaeolum majus* leave extract (gallic acid, p-coumaric, chlorogenic acid and rutin) possess good antibacterial activity against different types of bacterial strains (gram positive and gram negative bacteria) [20-23]. The current study's findings are promising and may improve the use of *Tropaeolum majus* leaves extract in the treatment of various bacterial infections especially (*Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*).

## CONCLUSIONS

In this study, we evaluated the antibacterial activity of the *Tropaeolum majus* extract (ethyl acetate fraction). And it displayed a good antibacterial activity with MIC<100 mg/mL due to the presence of phenolic compounds in ethyl acetate fraction at a much higher concentration than other fractions, indicating that these plants could be a good source for the antibacterial agents. More research is needed to investigate the additional antimicrobial effectiveness aspects of these powerful plant extracts (e.g. *in vivo* efficacy, toxicity and anti-mycobacterial, antiviral and anti-parasitic activity).

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