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Antibacterial Evaluation of *Volkameria inermis* Leaves Cultivated in Iraq

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ABSTRACT

Background: *Volkameria inermis* is a medicinal plant traditionally used for its antimicrobial, anti-inflammatory, and antioxidant properties. Owing to the increasing resistance of pathogenic bacteria to conventional antibiotics, there is a growing interest in exploring plant-derived compounds as alternative therapeutic agents. Ethanol extracts of medicinal plants, in particular, are known to contain a wide range of bioactive phytochemicals that may exhibit strong antibacterial effects**.**

Objectives: The objective of this study was to assess the effectiveness of an ethanolic extract of *Volkameria inermis* leaves by using an ethanolic solvent that was eighty percent strength.

Methods: Samples of leaves were gathered from the center area of Iraq (Al-Musayab), and washed, sorted, and dried in a shaded environment. Through the use of 800 milliliters of 80% ethanol, an extract of *Volkameria inermis* leaves was created and dried using a rotary evaporator, it was stored in petri dishes that had been sterilized, and one milligram of the extract was dissolved in one milliliter of dimethyl sulfoxide (DMSO). To conduct the antibacterial test using the agar well diffusion technique, the solution was serially diluted to a concentration of 1 mg/ml. The efficacy of an ethanol extract against bacteria as assessed by well diffusion method.

Results: When tested against both (*Staphylococcus aureus*) and (*Salmonella typhi*) bacteria, the ethanolic extract of *Volkameria inermis* demonstrated a notable antibacterial effect at a concentration of 500 µg/ml, which resulted in more inhibition zones than the antibiotic ciprofloxacin.

Conclusion: The outcomes of this study demonstrated that the *Volkameria inermis* plant outperformed the drug ciprofloxacin in its antibacterial activity against *Staphylococcus aureus* and *Salmonella typhi.*

Keywords: Volkameria inermis, Gram-positive, Gram-negative, Inhibition zone, Antibiotic Ciprofloxacin.

INTRODUCTION

Since ancient times, people have turned to natural remedies for a wide range of health issues. A wide variety of bioactive secondary metabolites may be found in both marine and terrestrial environments via the use of natural product chemistry methods. As a result, many diseases' potential treatments have their roots in natural ingredients [(1)]. Nearly all pharmacopoeias now contain medicinal plants for either self-medication or prescription use. You may use them on their own or combine them with other therapies [(2)].

As a member of the Lamiaceae family, *Volkameria inermis* is highly prized for its culinary and medicinal uses [3]. Glory bower or Garden quinine is the name of this plant, which grows wild in the central area of Iraq and has traditional medicinal and culinary uses [4, 5].

Plants of this species are most often found in the tropics and subtropics. Shrub *Volkameria inermis* is native to the islands of the Pacific, Australia, and southern and eastern Asia [6].

The straggly shrub *Volkameria inermis* has many branches. It might grow to be 2–9 meters in length. At times, it could become scandent. It releases an odor similar to that of a fetus when crushed. A bright brown bark coats the twiggy branches. Elliptic or obovate leaves are glabrous, whole, and have an obtuse or emarginated tip. Glabrous, opposite, elliptic or obovate, whole, obtuse or emarginated at apex, and measuring up to 5*3.8 cm, they are seldom alternating. A petiol's length may reach one centimeter. The blossoms are pedunculate cymes with three petals, a white corolla with a long, narrow tube, and a minutely serrated calyx. Lobes that are sub-equal, oblong, and obtuse. "Fruits" are oblong drupes [7].

Traditional medicine practitioners have relied on this plant—often in conjunction with other plant leaves—to treat a wide range of ailments, including fever, cough, skin rashes, and boils, for generations. Umbilical cord infections and uterine cleansing are two further local medical uses for them [8]. Some of the many pharmacological effects of this chemical include antimicrobial, anti-cancer, anti-oxidant, anti-diabetic, anti-malarious, analgesic, and anti-inflammatory actions [9]. The taxonomic classification of this plant has been reported in Table 1 [10].

Table 1: Taxonomic classification of *Volkameria inermis* [10]

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	*Clerodendrum L.*
Species	*Clerodendrum inerme*

MATERIALS AND METHODS

Sample collection

We washed and dried the leaf samples in shade for a few days after collecting them from the Al-Musayyab area in the center of Iraq.

Extraction method

After drying 100 grams of *Volkameria inermis* leaves powder (800 ml), the fat was extracted using hexane solvent. The defatted plant material was extracted using an additional 800 cc of 80% ethanol using the Soxhlet

device. After the plant material was defatted, the resulting 80% ethanol extract was dried using a rotary evaporator. Then, it was stored in sterile petri dishes in the refrigerator, covered with aluminum foil and Para film. The following was the procedure for preparing the ethanol concentration stock solutions: 1 milligram of extract in 1 milliliter of dimethyl sulfoxide (DMSO). The following concentrations were used for the 1 mg/ml serial dilutions: The agar well diffusion technique was used to create concentrations of 500 µg/ml, 250 µg/ml, and 125 µg/ml for an antibacterial experiment [(11)].

Preparation of laboratory media

Mueller-Hinton was produced per the factory-provided directions and is the preferred ready culture medium. The ready culture medium was autoclaved for 15 minutes at 121 degrees Celsius and 1 bar after preparation.

Sources of pathogenic bacterial isolates

The five gastrointestinal pathogenic bacterial isolates that were detected are included in Table 2. These isolates were obtained from the bacteriology laboratory at the College of Science - Al-Nahrain University. These isolates were obtained from patients who were infected at local hospitals in Baghdad, Iraq.

Table 2:Sources of pathogenic bacterial isolates

No.	Bacterial name	Bacterial type	Specimen
1	*Salmonella typhi*	Gram negative bacteria	Stool
2	*Escherichia coli*	Gram negative bacteria	Stool
3	*Staphylococcus aureus*	Gram Positive bacteria	Stool
4	*Pseudomonas aeruginosa*	Gram negative bacteria	Stool

Antibiotic sensitivity test:

Antibiotic sensitivity testing is performed with the purpose of determining whether or not a certain medicine is resistant to a particular disease and ensuring that the infection is susceptible to the drug of choice. The well diffusion technique was used in order to ascertain the antibiotic susceptibility of the organisms that were under investigation [(12)].

Ciprofloxacin (0.05) mg/ml was the antibiotic of choice to be used for antibacterial sensitivity for the reason that it is effective against both Gram-negative and Gram-positive bacteria and has a broad range of activity [(13)].

Antibacterial Activity of ethanolic extract by well diffusion method against different types of pathogenic bacteria:

The ethanolic extract's inhibitory efficacy was assessed against five pathogenic bacteria on nutrient agar using the well diffusion technique. After that, five wells were created in each agar plate of the studied bacteria, and (100 µl) of dilutions of the extracts (1 mg/ml, 500, 250, 125 µg/mL) were poured into the

wells. Wells (5 mm) in diameter were drilled for each plate containing the three extract dilutions, and DMSO solvent was used as a negative control, while Ciprofloxacin (0.05 mg/ml antibiotic was used as a positive control [14].

Overnight (24 hours), the plates were placed in an incubator set at 37 degrees Celsius. We assessed the breadth of the inhibitory zone to assess the antibacterial sensitivity. The antibacterial activity of the ethanol extracts of *Volkameria inermis* leaves was tested using the well diffusion technique. One gram-positive bacterium, *Staphylococcus aureus*, and four gram-negative bacterium, *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*, were used to assess the antimicrobial efficacy of the extracts [14]. Our antibiotic sensitivity test used Ciprofloxacin at a concentration of 0.05 mg/ml, with DMSO serving as the negative control [15, 16]

RESULTS

The concentrations of 500, 250, and 125 µg/mL were used to study the ethanolic extracts [17, 18] as shown in Table 3. The Results of Antibacterial activity of *Volkameria inermis* ethanolic extracts against gram positive and gram-negative bacteria is shown below in table 3 and Figure 1.

Table 3: Antibacterial activity of Volkameria inermis ethanolic extract with different bacterial species measured in millimeter by well diffusion method. L: highest concentration (500 µg/ml), M: medium concentration (250 µg/ml), S: lowest concentration (125 µg/ml).

BACTERIAL ISOLATES	CONCENTRATIONS OF ETHANOL EXTRACTS (MG/ML)/INHIBITION ZONE IN MILLIMETERS				
	500 (L)	**250 (M)**	**125 (S)**	**+VE Control**	**-VE CONTROL**
STAPHYLOCOCCUS AUREUS	23 mm	20 mm	17 mm	20 mm	ZERO
SALMONELLA TYPHI	30 mm	19 mm	11 mm	25 mm	ZERO
PSEUDOMONAS AERUGINOSA	15 mm	13 mm	11 mm	34 mm	ZERO
ESCHERICHIA COLI	12 mm	11 mm	8 mm	32 mm	ZERO

INTERNATIONAL SENSITIVITY CHART (CIPROFLOXACIN)		
SENSITIVE (S)	**INTERMEDIATE (I)**	**RESISTANT (R)**
≥ 21 mm	16-20 mm	≤ 15 mm

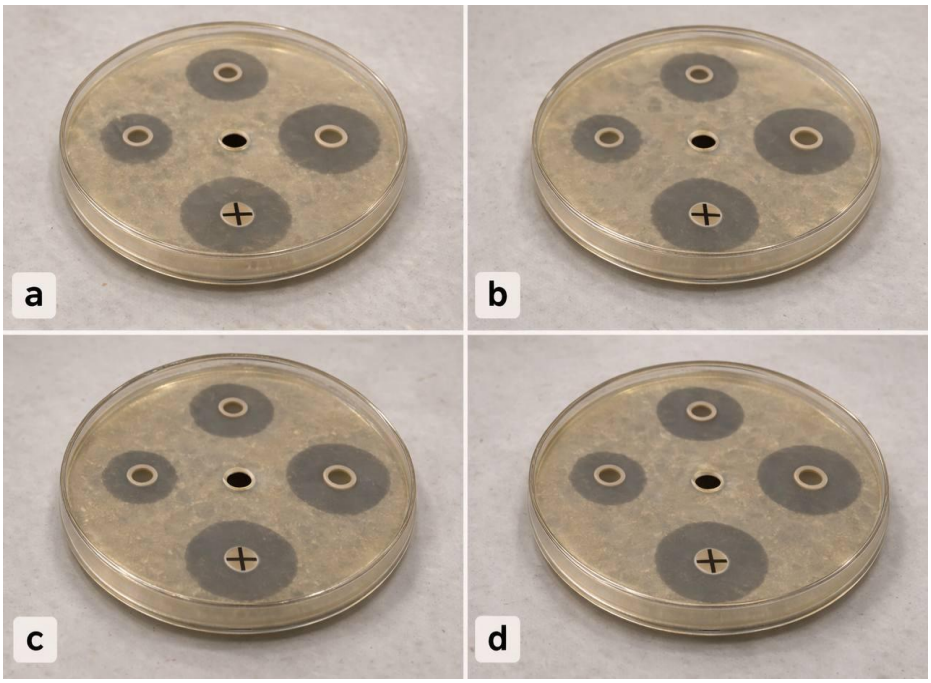

Figure 1: Results of Antibacterial activity of *Volkameria inermis* ethanolic extracts against gram positive and gram-negative bacteria; (a) Staphylococcus aureus, (b) Salmonella typhi, (c) Escherichia coli and (d) Pseudomonas aeruginosa.

COMPARISON AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF THE IRAQI PLANT EXTRACT VS INDIAN PLANT EXTRACT BY WELL DIFFUSION METHOD AGAINST DIFFERENT TYPES OF PATHOGENIC BACTERIA:

We compared the antibacterial properties of the Iraqi plant to those of an Indian species. The inhibitory zones against *Staphylococcus aureus* were 3 mm and 4 mm, respectively, when the Indian plant leaves were extracted with methanol and ethyl acetate at a concentration of 500 µg/ml [(19)].

whereas the Iraqi plant showed a suppression of (23 mm) using the same 500 µg/ml extract concentration. When tested against gram-negative bacteria, the Indian methanol extract inhibited growth at concentrations of 5 mm, 3 mm, and 0 mm, respectively. In contrast, the ethyl acetate extract had no effect on growth at concentrations of 5 mm and 1 mm, respectively, against *Pseudomonas aeruginosa* and *Salmonella typhi* [(20)]. whereas, at the same dosage, the Iraqi ethanol extract inhibited the growth of *Escherichia coli* (12 mm), *Pseudomonas aeruginosa* (15 mm), and *Salmonella typhi* (30 mm).

Discussion and conclusion:

According to the findings, the antibacterial activity of *Volkameria inermis* ethanolic leaves extract against gram-positive bacteria (*Staphylococcus aureus*) demonstrated a noteworthy activity associated with its higher concentration (500 µg/ml). This concentration resulted in a greater inhibition zone compared to the antibiotic that was utilized, leading to the conclusion that it was the most effective concentration. On the other hand, its other two concentrations (250 µg/ml and 125 µg/ml) were reported as having intermediate activity, as indicated by the international antibiotic sensitivity chart.

Ciprofloxacin, a medication, demonstrated a level of action that was intermediate against this particular strain of bacteria. It was seen that the extract exhibited a higher level of activity against gram-negative bacteria (*Salmonella typhi*) when it was at the same concentration. Furthermore, it was established that the extract was more sensitive in comparison to Ciprofloxacin. Furthermore, the extract with a lower concentration of 250 µg/ml had an intermediate level of activity. It was observed that the positive control, Ciprofloxacin, had a greater inhibitory zone compared to the other three species of gram-negative pathogenic bacteria, namely *Pseudomonas aeruginosa*., and *Escherichia coli*, which exhibited intermediate and resistant activity, respectively.

Staphylococcus aureus was the only pathogenic bacterium that exhibited poor action against the ethanolic extract of the lowest concentration (125 µg/ml). The other four pathogenic bacteria in question showed poor performance.

In addition, it was shown that the antibacterial activity of the extract of *Volkameria inermis* leaves from Iraqi soil may be just as efficient as the antibacterial activity of plant leaves from other soils, which has been the subject of several studies about its activity [18]. In addition, the findings of the antibacterial activity of the ethanolic extracts of *Volkameria inermis* against gram-positive bacteria as well as certain gram-negative bacteria are shown in table (3).

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CONFLICTS OF INTEREST

The only one author declare no conflict of interest.

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ETHICS STATEMENTS

The study doesn’t need ethical approval from an ethics committee.

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التقييم المضاد للبكتيريا لأوراق نبات فولكاميريا إنيرميس المزروع في العراق

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الخلاصة

الخلفية: يُعدّ نبات كليرودندروم إنيرمي (Volkameria inermis.) نباتًا طبيًا يُستخدم تقليديًا لخصائصه المضادة للميكروبات والالتهابات والأكسدة. ونظرًا لتزايد مقاومة البكتيريا الممرضة للمضادات الحيوية التقليدية، يتزايد الاهتمام باستكشاف المركبات النباتية كبدائل علاجية. ومن المعروف أن مستخلصات الإيثانول من النباتات الطبية، على وجه الخصوص، تحتوي على مجموعة واسعة من المركبات الكيميائية النباتية النشطة بيولوجيًا والتي قد تُظهر تأثيرات قوية مضادة للبكتيريا. الأهداف: هدفت هذه الدراسة إلى تقييم فعالية مستخلص إيثانولي لأوراق نبات كليرودندروم إنيرمي (Volkameria inermis.) باستخدام مذيب إيثانولي بتركيز 80%. الطرق: جُمعت عينات من الأوراق من منطقة المسيب في وسط العراق، وغُسلت وفُرزت وجُففت في بيئة مظللة. تم تحضير مستخلص أوراق نبات كليرودندروم إنيرمي باستخدام 800 مل من الإيثانول بتركيز 80%، ثم جُفف باستخدام المبخر الدوراني. حُفظ المستخلص في أطباق بتري معقمة، وأُذيب 1 مليغرام منه في 1 مل من ثنائي ميثيل سلفوكسيد (DMSO). ولإجراء اختبار مضاد للبكتيريا باستخدام تقنية الانتشار في الآبار على وسط الآجار، خُفف المحلول بشكل متسلسل إلى تركيز 1 مليغرام/مل. قُيّمت فعالية مستخلص الإيثانول ضد البكتيريا باستخدام طريقة الانتشار في الآبار. النتائج: عند اختباره ضد بكتيريا المكورات العنقودية الذهبية (Staphylococcus aureus) والسالمونيلا التيفية (Salmonella typhi)، أظهر مستخلص الإيثانول من نبات كليرودندروم إنيرمي فعالية ملحوظة كمضاد للبكتيريا عند تركيز 500 ميكروغرام/مل، مما أدى إلى تكوين مناطق تثبيط أكثر من المضاد الحيوي سيبروفلوكساسين. الخلاصة: أظهرت نتائج هذه الدراسة أن نبات Volkameria inermis تفوق على دواء سيبروفلوكساسين في نشاطه المضاد للبكتيريا ضد المكورات العنقودية الذهبية والسالمونيلا التيفية.

الكلمات المفتاحية: فولكاميريا إنيرميس، موجبة جرام، سالبة جرام، منطقة التثبيط، مضاد حيوي سيبروفلوكساسين.