

## Bio-effect of aqueous extract of *Cyclamen rohlfsianum* on some pathogenic bacteria

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### Abstract:

*Cyclamen rohlfsianum* is a species endemic to Libya, antimicrobial biological activities have not been studied, Therefore, this study was conducted to test a biological effect of an aqueous extract of leaves and tubers of *Cyclamen rohlfsianum* at concentrations (25, 50 and 100) mg/ml against some types of pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) to a sensitivity test by a disk diffusion method. The results showed *Cyclamen rohlfsianum* extracts have good inhibitory activity against all tested bacteria except for *E.coli*, a concentration of 100 mg/ml was the most effective to control bacteria, the results showed that tuber extracts are more efficient than leaf extracts, *E.coli* was the most resistant to aqueous extracts and Gentamicin.

**Key words:** *Cyclamen rohlfsianum*, biological effect, pathogenic bacteria.

التأثير الحيوي للمستخلص المائي لنبات بخور مريم *Cyclamen rohlfsianum* على بعض أنواع البكتيريا الممرضة

الملخص :

يعتبر نبات بخور مريم *Cyclamen rohlfsianum* من الأنواع المستوطنة في ليبيا، والذي لم تتم دراسة أنشطته البيولوجية المضادة للميكروبات، لذلك أجريت هذه الدراسة لاختبار التأثير الحيوي للمستخلص المائي لأوراق ودرنات بخور مريم بتركيز (25، 50، 100) ملجم / مل ضد بعض أنواع البكتيريا الممرضة (*Escherichia coli* و *Staphylococcus aureus*، *Proteus vulgaris*، و *Pseudomonas aeruginosa*) واختبار حساسيتها بطريقة الأقراص. أظهرت النتائج أن مستخلصات بخور مريم *Cyclamen rohlfsianum* لها فعالية تثبيطية جيدة ضد جميع أنواع البكتيريا المختبرة باستثناء بكتيريا *E.coli*، وكان التركيز 100 ملجم / مل هو الأكثر فعالية في السيطرة على البكتيريا، وأظهرت النتائج أيضا أن مستخلصات الدرنات أكثر كفاءة من مستخلصات الأوراق، وكانت *E.coli* هي أكثر أنواع البكتيريا مقاومة للمستخلصات المائية والمضاد الحيوي الجنتاميسين.

الكلمات المفتاحية: بخور مريم، التأثير الحيوي، البكتيريا الممرضة.

## 1. Introduction:

The Middle East region is suffering from phenomenon misuse of antibiotics, and the emergence of microbial strains are resistant [5, 10]. Which prompted a search for natural therapeutic alternatives represented in medicinal plants, which is a synopsis of traditional experiences over years, It's characterized by containing active substances can overcome microbes, free from side effects [2].

*Cyclamen rohlfsianum* is an endemic strain, only grows in Libya, known locally as Al-Rakhf [9], a perennial tuberous herbaceous plant, belonging to the family Primulaceae [3], It's classified as a poisonous plant because it contains Cyclamen glycoside [6], it's traditionally used in the therapy of diabetes, It's tubers are used in the process of fermenting milk to produce cheese [8].

*Cyclamen.sp* is among the medicinal plants are famous for their effective ability, despite their different types, locations, the type of part used against many microbial pathogens, The results of a study conducted in Jordan confirmed the effectiveness of the alcoholic and acetone extracts of *C.persicum* tubers in inhibiting positive and negative bacteria, and all species of *Candida* [4]. The results of a study in Iran demonstrated the possibility of using a mix of *C.coum* tuber extract and ciprofloxacin to prevent the formation of biofilm membranes of *Pseudomonas aeruginosa* [1]. In Serbia, the results of a study [14] indicated that *C.purpurascens* tuber extract is a potential source of anti-*Staphylococcus aureus*. In Turkey, ethanolic extracts of *C.mirabile* showed strong activity against *Staphylococcus aureus* causing bovine mastitis [13]. Therefore, the study aimed to evaluate the efficacy of aqueous extracts of *Cyclamen rohlfsianum* bio-control against some pathogenic bacteria, which had never hitherto been established.

## 2. Materials and Methods:

The study was carried out in Biology Department / Faculty of education / Omar Al-Mukhtar University. Plant washing (leaves- Tubers) with distilled water and dried inside the laboratory under room temperature, grinded by an electric grinder and saved for use.

## 2.1. Preparation aqueous extraction:

10 g of powder dry of the plant was added to 100 ml of sterile distilled water in a glass flask, put on a vibratory shaker for 24 hours at 35 ° C, then filter and shaken in a centrifuge at 3000 rpm for 10 minutes. The next step, was filtered with Whitman No.1 filter paper, and drying in Rotary evaporator to get dry powder [11].

The concentration of 100 mg/ml was prepared by dissolving 1g of powder in 10 ml distilled water.

### 2.1.1. Bacterial Isolates:

(*Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) were provided by bacterial collection at Department of microbiology, Omar Al-Mukhtar University.

### 2.1.2. Antibacterial susceptibility testing:

The disk diffusion method was tested, and bacteria were grown on Mueller-Hinton agar medium, and 6 ml tablets of plant extracts put were placed on bacteria with equal distances. The dishes were incubated for 24 hours at 37 ° C with three replications per dish. Inhibition zone diameters were measured and compared to Gentamicin (GN 10 µg), then a measure of diameters of inhibitory zones [7].



Fig(1): *Cyclamen rohlfsianum*.

## 2.2. Statistical Analysis

The experiments were designed according to the complete random design (CRD). Statistical analysis was performed using Minitab 17 program and ANOVA variance analysis tables. The averages were compared using Tukey's test at  $P < 0.05$ .

## 3. Results:

The results from table (1) showed were significant differences in the inhibitory activity of extracts of leaves and tubers of *Cyclamen rohlfsianum* against pathogenic bacteria, according to the type of microbe, concentration and type of part used. The leaf extract at a concentration of 100 mg/ml recorded weak inhibitory activity with a diameter of (2.0, 1.5 and 1.0 mm) against *P.vulgaris*, *S.aureus* and *Ps.aeruginosa*, respectively, the concentration of 25 and 50 mg/ml had no inhibitory effect. The results showed the tuber extract at a concentration of 25 mg/ml did not record any inhibitory activity against the tested bacteria, while a concentration of 50 mg/ml had a weak inhibitory activity did not exceed a diameter (2.1 mm) against all bacteria types excepted of *E.coli*, a concentration of 100mg/ml was had the best inhibitory diameters with (5.4, 3.9, 3.0 mm) against *P.vulgaris*, *S.aureus* and *Ps.aeruginosa*, respectively. The results also showed the extent of an effect of Gentamicin on tested bacteria, as *E.coli* showed high resistance with a diameter of (1.0 mm), while *Ps.aeruginosa* was middling sensitive with a diameter of (4.5 mm), *P.vulgaris* and *S.aureus* were the most sensitive with a diameter of (6.5, 7.0 mm), respectively.

**Table 1. Antibacterial activity of *Cyclamen rohlfsianum* aqueous extracts (mm).**

| Extract    | Concent.<br>mg/ml | <i>P.vulgaris</i> | <i>S.aureus</i> | <i>Ps.aeruginosa</i> | <i>E.coli</i> |
|------------|-------------------|-------------------|-----------------|----------------------|---------------|
| Leaves     | 25                | 0.0 d             | 0.0 d           | 0.0 d                | 0.0 b         |
|            | 50                | 0.0 d             | 0.0 d           | 0.0 d                | 0.0 b         |
|            | 100               | 2.0±0.2 c         | 1.5±0.5 c       | 1.0±0.0 c            | 0.0 b         |
| Tubers     | 25                | 0.0 d             | 0.0 d           | 0.0 d                | 0.0 b         |
|            | 50                | 2.1±0.3 c         | 1.4±0.3 c       | 0.8±0.1 c            | 0.0 b         |
|            | 100               | 5.4±0.5 b         | 3.9±0.1 b       | 3.0±0.4 b            | 0.0 b         |
| Gentamicin |                   | 6.5±0.2 a         | 7.0±0.4 a       | 4.5±0.6 a            | 1.0±0.1 a     |



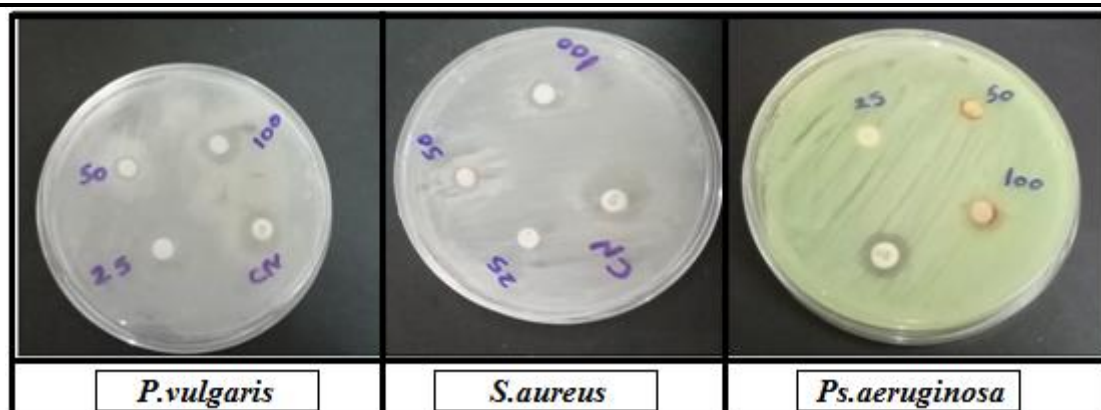


Fig (2): Effect of tubers extract of *Cyclamen rohlfsianum* against pathogenic bacteria.

#### 4. Discussion:

Due to a lack of research on the biological activities of endemic Libyan plants, this study was conducted, which showed of *Cyclamen rohlfsianum* extracts possess good inhibitory activity against some types of pathogenic bacteria, this result agreed with [4], this result disagrees with [12]. The results also showed the tuber extract was more efficient than the leaf extract, agreed with what was found [14], the concentration of 150 mg/ml was the most effective inhibitor of all extracts, *E.coli* was the most resistant to an extract and Gentamicin, *P. vulgaris* was It is most affected by the extract, *S.aureus* was the most sensitive to Gentamicin. The Inhibitory activity of *Cyclamen rohlfsianum* may be due to its content of alkaloids the presence of phenolics, triterpenoids, saponins, steroidal compounds, Kaempferol, Genistein, Hesperetin, Oleanolic acid and 4, 7, 8-Trihydroxyflavone [8].

#### 5. Conclusion:

This study concludes that *Cyclamen rohlfsianum* extracts can be used as antimicrobials, because have effective activities against some types of bacteria, so research recommends conducting more studies on different types of Libyan medicinal plants, interest in the possibility of using them as a new source for compounds, with diverse biological activities, especially in a field of biological control.

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