



# In vitro Antioxidant and Antibacterial Evaluation of Essential Oils Derived from Two Endemic Medicinal Apiaceae Species Growing Wild in Halgurd Mountain, Iraqi Kurdistan

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## Abstract

**Background:** *Prangos platychlaena* Boiss. and *Cachrys scabra* (Fenzl) Meikle are two distinct genera from the Apiaceae family that grow naturally in the Halgurd Mountains of Iraqi Kurdistan. These genera are traditionally used in medicine and as food flavoring agents across the world, particularly in the Middle East.

**Objectives:** This study aimed to investigate the antimicrobial and antioxidant potential of essential oils (EOs) obtained from two endemic plants of Iraqi Kurdistan, *P. platychlaena* and *C. scabra*.

**Methods:** The antioxidant and antibacterial activities of EOs extracted by steam distillation from the leaves and flowers of *P. platychlaena* and *C. scabra* collected from the Halgurd Mountains were evaluated.

**Results:** Overall, the EOs exhibited stronger inhibitory effects against ABTS radicals than against DPPH radicals. *Cachrys scabra* flower essential oil (CSFEO) and leaf essential oil (CSLEO) showed the weakest radical scavenging activity, with  $IC_{50}$  values of  $25.34 \pm 0.73$  mg/mL and  $25.09 \pm 1.31$  mg/mL, respectively. Furthermore, the EOs from both plants demonstrated good antibacterial efficacy against all tested microorganisms, except *Pseudomonas aeruginosa* (ATCC 27852), which was not affected by any of the EOs. The growth inhibition zones (GIZ) of sensitive strains ranged from  $10 \pm 0.5$  mm to  $20 \pm 1.8$  mm.

**Conclusions:** The EOs of *P. platychlaena* and *C. scabra* possess antioxidant activity capable of scavenging DPPH and ABTS radicals and exhibit antibacterial effects against both gram-positive and gram-negative bacteria. These findings suggest that these EOs may serve as promising natural preservatives for use in the pharmaceutical and food industries.

**Keywords:** Apiaceae, *Prangos platychlaena*, *Cachrys scabra*, Essential Oils, Antioxidant, Antibacteria

## 1. Background

*Prangos* and *Cachrys* are two genera belonging to the deep-rooted Apiaceae family and are distributed worldwide, particularly in the Irano-Turanian phytogeographic area (1, 2). *Prangos platychlaena* Boiss. and *Cachrys scabra* (Fenzl) Meikle are perennial herbaceous taxa that are endemic to the Iraqi Kurdistan region (3, 4) and are locally known as "Chewre" and "Xwerik", respectively, in Kurdish. Different parts of both plants have been used in folk medicine across numerous communities for treating a variety of illnesses, including antispasmodic, antihemorrhoidal, rheumatic,

carminative, diuretic, gout, and wound-healing conditions (1, 2, 5).

The widespread use of *Prangos* and *Cachrys* in folk medicine, together with the recognition of their terpene-rich essential oils (EOs) by the pharmaceutical industry (3, 6), underscores the need for a deeper understanding of their various properties, including phytochemical, toxicological, and pharmacological characteristics (1, 2, 7).

## 2. Objectives

This study aimed to investigate the antioxidant and antimicrobial potency of EOs obtained from *P.*

*platychlaena* and *C. scabra*, belonging to the Apiaceae family, which are indigenous to Iraqi Kurdistan.

### 3. Methods

#### 3.1. Plant Material

Leaves and flowers of *P. platychlaena* and *Cachrys scabra* were collected from the Halgurd Mountain in the Kurdistan Region of Iraq during June - July 2023 (altitude: 2268 m; Figure 1). The plant material was identified by Assistant Professor Abdullah Shukur Sardar, a botanist from the Department of Biology, Salahaddin University-Erbil. The collected plant parts were sorted, dried, and ground into a fine powder. The powdered samples were stored in air-tight containers at room temperature ( $25.00 \pm 2.00^{\circ}\text{C}$ ), protected from light until further use.

#### 3.2. Isolation of the Essential Oils

The EOs were extracted using the hydro-distillation method with a Clevenger apparatus for three hours. The obtained EOs were dried over anhydrous sodium sulfate and stored in dark vials at  $4^{\circ}\text{C}$  until analysis. The EO of *P. platychlaena* was pale yellowish in color, while that of *C. scabra* appeared clear.

#### 3.3. DPPH Radical Scavenging Activity

The antioxidant activity was measured using the DPPH free radical scavenging assay, following the procedure described by Dahal et al. (8), with slight modifications. First, 100  $\mu\text{L}$  of DPPH (90  $\mu\text{M}$ ) solution prepared in methanol (95% v/v) was added to each well. Then, 100  $\mu\text{L}$  of serial dilutions of the EOs (0.08 - 10 mg/mL), as well as the positive control (vitamin C), were added to 100  $\mu\text{L}$  of DPPH in methanol. The reaction mixture was shaken vigorously and incubated for 30 minutes. The absorbance of the solutions was then measured at 516 nm, and the radical scavenging activity (%) was calculated using the following equation: Radical scavenging activity% =  $(\text{Abs blank} - \text{Abs sample})/(\text{Abs blank}) \times 100$ .

The  $\text{IC}_{50}$  value was determined as the concentration of the sample required to inhibit 50% of the initial DPPH radical.

#### 3.4. ABTS<sup>+</sup> Radical Scavenging Assay

The ABTS<sup>+</sup> assay was performed according to the method described by Zengin et al. (9). The ABTS<sup>+</sup> cationic radical was generated by mixing 100  $\mu\text{L}$  of 2.45

mM potassium persulfate with 10 mL of 7 mM ABTS solution. The resulting mixture was incubated at room temperature in the dark for 16 hours. The ABTS<sup>+</sup> solution was then diluted with methanol to obtain an absorbance of  $0.70 \pm 0.002$  at 734 nm.

Next, 100  $\mu\text{L}$  of the ABTS<sup>+</sup> cationic radical solution was mixed with 100  $\mu\text{L}$  of serial concentrations of EOs (0.08 - 10 mg/mL) or vitamin C (positive control). After a 30-minute incubation in the dark, the absorbance of the mixture was measured at 734 nm. The antiradical activity of the samples was expressed as the percentage inhibition of the ABTS<sup>+</sup> radical, calculated using the same equation described previously for the DPPH assay.

#### 3.5. Antimicrobial Activity

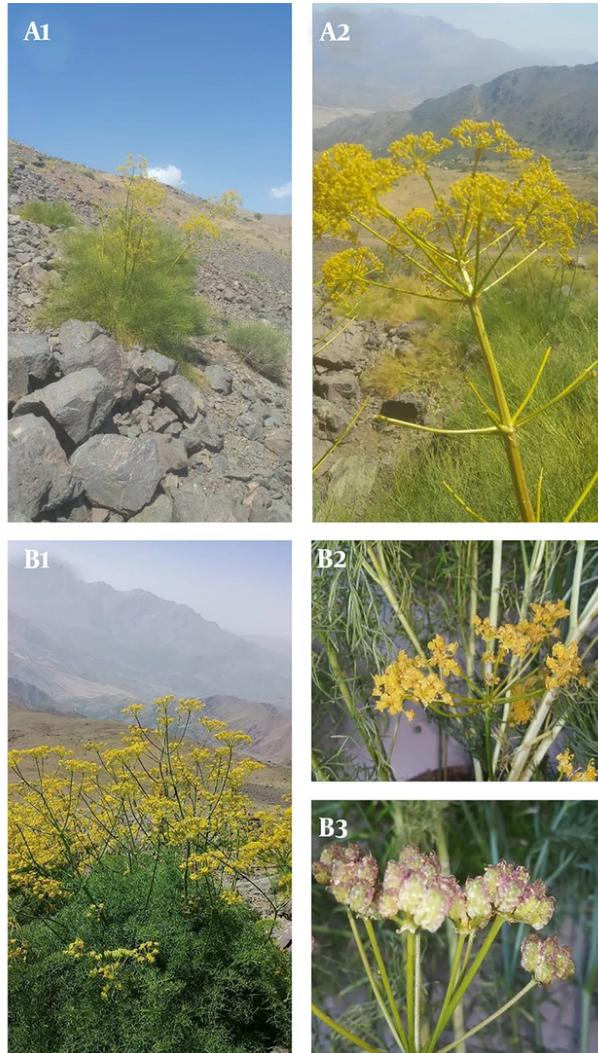
Nine bacterial strains were tested for antimicrobial activity, including six gram-positive species – *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 15753), *Bacillus pumilus* (PTCC 1274), *B. cereus* (PTCC 1015), and *B. subtilis* (ATCC 9372) – and three gram-negative species – *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27852), and *Klebsiella pneumoniae* (ATCC 3583). Tetracycline (30  $\mu\text{g}$ ), gentamicin (10  $\mu\text{g}$ ), and ampicillin (10  $\mu\text{g}$ ) were used as positive controls.

#### 3.6. Disc Diffusion Assay

The antimicrobial activity of the EOs was tested against pathogenic strains using the agar disc diffusion method described by Dahal et al. (8). Sterile Whatman filter paper discs (6 mm in diameter) were placed in the center of Petri dishes and impregnated with 20  $\mu\text{L}$  of the EOs at a concentration of 1 mg/mL. The inoculated Petri dishes were incubated at  $37^{\circ}\text{C}$  for 24 - 48 hours. Following incubation, the diameters of the growth inhibition zones (GIZ) were measured in millimeters (mm). The inhibition zones (IZ) were categorized as very strong ( $> 20$  mm), strong (10 - 20 mm), moderate (5 - 10 mm), or weak ( $< 5$  mm) (10).

#### 3.7. Microdilution Assay

The EOs that exhibited notable IZ against indicator strains in the agar disc diffusion assay were further analyzed quantitatively to determine their minimum inhibitory concentration (MIC) values. The broth microdilution method was employed to determine MIC, following the procedure described by Rahman et al. (11). Various concentrations of EOs (120 - 3.5 mg/mL) were added to 5 mL of appropriate broth tubes containing  $10^7$  CFU/mL of live bacterial cells. To ensure uniform



**Figure 1.** Morphology of the whole plant: A1, *Prangos platychnaena*; A2, *P. platychnaena* inflorescence; B1, *Cachrys scabra*; B2, *C. scabra* inflorescence; B3, *C. scabra* fruits.

dispersion of the oil throughout the broth, the tubes were incubated on a shaker incubator and then examined for visible microbial growth. The MIC was defined as the lowest concentration of EOs showing no visible bacterial growth after incubation.

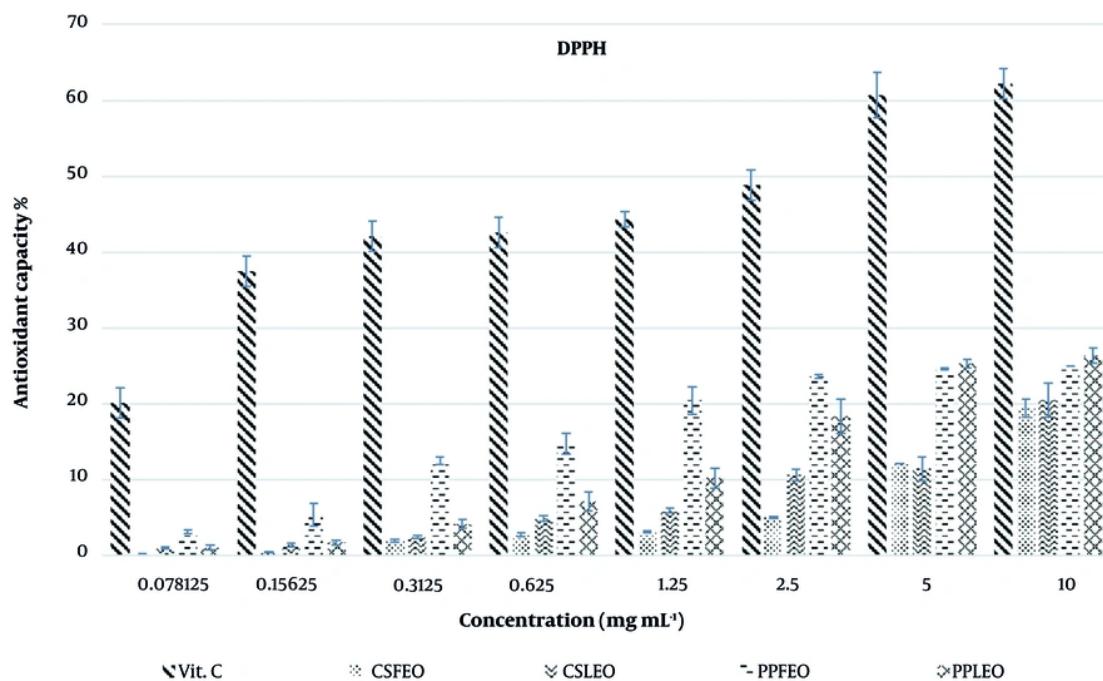
### 3.8. Statistical Analysis

All antioxidant and antibacterial assays were performed in triplicate. The results are presented as mean values  $\pm$  standard deviation (SD). The  $IC_{50}$  values for antioxidant tests were calculated from linear regression equations using Microsoft Excel 2021.

## 4. Results and Discussion

### 4.1. DPPH Radical-Scavenging Assay of Essential Oils

The DPPH radical scavenging activity of the EOs from *P. platychnaena* and *C. scabra* is presented in **Figure 2** and **Table 1**. Both the EOs and the positive control exhibited dose-dependent inhibition of the DPPH radical. All tested samples demonstrated significant antioxidant potential, with  $IC_{50}$  values of  $16.55 \pm 0.27$  mg/mL and  $25.34 \pm 0.73$  mg/mL, which were comparable to that of the positive control (vitamin C,  $IC_{50} = 4.76 \pm 0.10$



**Figure 2.** Antioxidant capacity (%) of the essential oils (EOs) obtained from *Prangos platychnaena* and *Cachrys scabra* compared with the standard (vitamin C)

mg/mL). PPLEO and PPFO exhibited markedly higher antioxidant activity than *Cachrys scabra* leaf essential oil (CSLEO) and *Cachrys scabra* flower essential oil (CSFEO).

**Table 1.**  $IC_{50}$  (mg/mL) Values for *Prangos platychnaena* and *Cachrys scabra* Essential Oils Against DPPH and ABTS Radicals

Sample No.	Sample Code	$IC_{50} \pm SD$ [mg mL⁻¹]	
		DPPH	ABTS
1	CSFEO	25.34 ± 0.73	9.95 ± 0.16
2	CSLEO	25.09 ± 1.31	10.80 ± 0.41
3	PPFO	19.39 ± 0.09	6.63 ± 0.25
4	PPLEO	16.55 ± 0.27	10.90 ± 0.34
5	Vitamin C	4.76 ± 0.10	1.81 ± 0.07

Abbreviations: SD, standard deviation; CSFEO, *Cachrys scabra* flower essential oil; CSLEO, *Cachrys scabra* leaf essential oil.

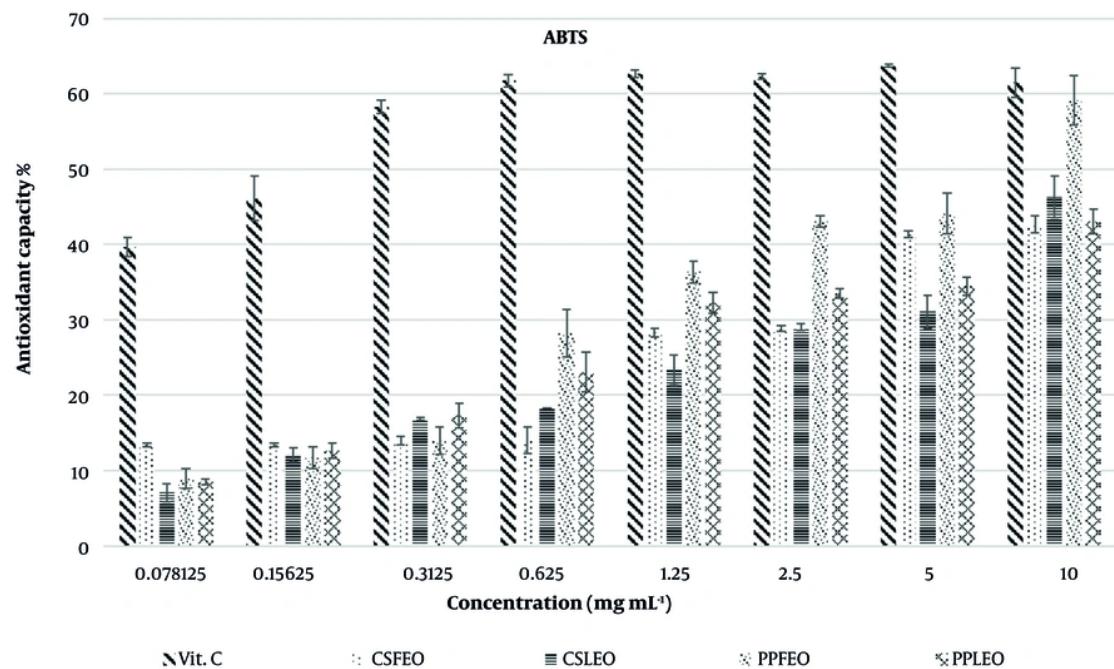
Within the concentration range tested (0.0785 - 10 mg/mL), the antioxidant potential of the EOs followed the order: PPLEO > PPFO > CSLEO > CSFEO.

#### 4.2. ABTS Radical Scavenging Activity of Essential Oils

As shown in Figure 3, the ABTS radical scavenging activity of the EOs and vitamin C was concentration-dependent, similar to the DPPH assay. At the highest concentration (10 mg/mL) of all EOs, the antioxidant capacity ranged between  $41.43 \pm 1.60\%$  and  $59.79 \pm 3.30\%$ , while that of vitamin C (positive control) was  $63.75 \pm 1.98\%$  (Table 1). Table 1 also presents the concentrations of the EOs required to inhibit 50% of the ABTS radicals, expressed as  $IC_{50}$  values.

Among the tested oils, PPLEO exhibited the lowest anti-ABTS radical activity with an  $IC_{50}$  value of  $10.90 \pm 0.34$  mg/mL, while PPFO demonstrated the highest anti-radical potency with an  $IC_{50}$  value of  $6.63 \pm 0.25$  mg/mL. Considering the tested concentration range (0.0785 - 10 mg/mL), the antioxidant potential of the EOs followed the order: PPFO > CSFEO > CSLEO > PPLEO.

Our findings are consistent with previous studies investigating the inhibitory effects of EOs from *Prangos* and *Cachrys* genera against DPPH and ABTS radical scavenging (5, 12). In a study conducted in Iraq, Rahman and Safar (3) reported that the EOs of *C. scabra* exhibited



**Figure 3.** Inhibitory percentage effect of the essential oils (EOs) of *Prangos platychlaena* and *Cachrys scabra* and vitamin C on ABTS radicals

higher DPPH free radical scavenging activity compared to other extracts, with  $IC_{50}$  values ranging from 6 to 8.6 mg/mL. Similarly, Matejic et al. (13) from Serbia demonstrated significant free radical scavenging properties in an aqueous extract of *C. cristata* fruits, with an  $IC_{50}$  value of 1.784 mg/mL, followed by methanolic extracts of fruits and aerial parts with  $IC_{50}$  values of 3.347 and 4.058 mg/mL, respectively.

Rahman et al. (12) from Iraq found that the petroleum ether extract of the flower of *P. platychlaena* exhibited higher DPPH antioxidant scavenging activity ( $IC_{50} = 0.19 \pm 0.01$  mg/mL) than the root, leaves, and stem. Furthermore, Baghiani et al. (5) from Algeria reported that the crude extract of *C. libanotis* roots displayed strong DPPH scavenging activity, with an  $IC_{50}$  of  $0.41 \pm 0.01$  mg/mL, followed by the ethyl acetate extract ( $IC_{50} = 0.57 \pm 0.01$  mg/mL). In addition, *P. uechtritzii* EO from Turkey, studied by Zengin et al. (9), demonstrated higher activity for DPPH free radical scavenging ( $IC_{50} = 1.74 \pm 0.10$  mg TE/g) among three *Prangos* species, while *P. heyniae* EO exhibited the most significant ABTS scavenging activity ( $IC_{50} = 92.99 \pm 1.29$  mg TE/g).

#### 4.3. Antibacterial Activity of Essential Oils

The antimicrobial activity of the EOs from *P. platychlaena* and *C. scabra* against nine bacterial strains is summarized in Table 2. All tested bacterial strains were sensitive to both *P. platychlaena* and *C. scabra* EOs, with GIZ diameters ranging from  $10 \pm 0.5$  to  $20 \pm 1.8$  mm. However, *P. aeruginosa* (ATCC 27852) showed complete resistance, displaying no inhibition by any of the oils.

The flower and leaf EOs of *P. platychlaena* exhibited notable activity against three pathogenic strains – *S. aureus*, *S. epidermidis*, and *E. coli* – with IZ diameters ranging from  $14 \pm 1.73$  to  $18 \pm 1.0$  mm. For *C. scabra*, both CSLEO and CSFEO displayed moderate to strong inhibitory effects against all tested strains (except *P. aeruginosa*), with IZ values between  $10 \pm 0.87$  and  $20 \pm 1.8$  mm.

The MIC results indicated that the EOs of both plants exerted substantial bactericidal potency, with MIC values greater than 7 mg/mL across all tested microorganisms. Interestingly, *P. aeruginosa* (ATCC 27852) remained unaffected by any of the EOs.

These findings are consistent with previously reported results for *Prangos* and *Cachrys* species (5, 13,

**Table 2.** Antibacterial Activity of *Prangos platychnaena* and *Cachrys scabra* Essential Oils Against Pathogenic Bacteria, Recorded as Inhibition Zones (mm) and Minimum Inhibitory Concentration (mg/mL)<sup>a</sup>

Samples	<i>Bacillus pumilus</i> (ATCC 1274)	<i>B. subtilis</i> (ATCC 9372)	<i>Staphylococcus aureus</i> (ATCC 25923)	<i>B. cereus</i> (ATCC 1015)	<i>Enterococcus faecalis</i> (ATCC 15753)	<i>S. epidermidis</i> (ATCC 12228)	<i>Klebsiella pneumonia</i> (ATCC 3583)	<i>Escherichia coli</i> (ATCC 25922)	<i>Pseudomonas aeruginosa</i> (ATCC 27852)
<b>PPFO</b>									
IZ $\pm$ SD (mm)	11 $\pm$ 1.32 <sup>b</sup>	12 $\pm$ 0.5	18 $\pm$ 0.5	12 $\pm$ 0.5	11 $\pm$ 1.8	18 $\pm$ 10	10 $\pm$ 0.5	17 $\pm$ 0.5	-
MIC (mg mL <sup>-1</sup> )	> 15 <sup>c</sup>	15	7.5	15	15	7.5	15	7.5	-
<b>PPLEO</b>									
IZ $\pm$ SD (mm)	12 $\pm$ 0.5	12 $\pm$ 1	18 $\pm$ 1.5	11 $\pm$ 1.32	11 $\pm$ 1.8	17 $\pm$ 0.87	12 $\pm$ 1.73	14 $\pm$ 1.73	-
MIC (mg mL <sup>-1</sup> )	15	15	7.5	15	> 15	7.5	15	15	-
<b>CSLEO</b>									
IZ $\pm$ SD (mm)	18 $\pm$ 0.87	10 $\pm$ 1.32	20 $\pm$ 1.8	11 $\pm$ 0.5	14 $\pm$ 0.5	10 $\pm$ 0.87	20 $\pm$ 1.73	14 $\pm$ 0.5	-
MIC (mg mL <sup>-1</sup> )	7.5	15	7.5	15	15	15	7.5	15	-
<b>CSFEO</b>									
IZ $\pm$ SD (mm)	17 $\pm$ 1	12 $\pm$ 0.87	14 $\pm$ 1	12 $\pm$ 0.87	11 $\pm$ 0.87	14 $\pm$ 2	18 $\pm$ 1.8	14 $\pm$ 0.87	-
MIC (mg mL <sup>-1</sup> )	7.5	15	15	15	> 15	15	7.5	15	-
<b>Tetracycline<sup>d</sup></b>									
IZ $\pm$ SD (mm)	-	14 $\pm$ 12.12	20 $\pm$ 1.73	Nt	Nt	34 $\pm$ 1.73	Nt	0	Nt
MIC (mg mL <sup>-1</sup> )	-	3.2	3.2	-	-	1.6	-	Nt	-
<b>Gentamicin<sup>e</sup></b>									
IZ $\pm$ SD (mm)	-	-	-	Nt	Nt	0	Nt	23 $\pm$ 1.8	Nt
MIC (mg mL <sup>-1</sup> )	-	Nt	Nt	-	-	Nt	-	3.2	-
<b>Ampicillin<sup>e</sup></b>									
IZ $\pm$ SD (mm)	15 $\pm$ 0.5	14 $\pm$ 0.87	13 $\pm$ 0.87	Nt	Nt	19 $\pm$ 0.87	Nt	12 $\pm$ 0.87	Nt
MIC (mg mL <sup>-1</sup> )	15	15	15	-	-	15	-	15	-

Abbreviations: IZ, inhibition zones; SD, standard deviation; MIC, minimum inhibitory concentration; CSLEO, *Cachrys scabra* leaf essential oil; CSFEO, *Cachrys scabra* flower essential oil; Nt, not tested.

<sup>a</sup>Values are expressed as mean  $\pm$  SD.

<sup>b</sup>Zone of inhibition (in mm) includes diameter of the disc (6 mm) with concentration 1 mg/disc.

<sup>c</sup>MIC values as mg mL<sup>-1</sup>, (-): Inactive, (7-13): Moderately active, (>14): Highly active.

<sup>d</sup>Tested at 30  $\mu$ g/disc.

<sup>e</sup>Tested at 10  $\mu$ g/disc.

14). For instance, Rahman et al. (12) reported that the petroleum ether extract of *P. platychnaena* leaves exhibited strong bactericidal activity against *S. aureus* and *P. aeruginosa*, with MIC values of 0.23 and 1.33 mg/mL, respectively—surpassing those of other extracts evaluated in their study.

A previous report from Iraq by Rahman and Safar (3) indicated that different concentrations (5.00 to 10.00 mg/mL) of the petroleum ether extract obtained from the leaves and flowers of *C. scabra* exhibited significant bactericidal activity against all tested strains. In contrast, the EOs and ethanol extracts showed weak antibacterial activity against *P. aeruginosa*, even at the highest tested concentrations. However, Rahman et al. (11) reported that the EOs extracted from the flowers and leaves of *P. platychnaena*, which are rich in terpenes, demonstrated strong bactericidal potency against *S.*

*aureus* and *P. aeruginosa*, with MIC values of  $0.86 \pm 0.13$  and  $1.43 \pm 0.06$  mg/mL for the flower EO, and  $2.54 \pm 0.32$  and  $1.16 \pm 0.08$  mg/mL for the leaf EO, respectively.

Conversely, Uzel et al. (15) from Turkey reported that *P. platychnaena* EO, with MIC values reaching up to 144 mg/mL, exhibited weak bactericidal efficacy against *S. aureus*, *E. faecalis*, *B. subtilis*, *E. coli*, *P. aeruginosa*, and *Salmonella typhimurium*, compared to *P. uechtritzii* EO.

#### 4.4. Conclusions

Plant-derived natural products, particularly EOs, are increasingly valued in the pharmaceutical, food, cosmetic, and perfumery industries. Their extensive use as antioxidants and antimicrobials highlights their potential as effective herbal medicinal alternatives. In this study, the EOs of *P. platychnaena* and *C. scabra*

exhibited significant antioxidant potential, as demonstrated by both DPPH and ABTS assays. Additionally, these EOs showed considerable antibacterial activity against all tested microorganisms, except for *P. aeruginosa*, which was resistant to their effects.

However, further studies are warranted to comprehensively evaluate the *in vitro* and *in vivo* biological activities of these EOs, particularly concerning their safety, toxicity, and potential mechanisms of action.

## Footnotes

**Authors' Contribution:** A. S. is the only author of the article and the study was solely carried out by the author.

**Conflict of Interests Statement:** The authors declare no conflict of interest.

**Data Availability:** The dataset presented in the study is available on request from the corresponding author during submission or after publication.

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