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(RESEARCH ARTICLE)

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Phytochemical screening and evaluation of antibacterial activity of *Olea europaea* L.Var. *sylvestris* (Miller) Lear. leaves

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Abstract

Olea europaea L.var. *Sylvestris* (Miller) Lehr., of the Oleaceae family, is commonly known as the wild olive tree. It is a tree used in traditional medicine in the Mediterranean region for its leaves, which contain numerous constituents with pharmacological properties such as hypoglycemic and anti-inflammatory properties. The objective of our work is to determine the qualitative chemical composition of the leaves of this species and to evaluate their antibacterial activity. The results we obtained after performing phytochemical screening of the methanolic extract of *Olea europaea* L.var. *sylvestris* leaves showed the presence of total polyphenols such as flavonoids and tannins, as well as sterols, triterpenes, and saponins. We evaluated the antibacterial activity of the methanolic extract of Olea europaea L.var. sylvestris using the agar diffusion method. The results obtained show that the leaves have moderate antibacterial activity. The most sensitive strains are *Bacillus cereus* ATCC 10876 and *Pseudomonas aeruginosa* ATCC 27853. Olea europaea L. var. sylvestris could be a solution to overcome bacterial resistance and is therefore of interest to public health.

Keywords: *Olea europaea* L. var. *sylvestris* (Miller) Lehr; Methanolic leaf extract; Phytochemical screening; phenolic compounds; Antibacterial activity

1. Introduction

Olea europaea L.var. *sylvestris* (Miller) Lehr. is widely used in traditional medicine for its therapeutic properties. It has been used to treat various conditions, including hypertension and diabetes [1], as well as fever and certain infectious diseases such as malaria [2]. The biological activity of its standardized extracts has been demonstrated in numerous experimental studies, both *in vitro* and *in vivo* [3]. The leaves of *Olea europaea* L.var. *sylvestris* (Miller) Lehr. are particularly rich in polyphenolic compounds, mainly oleuropein, hydroxytyrosol, and various flavonoids [4], which are known for their antioxidant and antimicrobial effects. The aim of the present study is therefore to characterize the phytochemical composition of the leaves of this species and to evaluate their antibacterial activity. Antibacterial activity was assessed using the agar diffusion method, employing a methanolic leaf extract tested against different bacterial strains.

2. Materials and methods

2.1. Plant material

The plant material was collected in the Collo region, in the province of Skikda. The part used consisted of the dried leaves of *Olea europaea* L. var. *sylvestris* (Miller) Lehr. The leaves were dried away from direct sunlight, at room

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temperature, in a dry and well-ventilated environment. The dried leaves were then crushed and sieved, and their water content was verified to be below 10%. The resulting leaf powder was stored in a glass container at room temperature and protected from light

2.2. Preparation of the methanolic extract

In an Erlenmeyer flask, 10 g of dried leaves were macerated in 100 mL of methanol at room temperature. After 48 hours, the mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure using a rotary evaporator. The dry residue was then dissolved in 10 mL of methanol and stored in amber bottles at +4 °C. The dry residue obtained was measured to determine the initial concentration of the extract (MElea = 316 mg/mL).

2.3. Phytochemical screening protocol

Phytochemical screening highlights the presence of active molecules in the plant. It is a qualitative study aimed at identifying the various chemical components (polyphenols, coumarins, alkaloids, etc.). To carry out this identification, an extract must first be obtained from dried leaves. The search for chemical components is carried out using these extracts, applying different reagents depending on the components to be identified.

2.2.1. Identification of polyphenols

Polyphenols are identified using ferric chloride (FeCl3)

- Add 200 mg of powder to 10 ml of water and boil for 2 minutes.
- Take 1 ml of the decoction and add a few drops of 10% FeCl3.
- The appearance of a blue-black or green coloration of varying intensity indicates the presence of polyphenols [211].

2.2.2. Identification of tannins

Condensed tannins

- **Stiasny reaction**

In a test tube, add 5 ml of the decoction, then add 2 ml of Stiasny's reagent (hydrochloric formaldehyde) , and heat for 10 to 15 minutes at 90°C.

A flocculent precipitate will appear [5].

- **Bate-Smith reaction**
  - Place 0.2 g of powder plant material in a test tube, add 2 ml of Bate-Smith reagent (hydrochloric butanol) and heat for 10 to 15 minutes at 90 °C.
  - A reddish-brown coloration is observed, which is intensifies by agitation (oxidationair in air) [6].

Hydrolyzable tannins

- Filter the decoction after treatment with Stiasny's reagent.
- Neutralize by adding sodium acetate, then add 3 drops of FeCl3.
- A blue-black precipitate appears [5].

2.2.3. Identification of flavonoids

Cyanidin reaction

Flavonoid glycosides in alcoholic solution, when exposed to hydrogen, produce a red color [7].

- Extraction: prepare a hydroalcoholic solution.
- Weigh 0.2 g of the drug in a test tube, then add 4 ml of ethanol and 1 ml of water.
- Place the tube in a water bath at 65°C for at least 10 minutes and filter the alcoholate while hot.
- Take 2 ml of the filtrate and add 0.5 ml of concentrated hydrochloric acid and 1 magnesium chip.
- Observe the appearance of a cherry red color with flavonols, orange with flavones, purplish red with flavanones, and no color with chalcones.

2.2.4. Identification of coumarins

Coumarins are characterized by UV fluorescence at 365 nm.

- Take 2 g of the powder and add 20 ml of methanol, then leave to macerate for 24 hours.
- Filter to recover the extract and divide the extract into two test tubes
- Use one tube as a negative control, and add a few drops of concentrated ammonia to the other.
- Observe under a UV lamp at 365 nm and compare with the control tube [8].

2.2.5. Identification of anthocyanins

Anthocyanins are detected based on their ability to change color depending on pH.

- Place 0.5 g of the drug in a test tube and add 5 ml of methanol.
- Mash with a spatula for about 5 minutes and filter through filter paper.
- The filtrate is acidified by adding concentrated hydrochloric acid (under a fume hood).
- A change in color from bright red to deep red is observed [9].
- Add increasing amounts of NaOH at 200 g/l to the acidified filtrate until a yellow color appears.

2.2.6. Identification of quinones

- Add 1 g of plant powder to 15 to 30 ml of petroleum ether.
- Shake and leave to stand for 24 hours.
- Filter through paper in a test tube and add a few drops of 1/10 NaOH to the filtrate.
- The aqueous phase turns yellow [10].

2.2.7. Identification of anthraquinones

- Add 100 mg of powder to 5 ml of dichloromethane in an Erlenmeyer flask, allow to macerate for 15 minutes and shake occasionally.
- Filter through paper in a test tube and add 2 ml of NH4OH diluted to ½ to the filtrate, stirring.
- After decanting, the aqueous phase turns pink [11].

2.2.8. Identification of saponins

The presence of saponins is determined by calculating the foam index [9].

- In a 500 ml beaker containing 100 ml of boiling water, add 1 g of powdered drug and maintain a moderate boil for 30 min.
- Filter and adjust to 100 ml after cooling.
- In 11 identical test tuibes, prepare dilutions from this filtrate according to Table 20.
- Close the tubes with your thumb and shake for 10 seconds.
- After 15 minutes, measure the height of the foam in each tube.
- Identify the tube that shows a persistent foam height of 1cm.
- Calculate the foam index using the following formula: FI = 10 / weight of the materiel tested

Table 1 Dilutions prepared from the filtrate

Tube No	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Decoction (ml)	0	0.5	1	1 .5	2	2.5	3	3.5	4	4.5	5
Distilled water ((ml)	1	9.5	9	8	8.5	7.5	7	6.5	6	5.5	5
Weight of the drug g	0	0.005	0.00	0.01	0.02	0.025	0.03	0.035	0.04	0.045	0.05

2.2.9. Identification of sterols and triterpenes

- Take 0.5g of plant powder, add 10ml of ether, and leave to macerate for 24 hours.
- Evaporate the ether extract to dryness and add 1ml of acetic anhydride.
- Add 1 ml of chloroform to a tube containing the extract and sulfuric acid.

- A reddish-brown or purple ring forms at the contact zone between the two liquid phases [8].

2.2.10. Identification of terpenoids "Slakowski test"

- Add 1 g of plant powder to 10 ml of methanol, shake and leave to stand for 24 hours.
- Filter the solution through paper in a test tube.
- Take 2.5 ml of extract and add 1 ml of chloroform.
- After homogenization, add 1.5 ml of concentrated sulfuric acid H2SO4.
- The appearance of a reddish-brown color at the interface of the two layers indicates the presence of terpenoids [12].

2.2.11. Identification of cardiotonic glycosides

- Add 1 g of plant powder to 20 ml of 50% v/v alcohol.
- Boil for 2 minutes, then add a few drops of basic Pb acetate.
- Filter and place the filtrate in a separating funnel.
- Add 15 ml of chloroform and shake.
- Collect the organic phase on anhydrous Na sulfate and divide it into 3 capsules.
- Evaporate to dryness [13].

Libermann reaction:

- Add a few drops of acetic anhydride and a few drops of concentrated sulfuric acid while cooling the residue.
- The appearance of a reddish-brown color that turns olive green after a few moments indicates the presence of steroid-ring substances [14].

Keller-Kiliani reaction:

This is a reaction used to characterize deoxyhexoses.

- Add 2 ml of glacial acetic acid and 1 drop of 2% FeCl3 to the residue.
- Carefully place this solution on 2 ml of sulfuric acid H2SO4.
- After a few moments, a brownish-red ring forms at the contact surface of the two liquids and the upper acetic layer turns blue-green, indicating the presence of 2,6-dideoxyhexoses [15].

Baljet reaction:

- Add a few drops of 10% soda and a few drops of 1% picric acid to the evaporation residue.
- A stable red-orange color develops.

2.2.12. Identification of alkaloids

Alkaloids precipitate under the action of certain reagents known as "general alkaloid reagents" [9].

- Place approximately 0.2 g of plant powder in a conical flask.
- Add 2 ml of 10% sulfuric acid, shake for 2 minutes, and filter.
- Add a few drops of Dragendorff's reagent (iodo-iodide reagent) to the filtrate, which in the presence of alkaloids an orange-red precipitate is formed.

2.3. Antibacterial activity protocol

The evaluation of the antibacterial activity of the methanolic extract of *Olea europaea* L.var. *sylvestris* (Miller) Lehr. Leaves is performed using the solid-phase diffusion technique [16, 17]. This method is similar to the antibiogram and consists of determining the sensitivity of a microbial strain to one or more substances.

The antibacterial activity of our extract is estimated in terms of the diameter of the inhibition zone around the discs containing the extract to be tested at different concentrations against the tested germs [18].

2.4.1. Bacterial strains tested

The methanolic extract of *Olea europaea* L.var. *sylvestris* (Miller) Lehr. leaves is tested for its antibacterial activity against the strains used.

These strains are pure known references, provided by the microbiology laboratory of Badji Mokhtar University in the city of Annaba, Algeria.

Table 2 The names and references of these strains

Souches	Références	Gram
Staphylococcus aureus	ATCC 25923	Gram positif
Bacillus cereus	ATCC 10876	Gram positif
Escherichia coli	ATCC 25922	Gram négatif
Pseudomonas aeruginosa	ATCC 27853	Gram négatif

2.3.1. Preparation of test solutions

From the concentrated methanol extract prepared beforehand, dilutions are prepared in methanol, which allows five concentrations to be obtained for each extract: Cmère, C ½, C¼, C⅛, and C1/16.

2.3.2. Preparation of the culture medium

Müller-Hinton medium was used. The agar melted in a water bath is poured into Petri dishes 90 mm in diameter to obtain a 4 mm thick layer of agar. The dishes are cooled and then stored in a refrigerator at +4°C. The prepared media are dried in an oven at 37°C before use.

2.3.3. Transferring bacterial strains

The bacterial strains are maintained by transferring them to nutrient agar incubated for 24 hours at 37°C to obtain young, isolated colonies that will be used to prepare the inoculum.

2.3.4. Preparation of bacterial suspensions

Antibacterial tests are performed using young colonies aged 18 to 24 hours in the exponential growth phase. The bacterial suspension (inoculum) is prepared as follows: using a sterile swab, 2 to 3 well-isolated and perfectly identical colonies are scraped off and the swab is discharged into approximately 5 ml of sterile 0.9% physiological saline for each strain.

2.3.5. Seeding

Once drying is complete, the freshly prepared bacterial suspension is spread three times over the surface of the Muller-Hinton agar using a swab, turning the plate by approximately 60° after each application and remembering to rotate the swab itself. For the final stage of seeding, the swab is passed over the periphery of the agar to ensure even distribution of the inoculum.

2.3.6. Application of discs

- Using sterile forceps, discs previously impregnated with extracts are placed on the agar.
- Each plate contains six discs, five of which contain extracts (one concentrated extract and four serial dilutions: 1/2, 1/4, 1/8 and 1/16).
- The negative control is a disc soaked in methanol (solvent without extract).
- The plates are incubated at 37°C for 24 hours.

2.3.7. Expression of results

Antibacterial activity is assessed by measuring the diameters of the inhibition zones (mm) formed around the discs using a caliper. The sensitivity of the target bacteria to the different compounds is classified according to the diameters of the inhibition halos. Each test is repeated three times under the same operating conditions.

According to Konan et al [19], the diameters of the antibacterial activity inhibition zones are classified into four classes, namely:

- Non-sensitive (-) or resistant: diameter <8 mm.
- Sensitive (+): diameter between 8 and 14 mm.
- Very sensitive (++): diameter between 15 and 20 mm.
- Extremely sensitive (+++): diameter >20 mm.

3. Results and discussion

3.1. Phytochemical screening

3.1.1. Results

Phytochemical screening of the leaves of *Olea europaea* L.var. *sylvestris* (Miller) Lehr. revealed the presence of phenolic compounds such as flavonoids, tannins, coumarins, and free quinones. However, anthraquinones and anthocyanins were not detected.

Regarding saponins, the foam index measurement based on their aphrogenic capacity, confirmed their presence in the plant material.

The leaves also tested positive for sterols and triterpenes, indicating their presence, and the positive Salkowski test confirmed the presence of terpenoids.

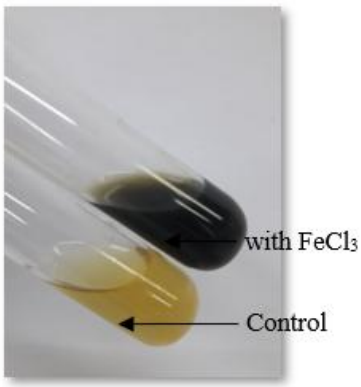

Figure 1 Identification result of polyphenols by the FeCl3 test

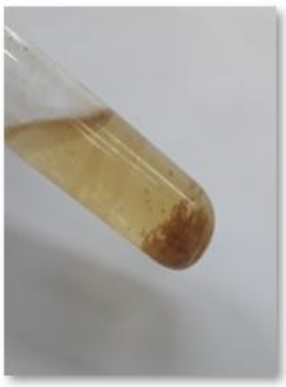

Figure 2 Identification results of condensed tannins by the Stiasny reaction

Figure 3 Identification results of tannins condensed by the Bate-smith reaction

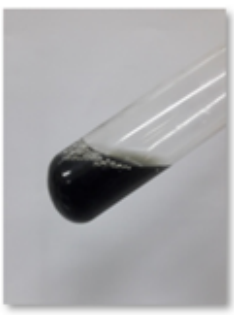

Figure 4 Identification results of hydrolyzable tannins

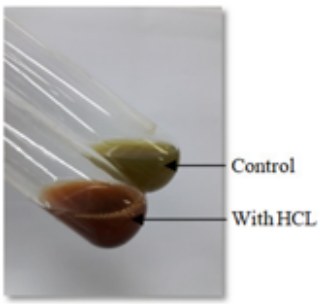

Figure 5 Identification results of flavonoïds by the cyanidine Reaction

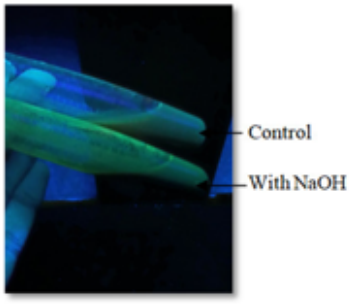

Figure 6 Identification results of coumarins by the fluorescence

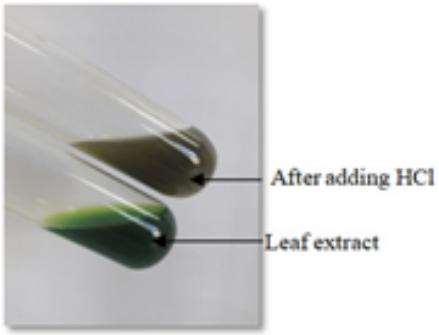

Figure 7 Identification results of antocyanins

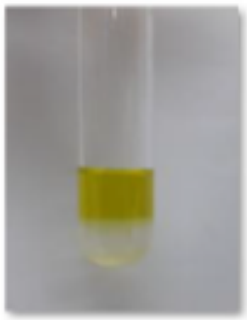

Figure 8 Identification results of quinones

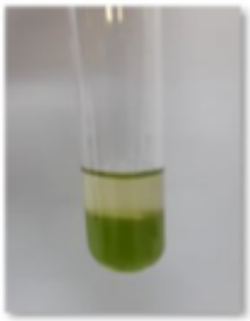

Figure 9 Identification result of anthraquinones by the Bornträger reaction

Figure 10 Identification results of sterols and tri terpenes

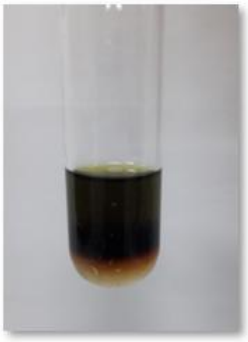

Figure11 Identification results des terpenoïde

Figure 12 Identification results of alkaloids by the Dragendroff reaction

Figure 13 Identification results of saponins By the foam index

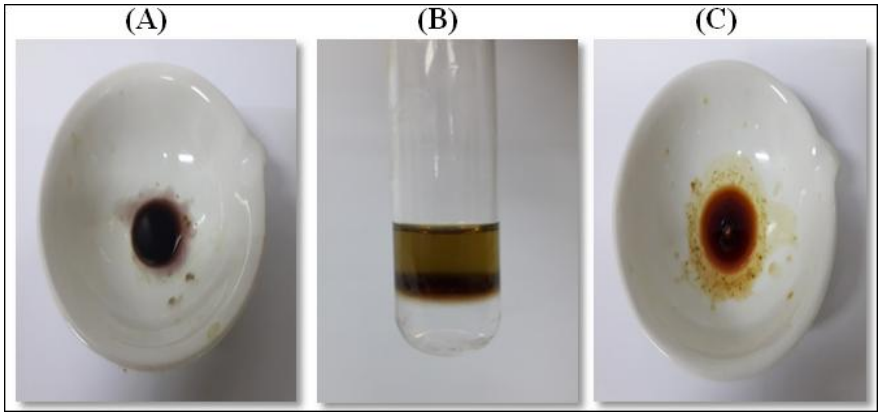

Figure 14 Identification results of cardiotonic glycosides by the reactions of :(A) Libermann (B)keller-kiliani (C) Baljet

Table 3 Results of phytochimical screening

Compounds sought		**Results**	
Polyphenols		Positive reaction	Greenish-brown precipitate
Condensed tannins	Stiasny	Positive reaction	Flocculating precipitate
	Bate-smith	Positive reaction	Red-brown coloration
Hydrolysable tannins		Positive reaction	Blue-black precipitate
Flavonoids		Positive reaction	Red coloration
Coumarins		Positive reaction	Fluorescence under UV light
Anthocyanins		Negative reaction	No red coloration
Quinones		Positive reaction	Yellow coloration of the aqueous phase
Anthraquinones		Negative reaction	No pink coloration of the aqueous phase
Sterols/triterpenes		Positive reaction	Anneau rouge brunâtre
Terpénoides		Positive reaction	Brownish red ring
Saponosides		Positive reaction	Reddish-brown coloration at the interface
Alkaloids	Dragendroff	Positive reaction	Orange-red precipitate
Glycosides Cardiotonics	Liebermann	Positive reaction	Brownish-red coloration
	Keller-kiliani	Positive reaction	Red-brown ring Blue-green upper layer
	Baljet	Positive reaction	Stable red-orange coloration

3.1.2. Discussion

"Our results are consistent with those of Zaouani [20], who demonstrated the presence of flavonoids, tannins, coumarins, sterols, triterpenes, and terpenoids. Furthermore, recent studies conducted by Harouak [21] and Mezouar [22] on oleaster leaves have also revealed the presence of flavonoids, tannins, sterols and triterpenes, saponosides, and the absence of anthraquinones and anthocyanins. Kaskoos [23] also indicated the presence of alkaloids in wild olive leaves, which confirms our results".

The difference in results between different researchers can be explained by the fact that the composition of olive leaves in bioactive compounds varies according to their origin, climatic conditions, drying method, time and types of extraction solvents, and storage conditions [24].

3.2. Antibacterial activity

3.2.1. Results

The antibacterial activity results we obtained showed that the methanolic extract of wild olive leaves has antibacterial activity against *Bacillus cereus* ATCC 10876, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923, but this extract has no activity against *Escherichia coli* ATCC 25922.

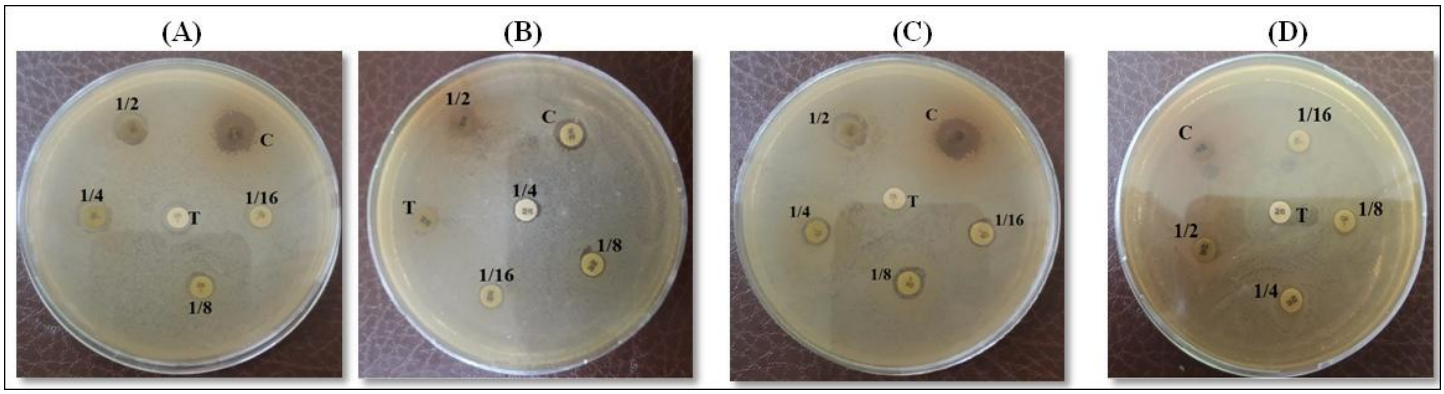

Figure 15 Antibacterial action of the methanolic extract of wild olive leaves on : *Bacillus cereus* (B)*Staphylococcus aureus* (C)*Pseudomonas aeruginosa* (D)*Escherichia coli*

Table 4 Inhibition zones of the methanolic extract of wild olive leaves on the bacterial strains tested

Strain	**Diameters of inhibition zones (mm)**					
	T-	**$C_{mère}$**	**C½**	**C¼**	**C⅛**	**$C^1/_{16}$**
Bacillus cereus ATCC 10876	0	12±0	11,33±0,44	10,66±0,88	0	0
*Staphylococcus aureus*ATCC25923	0	9±0	7±0	0	0	0
Escherichia coli ATCC 25922	0	0	0	0	0	0
Pseudomonasaeruginosa ATCC 27853	0	12,66±0,44	11±0,44	9±0,66	8±0	7±0

Table 5 Sensitivity test results for methanolic extract of wild olive leaves

Strain	**Antibacterial activity result**				
	$C_{mère}$	**C½**	**C¼**	**C⅛**	**$C^1/_{16}$**
Bacillus cereus ATCC 10876	+	+	+	-	-
Staphylococcus aureus ATCC25923	+	-	-	-	-
Escherichia coli ATCC 25922	-	-	-	-	-
Pseudomonas aeruginosa ATCC 27853	+	+	+	+	-

3.2.2. Discussion

"Our results are consistent with those obtained by Ben-Amor [25], who showed that hydroethanolic extract of wild olive leaves exhibited antibacterial activity against *Staphylococcus aureus*, with no activity against *Escherichia coli*. Furthermore, the antibacterial activity of the methanolic extract of wild olive leaves against *Pseudomonas aeruginosa* and *Staphylococcus aureus* reported in the study by Hussain [26] is comparable to that observed in our work. Djenane [27] and Nahalbouderba [28] also demonstrated the antibacterial activity of the aqueous extract of *Olea europaea* L. leaves against *Pseudomonas aeruginosa* and *Staphylococcus aureus".*

The differences in inhibition diameter may be attributed to variations in the microbial strains tested, the origin of the plant material, and the extraction technique employed (duration, agitation, solvent purity,etc) [29].

4. Conclusion

This work is part of the effort to promote the use of medicinal plant resources used in traditional medicine in Algeria. It focused on the phytochemical study and evaluation of the antibacterial activity of the leaves of the wild olive tree: *Olea europaea* L.var. *sylvestris* (Miller) Lehr., belonging to the Oleaceae family.

The results of the phytochemical screening revealed the presence of a wide range of metabolites in wild olive leaves, including polyphenols, tannins, flavonoids, coumarins, free quinones, sterols and triterpenes, terpenoids, saponins, alkaloids, and cardiotonic glycosides.

Through the study of antibacterial activity using the solid-phase diffusion method, the results obtained show that the methanolic extract of the leaves of the species studied has significant antibacterial activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, with inhibition zones of 12, 12.66, and 9 mm, respectively. However, *Escherichia coli* proved resistant to all concentrations of the extract tested.

In conclusion, the results highlight the notable phytochemical and antibacterial potential of *Olea europaea L*. var. *sylvestris* (Miller) Lehr. This species could be a promising source of bioactive compounds of pharmaceutical interest.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

I declare and all co-authors, that we participated in the design, execution, and analysis of the document and that I approve the final version. In addition, there is no conflict of interest in connection with this document, and the materiel described is not the process of being published nor is it intended for publication elsewhere.

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