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PHENOLIC COMPOUNDS, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF THE METHANOLIC EXTRACT FROM THE SAHARAN ENDEMIC PLANT *HALOXYLON SCOPARIUM* POMEL

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This study aimed to investigate the phenolic profile, antioxidant, and antibacterial activities of the methanolic extract of Haloxylon scoparium Pomel, an endemic plant of the Algerian Sahara. Quantitative analysis revealed total phenolic content of 54.77 mg GAE/g DM, flavonoids at 9.39 mg CE/g DM, and total tannins at 5.36 mg TAE/g DM. High-Performance Liquid Chromatography identified several phenolic compounds, including chlorogenic acid, caffeic acid, quercetin, catechin, rutin, and kaempferol. Antioxidant activity, assessed using the DPPH radical scavenging assay, demonstrated a strong activity with an IC_{50} value of 68.64 µg/ml. The methanolic extract exhibited significant antibacterial activity against various pathogenic bacteria, including Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae, with inhibition zones ranging from 19.00 to 22.10 mm. The extract also showed promising activity against two multidrug-resistant strains. These findings support the potential use of H. scoparium as a valuable source of bioactive compounds, offering significant antibacterial properties that could be further explored for the development of alternative therapeutic agents.

Keywords: *Haloxylon scoparium, methanolic extract, HPLC analysis, antioxidant, antibacterial*

INTRODUCTION

Scientific studies on medicinal plants based on ethnobotanical research often yield significant results in terms of therapeutic properties. This is largely due to the extensive experience of indigenous populations, which has guided the selection of specific species for the treatment of various ailments. Traditional medicine based on herbal remedies is deeply

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rooted and highly respected in Algeria, particularly in the Saharan regions, where many remedies have been validated by phytochemical studies.

Among the most widely used endemic plants, *Haloxylon scoparium* Pomel, locally known as "Remth", is especially valued in the southwestern region for its therapeutic properties. This halophytic shrub belongs to the *Amaranthaceae* family and is endemic to the southwestern Sahara of Algeria, as well as other semi-arid regions across North Africa and the Middle East[1]. *H. scoparium* thrives in arid environments, capable of colonizing regions with harsh conditions, including drought, salinity, and low rainfall, making it an important species for soil conservation in steppe planting[2]. This species was used for centuries by local communities to treat conditions such as cancer, inflammation[3], common cold, poison, snake stings[4], diabetes, and wounds[5].Studies have highlighted its potential as an antidiabetic[6], antioxidant[7], and anti-leukemic agent [8].

Given the wide range of biological activities attributed to *H. scoparium*, our study aims to investigate the phenolic, flavonoid, and tannin contents of its methanolic extract, and identify phenolic compounds via HPLC analysis. Additionally, we assess the antioxidant activity of the extract and evaluate its antibacterial potential against a selection of pathogenic bacterial strains.

MATERIAL AND METHODS

Plant material

Haloxylon scoparium Pomel, from the *Amaranthaceae* family, was collected in 2022 in Bechar Province. The plant was identified by Dr. A. Abdelhakem from the National Nature Agency, and the voucher specimen (MPE-12-191) was deposited in the Chiral Separation Laboratory (BMCS) and the Herbarium of Bioactive Molecules in the Medicinal Plant Encyclopedia, Bechar, Algeria. The aerial parts of the plant were isolated, cleaned, dried, and ground to obtain the plant powder.

Methanolic extraction

The plant powder was macerated in methanol (80%) at 10% for 24 hours at room temperature. After filtration, the mixture was evaporated using a rotary evaporator at 40°C. The obtained crude extract was then stored at 4°C until use.

Total Phenolic Content (TPC)

The total polyphenol content was assessed using the Folin-Ciocalteu method [9], with some modifications. A 0.2 mL aliquot of each sample dilution was mixed with 1 mL of Folin-Ciocalteu reagent (diluted tenfold). After 4 minutes, 0.8 mL of 7.5% sodium carbonate (Na_2CO_3) was added. The mixture was incubated for 2 hours at room temperature in the dark. The absorbance of each solution was measured at 765 nm. A calibration curve was constructed using gallic acid (20-100 µg/mL), and the results are expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

Total Flavonoid Content (TFC)

A 1 mL aliquot of the sample was mixed with 4 mL of distilled water, followed by the addition of 300 µL of 5% $NaNO_2$. After 5 minutes of incubation, 300 µL of 10% $AlCl_3$ was added. 6 minutes later, 2 mL of 1M NaOH was introduced into the mixture. The volume of the solution was then adjusted to 10 mL with distilled water, and the absorbance was measured at 510 nm[10]. A calibration curve was constructed using catechin, and the results were expressed as milligrams of catechin equivalent per gram of dry matter (mg CE/g DM).

Total Tannins Content (TTC)

5 mL of Folin-Denis reagent and 1 mL of 0.5% sodium carbonate solution were added to calibrated 5 mL volumetric flasks. Subsequently, 100 µL of the methanolic extract dilution was introduced, and the total volume was adjusted to 5 mL with distilled water. The mixture was incubated for 30 minutes[11]. The absorbance was then measured at 775 nm using a UV-Visible spectrophotometer. The results were expressed as milligrams of tannic acid equivalent per gram of dry plant material (mg TAE/g DM).

Chromatographic analysis

The Chromatographic analysis was conducted using a Shimadzu HPLC system equipped with a UV detector and a C18 chromatographic column (250 mm x 4.6 mm).

The flow rate was set at 1 mL/min, with an injection volume of 10 µL, and the column temperature was maintained at 40°C. All analyses used high-purity HPLC-grade solvents, and detection was performed using a UV-Visible detector at a wavelength of 350 nm [12]. Product identification was based on comparing retention times with reference standards. The total run time for the analysis was 60 minutes.

Free radical scavenging activity (DPPH assay)

A 2 mL aliquot of DPPH solution was added to 2 mL of the sample, and the mixture was incubated in the dark for 30 minutes at room temperature[13]. The absorbance was measured at 517 nm, and the inhibition percentage was calculated using the following formula:

$$\text{Inhibition } \% = (A_{control} - A_{sample}) / (A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample.

Antibacterial activity

The antibacterial efficacy of the methanolic extract was evaluated using the disc diffusion method. The assay included four American Type Culture Collection (ATCC) strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853. In addition, two multidrug-resistant strains isolated from urine samples were also tested. Before antibacterial tests, the strains were cultured for 24 hours, and microbial suspensions were standardized to 0.5 McFarland turbidity. Microbial suspensions (100µL) were spread on the surface of Mueller-Hinton agar plates. Discs impregnated with 30 µL of the methanolic extract at concentrations of 100 mg/mL and 50 mg/mL were placed on the inoculated agar plates. Dimethyl sulfoxide (DMSO) was used as a negative control. After incubation for 24h at 37°C, inhibition diameter zones were measured.

Statistical analysis

All experiments were performed in triplicate, with data presented as mean ± standard deviation and analyzed using Excel (2016).

RESULTS AND DISCUSSION

Total phenolic, flavonoid and tannin contents

The results of the phenolic, flavonoid, and tannin content measurements in the methanolic extract of *H. scoparium* are shown in **Table 1**.

The total phenolic content (TPC) in the methanolic extract of *Haloxylon scoparium* yielded 54.77 mg GAE/g DM. This is comparable to a TPC value of 59.75 mg GAE/g DM found in a methanolic extract of *H. scoparium* leaves[7]. However, other studies reported significantly higher values of phenolic content of 228.58 mg GAE/g DMand259.44 mg GAE/g DM respectively[6,14]. This variability in phenolic concentrations may be attributed to various factors, including the origin of the samples and the extraction methods.

Regarding flavonoids content, the extract exhibited a value of 9.39 mg CE/g DM, which is notably higher than the value reported for *Haloxylon scoparium* leaves from the northeastern region of the Algerian Sahara (0.95 mg QE/g DM)[15].Nonetheless, it is comparable to the result of 5.91 mg EQ/g DM found in the methanolic extract of *Haloxylon scoparium* leaves from Tunisia[7].For total tannin content, the extract showed a concentration of 5.36 mg TAE/g DM, which is close to the value reported by another study[14] estimated at 4.12 mg CE/g DM. In contrast, distinct values were reported for condensed tannins (2.55 mg CE/g DM) and hydrolysable tannins (24.28 mg TAE/g DM)[16].

Table 1: Quantitative measurements of total phenolics (TPC), flavonoids (TFC) and total tannins (TTC) contents.

TPC (mg GAE/g DM)	**TFC** (mg CE/g DM)	**TTC** (mg TAE/g DM)
54,77 ±1,93	9,39 mg ±2,76	5,36 mg ± 2,66

HPLC analysis

The chromatographic analysis of the extract, presented in **Table 2**, revealed the presence of some phenolic compounds (**Fig. 1**).

The methanolic extract of the aerial parts of *Haloxylon scoparium*, analyzed by HPLC-UV, reveals a significant presence of phenolic compounds, including chlorogenic acid, caffeic acid, quercetin, catechin, rutin, and kaempferol, all of which are well-known for their antioxidant and antibacterial properties. These findings are consistent with those found in the aerial parts of *Haloxylon scoparium* from southeastern Algeria[6], which contain caffeic acid, quercetin, and rutin, though their study highlighted a wider range of phenolic compounds, including gallic acid. Similarly, another study[17] reported the presence of some overlapping compounds, such as chlorogenic acid and quercetin. The presence of these bioactive compounds may contribute to the biological activities of the plant extract.

Antioxidant activity

The antioxidant activity of the methanolic extract of *Haloxylon scoparium*, as estimated by the DPPH test, is presented in **Fig. 2**.

The extract of *H. scoparium* demonstrates notable antioxidant activity, showing a gradual increase in inhibition reaching approximately 90% at a concentration of around 125 mg/mL. Half-maximal inhibitory concentration (IC_{50}) value was determined at 68.64 ± 0.065 µg/mL, indicating a strong antioxidant activity of the methanolic extract. This value is lower than the IC_{50} of 660 µg/mL reported for an aqueous extract[16] and closer to the IC_{50} of 8.8 µg/mL reported for a methanolic extract[14], indicating the impact of the solvent type on antioxidant efficacy.

Table 2: HPLC-UV determined phenolic compounds of *Haloxylon scoparium* Pomel. extract.

Peak	**Ret. Time**	**Compound**
01	5,227	Chlorogenic acid
02	6,109	Caffeic acid
03	9,535	Rutin
04	15,332	Quercetin
05	26,664	Kaempferol
06	34,625	OH-methoxy flavone
07	37,690	Catechin

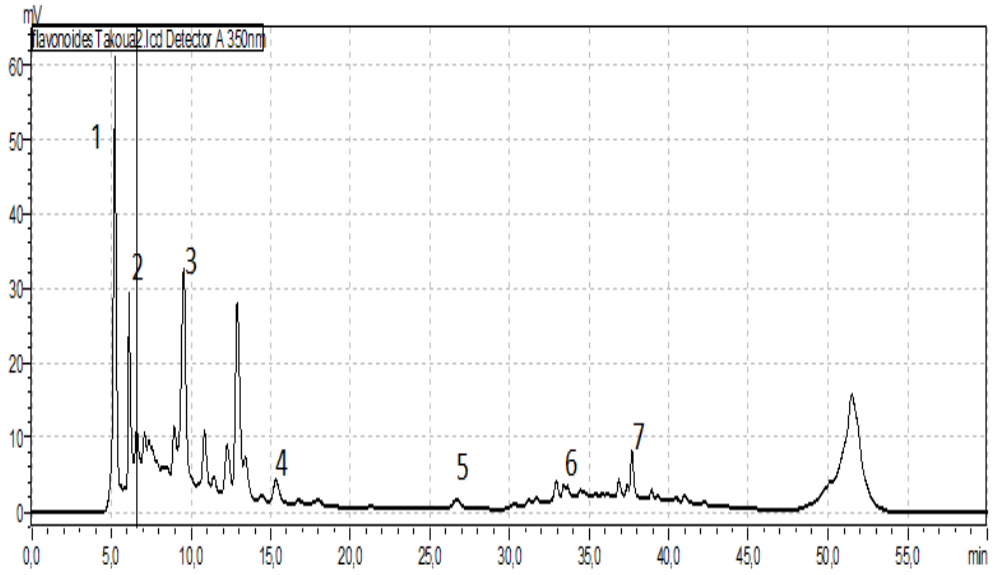

Fig. 1: HPLC chromatogram of phenolic compounds (60 nm).
Peaks: Chlorogenic acid (1), Caffeic acid (2), Rutin (3), Quercetin (4), Kaempferol (5), OH-methoxy flavone (6) and Catechin (7)

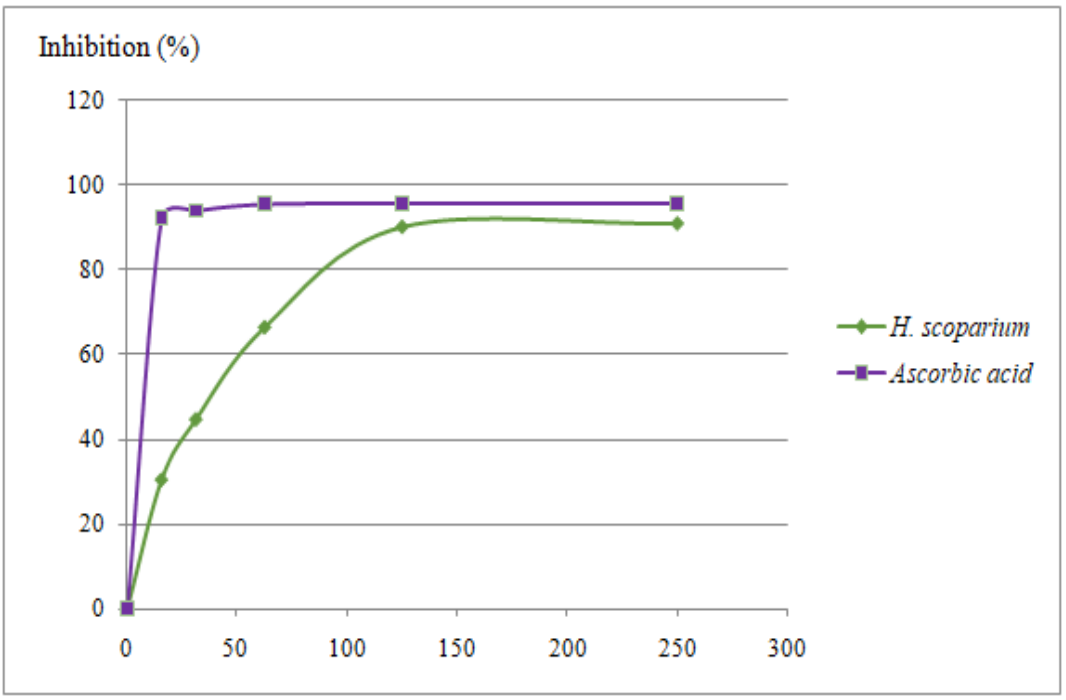

Fig. 2: DPPH radical scavenging of *H. scoparium* methanolic extract and ascorbic acid.

Antibacterial activity

The results of the antibacterial test of the methanolic extract of *H. scoparium* against pathogenic bacteria are presented in **Fig. 3**.

The methanolic extract of *Haloxylon scoparium* exhibited significant activity against *E. coli* ATCC 25922 (19.50 mm), *S. aureus* ATCC 25923 (20.1 mm), *P. aeruginosa* ATCC 27853(22.10 mm), and *K. pneumoniae* ATCC 700603 (19.00 mm). These values are comparable to the inhibition diameters reported by another study [7] with zones ranging from 18 to 25 mm for the same strains, with the highest result observed for *S. aureus* (25 mm). In

contrast, it was reported that the methanolic extract of *H. scoparium* at a concentration of 500 mg/ml showed no antibacterial activity against the four strains, and only the ethyl acetate extract exhibited moderate antibacterial activity against *S. aureus*[18]. Variations in the effect of the extracts may be due to several extraction parameters[19].Furthermore, our study also demonstrated promising activity against multidrug-resistant strains, with inhibition zones of 14.55 mm for *E. coli* and 16.99 mm for *P. aeruginosa*. This result indicates the potential of *H. scoparium* to be effective against resistant pathogens and suggests that the extract may contain antibacterial compounds capable of inhibiting the tested strains. Indeed, some phenolic compounds detected by HPLC in the methanolic extract of *H. scoparium* were previously documented by several studies to exhibit significant antibacterial properties. For example, caffeic acid is known for its strong bactericidal effects by increasing the permeability of the outer and plasma membranes of *Pseudomonas aeruginosa*[20]. Quercetin has been shown to enlarge bacterial cell diameter and alter membrane fluidity, causing structural disorganization that underlies its antibacterial action against *Staphylococcus aureus*[21]. Furthermore, kaempferol demonstrates effective antibacterial activity against *Escherichia coli* by damaging the cell membrane and inducing bacterial protein leakage[22]. Rutin exhibits similar antibacterial effects against both Gram-positive and Gram-negative bacteria[23], while catechins, especially epicatechin and epigallocatechin, show potent bactericidal activity by targeting bacterial lipid membranes and disrupting the lipid bilayer[24]. These mechanisms may explain the promising antibacterial results observed with the methanolic extract of *H. scoparium*, supporting the search for resources of therapeutic agents, including antimicrobial compounds, that could serve as alternatives to antibiotics in the treatment of antibiotic-resistant bacteria [25].

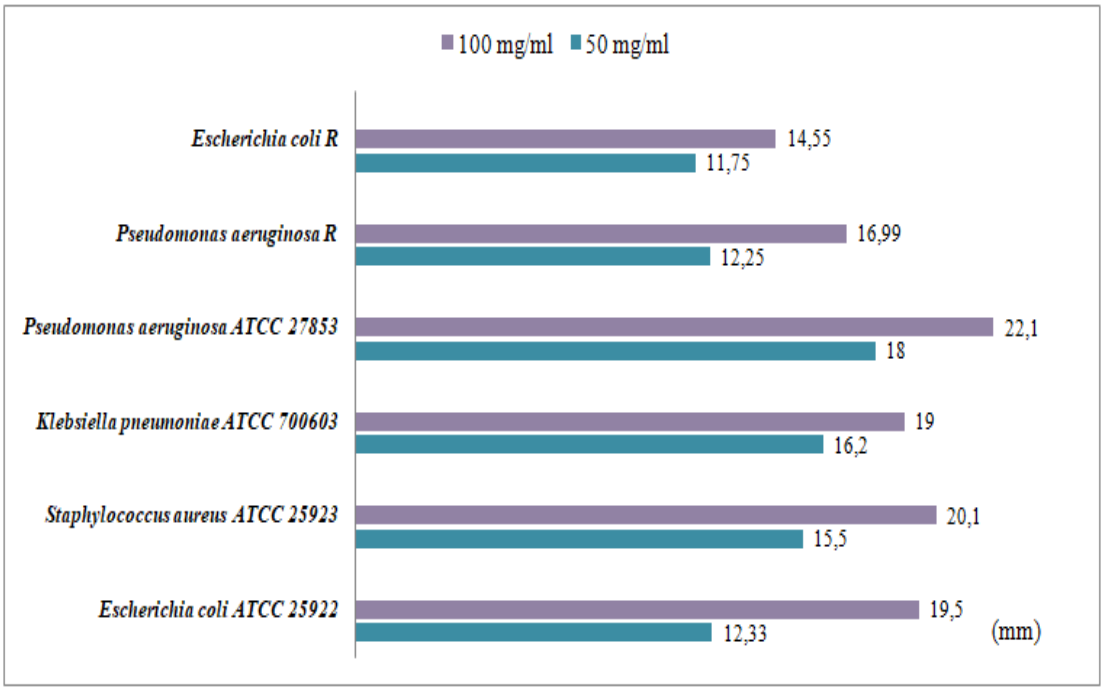

Fig. 3: Mean inhibition zones for the disc diffusion assay of *H. scoparium* extract.

Conclusion

In conclusion, the methanolic extract of *Haloxylon scoparium* demonstrates a rich composition of phenolic compounds associated with notable antioxidant and antibacterial activities. The total phenolic content, flavonoid levels, and the presence of specific phenolic compounds such as caffeic acid, quercetin, and kaempferol contribute to its biological efficacy. The extract demonstrated significant inhibition of pathogenic bacteria, including multidrug-resistant strains, highlights its potential for medicinal applications. These results underscore the importance of *Haloxylon scoparium* as a valuable source of natural compounds that could be further explored for developing effective therapeutic agents against microbial infections.

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نشرة العلوم الصيدليـــة
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المركبات الفينولية والأنشطة المضادة للأكسدة والمضادة للميكروبات للمستخلص الميثانولي للنبات الصحراوي المتوطن *Haloxylon scoparium Pomel*

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[٩] مخبر الكيمياء التحليلية، قسم الصيدلة، كلية الطب، جامعة باجي مختار، ٢٣٠٠٠، عنابة، الجزائر

تهدف هذه الدراسة إلى الكشف عن التركيب الفينولي والنشاطات المضادة للأكسدة والمضادة للبكتيريا للمستخلص الميثانولي من *Haloxylon scoparium* Pomel، وهو نبات مستوطن في صحراء الجزائر. كشفت التحليلات الكمية عن المحتوى الإجمالي من الفينولات البالغ ٥٤,٧٧ ملغم/GAE غرام من المادة الجافة، والفلافونويدات بنسبة ٩,٣٩ ملغم/CE غرام من المادة الجافة ، والتانين الإجمالي بنسبة ٥,٣٦ ملغم/TAE غرام من المادة الجافة، وقد حددت كروماتوغرافيا السائل عالية الأداء العديد من المركبات الفينولية، بما في ذلك حمض الكلوروجينيك، حمض الكافييك، كيرسيتين، كاتيشين، روتين، وكامبفيرول.

أظهر النشاط المضاد للأكسدة، الذي تم تقييمه باستخدام اختبار امتصاص الجذور الحرة DPPH، نشاطًا قويًا مع قيمة التركيز المثبط بنسبة ٥٠٪ تساوي ٦٤.٦٨ ميكروغرام/مل. كما أظهر المستخلص الميثانولي نشاطًا مضادًا للبكتيريا فعالا ضد العديد من البكتيريا الممرضة، بما في ذلك *Escherichia coli* ، *Staphylococcus aureus*، *Pseudomonas aeruginosa*، و *Klebsiella pneumoniae*، مع مناطق تثبيط تتراوح بين ٠٠.١٩ إلى ١٠.٢٢ مم. كما أظهر المستخلص نشاطًا

واعدًا ضد سلالتين مقاومتين لعدة أدوية. تدعم هذه النتائج الاستخدام المحتمل لـــ *H. scoparium* كمصدر للمركبات النشطة بيولوجيًا ذات خصائص مهمة مضادة للبكتيريا.