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Research Article

PHYTOCHEMICAL SCREENING AND EVALUATION OF THE ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF SAHARAN *Asphodelus tenuifolius* CAV

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ABSTRACT. *Asphodelus tenuifolius* Cav. is a traditional medicinal plant used in arid regions for its therapeutic properties. The present study aimed to investigate the phytochemical composition, antioxidant capacity, and antibacterial activity of ethanolic extracts (EtOH/H_2O, 75:35, v/v) from the aerial parts and roots of *A. tenuifolius* Cav. harvested in the region of Ghardaïa (Algerian Sahara). Phytochemical screening showed significant levels of polyphenols, flavonoids, and tannins. The antioxidant activity was determined using DPPH free radical assay and revealed a significant concentration-dependent scavenging capacity. The aerial parts extract exhibited significantly higher antioxidant activity (IC_{50} = 51.22 ± 4.15 µg/ml) compared to the root extract (IC_{50} = 88.33 ± 4.15 µg/ml; $p < 0.05$). Furthermore, the extracts also exhibited good antibacterial activity against the tested bacterial pathogens. *A. tenuifolius* from Ghardaïa proves to be a promising candidate for bio-sourced antimicrobial and antioxidant agents.

Keywords: *Asphodelus tenuifolius, Ghardaïa, Phytochemical screening, Antioxidant activity, DPPH, Antibacterial activity*

INTRODUCTION

The use of traditional medical systems, such as Ayurveda, Traditional Chinese Medicine (TCM), and African herbal medicine, has substantially helped in the identification of bioactive compounds. These systems provide a wealth of information on therapeutic uses and provide insight into possible bioactive compounds that may be turned into medications [1]. To this end, Algeria possesses a unique floristic biodiversity, complemented by an age-old heritage of traditional pharmacopeia [2].

In this context, *Asphodelus tenuifolius* Cav., a prominent medicinal species from the Saharan flora of Ghardaïa, represents a promising candidate for exploring new natural bioactive molecules. This plant is found in North Africa and in the southwestern regions of Asia and Europe [3]. In Algeria, it grows in dunes, arid pastures, and steppes. It is common in the Oran coastal sahels, the High Plateaus, and the Sahara. It is an acaulescent plant with fasciculate and nearly napiform roots. The leaves are linear, 20–30 cm long, with ciliated margins. The flowers are large and white, with dark-veined tepals. Capsules are borne on an elongated peduncle, and the seeds are finely pitted [4].

Beyond its botanical features, *A. tenuifolius* holds a significant place in Saharan folk knowledge, where it is valued for both its nutritional and traditional medicinal uses. Among the Tuareg, the leaves of *A. tenuifolius*, which resemble onion seedlings, are consumed boiled, with the cooking water discarded. The seeds, known as *Tebhit*, are highly prized and eaten like other seeds in porridges, flatbreads, or combined with crushed dates. A Touareg proverb states: *"COUD IOUNER OUL IZEAN"* ("When I am full, I would not even eat an *Izean*"). During a severe food scarcity in Mertoûtek (southern Algeria) around the turn of the century, people ingested significant amounts of *A. tenuifolius* Cav.'s flower stalks, which are delicious when young [5].

The aim of our study is to evaluate the antibacterial and antioxidant activities of the hydroethanolic extract (ethanol/water: 75/35, v/v) of *Asphodelus tenuifolius* Cav. harvested from the region of Ghardaïa (Algeria).

MATERIALS AND METHODS

Plant material

A. tenuifolius Cav. was harvested in March 2021 from the Oued Zergoun site, a valley or ravine bounded by relatively steep banks that turns into a watercourse during the rainy season, located in the Wilaya of Ghardaïa (Northern Saharan Algeria), at an altitude of 822 meters. The plant species was identified using the flora key of Quézel and Santa [4]. A voucher specimen was deposited in the herbarium of the Laboratory of Medical Botany and Cryptogamy, Department of Pharmacy, Faculty of Medicine, University of Tizi Ouzou, Algeria.

The extraction of dry, powdered aerial parts and roots of *A. tenuifolius* (100 g), following defatting with petroleum ether, was performed by ultrasound-assisted extraction using aqueous ethanol ($EtOH/H_2O$, 75:35, v/v) for 30 min, repeated three times. After evaporation to dryness under reduced pressure at 50 °C, a crude extract of 23 g was obtained.

Phytochemical Screening

The qualitative chemical analysis of powders obtained from various plant organs was performed to identify the main chemical groups. Identification is based on either precipitation reactions (yielding insoluble complexes) or color-forming reactions associated with molecular conjugation or unsaturation [6, 7, 8, 9].

The results are classified according to their appearance as:

Strongly positive reaction: +++;

Positive reaction: ++ ;

Moderately positive reaction: +;

Doubtful reaction: ± ;

Negative reaction: -.

Antioxidant Activity (DPPH Free Radical Scavenging Assay)

Stock solutions of each extract were prepared by serial dilution in methanol. In brief, 0.1 ml of each dilution was mixed with 3.9 ml of a 60 μM methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl). The reaction mixtures were incubated at room

temperature in the dark for 30 min. Absorbance was then measured at 517 nm using a spectrophotometer.

Ascorbic acid was employed as a positive standard. A control was prepared under similar operational conditions with pure methanol instead of plant extract [10]. All measurements were performed in triplicate. Radical scavenging activity was reported as percentage inhibition using Eqn. 1:

Eqn. 1 Radical Scavenging Activity (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

$A_{control}$ = Absorbance of the control at 517 nm.

A_{sample} = Absorbance of the extract or standard at 517 nm.

The IC_{50} value, defined as the concentration of extract required to scavenge 50% of the DPPH radicals, was determined graphically.

The results of the present study were analyzed using Minitab 18 software, applying a one-way analysis of variance (one-way ANOVA) with a significance level of 5%. This test allowed us to determine whether the samples originated from the same population or exhibited significant differences.

Evaluation of antibacterial activity

In this study, agar disc diffusion and broth microdilution assays were used to assess the potential antibacterial activity of the plant extracts. All tests were conducted in accordance with CLSI (Clinical and Laboratory Standards Institute) guidelines.

The bacterial strains used were reference strains from the American Type Culture Collection (ATCC), provided by the microbiology laboratory of the Tizi Ouzou University Hospital Center (CHU Tizi Ouzou), and were subjected to preliminary identification by Gram staining. The morphological characteristics and origins of the selected bacteria are summarized in Table 1.

Table 1. *Characteristics of the Bacterial Strains Used in This Study*

Bacteria type and Gram morphology	Species	ATCC	Pathogenicity / Associated infections
Gram-negative bacillus	*Escherichia coli*	25922	Urinary tract infections, septicemia, respiratory tract infections.
	Pseudomonas aeruginosa	27853	Urinary and pulmonary infections, burns, wounds, septicemia.
	Klebsiella pneumoniae	4392	Urinary and pulmonary infections, wounds, septicemia.
Gram-positive coccus	*Staphylococcus aureus*	43300	Skin infections, wounds, burns, septicemia, food poisoning, osteitis, pulmonary infections, endocarditis.
Gram-positive bacillus	*Bacillus cereus*	10876	Food poisoning, septicemia in immunocompromised patients.
Gram-positive coccus	*Enterococcus faecalis*	494502	Endocarditis, urinary tract infections, prostatitis, cellulitis, wounds, septicemia.

Agar Diffusion Procedure

Stored bacterial strains were inoculated into tubes containing Brain Heart Infusion Broth (BHIB) and incubated at 37 °C for 24 h to stimulate their growth. Subculturing was performed on Mueller-Hinton agar plates, which were then incubated at 37 °C for 24 h.

To prepare the inoculum, four to five well-isolated colonies were selected, transferred with a loop into a tube of sterile 0.9% saline solution, and homogenized using a vortex mixer. The optical density of the suspension was measured with a spectrophotometer (1 cm path length). An absorbance of 0.08 to 0.13 at 625 nm was obtained, corresponding to a 0.5 McFarland standard (~ 10^8 CFU/ml).

The bacterial inoculum was prepared and seeded onto Mueller-Hinton agar plates within 15 minutes. A sterile cotton swab was immersed in the bacterial suspension, with excess liquid removed by pressing and rotating the swab against the inner tube wall. The agar surface was thoroughly swabbed in three directions, including the outer perimeter of the plate.

Sterile dimethyl sulfoxide (DMSO) was used to dissolve dry plant extracts at a concentration of 200 mg/ml. A volume of twenty microliters (20 µl) of each extract was applied onto 6-mm diameter filter paper discs, which were then placed on the surface of the inoculated agar.

Sterile disks impregnated with DMSO (20 µl) and chloramphenicol (30 µg), which served as negative and positive controls respectively, were also placed on the inoculated agar surface. The plates were then incubated at 37 °C for 18 hours. All tests were performed in triplicate for each extract.

Plant extracts were considered active when an inhibition zone exceeding 6 mm in diameter was observed around the disc. Measurements were recorded with a ruler on the bottom of the Petri dish.

Determination of Minimum Inhibitory Concentration (MIC) by the Broth Microdilution Method

The MIC values of the plant extracts against different bacterial strains were determined using the resazurin microdilution assay, as outlined by Drummond and Waigh [11]. This resazurin assay is simple, sensitive, rapid, robust, and reliable [11].

The plates were prepared under aseptic conditions. Each well of the 96-well microplates received 100 µl of Mueller-Hinton broth, 100 µl of plant extract (or chloramphenicol control), 10 µl of inoculum, and 10 µl of resazurin.

For each plate, a sterility control (plant extract, MH broth, and resazurin), a positive control (chloramphenicol, inoculum, MH broth, and resazurin), and a negative control (inoculum, MH broth, and resazurin) were included.

Stock solutions of plant extracts (200 mg/ml) and chloramphenicol (0.001 mg/ml) were prepared in DMSO.

To determine the MIC values of the extracts and the positive control, twelve serial dilutions were prepared. A volume of 100 µl of plant extract or chloramphenicol and 100 µL of MH broth were added and mixed in the first well. Serial dilutions were carried out by transferring 100 µl down eleven successive wells containing 100 µl of MH broth. Finally, 100 µl was discarded from the last well. Each well was subsequently inoculated with 10 µl of bacterial suspension and supplemented with 10 µL of resazurin solution. The contents of each well were thoroughly mixed using a micropipette, and the microplates were incubated at 37 °C for 18–24 h.

The color change was then assessed visually. Any color variation from purple to pink or colorless was recorded as positive for bacterial growth. Microplates were prepared in triplicate. The MIC value was established as the lowest extract concentration preventing visible color change (the final well in which no color change was observed).

Determination of Minimum Bactericidal Concentration (MBC)

Following MIC determination, 5 µl aliquots from all wells exhibiting no visible bacterial growth were subcultured onto nutrient agar. Bacterial viability was assessed after 24 h of incubation at 37 °C. The MBC of the antibacterial extracts was defined as the lowest concentration yielding no visible growth. All MBC assays were performed in triplicate.

RESULTS AND DISCUSSION

Phytochemical Screening

The results of the phytochemical screening are summarized in Table 2.

Table 2. *Phytochemical Screening Results of Different Organs of A. tenuifolius*

Phytochemical Compounds	Aerial Parts	Roots
Alkaloids	+	+
Tannins		
Catechin tannins	++++	++++
Gallic tannins	-	-
Flavonoids	++++	++
Saponins	+	+
Foam index	<100	<100
Anthracene derivatives	++++	++++
Cardiac glycosides	-	-
Sterols and triterpenes	++++	++++
Coumarins	-	-

The phytochemical screening of *A. tenuifolius* revealed the presence of anthraquinones, a major class of chemical constituents, in both aerial parts and roots. This finding is consistent with the established chemotaxonomic traits of the Asphodelaceae family, in which these specialized metabolites serve as essential taxonomic markers [12].

Additionally, secondary metabolite screening showed strongly positive results for polyphenolic compounds (flavonoids and tannins), triterpenoids, and sterols, which have noteworthy biological activities. In contrast, tests for alkaloids and saponins yielded weakly positive reactions, whereas cardiac glycosides and coumarins were not detected. The total absence of cardiac glycosides significantly reduces the toxicological risks associated with the use of *A. tenuifolius*, providing a rationale for its culinary and ethnomedicinal applications as a food plant and condiment in southern Algeria. These results fully align with previous reports [13].

Antioxidant Activity

The kinetic study of this activity permitted the determination of the half-maximum inhibitory concentration (IC_{50}) values for both extracts and ascorbic acid, in µg/ml. Figs. 1–3 display the variation in % inhibition as a function of the concentration of ascorbic acid and each *A. tenuifolius* extract. The percentage of free radical scavenging activity increased with a proportionally increasing concentration.

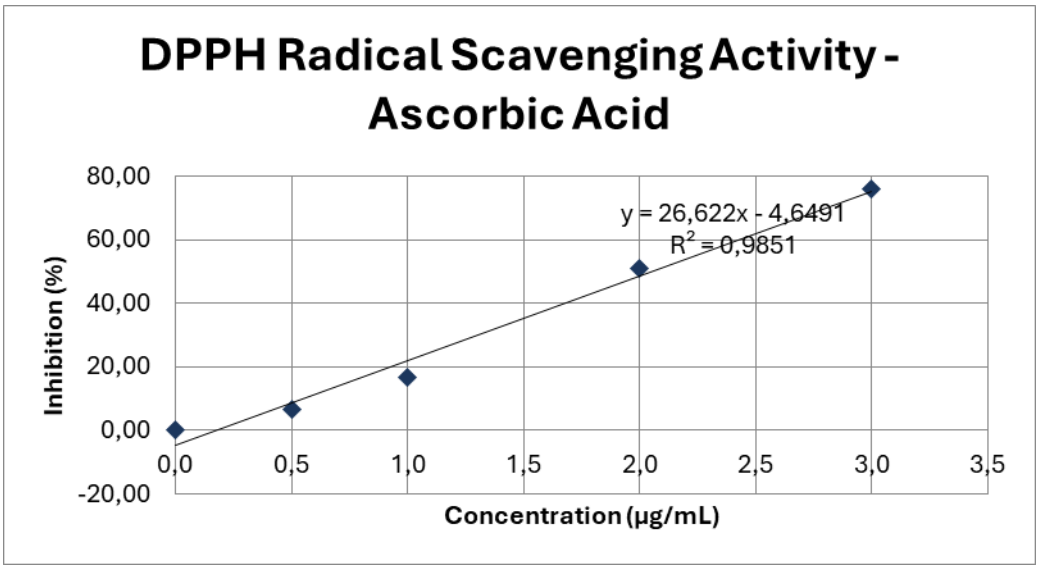


Fig. 1. *DPPH free radical inhibition percentages as a function of ascorbic acid concentration.*

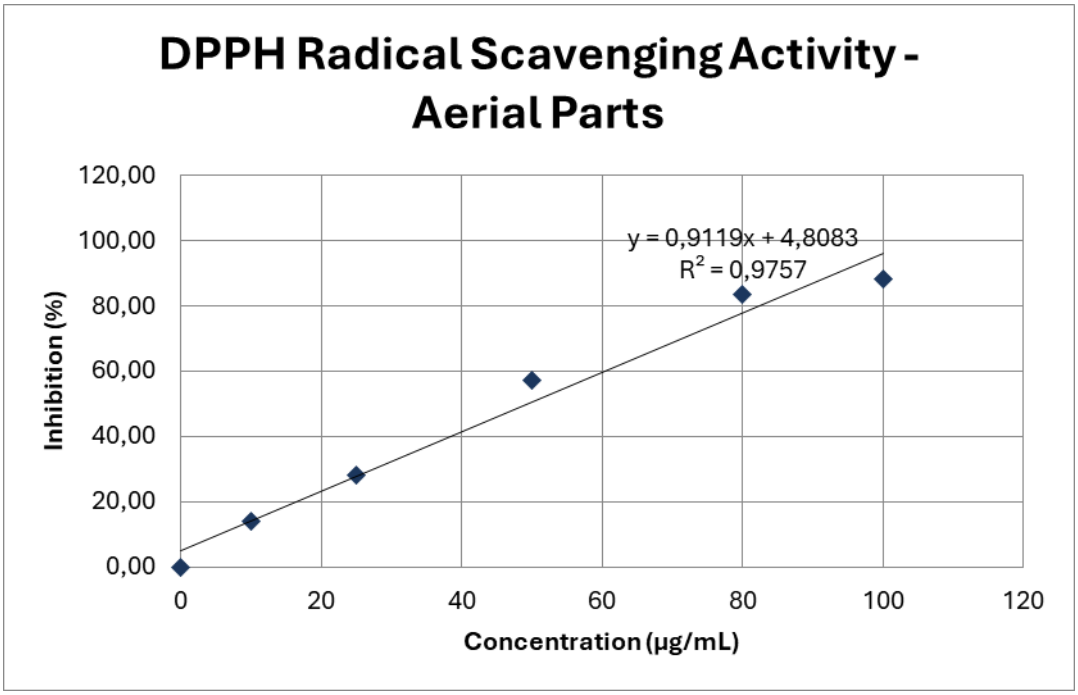


Fig. 2. *DPPH free radical inhibition percentages as a function of concentration for the ethanolic extract (EtOH/H_2O, 75:35, v/v) of A. tenuifolius Cav. aerial parts.*

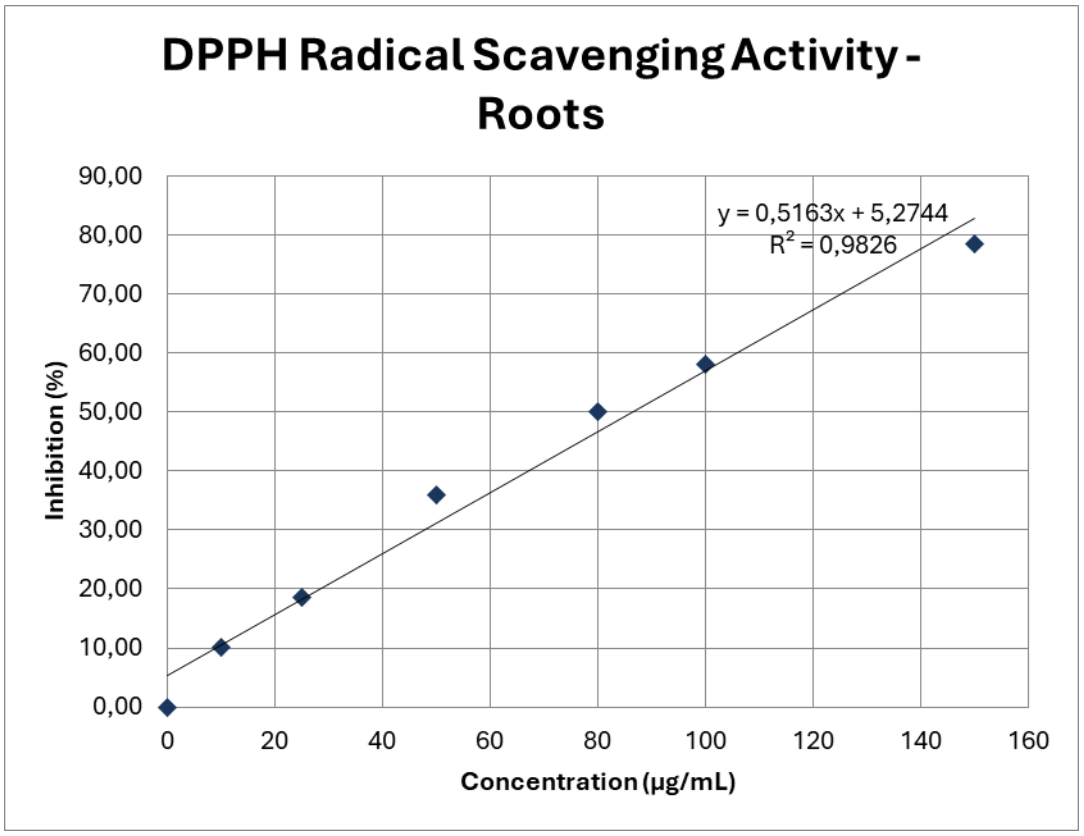


Fig. 3. *DPPH free radical inhibition percentages as a function of concentration for the ethanolic extract (EtOH/H_2O, 75:35, v/v) of A. tenuifolius Cav. roots.*

The IC_{50} values and radical scavenging activities of the extracts were calculated from the linear regression equations of the respective graphs. The obtained values are shown in Table 3.

Table 3. *IC_{50} values of A. tenuifolius Cav. extracts and ascorbic acid.*

Extracts / Standards	IC_{50} (µg/ml)	AAR (1 / IC_{50})
Ethanolic extract of aerial parts	51.22 ± 4.06 ^{B}	0.0195
Ethanolic extract of roots	88.33 ± 4.15 ^{C}	0.0113
Ascorbic acid	2.05 ± 0.04 ^{A}	0.48

Values represent the mean of three measurements ± SD (Standard Deviation). ANOVA test, $p < 0.05$. A, B, and C indicate significant differences according to Tukey's test.

The aerial parts and roots exhibited distinct antioxidant activities, with IC_{50} values of 51.22 µg/ml and 88.33 µg/ml, respectively. ANOVA statistically corroborated this difference ($p < 0.05$). In addition, Tukey's post-hoc test for multiple comparisons revealed significant differences for all measured values.

In a study on the same plant species in India, the root methanolic extract was reported to have an IC_{50} value of 2006 µg/ml. This value is much larger than that found in the present investigation, which indicates a reduced radical scavenging activity [14].

Evaluation of antibacterial activity

Table 4. *Antibacterial activity (inhibition zone diameter in mm) of Asphodelus tenuifolius extracts and Chloramphenicol.*

Bacterial species	Aerial parts (*A. tenuifolius*)	Roots (*A. tenuifolius*)	Chloramphenicol (Standard control)
Escherichia coli	6C	6D	32.66 ± 1.15^{A}
Pseudomonas aeruginosa	6C	6D	14.66 ± 1.15^{C}
Klebsiella pneumoniae	15.33 ± 1.15A	13.66 ± 0.57B	28.66 ± 1.15^{AB}
Bacillus cereus	10.33 ± 1.52B	14.33 ± 0.57B	29.33 ± 2.30^{AB}
Staphylococcus aureus	9.66 ± 0.57B	23.33 ± 1.15A	31.33 ± 2.30^{A}
Enterococcus faecalis	9.66 ± 0.57B	10.66 ± 1.15C	28^{B}

Values represent the mean of three measurements ± SD (Standard Deviation). Means sharing different superscript letters (A, B, C, D) within the same assay are significantly different according to Tukey's test (ANOVA, $p < 0.05$).

Table 5. *Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Asphodelus tenuifolius extracts and Chloramphenicol.*

Bacterial species	Aerial parts (mg/ml)MIC / MBC	Roots (mg/ml)MIC / MBC	Chloramphenicol (µg/ml)MIC / MBC
Escherichia coli	12.5 / 12.5	12.5 / 12.5	0.003 / 0.003
Pseudomonas aeruginosa	6.25 / 6.25	3.125 / 25	0.125 / 0.250
Klebsiella pneumoniae	0.781 / 0.781	6.25 / 6.25	0.003 / 0.003
Bacillus cereus	0.390 / 0.390	0.390 / 0.390	0.062 / 0.062
Staphylococcus aureus	0.390 / 0.390	0.390 / 6.25	0.003 / 0.003
Enterococcus faecalis	0.390 / 0.390	0.390 / 0.390	0.003 / 0.062

MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration.

According to the results of the agar diffusion assays, *A. tenuifolius* extracts displayed antibacterial activity against all tested Gram-positive strains. However, all Gram-negative bacteria were resistant except Klebsiella pneumoniae.

The highest inhibition zones were demonstrated with the root extract against *Staphylococcus aureus* (23.33 mm), *Bacillus cereus* (14.33 mm), and *Klebsiella pneumoniae* (13.66 mm), as well as with the aerial parts extract against *Klebsiella pneumoniae* (15.33 mm). Furthermore, acceptable antibacterial activity was obtained for aerial parts extract against Bacillus cereus (10.33 mm), *Staphylococcus aureus* (9.66 mm) and *Enterococcus faecalis* (9.66 mm) and for root extract against *Enterococcus faecalis* (10.66 mm).

The determination of MIC and MBC values demonstrated that all tested bacterial strains were susceptible to the extracts. Both extracts showed similar inhibitory activities against the tested pathogens. Treatment of the bacteria with the different extracts revealed that the MIC and MBC values of the *A. tenuifolius* aerial parts extract were equal for all tested strains. However, MIC and MBC values of the root extract differed for *P. aeruginosa* and *S. aureus.* All the antibacterial activity observed was lower than those of Chloramphenicol which was utilized as positive control in this

experiment. The agar disc diffusion test results indicated that Gram-positive bacteria and *E. coli* were very sensitive with MIC values of 0.003 µg/ml and 0.062 µg/ml, respectively, whereas *P. aeruginosa* had a MIC of 0.125 µg/ml.

The methanolic extract (methanol/water: 80/20) of *A. tenuifolius* was investigated previously for antibacterial activity against *S. aureus, E. coli, P. aeruginosa, K. pneumoniae and S. typhi* showing effectiveness against all tested organisms [15]. In another study, aerial portions of *A. tenuifolius* extracts obtained from dichloromethane, ethyl acetate and butanol showed antibacterial activity against Gram-positive (S. aureus, E. faecalis) and Gram-negative (*E. coli, P. aeruginosa*) bacteria [16]. Moreover, various extracts of *A. tenuifolius* displayed different levels of antibiotic activity against many organisms (*S. aureus, S. epidermidis, Proteus vulgaris, Proteus mirabilis, E. coli, Klebsiella pneumoniae and Pseudomonas aeruginosa*) [17].

Our results are consistent with previous results and showed that the ethanol/water extract (75/35) was efficacious against Gram-positive bacteria, whereas it was inactive against Gram-negative bacteria (except for K. pneumoniae and S. aureus). This inequality may be due to the different penetration methods of active substances between Gram-positive and Gram-negative bacterial cell walls.

CONCLUSION

The present study highlights the pharmacological interest of *Asphodelus tenuifolius* Cav., a Saharan medicinal plant gathered from Ghardaïa (Algeria). Phytochemical screening confirmed the abundance of key secondary metabolites, notably anthraquinones (characteristic chemotaxonomic markers of the Asphodelaceae family), polyphenols, flavonoids, catechin tannins, sterols, and triterpenes and thereby supporting its safety in traditional food and herbal preparations. The biological assay results showed a strong antioxidant capacity in a concentration-dependent manner demonstrated by DPPH scavenging activity, a higher activity was found in the aerial parts (IC_{50} = 51.22 µg/ml) than in the roots (IC_{50} = 88.33 µg/ml). Moreover, hydroethanolic extracts of the two organs exhibited strong antibacterial activity against clinically relevant Gram-positive pathogens (B. cereus, S. aureus, E. faecalis) and K. pneumoniae. To summarize, *A. tenuifolius* Cav. from the arid region of Ghardaïa is a good option for the development of natural antioxidants and antimicrobials of biological origin. Future research should include chromatographic bioguided isolation and identification of particular active compounds and evaluation of their mechanisms of action and in vivo efficacy.

REFERENCES

[1] Latif, R., Nawaz, T. (2025): Medicinal plants and human health: a comprehensive review of bioactive compounds, therapeutic effects, and applications. – Phytochemistry Reviews, DOI: 10.1007/s11101-025-10194-7.

[2] Maiza K. (2007): Pharmacopée traditionnelle saharienne. PhD thesis in Medical Sciences, Youssef Ben Khedda University, Algiers.

[3] Govaerts, R. (2003): World Checklist of Selected Plant Families Database in ACCESS: 1-216203. – The Board of Trustees of the Royal Botanic Gardens, Kew. Available at: http://wcsp.science.kew.org/qsearch.do (Accessed 28 November 2020).

[4] Quézel, P., Santa, S. (1962): New Flora of Algeria and Southern Desert Regions. – CNRS, Paris.

[5] Gast, M. (2000): Food and Nutrition of the Ahaggar Populations. – Éditions du CNRS, Paris.
[6] Faugeras, G., Lavenir, R. (1965): On the presence of alkaloids in Papilionaceae. – Plantes Médicinales et Phytothérapie 2: 12-20.
[7] Farnsworth, N. R. (1966): Biological and phytochemical screening of plants. – Journal of Pharmaceutical Sciences 55(3): 225-276.
[8] Paris, R. R., Moyse, H. (1976): Treatise of Materia Medica. – Masson, Paris.
[9] Bruneton, J. (2016): Pharmacognosy – Phytochemistry, Medicinal Plants. 5th edition, Lavoisier, Paris.
[10] Coelho, M. T., Goncalves, J. C., Alves, V., Moldao-Martins, M. (2011): Antioxidant activity and phenolic content of extracts from different *Pterospartum tridentatum* population growing in Portugal. – Procedia Food Science 1: 1454-1458.
[11] Drummond, O. S., Waigh, R. D. (2000): The development of microbiological assay techniques using resazurin. – Journal of Pharmacy and Pharmacology 52: 311-318.
[12] Safdar, N. F., et al. (2014): Chemotaxonomic significance of anthraquinones in Asphodelaceae. – Phytochemistry 98: 102-110.
[13] Malmir, M., et al. (2018): Phytochemical profile and safety evaluation of Saharan *Asphodelus* species. – Journal of Herbal Medicine 12: 45-52.
[14] Kalim, M.D., Bhattacharyya, D., Banerjee, A., Chattopadhyay, S., 2010. Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine. BMC Complementary and Alternative Medecine. 10, 77.
[15] Ahmed, A., Howladar, S., Mohamed, H., Al-Robai, S. (2016): Phytochemistry, antimicrobial, antigiardial and antiamoebic activities of selected plants from Albaha area, Saudi Arabia. – British Journal of Medicine & Medical Research 18: 1-8.
[16] Faidi, K., Hammami, S., Salem, A. B., El Mokni, R., Mastouri, M., Gorcii, M., Ayedi, M. T. (2014): Polyphenol derivatives from bioactive butanol phase of the Tunisian narrow-leaved asphodel (*Asphodelus tenuifolius* Cav., Asphodelaceae). – Journal of Medicinal Plants Research 8: 550-557.
[17] Panghal, M., et al. (2011): Evaluation of antibacterial activity of Saharan medicinal plants against multidrug resistant pathogens. – Annals of Clinical Microbiology 10: 24.