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Anabasis oropediorum Maire. as a health-promoting source: Phytochemical content, *in vitro* antioxidant, antidiabetic, antibacterial, and anti-inflammatory potential

Noura GHERAISSA [1,2 *], Ahmed Elkhalifa CHEMSA [2,3], Nezar CHERRADA [1,2], Erol EBRU [4], Eman Ramadan ELSHARKAWY [5]

1 Department of Cellular and Molecular Biology, Faculty of Life and Natural Sciences, University of El Oued, 3900 El Oued, Algeria
2 Laboratory of Biodiversity and Application of Biotechnology in Agriculture, El Oued University, 3900 El Oued, Algeria
3 Department of Biology, Faculty of Life and Natural Sciences, University of El Oued, 3900 El Oued, Algeria
4 Department of Analytical Chemistry, Faculty of Pharmacy, Bezmialem Vakif University, Adnan Menderes Bulvarı (Vatan Cad.) P.K. 34093 Fatih / İstanbul, Turkey
5 Department of Chemistry, Faculty of Science, Northern Border University, Arar, 9280, Ar Ar 73213, Arabie saoudite, Saudi Arabia
* Corresponding Author. E-mail: nouragherr@gmail.com/gheraissa-noura@univ-eloued.dz (N.G.) Tel. +213-669-000-583.

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ABSTRACT: Phytochemicals, which are necessary for plants to adapt to their environment, offer an exciting source of medicinal products. This study focuses on the desert plant *Anabasis oropediorum* (*Chenopodiaceae*), which is native to calcareous sandy regions in North African countries and Palestine. This investigation is the first to shed light on the therapeutic nature of the methanolic extract of the aerial parts of *A. oropediorum*. A phytochemical screening analysis was conducted, including a quantitative estimate of the total phenols, flavonoids, flavonols, anthocyanins, hydrolyzable tannins, and condensed tannins. Antioxidant activity was evaluated *in vitro* using three methods: DPPH• scavenging, β-carotene bleaching, and anti-hemolytic assay. The antidiabetic activity was tested using two assays: non-enzymatic hemoglobin glycosylation and glucose uptake by yeast cells assay. Antibacterial activity was evaluated by the disc diffusion method, and anti-inflammatory activity was evaluated by the protein anti-denaturation method. Phytochemical screening revealed the presence of alkaloids, coumarins, cardiac glycosides, leuco-anthocyanins, mucilage, phenols, saponins, sterols, and terpenes. The quantitative analysis showed that the methanolic extract provided a high level of flavonoids (17.0±0.50 µg QE/mg) and the total contents of tannins (5.3±0.04 µg GAE/mg, 7.3±0.14 µg CE/mg). Chlorogenic acid, *p*-coumaric acid, quercetin, and rutin were the phenolic compounds detected by RP-HPLC analysis. FTIR spectroscopy confirmed the presence of alkanes, aromatic compounds, and aliphatic amines in the methanolic extract. Biologically, this medicinal plant exhibited medium antioxidant activity, good in vitro antidiabetic activity, antibacterial activity against only *Staphylococcus aureus*, *Listeria innocua*, and *Escherichia coli*, and very good albumin protection activity from heat denaturation.

KEYWORDS: *Anabasis oropediorum*; methanolic extract; phytochemical content; RP-HPLC; biological activity.

1. INTRODUCTION

The family *Chenopodiaceae* comprises 102 genera and around 1425 species, most of which are halophytes, particularly capable of tolerating salt conditions and environmental pressures. They are widespread in temperate and subtropical regions, such as salt marshes, deserts, and semi-deserts. these species are known to be rich in bioactive molecules, such as flavonoids, phenolic acids, glycosides, sesquiterpenes, diterpenes, triterpenes, and saponins [1].

The genus *Anabasis* is one of the most prominent genera in the family *Chenopodiaceae*, comprising 29 species distributed worldwide in tropical, temperate, brackish, arid, and semi-arid ecosystems [2]. Plants of this genus are used as herbs. In Algeria, the medicinal plant *A. articulata* is used to treat skin diseases, diabetes,

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fever, and headaches [1]. The leaves of *A. aretioides* are used in Morocco as antihypertensive, antidiabetic, antirheumatic, diuretic, and as an antidote against poison [3]. Moreover, *A. syriaca* is used to manage childbirth and menstruation, activates the respiratory system, and is used in the treatment of liver diseases and ulcers in Jordan [4]. *A. aphylla* is used as a botanical insecticide by the population of northwest China [5].

Biological studies have shown that some species of the genus *Anabasis* have antioxidant, antibacterial, and antidiabetic properties, due to their content of active secondary metabolites [1, 3, 6]. In previous studies on *Anabasis* species, several secondary metabolites have been reported: the isoflavonoid compounds (2′-hydroxy-5,6,7-trimethoxyisoflavonoid) and five glucosidic (2-O-(2)-*β*-D glucopyranosyloxy-4, 2-*O*-*β*-D glucopyranosyloxy-4,6-dimethoxy phenylenthanone, 3-methyl-but-2-enoic acid-[2-(4-methoxy phenyl)-ethyl]-amide, 5,6,7,2′- tetramethoxy isoflavonoid, and 6-dimethoxy phenylenthanone) isolated from the *A. salsa* and *A. brevifolia* [7]. Also, picraquassioside C, syringin, piceoside, tortoside A, and phytolaccagenicacid-3-*O*-*β*-D-glucopyranuronide 28-*β*-D-glucopyranosyl ester were isolated for the first time from the *n*-butanol extract of *A. salsa* [8]. Gamal et al. isolated and identified seven triterpenoids from aerial parts of *A. articulata*, and chemical analysis by Abdou et al. showed that the *n*-butenol extract of *A. setifera* contains a triterpene saponin (sophradiol 3-O-α-L-1C4-rhamnopyranosyl- (1‴→4″)-O-β-D-4C1-galactopyranosyl (1″→6′)-O-β-D-4C1-glucopyranoside) [9,10]. Moreover, Shakeri et al. and Mohammadi et al. demonstrated that the two species, *A. aphyll* and *A. setifera* are a source of secondary metabolites; saponins, alkaloids, phenols, terpenes, and sterols [11,12].

Anabasis oropediorum Maire (Arabic name: Ajram) is a desert pastoral shrub, that is distinguished by its dark green color and articulated twigs without leaves [13]. İt is native to Palestine and North Africa, found in rocky hills and sandy areas. In North Africa, this plant is used to treat skin diseases, especially eczema [6]. With the exception of research by Bouaziz et al., which examined the antioxidant and antibacterial effects of this plant, it has not been the subject of chemical analysis or biological investigations before [6].

To our knowledge, this study is the first to investigate the potential benefits of the medicinal plant *A. oropediorum*. This work presents the characterization of the phytochemical content by performing primary phytochemical analysis, quantitative estimation of several classes of phenolic compounds, RP-HPLC chromatography, and FTIR spectroscopy of the *A. oropediorum* samples. Additionally, the biological activity of the aerial part of *A. oropediorum* was evaluated by testing *in vitro* the anti-inflammatory, antibacterial, antidiabetic, and antioxidant activities of the methanolic extract.

2. RESULTS

2.1. Phytochemical profile

According to Table 1, it is clear that all the bioactive compounds tested using phytochemical screening were present in the methanolic extract of *A. oropediorum*.

Table 1. Qualitative phytochemical analysis of the methanolic extract of *A. oropediorum*.

Phytochemical compounds		Observation
Alkaloids	**Dragendorff**	(+)
	Wagner	(+)
Coumarins		(+)
Cardiac glycosides		(+)
Flavonoids		(+)
Leuco-anthocyanins		(+)
Mucilage		(+)
Phenols		(+)
Saponins		(+)
Sterols and terpenes		(+)
Tannins		(+)

Figure 1 shows that the extract has abundant and varied phenolic content, including phenols (20.22±0.35 µg gallic acid/mg), flavonoids (17±0.50 µg quercetin/mg), hydrolyzable tannins (5.3±0.04 µg gallic acid/mg), and condensed tannins (3.67±0.14 µg catechin/mg). Where the flavonoid content is divided into two classes: flavonols (6.3±0.10 µg gallic acid/mg) and anthocyanins (1.75±0.83 µg cyanidin-3-glucoside/mg).

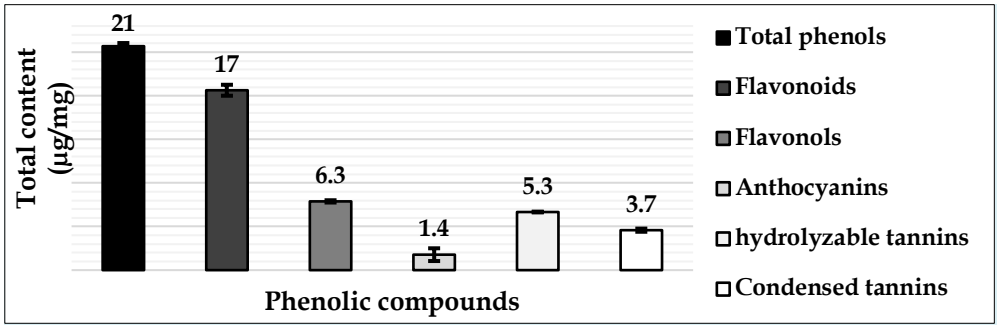

Figure 1. Total phenolic, flavonoid, flavonol, anthocyanin, hydrolysable, and condensed tannin contents in the extract of *A. oropediorum.*

There are 69 compounds in the methanolic extract of *A. oropediorum*, according to the graphical curve for RP-HPLC analysis (Figure 2), four of which have been identified as chlorogenic acid, *p*-coumaric acid, rutin, and quercetin. Table 2 shows that quercetin is the main compound of the compounds identified by RP-HPLC followed by chlorogenic acid (1.21 µg/mg), rutin (0.80 µg/mg), and then *p*-coumaric acid (0.30 µg/mg) with low content (0.03 µg/mg).

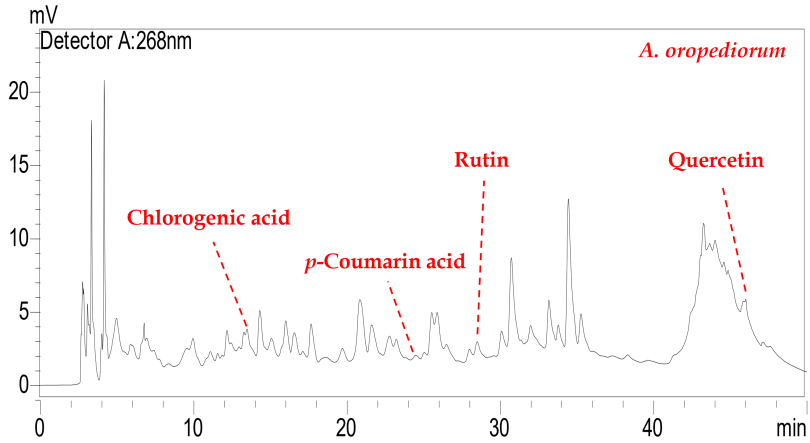

Figure 2. RP-HPLC chromatogram of methanolic extract of *A. oropediorum.*

Table 2. Retention time, equation and content of compounds determined by RP-HPLC analysis of plant extract.

Phenolic compounds	Rt(min)	Equation	Content (µg/mg ED)
Phenolic acid			
Chlorogenic acid	13.392	y=21665x	0.80
p-Coumaric acid	23.817	y=49495x	0.03
Flavonoids			
Quercetin	45.047	y=45378x	1.21
Rutin	28.37	y=28144x	0.30

The methanolic extract of *A. oropediorum* gave the following characteristic absorption peaks as shown in Figure 3 and Table 3. The peaks in the range of 3400-3200 cm^{-1} and 2900-2800 cm^{-1} indicate O–H and C–H stretching which corresponds to a phenol group and an alkane function. The peak at 1600 cm^{-1} and 1400 cm^{-1} represents the presence of aromatic compounds as functional groups. In addition, the peak at 1250 cm^{-1}, 1050 cm^{-1}, and 850 cm^{-1} confirms the presence of aromatic amine and aliphatic amine and aromatics respectively [14].

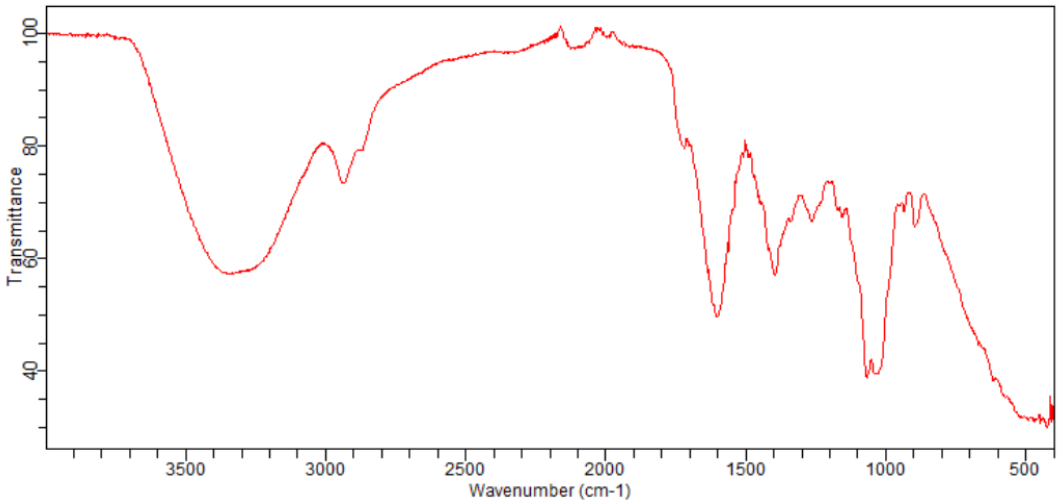

Figure 3. FTIR spectrum of methanolic extract of *A. oropediorum*.

Table 3. FTIR peak values and functional groups of methanolic extract of *A. oropediorum*.

Stem peak value	Bond	Characteristics
3400-3200 cm^{-1}	O–H stretching	Phenol
2900-2800 cm^{-1}	C–H stretching	Alkane
1600 cm^{-1}	C=C stretching	Aromatic compounds
1400 cm^{-1}	C–C stretching	Aromatic compounds
1250 cm^{-1}	C–N stretching	Aromatic amine
1050 cm^{-1}	C–N	Aliphatic amines
850 cm^{-1}	C–H	Aromatic

2.2. Bioactivities

The antioxidant capacity of the extract was determined using three different *in vitro* assays: DPPH$^{\bullet}$, *β*-carotene, and hemolysis. The tIC$_{50}$ values presented in Table 4 were compared with those of the reference compounds (ascorbic acid and *α*-tocopherol), revealing that the concentration of the *A. oropediorum* extract capable of inducing 50% of the activity was more than 150 µg/mL, while ascorbic acid/ *α*-tocopherol did not exceed 95 µg/mg for the three studied assays.

Table 4. Antioxidant activity of methanolic extract of *A. oropediorum*.

	IC$_{50}$ (µg/mL)		
	Extract	**Ascorbic acid**	***α*-Tocopherol**
DPPH$^{\bullet}$	256±3.16	1.87±0.03	-
***β*-carotene bleaching**	154±1.24	-	2.10 ± 0.08
Anti-hemolytic	241±2.90	90.5±0.63	-

Based on the percentages of hemoglobin binding inhibition and increased glucose uptake by yeast cells in the presence of the *A. oropediorum* plant extract, it appears to have an antidiabetic effect. Moreover, there is a clear correlation between the concentration of the extract and the degree of inhibition of hemoglobin binding and increase in glucose uptake by yeast cells (Figure 4). Through statistical analysis (p-value<5%), the extract outperformed the drug Metformine in the hemoglobin glycosylation inhibitory activity and was similar to it in enhancing the uptake of glucose by the yeast cells (Figure 5).

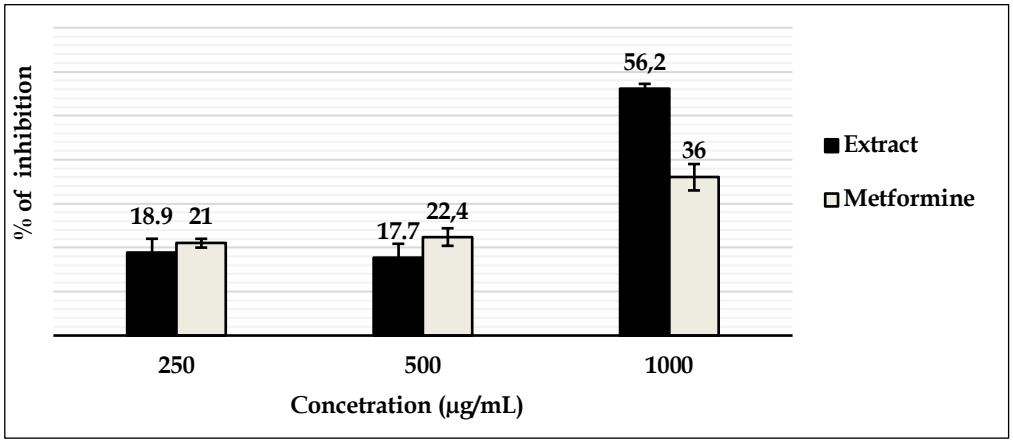

Figure 4. Non-enzymatic glycosylation of methanolic extract of *A. oropediorum* with Metformine.

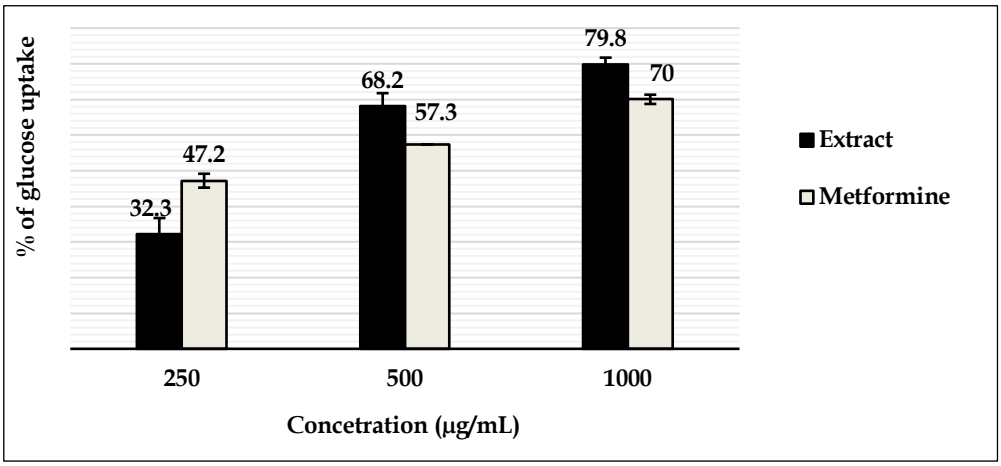

Figure 5. Glucose uptake by yeast cells activity of methanolic extract of *A. oropediorum* with metformine.

According to the results shown in Figure 6, the *A. oropediorum* extract has moderate sensitivity to *Staphylococcus aureus*, with a zone of inhibition exceeding 15 mm in diameter at a dose of 4 mg, weak sensitivity to *Listeria innocua* and *Escherichia coli* and little or no sensitivity to *Pseudomonas aeruginosa, Salmonella typhimurium, and Bacillus subtilis.*

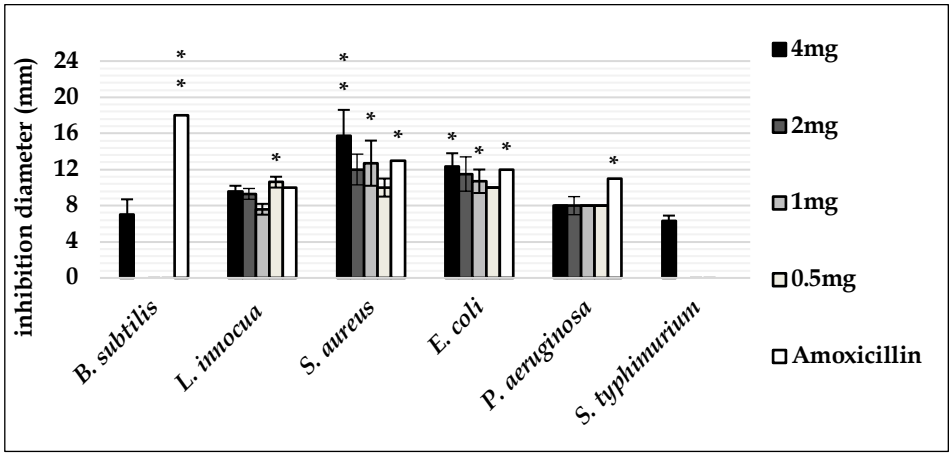

Figure 6. Antibacterial activity of methanolic extract of *A. oropediorum*. Zone diameter: ≥21mm; strong sensibility***, 15–20 mm; moderate sensibility**, 10–15 mm; weak sensibility*, ≤10 mm; little or no sensibility.

The methanol extract of *A. oropediorum* showed concentration-dependent activity in protecting albumin from denaturation (Figure 7). The use of Aspirin as positive control, showed similar protection to that of the extract at the concentration of 500 µg/mL (55.2±0.51, 54±0.57% respectively) (*p*-value<5%), while it was

superior to the extract at the concentrations of 250 µg/mL (60.9±0, 46±1.02%) and 125 µg/mL (61.8±1.32, 16.66±0%).

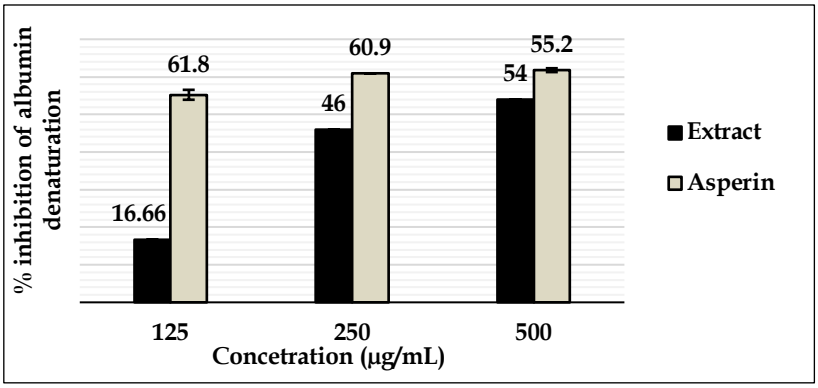

Figure 7. Anti-inflammatory activity of methanolic extract of *A. oropediorum*

3. DISCUSSION

Results of preliminary phytochemical screening (Table 1) indicate that *A. oropediorum* extract is a source of a beneficial classes of phytochemicals such as tannins, phenols, alkaloids, terpenoids, saponins, and flavonoids. These chemical compounds are usually found in all higher plants, and they play defensive roles, enabling the plant to acquire medicinal properties. These results are consistent with a phytochemical screening of *A. aphyll* [11] and *A. setifera* [12].

According to quantitative and quantitative determination by using RP-HPLC, it is clear that the aerial part of *A. oropediorum* has a varied content of excitatory phenolic compounds, including both simple and complex phenols, where the flavonoids occupied 80% (Figure 1), and quercetin found to be the dominant among the phenolic compounds identified through chromatographic analysis, with a value of 1.21 µg/mg (Table 2). FTIR spectroscopy, a reliable and sensitive method for detecting biomolecular structure by defining functional groups [15], confirmed the presence of phenolic compounds, alkanes, aromatic and non-aromatic compounds such as aliphatic amines in the methanolic extract of *A. oropediorum*.

The DPPH• assay expresses the presence of antioxidant compounds capable of changing the color of the DPPH radical from violet to yellow [16], while the *β*-carotene bleaching test demonstrates the ability of these compounds to slow the rate of *β*-carotene loss of yellow color due to its interaction with free radicals caused by the thermal oxidation of linoleic acid [17]. Antioxidant activity is attributed to phenolic substances that free radical quenching and inhibit lipid peroxidation by donating an electron or hydrogen through hydroxyl groups to active molecules with oxidative disruptive activity [18]. Based on the IC_{50} values (Table 4), the *A. oropediorum* extract showed an antioxidant activity consistent with a total phenolic content of 21±0.29 µg GAE/mg. This is consistent with what Tosun et al. found through their study on eight plants (*Lamiaceae*) [19]. They observed a positive relationship between phenolic content and DPPH• radical scavenging activity and *β*-carotene bleaching, where the higher the phenolic content gives the higher the antioxidant activity. However it contradicts what Bouaziz et al. found for the aqueous and methanol extracts of the same plant (*A. oropediorum*) grown in Tunisia [6]. They observed good scavenging activity of DPPH• (1.12±0.19 and 3.72±0.63 µg/mL respectively) with weak phenolic content (45±5 and 159±19 mg PgE/100g, respectively). The difference between the two studies can be attributed to the nature of the phenols present in the extract, which may depend on the extraction method used. Zheng et al. found that the phenolic structure with ortho-dihydroxyl structure had the best scavenging activity [20]. They also discovered that the presence of a third hydroxyl group at C-5', methylation of the hydroxyl, glycosylation and phloroglucinol structures, or 5-hydroxyl or 7-hydroxyl in the A-ring had lower scavenging activities. Through the results of RP-HPLC (Table 2) and quantitative estimates (Figure 1), it is clear that the extract of *A. oropediorum* contains the phenolic structure with ortho-dihydroxyl structure represented by quercetin and some anthocyanins. It contains rutin, a glycoside that combines the flavonol quercetin with the polysaccharide rutinose.

The anti-hemolysis assay is one of the criteria included in evaluation of the antioxidant activity of the tested substances, it tests their ability to protect red blood cells from damage by free radicals [21]. According to the value of IC_{50} = 482±5.46 µg/mL (Table 3), the methanolic extract of the aerial parts of *A. oropediorum* has anti-hemolytic activity. The anti-hemolytic activity of plant extracts can be attributed to their content of flavonoids due to their remarkable ability to scavenge oxidants. This is due to their chemical structure, which

provides them an abundant number of hydroxyl radicals are primarily responsible for scavenging free radicals [22].

The assays of non-enzymatic glycosylation of hemoglobin and glucose uptake by yeast cells do not give an accurate description of the mechanism of the antidiabetic activity of the tested substances, but it indicates whether these substances have a role in regulating the level of glucose in the blood. Diabetes is a metabolic disease represented by a defect in the general mechanism of glucose regulation. The reaction of glucose with hemoglobin is a severe complication of diabetes. Some medicinal herbs are used to reduce the formation of glucose and hemoglobin, and thus increase the amount of free hemoglobin [23]. According to the percentages of inhibiting hemoglobin binding to glycosylation provided by the methanolic extract, which was more than 50% and superior to metformine (36±3%) at a concentration of 500 µg/mL (Figure 4). Also, according to its effects in promoting glucose uptake by yeast cells, which was similar to that of metformine (Figure 5). Therefore, *A. oropediorum* is a herbal medicine with hypoglycemic properties. Two plants of the same genus, *A. articulata* and *A. aretioides*, are traditionally used for the treatment of diabetes [1, 3].

According to the results of the bacterial susceptibility test (Figure 6), the wild grass *A. oropediorum* has promising antibacterial properties against *S. aureus*, *L. innocua*, and *E. coli*, while it has no inhibitory effect on the growth of *P. aeruginosa*, *S. typhimurium*, and *B. subtilis*. These results are consistent with the findings of Bouaziz et al. where the methanolic extract of the same plant showed activity against *S. aureus* and showed no activity against *P. aeruginosa* and *B. subtilis*, as well as the aqueous extract showed no activity against all tested strains [6].

Direct skin exposure to toxic substances such as synthetic detergents causes denaturation of skin proteins, which leads to many skin problems such as eczema, cracks, and scratches [24]. The medicinal plant *A. oropediorum* is traditionally used by the peoples of North Africa in the treatment of skin diseases, especially eczema [6]. Therefore, the extract had amazing anti-inflammatory activity through the anti-denaturation assay (Figure 7). This activity can be attributed to the active compounds detected by phytochemical scanning analysis (Table 1), but it requires further research to understand and determine the mechanism of the anti-inflammatory activity of plant extracts in general.

The impact of each class of compounds analyzed on the antioxidant, antidiabetic, antibacterial, and anti-inflammatory potential of the extracts was investigated in our study. We found that rutin, a flavonoid present in the extracts, exhibited antidiabetic, anti-inflammatory, and antimicrobial activities. This is consistent with the findings of a previous study by Ganeshpurkar et al., which reported that rutin has pharmacological potential in these areas [25]. Additionally, quercetin, another flavonoid present in the extracts, has been reported to have antioxidant activity *in vitro* by inhibiting the oxidation of other molecules. In addition, quercetin was found to exhibit antibacterial and anti-inflammatory activities, which is consistent with the findings of a review article by David et al. on the biological importance of quercetin. Therefore, our results suggest that the flavonoids present in the extracts may contribute to their antioxidant, antidiabetic, antibacterial, and anti-inflammatory activities [26].

4. CONCLUSION

Researchers seek, through phytochemical and biological studies, to reach active compounds from natural sources that can be exploited in various fields such as food, pharmaceutical, cosmetics, and others. The data of this study, which is the first study of the mentioned desert herb *Anabasis oropediorum* for the treatment of skin diseases, shows that the methanolic extract of the aerial part of *Anabasis oropediorum* has a diverse content of phytochemical compounds such as alkaloids, coumarins, cardiac glycosides, flavonoids, leuco-anthocyanins, mucilage, phenols, and saponins, sterols, terpenes, and tannins. It also shows promising properties as an antioxidant, antibacterial, and anti-protein denaturant, in addition to its activity in regulating blood sugar by inhibiting hemoglobin glycosylation and increasing glucose uptake. Therefore, the results of this research are important for the development of health-promoting ingredients and functional foods for the prevention and control of diabetes and other chronic diseases. However, more studies on this plant are needed in order to isolate, identify, characterize, and elucidate the structure of the bioactive compounds, as well as determine their biological activity *in vitro* and *in vivo*.

5. MATERIALS AND METHODS

5.1. Collection of the plant

The aerial parts of *Anabasis oropediorum* were collected in October 2020 during the flowering period in Algeria's Ben Ghasha district (34°20′N, 7°61′E). After being rinsed and dried; they were crushed with a grinder.

The plant was identified taxonomically by Prof. Noureddine Slimani (Higher School of Desert Agriculture, El Oued University).

5.2. Preparation of the plant extract

The plant matter powder was soaked (20 g) in 100 mL of methanol diluted with distilled water (70%) for 24h. Then the extract was filtered, dried and stored at 4°C.

5.3. Phytochemical analysis

5.3.1. Phytochemical screening

Both phytochemicals were detected; Alkaloids, coumarins, cardiac glycosides, flavonoids, leuco-anthocyanins, mucilage, phenols, saponins, sterols, terpenes, and tannins according to usual phytochemical screening [27]. The sign (+) indicates the presence of the phytochemical and the sign (-) indicates its absence.

5.3.2. Quantitative estimations of phenolic content

The total phenolic content of the crude extract was determined according to the Folin-Ciocalteu method [17]. 200 µL of the extract or gallic acid concentrates with 1000 µL of Folin-Ciocalteu reagent (10%) were incubated for three minutes. Then 0.8 mL of Na_2CO_3 (7.5%) was added and the mixture was incubated for 30 min, the absorbance was measured at 765 nm. Content was expressed as µg gallic acid/mg dry extract.

Flavonoids are phenolic compounds that can be chemically identified through the following steps as described by Chekroun-Bechlaghem et al. [17]. Quercetin was used as a standard compound for the determination of total flavonoid content (µg QE/mg dry extract). The reaction mixture contained 250 µL of the extract or quercetin concentrate, 255 µL of methanol, 100 µL of CH_3COOK (1 M), and 100 µL of $Al(NO_3)_3$ (10%). The mixture was incubated for 40 minutes. The absorbance of the mixture was determined at 415 nm.

The content of flavonols was performed as described by Chekroun-Bechlaghem et al. [17]. 250 µL of extract or concentration of quercetin were mixed with 250 µL $AlCl_3$ (2 mg/mL) and 1500 µL $C_2H_3NaO_2$ (50 mg/mL). The absorbance at 440 nm was recorded after two and half hours of incubation. The content of flavonols was expressed as milligrams of quercetin equivalents per gram of dry weight (µg QE/mg).

Anthocyanin content was determined according to the pH differential method [28]. Briefly, 400 µL of the extract was mixed with 3600 µL of KCl buffer (0.0025M, pH=1) and $C_2H_3NaO_2$ buffer (0.4M, pH=4.5) was added each one by one. After incubation for 30 minutes, the absorbance was read at 700 nm and 510 nm. By applying the equation 1, anthocyanin content was expressed in µg equivalent to cyanidin-3-glucoside per milligrams of dry extract.

$$\text{(Eq. 1) Anthocyanin pigment (µg C-3-GE/mg)} = ((A \times MW \times DF \times 100)/MA)/100$$

Where the value of "A" is calculated by applying equation:

$$\text{(Eq. 2) } A = (Abs_{510} - Abs_{700})pH1.0 - (Abs_{510} - Abs_{700})pH4.5$$

Where: Abs_{510}; absorbance at 510 nm. Abs_{700}; absorbance at 700 nm MW; cyanidin-3-glucoside molecular weight (449.2 g/mol). DF; dilution factor. MA; molar extinction coefficient of cyanidin-3 glucoside (26.9 l/mol.cm).

Determination of hydrolyzed tannin content was performed according to the Follin-Dennis method [29]. 500 µL of the extract or gallic acid concentrates were mixed with 1000 µL of Folin-Denis reagent and 500 µL of Na_2CO_3 (35%), then distilled water was added to the mixture up to 10 mL. After 30 minutes of incubation in the dark the absorbance was determined at 700 nm. Results were expressed as µg catechin equivalents (CE)/mg sample.

The condensed tannin content was determined by the vanillin HCl method [17]. To 500 µL of extract or different concentrations of catechin were added 3000 µL of vanillin reagent (4%) and 1500 µL of 1N HCl and the mixture was mixed well. After incubation in the dark at 20 °C for 15 minutes, the absorbance was read at 760 nm. Results were expressed as micrograms of catechin equivalents (CE) per milligram of extract weight (µg CE/mg).

5.3.3. Chromatographic analysis

The most important simple phenols compounds in the methanolic extract of *A. oropediorum* were determined by reverse-phase high-performance liquid chromatography (RP-HPLC) using a Shimadzu LC-20 HPLC system with UV (λ = 268 nm), connected to a C18 column (5 µm, 4.6×250 mm), and injection volume (20 µL), the mobile phase is acetonitrile (A) and 0.2% acetic acid in water (B). With a flow rate of 1 mL/min,

the gradient method was developed by starting with 90% B, reducing to 86% B in 6 min, 83% B in 16 min, 81% B in 23 min, 77% B in 28 min, holding at 77% B in 28-35 min, 60% B in 38 min, and 90% B in 50 min. The compounds were determined using the retention time equation and the reference compounds (gallic acid, chlorogenic acid, vanillic acid, caffeic acid, vanillin, coumaric acid, rutin, naringin, and quercetin).

5.3.4. FTIR spectroscopy

The samples were analyzed by Agilent Cary 630 Fourier transform infrared spectroscopy (Agilent Technologies) in order to identify the characteristic functional groups in the methanolic extract of *A. oropediorum*. The sample was placed directly on the platform, uniform pressure applied, and the spectra recorded as the average of 8 scans at 16 cm^{-1} resolution in the region of (400-4000 cm^{-1}).

5.4. Bioactivities tests

5.4.1. Evaluation of antioxidant activity

DPPH• assays. The antioxidant capacity was evaluated by determining the value of IC_{50}, which expresses the concentration of the samples capable of inhibiting 50% of free radicals. The DPPH• test was applied as mentioned by Chekroun-Bechlaghem, *et al* [17], where the absorbance was read at 510 nm for the sample mixture with the DPPH• solution (after incubation in the dark for 30 min). The percentage of DPPH• inhibition was calculated using equation 2:

$$\text{(Eq. 2) Percentage of inhibition} = \left[{}^{(Ac - As)}/_{Ac}\right] \times 100$$

As = absorbance of the sample, Ac = absorbance of the control

β-carotene bleaching assay. Antioxidant activity to inhibit lipid peroxidation was evaluated using the *β*-carotene bleaching method as described by Chekroun-Bechlaghem, *et al* [17]. The sample was mixed at different concentrations with the reaction mixture, which was prepared by mixing 0.5 mg of *β*-carotene with 1 mL of chloroform, 25 µL of linoleic acid and 200 mg of Tween 40, the chloroform was evaporated and the precipitate was dissolved in 100 mL of oxygenated water with rapid magnetic stirring. The absorbance is measured at 470 nm immediately after mixing, and it is called the absorbance at 0 min. Then the mixture was incubated at 50°C for 120 min, then the absorbance was measured and the absorbance was considered at 120 min. To determine the antioxidant activity, the equation 3 was applied:

$$\text{(Eq. 3) Percentage of antioxidant activity} = \left[1-\left({}^{As\ (0min)\ -\ As\ (120min)}/_{Ac\ (0min)\ -\ Ac\ (120min)}\right)\right] \times 100$$

The IC_{50} is the value that represents the concentration capable of inhibiting lipid peroxidation by 50%.

Anti-hemolytic assays. The efficiency of extract to the inhibition of human erythrocyte hemolysis was determined, by applying the following steps, described in [30]. 2 mL of graduated concentrations of extract or ascorbic acid (1-0.05 mg/mL) were mixed with 40 µL of 10% RBCs. After incubating for five minutes at 37°C these reagents are added to the mixture; 40 µL of 30 mM H_2O_2, 40 µL of 80 mM $FeCl_3$, and 40 µL of 50 mM ascorbic acid, respectively. After incubating for one hour at 37°C, the mixture was separated by centrifugation (700 rpm for 10 min). The absorbance is read at 540 nm. The percentage of hemolysis was calculated according to equation 4:

$$\text{(Eq. 4) Percentage of hemolysis} = \left[{}^{Ac}/_{As}\right] \times 100$$

IC_{50} is a value that represents the concentration capable of inhibiting 50% from hemolysis.

5.4.2. In vitro evaluation of antidiabetic activity

Non-enzymatic hemoglobin glycosylation assay. *The* efficiency of the extract in inhibiting the binding of hemoglobin with glycosylation was determined by following the method [23]. Briefly, 1 mL of the sample was mixed with 1 mL of 0.06% hemoglobin solution, 65 µL 0.02% gentamicin, and 1 mL of 2% glucose, prepared in 0.01 M phosphate buffer (pH = 7.4). After incubation for 72h in the dark at 37°C, the absorbance of the mixture was read at a wavelength of 520 nm. According to equation 5, the percentage inhibition of hemoglobin binding to glycosylation was calculated:

$$\text{(Eq. 5) Percentage inhibition of hemoglobin binding to glycosylation} = \left[{}^{(As - Ac)}/_{As}\right] \times 100$$

Glucose uptake by yeast cells assay. The test is done by applying the following steps [31]. First the yeast supernatant is prepared by repeated centrifugation (3000×g, 5min). 2 mL of sample and 2 mL of 10 mM glucose solution were mixed and incubated at 37°C for 10 min. Then 200 µL of 10% yeast suspension was added. The mixture was mixed by Vortex for 1min and then incubated for one hour at 37°C. Then the mixture was placed in a centrifuge for 15 min at 3000 rpm. Then the absorbance of the supernatant was measured at wavelength of 620 nm. Determination of the percentage increase of glucose uptake by the yeast cells was calculated using the following formula:

$$\text{(Eq. 6) Percentage of increase of glucose uptake} = \left[{}^{(Ac - As)}/_{Ac}\right] \times 100$$

The control is having all reagents except the test sample. Metformine® was used as an antidiabetic drug.

5.4.3. Evaluation of antibacterial activity

By following the method of Disk diffusion [32], the efficacy of the extract was tested against bacterial strains: *Bacillus subtilis* ATCC-6633, *Listeria innocua* CLIP-74915, *Staphylococcus aureus* ATCC-6633, *Escherichia coli* ATCC-8737, *Pseudomonas aeruginosa* ATCC-9027, and *Salamonella typhimuruim* ATCC-14028. Active bacteria strains were spread on the Mueller-Hinton agar using sterile cotton swab. Then the discs were placed on an agar plate containing 10 µL volume of extract at different doses (4-1 mg). The zone of inhibition (mm) was observed after 24 hours of incubation at 37 °C in the incubator.

5.4.4. Evaluation anti-inflammatory activity

The efficacy of the extract in protecting protein (albumin) from heat denaturation was determined by the following method: mixing 1 mL of 5% serum albumin, 1 mL of varying concentrations of the studied samples (0.125-0.5 mg/mL) and 20 µg/mL of 1N HCl. The mixture was incubated at 37°C for 20 min and then placed in a water bath at 57°C for three min. After cooling, 2.5 mL of phosphate buffer solution (0.1 M, pH = 6.4) was added. The absorbance was measured at 660 nm [30]. Aspirin was used as a reference drug. The percentage protection from denaturation is calculated by using the formula:

$$\text{(Eq. 7) Percentage of protection from denaturation} = \left[1-\left({}^{As}/_{Ac}\right)\right] \times 100$$

Where control is the solution having all reagents except the test sample.

5.5. Statistical analysis

The results were expressed as the mean ± standard deviation of three replicates. The statistical study was conducted using SPSS Statistic for Windows version 15.0. The differences between the two variables were determined using Student's t-test.

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