European Journal of Chemistry

View Journal Online
View Article Online

Antioxidant and antimicrobial activities of four medicinal plants from Algeria

Yuva Bellik [1,*] and Nasreddine Mekhoukh [2]

[1] *Department of Biology, Faculty of Nature and Life Sciences, University of Mohamed El Bachir El Ibrahimi, Bordj Bou Arreridj, 34000, Algeria*
[2] *Department of Physico-Chemical Biology, Faculty of Nature and Life Sciences, University of Bejaia, 06000, Algeria*

* *Corresponding author at: Department of Biology, Faculty of Nature and Life Sciences, University of Mohamed El Bachir El Ibrahimi, Bordj Bou Arreridj, 34000, Algeria.*
e-mail: y.bellik@univ-bba.dz (Y. Bellik).

RESEARCH ARTICLE

10.5155/eurjchem.14.1.121-128.2358

Received: 17 November 2022
Received in revised form: 17 January 2023
Accepted: 25 January 2023
Published online: 31 March 2023
Printed: 31 March 2023

KEYWORDS

Dittrichia viscosa
Globularia alypum
Juniperus oxycedrus
Antioxidant activity
Retama sphaerocarpa
Antimicrobial activity

ABSTRACT

Medicinal plants are used in folk medicine to cure several human diseases. This work was designed to evaluate the antioxidant and antimicrobial activities of different extracts of *Globularia alypum*, *Dittrichia viscosa*, *Juniperus oxycedrus,* and *Retama sphaerocarpa.* The total phenolic content (TPC), the total flavonoid content (TFC), and the condensed tannin content (CTC) were determined spectrophotometrically. The antioxidant activity was tested using TAC, DPPH and reducing power assays. The agar diffusion method was used to determine antimicrobial activity against four bacteria (*Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*) and one fungus (*Candida albicans*). *J. oxycedrus* acetone extract showed the highest extraction yield (35.56±0.45%), TPC (504.96±14.82 mg GAE/g DE) and TFC (43.91±0.87 mg QE/g DE). The same extract exhibited the highest TAC (350.67±6.05 mg GAE/g DE) and was the most effective against the DPPH free radical (IC_{50} = 0.21±0.01 mg/mL). In contrast, the *J. oxycedrus* methanol extract showed the highest reducing power ($A_{0.5}$ = 0.39 ± 0.09 mg/mL). All extracts tested showed antibacterial and anticandidal activities at different concentrations. The best antimicrobial effect was also observed with the acetone extract of *J. oxycedrus* against *P. aeruginosa* (26.77±0.06 mm), *B. cereus* (17.16±0.08 mm), *E. coli* (15.84±0.04 mm), and *C. albicans* (21.36±0.11 mm), while the ethanol extract of *D. viscosa* was the most active against *S. aureus* (24.54±0.03 mm). The results of this study provide a scientific basis for the traditional use of these local plants and demonstrate their potential as sources of natural antioxidant and antimicrobial bioactive compounds.

Cite this: *Eur. J. Chem.* **2023**, *14(1)*, 121-128 **Journal website**: www.eurjchem.com

1. Introduction

The use of plant substances for medical purposes is associated with earlier times of humankind. Today, plants are still the new primary source of structurally important chemical substances that lead to the development of innovative drugs [1,2]. Currently, approximately 500,000 to 800,000 plant species are used for medical purposes worldwide. There is an increase in the consumption of medicinal plants due to the proven safety and efficacy of medicinal plants in preventing and curing some diseases [3,4]. Plants continue to be a rich source of therapeutic agents, particularly secondary metabolites such as polyphenols, flavonoids, tannins, saponins, glucosides, *etc.*, involved in ecological functions to improve plant survival during environmental stress. These same plant molecules provide appreciable healing properties in humans [5,6]. In fact, these compounds can interact with reactive oxygen species (ROS) to protect different biological macromolecules (Proteins, lipids, and nucleic acids) from oxidative damage [7]. ROS are produced mainly by enzymatic and non-enzymatic reactions during physiological and pathological conditions. Oxidative stress results from an imbalance between ROS production and antioxidant defenses. In recent years, it has become apparent that oxidative stress plays a central role in various acute and chronic pathological processes such as cancer, neurodegenerative, cardiovascular, and inflammatory diseases [8]. In addition, a growing number of infections are becoming a major health problem because many bacteria and fungi have become increasingly resistant to multiple antibiotics [9]. Many studies have been conducted to investigate the therapeutic effects of antioxidants from natural sources for their wide variety of pharmacological activities, particularly antioxidant and antimicrobial properties. Phytochemical studies identified different compounds, such as quercetin, gallic acid, rutin, resveratrol, and caffeic acid, responsible for antioxidative reactions [10]. In addition, compounds from medicinal plants have been shown to inhibit the growth of a wide range of Gram-positive and Gram-negative bacteria, fungi, viruses, and protozoa through different mechanisms [1].

Algeria benefits from various climates that favour the development of a rich and diversified flora. Indeed, the Algerian territory has a diversified vegetation cover distributed on the coasts, plains, mountains, steppe, Sahara, and around the water points.

Globularia alypum L., also called globular, is a plant belonging to the Globulariaceae family, which includes the two genera *Globularia alypum* L. and *Poskea* Vatke and about thirty species widespread in Europe and North Africa. Species of the genus *Globularia* have been used as agents for various ailments. According to ethnopharmacological studies, *G. alypum* is one of

https://dx.doi.org/10.5155/eurjchem.14.1.121-128.2358

the plants most commonly used to prevent and heal different chronic diseases [11].

Juniperus oxycedrus L. is a plant species of the Cupressaceae family. It is a small dioecious tree highly valued for its richness in secondary metabolites and, in particular, essential oils [12]. This plant is widely used in traditional medicine. It is common in Mediterranean coastal regions and has remarkable resistance to hostile environments such as drought and cold [13]. This plant is used in traditional medicine to treat diseases such as hyperglycemia, obesity, tuberculosis, bronchitis, and pneumonia [14].

Dittrichia viscosa (L.) Greuter is a perennial plant of the Asteraceae (compositeae) family. It is common in the Mediterranean region, where it flowers in late summer and early fall, and is also present in temperate and cold regions of the world. It is considered the queen of medicinal plants because of its multiple biological and pharmacological properties. It is used in traditional medicine for its anti-inflammatory, antipyretic, astringent, and vulnerary properties [15].

Retama sphaerocarpa (L.) Boiss. is a broom-like shrub that occurs in the Mediterranean area of North East Africa and the Iberian Peninsula [16], growing on a wide variety of soil types and under an extensive range of climatic conditions [17]. Plants of the genus Retama Park. of the Mediterranean basin are used in traditional medicine to treat many disorders, including rabies, diabetes, hepatitis, jaundice, sore throat, skin diseases, joint pain, rheumatism, fever, and inflammation [18].

Several medicinal plants and pure compounds have been reported that are being investigated for preventive and therapeutic activities. However, many species of higher plants have not yet been fully described, much less surveyed for chemical or biologically active constituents. Therefore, the present study aimed to determine the phytochemical composition, antioxidant, and antimicrobial properties of four local Algerian plants.

2. Experimental

2.1. Chemicals

Folin-Ciocalteu, sodium carbonate, vanillin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT), ascorbic acid (Vitamin C), iron chloride (II), aluminum chloride, trichloroacetic acid (TCA), dimethylformamide (DMF), gallic acid, quercetin, catechin and other chemicals were purchased from Sigma Aldrich. All organic solvents and chemicals used in the present study were of analytical grade.

2.2. Plant material

Fresh leaves of *J. oxycedrus*, *D. viscosa*, *G. alypum* L., and stems of *R. sphaerocarpa* were collected during the flowering stage in March 2021 in the region of Teffreg, the city of Bordj Bou Arreridj, North-East Algeria. The plants were dried at 37 ° C in an oven prior to extraction. The dried plants were ground to a fine powder (<200 µm particles) using an electric mill (Siyolux, Italy).

2.3. Preparation of plant extracts

Briefly, 10 g of each plant powder were extracted with 150 mL of different solvents (Methanol 70%, acetone 70%, ethanol 70%, and ethyl acetate 70%) using a magnetic stirrer for three hours in the dark at room temperature. Subsequently, the extracts were filtered with a filter paper. A second extraction was performed under the same conditions to obtain a maximum amount of active substances. The filtrates obtained were then evaporated on a rotary evaporator to remove the solvent. The residue was dried in an oven at 40 °C. Finally, the dried extracts were stored at -4 °C until analysis. The extraction yield (%) was determined as the ratio between the amount of dry extract obtained and the initial amount of powder used for extraction.

2.4. Phytochemical study

2.4.1. Total phenolic content

The total phenolic content of the different extracts was estimated spectrophotometrically using the Folin-Ciocalteu test following the Singleton and Rossi protocol [19]. An aliquot of 1 mL of Folin's reagent (10 times diluted) was added to 200 µL of sample or standard with suitable dilutions. After 4 min, 800 µL of a sodium carbonate solution (7.5%) was added. The mixture was left to react for 2 h in darkness at room temperature, and then the absorbance was measured at 760 nm against a blank using a double beam UV-vis spectrophotometer (Shimadzu UV-1601, Japan). All operations were performed in triplicate. The total polyphenols concentration was calculated from the regression equation of the calibration range established with gallic acid. It was expressed in milligram gallic acid equivalent per gram of dry extract (mg GAE/$g_{Dry\ extract}$).

2.4.2. Total flavonoid content

The flavonoid content was evaluated according to the aluminum chloride colorimetric method [20]. A volume of 1 mL of each extract or standard solution (quercetin) at different concentrations was reacted with 1 mL of aluminum chloride (2%). The mixture was vigorously stirred and incubated for one hour at room temperature. The absorbance was read at 430 nm using a double beam UV-vis spectrophotometer (UV-1800 Shimadzu). All samples were analyzed in triplicate and the content of flavonoids was determined using the calibration curve realized using quercetin. Total flavonoid content was expressed as milligram quercetin equivalent per gram of dry extract (mg QE/$g_{Dry\ extract}$).

2.4.3. Determination of condensed tannins

The acidified vanillin method was used to determine the content of condensed tannins [21]. Briefly, 0.5 mL of each extract (1 mg/mL) was mixed with 3 mL of vanillin solution (4%) in methanol and 1.5 mL of hydrochloric acid (37%). The mixture was shaken well and incubated for 15 min at room temperature. The absorbance was read at 500 nm. The experiment was carried out in triplicate. The condensed tannin content was calculated using a standard catechin calibration curve and the concentration was expressed in milligram catechin equivalent per gram of dry extract (mg CatE/$g_{Dry\ xtract}$).

2.5. Antioxidant activities

2.5.1. Total antioxidant capacity (TAC)

The total antioxidant capacity of the extracts was evaluated using the phosphomolybdenum test described by Prieto *et al.* [22] with specific modifications. A volume of 0.3 mL of each extract or standard was mixed with 3 mL of the reaction mixture (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was then incubated at 95 °C for 90 min and the absorbance was recorded at 695 nm. The antioxidant activity was expressed as milligram gallic acid equivalent per gram of dry extract (mg GAE/$g_{Dry\ xtract}$).

2.5.2. DPPH free radical scavenging activity

The free radical scavenging activity of the different extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was evaluated as previously described [23]. DPPH was dissolved in 100 mL of

methanol to prepare a stock solution which was then diluted to give an absorbance of 0.98±0.02 at 517 nm. A volume of 100 µL of each extract at different concentrations or standards (ascorbic acid and BHT) was added to 2.5 mL of DPPH. The mixture was then allowed to stand in the dark for 30 min and the decolorization compared to the negative control containing only the DPPH solution was measured at 517 nm. The free radical scavenging activity was calculated according to Equation (1):

$$I\ \% = \frac{(Abs_{517\text{-Control}} - Abs_{517\text{-Sample}})}{Abs_{517\text{-Control}}} \times 100 \quad (1)$$

where I is the inhibition percentage. $A_{control}$ represents the absorbance of the control reaction (containing all reagents except the tested compound), and A_{sample} represents the absorbance of the sample. The values were expressed as IC_{50} (mg/mL), the concentration of samples that causes a 50% scavenging of the DPPH radical.

2.5.3. Reducing power activity

The reducing power of the extracts of both plants was determined using the methodology developed by Oyaizu [24]. A volume of 200 µL of each extract at different concentrations was mixed with 500 µL of phosphate buffer (0.2 M; pH = 6.6) and 2.5 mL of 1% potassium ferricyanate solution ($K_3Fe(CN)_6$) prepared in distilled water. The mixture was incubated at 50 °C for 20 min. Subsequently, 2.5 ml of 10% trichloroacetic acid (TCA) was added, followed by centrifugation at 650 rpm for 10 min. A volume of 500 µL of supernatant was taken to which 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ were added. The absorbance was measured at 700 nm. Ascorbic acid and BHT were used as antioxidant standards. Values were expressed as $A_{0.5}$ (mg/mL), the concentration of the sample that gives an absorbance of 0.5 at 700 nm ($A_{0.5}$) calculated from a linear regression analysis.

2.6. Antimicrobial activity

2.6.1. Microorganisms

The antimicrobial activity of plant extracts was evaluated against the following pathogens: four strains of bacteria (*Bacillus subtilis* ATCC 6633, *Streptococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853), and one fungus (*Candida albicans*).

2.6.2. Inoculums preparation

Before the experiment, the bacterial strains were inoculated on the surface of the nutrient agar medium. Inoculum suspensions were obtained by selecting 4-5 well-isolated colonies from 24-hour cultures. The colonies were suspended in nutrient broth (or sterile physiological water with 0.9% NaCl). The optical density was adjusted to a McFarland standard turbidity of 0.5 (equivalent to 1-5×10^8 CFU/mL). For *C. albicans*, the inoculum was prepared from a 48-hour culture of isolated fungi grown on potato dextrose agar (PDA). The optical density was also adjusted to 0.08-0.10 to obtain a solution corresponding to ×10^6 spores/mL.

2.6.3. Antimicrobial screening

To evaluate the antibacterial activity of different extracts, the agar well diffusion method was used as previously described [25]. Wells (6 mm in diameter) were punched on Mueller-Hinton agar plates pre-inoculated with young bacteria (1×10^8 CFU/mL) or yeast (5×10^6 cells/mL) and filled with 80 µL of each extract (prepared in DMF) at a concentration of 128 mg/mL. Gentamicin 40 mg/mL was used as standard antibiotic due to its wide spectrum of inhibition. After cooling at 4 °C for 2 hours, the plates were incubated at 37 °C for 24 hours (bacteria) or 48 hours (yeast). Antimicrobial activity was determined by measuring the diameter of the inhibition zone around the well.

2.6.4. Determination of the minimum inhibitory concentration (MIC)

To determine the minimum inhibitory concentration (MIC) of the different extracts, the macroscopic dilution method was used. Müller-Hinton agar was poured into Petri dishes and inoculated by the smear method with bacterial strains to be tested at a certain density (0.08-0.10 at λ = 628 nm). The sterile Pasteur pipettes were then used to form 6 mm wells in the agar. Extracts at different concentrations (128, 64, 32, 16, 8, 4, 2, and 1 mg/mL) were prepared in DMF by double dilution and then autoclaved at 121 °C for 15 minutes. Eighty microliters of each extract were added to each well. After incubation for 18 hours at 37 °C, the MIC was determined. The MIC is the concentration of the extract that causes the lowest zone of inhibition greater than 8 mm [26].

2.6.5. Determination of minimum bactericidal and fungicidal concentration (MBC/MFC)

The MBC is the concentration of an antibacterial substance with a bactericidal effect and can be obtained after 18-24 hours of incubation at 37 °C. To determine MBC and MFC, the surface of each inhibition zone recorded with different concentrations of extracts (128, 64, 32, 16, 8, and 4 mg/mL) was scraped using a Pasteur pipette. The nutrient broth tubes were then inoculated with the scraped agar pieces. The tubes were incubated at 37 °C for 24 hours. The absence of growth (no turbidity) in the inoculated tubes indicated a bactericidal/fungicidal effect and was declared to contain MBC/MFC, which is equal to the concentration where it was initially scraped [27].

2.7. Statistical analysis

All assays were performed in triplicate and the results were expressed as mean±SD. Statistical comparisons were made with the Student's t test or the ANOVA test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Extraction yields and total polyphenols contents

Figure 1 shows the extraction yields, total phenolics, flavonoids, and condensed tannins of extracts of *J. oxycedrus*, *D. viscosa*, *G. alypum* leaves, and *R. sphaerocarpa* stems from various extraction solvents. The yields of the bioactive compounds varied significantly according to the plant and solvent. The highest extraction yield was detected in the ethanol and acetone extract of *J. oxycedrus* (35.70±0.35% and 35.56±0.45%, respectively), while the lowest yield was recorded in the *G. alypum* ethyl acetate extract (12.30±0.45%) (Figure 1a).

Similarly, the results of phenolics content revealed that the acetone extract of *J. oxycedrus* exhibited the highest level of polyphenols (504.96±14.82 mg GAE/g DE), followed by the acetone extract of *D. viscosa* (454.60±18.06 mg GAE/g DE) and the acetone extract of *R. sphaerocarpa* (333.01±2.61 mg GAE/g DE). In the case of *G. alypum*, the highest level of polyphenol compounds was detected in the ethanol extract (197.51±3.21 mg GAE/g DE) (Figure 1b).

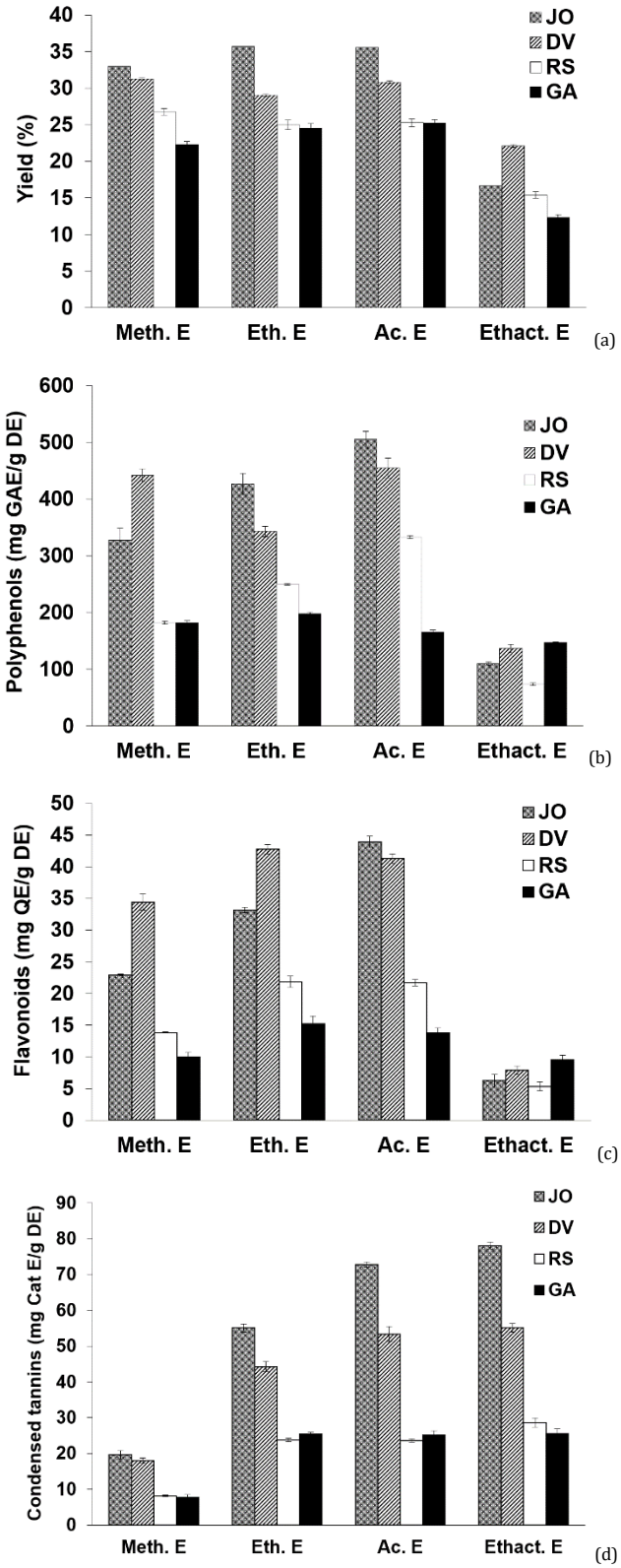

Figure 1. Extraction yields (a), polyphenols (b), flavonoids (c), and condensed tannins (d) contents of the four plant extracts. The results are reported per 1 g of extract and are presented as mean±SD (n=3). GA: *Globularia alypum,* DV: *Dittrichia viscosa,* JO: *Juniperus oxycedrus,* RS: *Retama sphaerocarpa*. Ac. E: Acetone extract, Eth. E: Ethanol extract, Ethact. E: Ethyl acetate extract, Meth. E: Methanol extract. Cat E = Catechin equivalent; DE = Dry extract; GAE = Gallic acid equivalent; QE = Quercetin equivalent.

According to the data in Figure 1c, the studied plant extracts showed the same flavonoids evolution profile as for phenolics content. The acetone extract of *J. oxycedrus* had the highest flavonoids concentration with an average value of 43.91±0.87 mg QE/g DE, followed by the acetone extract of *D. viscosa* (41.28±0.75 mg QE/g DE) and the acetone extract of *R. sphaerocarpa* (21.69±0.47 mg QE/g DE).

Table 1. Antioxidant activity of different extracts *.

Plants	Standards and extracts	DPPH, IC_{50} (mg/mL)	RP, $A_{0.5}$ (mg/mL)	TAC (mg GAE/g DE)
J. oxycedrus	Meth. E	0.32±0.01	0.39±0.09	340.23±4.13
	Eth. E	0.23±0.01	0.94±0.03	344.44±5.89
	Ac. E	0.21±0.01	0.59±0.05	350.67±6.05
	Ethact. E	1.99±0.06	1.80±0.06	222.03±3.74
D. viscosa	Meth. E	0.21±0.06	0.46±0.08	247.32±4.92
	Eth. E	0.27±0.01	0.44±0.08	282.53±4.07
	Ac. E	0.36±0.01	0.54±0.05	347.99±8.75
	Ethact. E	0.86±0.01	2.08±0.07	155.88±2.41
R. sphaerocarpa	Meth. E	0.89±0.02	5.22±0.12	247.89±3.62
	Eth. E	0.48±0.01	3.01±0.17	316.89±0.09
	Ac. E	0.39±0.02	3.66±0.15	299.87±3,09
	Ethact. E	2.18±0.05	5.68±0.19	103.27±1.53
G. alypum	Meth. E	0.89±0.03	5.31±0.11	116.72±0.79
	Eth. E	0.83±0.04	4.42±0.03	141.33±3.54
	Ac. E	0.82±0.02	4.02±0.09	65.02±3.13
	Ethact. E	0.90±0.01	4.73±0.10	29.70±2.84
-	Ascorbic acid	0.11±0.02	0.28±0.01	403.32±6.66
	2,6-Di-*tert*-butyl-4-methylphenol	2.21±0.10	1.09±0.09	214.45±5.32

* Ac. E: Acetone extract, Eth. E: Ethanol extract, Ethact. E: Ethyl acetate extract, Meth. E: Methanol extract. RP: reducing power, TAC: Total antioxidant capacity.

Table 2. Antimicrobial activity of different plant extracts at a concentration of 128 mg/mL *.

Plants	**Standard and extracts**	**Micro-organisms / Diameter of inhibition zone (mm)**				
		P. aeruginosa	***S. aureus***	***E. coli***	***B. cereus***	***C. albicans***
J. oxycedrus	Ac. E	26.77±0.06	12.01±0.03	15.84±0.04	17.16±0.08	21.36±0.11
	Meth. E	22.33± 0.04	12.03±0.05	15.37±0.02	16.34±0.12	20.66±0.12
	Eth. E	24.45±0.04	14.04±0.05	15.85±0.02	16.28±0.09	16.56±0.07
	Ethact. E	12.70±0.07	14.31±0.04	13.77±0.05	15.86±0.11	14.23±0.08
D. viscosa	Ac. E	22.78±0.04	22.42±0.11	09.22±0.02	13.11±0.03	15.35±0.11
	Meth. E	24.44±0.06	19.13±0.02	09.85±0.06	13.93±0.04	18.34±0.04
	Eth. E	20.13±0.04	24.54±0.03	08.65±0.03	21.91±0.02	20.44±0.06
	Ethact. E	18.91±0.02	24.25±0.04	07.35±0.07	13.42±0.04	19.74±0.11
R. sphaerocarpa	Ac. E	24.47±0.12	19.50±0.02	11.42±0.11	11.24±0.11	14.00±0.13
	Meth. E	24.60±0.20	20.18±0.10	11.10±0.09	14.10±0.11	11.16±0.34
	Eth. E	23.24±0.11	20.25±0.06	10.17±0.07	14.60±0.06	24.02±0.17
	Ethact. E	29.12±0.04	20.70±0.03	11.51±0.02	08.13±0.11	15.03±0.07
G. alypum	Ac. E	25.68±0.07	20.86±0.05	08.58±0.05	12.05±0.04	10.73±0.43
	Meth. E	23.63±0.06	21.09±0.10	15.11±0.10	12.83±0.05	15.11±0.11
	Eth. E	22.23±0.04	22.08±0.07	10.10±0.02	13.06±0.05	15.10±0.19
	Ethact. E	24.90±0.03	22.27±0.09	08.42±0.02	14.33±0.14	19.70±0.34
Antibiotic	Gentamycine	37.01±0.96	35.33±1.27	35.78±0.04	38.21±0.06	38.63±0.09

* Ac. E: Acetone extract, Eth. E: Ethanol extract, Ethact. E: Ethyl acetate extract, Meth. E: Methanol extract.

In the case of *G. alypum*, the highest level of flavonoids was detected in the ethanol extract (15.21±1.25 mg QE/g DE).

Analysis of condensed tannins showed that the highest concentrations for all plants studied were obtained with ethyl acetate extracts: 78.01±1.02 mg CatE/g DE for *J. oxycedrus*, 55.13±1.22 mg CatE/g DE for *D. viscosa*, 28.61±1.28 mg CatE/g DE for *R. sphaerocarpa* and 25.55±1.26 mg CatE/g DE for *G. alypum* (Figure 1d).

3.2. Antioxidant activity

In the present work, the antioxidant activity of plant extracts was evaluated by measuring the total antioxidant capacity (TAC), the free radical DPPH and the reducing power. The results are given as IC_{50} and $A_{0.5}$ values.

In general, acetone extracts exhibited higher antioxidant activity. The results in Table 1 showed that *J. oxycedrus* acetone extract displayed the highest TAC (350.67±6.05 mg GAE/g DE), followed by *D. viscosa* acetone extract (347.99±8.75 mg GAE/g DE), *R. sphaerocarpa* ethanol extract (316.89±0.09 mg GAE/g DE), and *G. alypum* ethanol extract (141.33±3.54 mg GAE/g DE).

With regard to DPPH scavenging activity, the acetone extract of *J. oxycedrus* and methanol extract of *D. vicosa* were the most active extracts against DPPH free radical with IC_{50} values of 0.21±0.01 and IC_{50} = 0.21±0.06 mg/mL, respectively. Furthermore, all extracts tested exhibited higher antioxidant activity than BHT (IC_{50} = 2.21 ± 0.10 mg/mL) (Table 1).

Analysis of metal iron-reduction revealed that *J. oxycedrus* methanol extract and *D. vicosa* ethanol extract displayed very potent antioxidant powers with A_{50} values of 0.39±0.09 and 0.44±0.08 mg/mL, respectively. The ethanol extract of *R. sphaerocarpa* and the acetone extract of *G. alypum* exhibited slight antioxidant activity with A_{50} values of 3.01±0.17 and 4.02±0.09 mg/mL, respectively (Table 1).

3.3. Antimicrobial activity

The bacteria and fungus used in this study are associated with various forms of diseases [28]. The antimicrobial activities of the different plant extracts are shown in Table 2.

The extracts tested exhibited different degrees of antimicrobial activity. The acetone extract of *J. oxycedrus* was the most active against almost all strains tested strains (26.77±0.06 mm for *P. aeruginosa*, 21.36±0.11 mm for *C. albicans*, 17.16±0.08 mm for *B. cereus*, 15.84±0.04 mm for *E. coli)*, except *S. aureus,* which was more sensitive to the action of the ethyl acetate extract (14.31±0.04 mm) (Table 2).

In the case of *D. viscosa*, methanol and ethanol extracts were the most active against the bacteria and fungi tested. Consequently, the mean diameter of the growth inhibition zone of methanol extract against *P. aeruginosa* was equal to 24.44±0.06 mm. The ethanol extract showed inhibition zones of diameters of 24.54±0.03, 21.91±0.02, and 20.44±0.06 mm against *S. aureus, B. cereus*, *C. albicans*, respectively (Table 2).

The ethyl acetate extract of *R. sphaerocarpa* showed the strongest antibacterial activity on *P. aeruginosa, S. aureus,* and *E. coli* (29.12±0.04, 20.70±0.03, and 11.51±0.02 mm, respectively), while the ethanol extract exhibited high antimicrobial activity against *C. albicans* and *B. cereus* (24.02±0.17 and 14.60±0.06 mm, respectively) (Table 2).

Table 3. Minimum inhibitory concentration (MIC) *.

Microorganisms	MIC (mg/mL)															
	Meth. E				Eth. E				Ac. E				Ethact. E			
	JO	DV	RS	GA	JO	DV	RS	GA	JO	DV	RS	GA	JO	DV	RS	GA
P. aeruginosa	16	8	8	8	16	32	8	8	8	16	8	8	16	16	8	8
B. cereus	16	8	8	32	16	32	32	8	8	16	16	16	8	16	32	8
S. aureus	8	8	8	8	8	8	8	8	8	8	8	16	8	8	8	8
E. coli	64	64	64	16	64	64	32	16	64	128	64	16	64	128	32	32
C. albicans	8	16	64	16	16	32	16	16	8	32	32	64	16	16	16	6

* DV: *D. viscosa*, GA: *G. alypum*, JO: *J. oxycedrus*, RS: *R. sphaerocarpa*.

Table 4. Minimum bactericidal/fungicidal concentrations (MBC/MFC) *.

Microorganisms	MBC and MFC (mg/mL)															
	Meth. E				Ethact. E				Ac. E				Ethact. E			
	JO	DV	RS	GA	JO	DV	RS	GA	JO	DV	RS	GA	JO	DV	RS	GA
P. aeruginosa	128	64	32	32	128	128	64	128	128	128	64	64	16	128	16	32
B. cereus	128	64	32	64	128	128	128	64	64	128	64	64	64	128	32	128
S. aureus	64	128	64	64	32	64	32	64	128	128	32	128	64	64	64	16
E. coli	128	128	128	64	128	128	128	64	128	128	128	64	128	128	128	128
C. albicans	64	128	128	64	128	128	64	64	32	128	64	128	64	128	64	64

* DV: *D. viscosa*, GA: *G. alypum*, JO: *J. oxycedrus*, RS: *R. sphaerocarpa*.

Regarding *G. alypum*, all extracts showed antimicrobial activity against the bacteria and fungi tested. The minimum inhibitory concentration (MIC) and the minimum bactericidal/ fungicidal concentrations (MBC and MFC) of the plant extracts are displayed in Tables 3 and 4.

4. Discussion

In this study, we investigated the phytochemical composition, antioxidant and antimicrobial activities of different extracts prepared from different plants including *G. alypum*, *J. oxycedrus*, *D. viscosa* and the stems of *R. sphaerocarpa* using various solvents of differing polarities. Quantitative analysis revealed that the extraction yields and contents of the phenolic compounds differed significantly depending on the plant and the extraction solvent [29]. In the present study, ethanol and acetone of *J. oxycedrus* recorded the highest extraction yields (35.70±0.35 and 35.56±0.45%, respectively). The results of this study are higher than those obtained by Chaouche *et al*. [30] who reported an extraction yield of 12.1%, but lower than that of Živić *et al.* [31], who reported an extraction yield of 49.80%. The extraction yield is mainly influenced by the chemical nature of the solvent used, the extraction time, the sample size, and the presence of interference substances [32]. Furthermore, the combined use of water and organic solvents can facilitate the extraction of soluble chemicals in water and/or organic solvents [33].

The polyphenols content ranged from 73.75±2.11 to 504.94±3.36 mg GAE/g (Figure 1b). The acetone extract of *J. oxycedrus* recorded the highest phenolic compounds among all extracts tested. Our results are significantly higher than those of of Taviano *et al.* [34] Chaouche *et al.* [30], and Živić *et al.* [31] with values of 253.29±3.16 mg GAE/g DW, 76.1±2.8 mg GAE/g DW, and 58.73±0.14 mg GAE/g DW, respectively. The acetone extract of *D. viscosa* also showed a high level of phenolic compounds (454.60±18.06 mg GAE/g DE). The result is much higher than that reported by Gökbulut *et al*. [35] on *D. viscosa* from Turkey (177.1±3.6 mg GAE/g DE) and Rhimi *et al*. [36] on *D. viscosa* from Tunisia (117.58±1.29 mg GAE/g). Phytochemicals are mainly extracted using an organic solvent. Several studies reported that acetone is the best solvent for polyphenols extraction in comparison to methanol, ethanol, and water [37].

The antioxidant activity of medicinal plant is attributed mainly to its richness in phenolic compounds, but also to other bioactive molecules [38,39]. Evaluation of the antioxidant activity of plant extracts using different assays, including TAC, DPPH, and reducing power, showed interesting antioxidant effects.

TAC indicates the general antioxidant activity of a food or plant and its ability to neutralise free radicals in the body. The results obtained showed that the TAC decreased in the following order: *J. oxycedrus* acetone extract (350.67±6.05 mg GAE/g DE) > *D. viscosa* acetone extract (347.99±8.75 mg GAE/g DE) > *R. sphaerocarpa* ethanol extract (316.89±0.09 mg GAE/g DE) > *G. alypum* methanol extract (141.33±3.54 mg GAE/g DE). The difference in total antioxidant capacity from one extract to another can be explained by the ability of substances to transfer electrons in the reaction medium [40]. The results of TAC regarding *D. viscosa* are much higher than those obtained by Rhimi *et al.* [36].

The antiradical activity results showed that the *J. oxycedrus* acetone extract was also the most active against the free radical DPPH with IC_{50} = 0.21±0.01 mg/mL, followed by the *D. viscosa* methanol extract (IC_{50} = 0.21±0.06 mg/mL) *R. sphaerocarpa* acetone extract (IC_{50} = 0.39±0.02 mg/mL) and the *G. alypum* acetone extract (IC_{50} = 0.82±0.02 mg/mL). Our results for *J. oxycedrus* are much higher compared to those obtained with *Juniperus thurifera* L extracts (IC_{50} = 29.348 mg/mL with ethyl acetate extract, IC_{50} = 37.538 mg/mL with aqueous extract, and IC_{50} = 52.573 mg/mL with methanol extract) [41]. The results of *D. viscosa* are lower than those recorded by Rhimi *et al.* [36] with value of 0.056±1.2 mg/mL. Regarding *R. sphaerocarpa,* the results of this study are higher than those of Edziri *et al*. [42] who reported an IC_{50} of 0.5 mg/mL. Our results are much higher than those of Khlifi *et al.* [43] who reported an IC_{50} of 15.58 mg/L with *G. alypum* methanol extract.

The reducing power of the studied extracts is classified in the following order: *J. oxycedrus* methanol extract ($A_{0.5}$ = 0.39±0.09 mg/mL) > *D. viscosa* ethanol extract ($A_{0.5}$ = 0.44±0.08 mg/mL) > *R. sphaerocarpa* ethanol extract ($A_{0.5}$ = 3.01±0.17 mg/mL) > *G. alypum* acetone extract ($A_{0.5}$ = 4.02±0.09 mg/mL). This difference between the different extracts can be explained by the differences in the polarities of the bioactive compounds of each extract, which can influence their solubility and their reducing power [44].

All plant extracts exhibited antibacterial and anticandidal activities, with MIC values ranging from 8 to 128 mg/mL (Table 2). All extracts were more active against Gram-positive bacteria and yeasts than Gram-negative ones. Several studies have shown that Gram-positive bacteria are much more sensitive than Gram-negative ones. It has also been reported that the resistance of Gram-negative bacteria such as *E. coli* to antimicrobial agents is linked to the presence of an envelope that includes a cell membrane rich in lipopolysaccharides and a wall that limits access to antimicrobial agents, unlike gram-positive bacteria which are less protected against external agents (detergents and antibiotics). According to Akiyama *et al*.

[45] and Funatogawa *et al.* [46], the inhibitory effect of phenolic compounds and flavonoids on the growth of bacteria can be associated with several mechanisms of action, among which the chelation of iron which is necessary for microbial growth, and the action on the membrane of microorganism leading to the loss of its structural integrity.

5. Conclusions

This work aimed to investigate the physicochemical, phytochemical, antioxidant and antimicrobial activities of various plant extracts, including *D. viscosa, J. oxycedrus, R. sphaerocarpa, and G. alypum,* prepared from different solvents. The screening of the phenolic compounds of the different extracts showed that the investigated plants were rich in antioxidant and antimicrobial compounds, including polyphenols, flavonoids, and condensed tannins. On the other hand, the study of antioxidant activity using complementary methods (total antioxidant capacity, DPPH free radical scavenging activity, and reducing power) showed that most of the extracts exhibited significant antioxidant potency. The antimicrobial activity results showed that all extracts tested have antibacterial and anticandidal activities at different concentrations. Our results demonstrate that the studied plants could be a consistent source of antioxidant and antimicrobial compounds and support the data from the previous literature and the widespread usage of the medicinal plants tested.

Acknowledgements

This work was funded by the Algerian Ministry of Higher Education and Scientific Research (PRFU project grant no D01N01UN340120180001).

Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
Ethical approval: All ethical guidelines have been adhered to.
Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement

Conceptualization: Yuva Bellik; Methodology: Yuva Bellik, Nasreddine Mekhoukh; Software: Yuva Bellik; Validation: Yuva Bellik; Formal Analysis: Yuva Bellik; Investigation: Yuva Bellik, Nasreddine Mekhoukh; Resources: Yuva Bellik; Data Curation: Yuva Bellik; Writing - Original Draft: Yuva Bellik, Nasreddine Mekhoukh; Writing - Review and Editing: Yuva Bellik; Visualization: Yuva Bellik; Funding acquisition: Yuva Bellik; Supervision: Yuva Bellik; Project Administration: Yuva Bellik.

ORCID and Email

Yuva Bellik
y.bellik@univ-bba.dz
https://orcid.org/0000-0002-5596-2771
Nasreddine Mekhoukh
n.mekhoukh@univ-bba.dz
https://orcid.org/0000-0001-6677-4381

References

[1]. Gurib-Fakim, A. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med.* **2006**, *27*, 1–93.
[2]. Newman, D. J.; Cragg, G. M. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J. Nat. Prod.* **2020**, *83*, 770–803.
[3]. Michel, J.; Abd Rani, N. Z.; Husain, K. A review on the potential use of medicinal plants from Asteraceae and Lamiaceae plant family in cardiovascular diseases. *Front. Pharmacol.* **2020**, *11*, 852.
[4]. Bellik, Y.; Selles, S. M. A. In vitro synergistic antioxidant activity of honey-Mentha spicata combination. *J. Food Meas. Charact.* **2017**, *11*, 111–118.
[5]. Fabricant, D. S.; Farnsworth, N. R. The value of plants used in traditional medicine for drug discovery. *Environ. Health Perspect.* **2001**, *109*, 69–75.
[6]. Kim, Y. S.; Young, M. R.; Bobe, G.; Colburn, N. H.; Milner, J. A. Bioactive food components, inflammatory targets, and cancer prevention. *Cancer Prev. Res. (Phila.)* **2009**, *2*, 200–208.
[7]. Salehi, B.; Azzini, E.; Zucca, P.; Maria Varoni, E.; V. Anil Kumar, N.; Dini, L.; Panzarini, E.; Rajkovic, J.; Valere Tsouh Fokou, P.; Peluso, I.; Prakash Mishra, A.; Nigam, M.; El Rayess, Y.; El Beyrouthy, M.; N. Setzer, W.; Polito, L.; Iriti, M.; Sureda, A.; Magdalena Quetglas-Llabrés, M.; Martorell, M.; Martins, N.; Sharifi-Rad, M.; M. Estevinho, L.; Sharifi-Rad, J. Plant-derived bioactives and oxidative stress-related disorders: A key trend towards healthy aging and longevity promotion. *Appl. Sci. (Basel)* **2020**, *10*, 947.
[8]. Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F.; Bonaduce, D.; Abete, P. Oxidative stress, aging, and diseases. *Clin. Interv. Aging* **2018**, *13*, 757–772.
[9]. Antimicrobial Resistance Collaborators Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* **2022**, *399*, 629–655.
[10]. Cai, Y.; Luo, Q.; Sun, M.; Corke, H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.* **2004**, *74*, 2157–2184.
[11]. Mohamed, T.; Souiy, Z.; Achour, L.; Hamden, K. Anti-obesity, anti-hyperglycaemic, anti-antipyretic and analgesic activities of Globularia alypum extracts. *Arch. Physiol. Biochem.* **2022**, *128*, 1453–1460.
[12]. Adams, R. P. The leaf essential oils and chemotaxonomy of Juniperus sect. Juniperus. *Biochem. Syst. Ecol.* **1998**, *26*, 637–645.
[13]. Vilar, L.; Caudullo, G.; de Rigo, D. Juniperus oxycedrus in Europe: distribution, habitat, usage and threats. In: San-Miguel- Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A. (Eds.), European Atlas of Forest Tree Species., Publ. Off. EU, Luxembourg, pp. e013abb+. https://ies-ows.jrc.ec.europa.eu/efdac/download/Atlas/pdf/Juniperus_oxycedrus.pdf (accessed January 1, 2023).
[14]. Swanston-Flatt, S. K.; Day, C.; Bailey, C. J.; Flatt, P. R. Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetologia* **1990**, *33*, 462–464.
[15]. Grauso, L.; Cesarano, G.; Zotti, M.; Ranesi, M.; Sun, W.; Bonanomi, G.; Lanzotti, V. Exploring Dittrichia viscosa (L.) Greuter phytochemical diversity to explain its antimicrobial, nematicidal and insecticidal activity. *Phytochem. Rev.* **2020**, *19*, 659–689.
[16]. Guerrouj, K.; Ruíz-Díez, B.; Chahboune, R.; Ramírez-Bahena, M.-H.; Abdelmoumen, H.; Quiñones, M. A.; El Idrissi, M. M.; Velázquez, E.; Fernández-Pascual, M.; Bedmar, E. J.; Peix, A. Definition of a novel symbiovar (sv. retamae) within Bradyrhizobium retamae sp. nov., nodulating Retama sphaerocarpa and Retama monosperma. *Syst. Appl. Microbiol.* **2013**, *36*, 218–223.
[17]. Villar-Salvador, P.; Valladares, F.; Domínguez-Lerena, S.; Ruiz-Díez, B.; Fernández-Pascual, M.; Delgado, A.; Peñuelas, J. L. Functional traits related to seedling performance in the Mediterranean leguminous shrub Retama sphaerocarpa: Insights from a provenance, fertilization, and rhizobial inoculation study. *Environ. Exp. Bot.* **2008**, *64*, 145–154.
[18]. León-González, A. J.; Navarro, I.; Acero, N.; Muñoz Mingarro, D.; Martín-Cordero, C. Genus Retama: a review on traditional uses, phytochemistry, and pharmacological activities. *Phytochem. Rev.* **2018**, *17*, 701–731.
[19]. Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.
[20]. Jain, D. P.; Pancholi, S. S.; Patel, R. Synergistic antioxidant activity of green tea with some herbs. *J. Adv. Pharm. Technol. Res.* **2011**, *2*, 177–183.
[21]. Oyedemi, S. O.; Afolayan, A. J. In vitro and in vivo Antioxidant Activity of Aqueous Leaves Extract of Leonotis leonurus (L.) R. Br. *Int. J. Pharmacol.* **2011**, *7*, 248–256.
[22]. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* **1999**, *269*, 337–341.
[23]. Burits, M.; Bucar, F. Antioxidant activity of Nigella sativa essential oil. *Phytother. Res.* **2000**, *14*, 323–328.
[24]. Oyaizu, M. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr. Diet.* **1986**, *44*, 307–315.
[25]. Daoud, A.; Malika, D.; Bakari, S.; Hfaiedh, N.; Mnafgui, K.; Kadri, A.; Gharsallah, N. Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. *Arab. J. Chem.* **2019**, *12*, 3075–3086.
[26]. Min; B. R; Pinchak; W. E; Merkel; R; Walker; S; Tomita; G; R. C Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens. *Sci. Res. Essays* **2008**, *3*, 066–073.
[27]. Salama, H. M. H.; Marraiki, N. Antimicrobial activity and phytochemical analyses of Polygonum aviculare L. (Polygonaceae), naturally growing in Egypt. *Saudi J. Biol. Sci.* **2010**, *17*, 57–63.
[28]. Nester, E. W.; Nester, M. T.; Evans Roberts, C.; Anderson, D. G. *Microbiology: A Human Perspective*; 7th ed.; McGraw Hill Higher Education: Maidenhead, England, 2012.

[29]. Bakhouche, I.; Aliat, T.; Boubellouta, T.; Gali, L.; Şen, A.; Bellik, Y. Phenolic contents and in vitro antioxidant, anti-tyrosinase, and anti-inflammatory effects of leaves and roots extracts of the halophyte Limonium delicatulum. *S. Afr. J. Bot.* **2021**, *139*, 42–49.
[30]. Chaouche, T. M.; Haddouchi, F.; Atik-Bekara, F.; Ksouri, R.; Azzi, R.; Boucherit, Z.; Tefiani, C.; Larbat, R. Antioxidant, Haemolytic Activities and HPLC–DAD–ESI–MSn Characterization of Phenolic Compounds from Root Bark of Juniperus Oxycedrus Subsp. Oxycedrus. *Ind. Crops Prod.* **2015**, *64*, 182–187.
[31]. Živić, N.; Milošević, S.; Dekić, V.; Dekić, B.; Ristić, N.; Ristić, M.; Sretić, L. Phytochemical and antioxidant screening of some extracts of Juniperus communis L. and Juniperus oxycedrus L. *Czech J. Food Sci.* **2019**, *37*, 351–358.
[32]. Stalikas, C. D. Extraction, separation, and detection methods for phenolic acids and flavonoids. *J. Sep. Sci.* **2007**, *30*, 3268–3295.
[33]. Do, Q. D.; Angkawijaya, A. E.; Tran-Nguyen, P. L.; Huynh, L. H.; Soetaredjo, F. E.; Ismadji, S.; Ju, Y.-H. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *J. Food Drug Anal.* **2014**, *22*, 296–302.
[34]. Taviano, M. F.; Marino, A.; Trovato, A.; Bellinghieri, V.; La Barbera, T. M.; Güvenç, A.; Hürkul, M. M.; Pasquale, R. D.; Miceli, N. Antioxidant and antimicrobial activities of branches extracts of five *Juniperus* species from Turkey. *Pharm. Biol.* **2011**, *49*, 1014–1022.
[35]. Gökbulut, A.; Özhana, O.; Satılmiş, B.; Batçioğlu, K.; Günal, S.; Şarer, E. Antioxidant and antimicrobial activities, and phenolic compounds of selected *Inula* species from turkey. *Nat. Prod. Commun.* **2013**, *8*, 1934578X1300800.
[36]. Rhimi, W.; Hlel, R.; Ben Salem, I.; Boulila, A.; Rejeb, A.; Saidi, M. *Dittrichia viscosa* L. ethanolic extract based ointment with antiradical, antioxidant, and healing wound activities. *Biomed Res. Int.* **2019**, *2019*, 4081253.
[37]. Liu, S.; Lin, J.; Wang, C.; Chen, H.; Yang, D. Antioxidant properties of various solvent extracts from lychee (Litchi chinenesis Sonn.) flowers. *Food Chem.* **2009**, *114*, 577–581.
[38]. Balasundram, N.; Sundram, K.; Samman, S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.* **2006**, *99*, 191–203.
[39]. Yousfi, F.; Abrigach, F.; Petrovic, J. D.; Sokovic, M.; Ramdani, M. Phytochemical screening and evaluation of the antioxidant and antibacterial potential of Zingiber officinale extracts. *S. Afr. J. Bot.* **2021**, *142*, 433–440.
[40]. Hinneburg, I.; Damien Dorman, H. J.; Hiltunen, R. Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem.* **2006**, *97*, 122–129.
[41]. Manel, M.; Nouzha, H.; Rim, M.; Imane, M.; Sana, A.; Yasmine, O.; Ammar, A. Antibacterial and antioxidant activity of Juniperus thurifera L. leaf extracts growing in East of Algeria. *Vet. World* **2018**, *11*, 373–378.
[42]. Hayet, E.; Maha, M.; Samia, A.; Mata, M.; Gros, P.; Raida, H.; Ali, M. M.; Mohamed, A. S.; Gutmann, L.; Mighri, Z.; Mahjoub, A. Antimicrobial, antioxidant, and antiviral activities of Retama raetam (Forssk.) Webb flowers growing in Tunisia. *World J. Microbiol. Biotechnol.* **2008**, *24*, 2933–2940.
[43]. Khlifi, D.; Hamdi, M.; Hayouni, A. E.; Cazaux, S.; Souchard, J. P.; Couderc, F.; Bouajila, J. Global chemical composition and antioxidant and anti-tuberculosis activities of various extracts of Globularia alypum L. (globulariaceae) leaves. *Molecules* **2011**, *16*, 10592–10603.
[44]. Jayaprakasha, G. K.; Patil, B. S. In vitro evaluation of the antioxidant activities in fruit extracts from citron and blood orange. *Food Chem.* **2007**, *101*, 410–418.
[45]. Akiyama, H. Antibacterial action of several tannins against Staphylococcus aureus. *J. Antimicrob. Chemother.* **2001**, *48*, 487–491.
[46]. Funatogawa, K.; Hayashi, S.; Shimomura, H.; Yoshida, T.; Hatano, T.; Ito, H.; Hirai, Y. Antibacterial activity of hydrolyzable tannins derived from medicinal plants against*Helicobacter pylori*. *Microbiol. Immunol.* **2004**, *48*, 251–261.