Pharmacogn. Res. A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products

In vitro Screening for Antioxidant and Antimicrobial Properties of Three Lebanese Medicinal Plants Crude Extracts

Josiane Alhage, Hoda Elbitar

AZM Centre for Research in Biotechnology and Its Applications, Laboratory of Applied Biotechnology for Biomolecules, Biotherapy and Bioprocess, Lebanese University, Tripoli, Lebanon

ABSTRACT

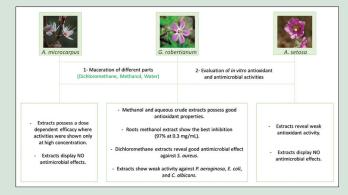
Background: Despite its small geographic area, Lebanon is characterized by a natural wealth in medicinal plants. Lebanese flora has about 2600 species, where >100 are endemic and are used in traditional medicine for their therapeutic effects. Objective: To evaluate the antioxidant and antimicrobial activities of dichloromethane, methanol, and aqueous crude extracts of three Lebanese medicinal plants: Geranium robertianum, Asphodelus microcarpus, and Alcea setosa. Materials and Methods: The antioxidant activity of different crude extracts was determined using the free radical, 1,1-diphenyl-2-picrylhydrazyl. Antimicrobial activity was evaluated in a plate-hole diffusion assay against two Gram-negative (Pseudomonas aeruginosa and Escherichia coli) bacterial strains, one Gram-positive (Staphylococcus aureus) bacterial strain, and a fungal strain (Candida albicans). Results: Methanol and aqueous extracts of G. robertianum possessed high scavenging activity (Radical scavenging activity (RSA) >85%) at 0.3 mg/mL followed by the extracts of A. microcarpus which showed a moderate activity. G. robertianum extracts exhibited good inhibition diameters against S. aureus growth. Conclusion: Obtained results give an overall view on the bioactivities of three Lebanese medicinal plants crude extracts. Key words: Alcea setosa, antimicrobial activity, antioxidant activity, Asphodelus microcarpus, crude extracts, Geranium robertianum

SUMMARY

- Geranium robertianum methanol and aqueous extracts possess a good ability to scavenge the free radical 2,2-diphenyl-2-picrylhydrazyl
- The best activity was observed by *G. robertianum* roots methanol extract which possessed an inhibition of 97% of the radical at 0.3 mg/mL
- Asphodelus microcarpus extracts possessed a dose-dependent efficacy where potent activities were shown at high concentration
- · Alcea setosa extracts revealed weak antioxidant activity
- G. robertianum dichloromethane extracts displayed good antimicrobial effect against Staphylococcus aureus

• A. microcarpus and A. setosa extracts did not display significant growth inhibition against the tested strains

• Further studies are required to identify the agent responsible for the activity of the active extracts.



 Abbreviations
 Used:
 DPPH:
 2,2-Diphenyl-1-picrylhydrazyl;

 CFU:
 Colony-forming unit;
 DMSO:
 Dimethyl sulfoxide;
 IZ:
 Inhibition zone;

 CH₂Cl₂
 Dichloromethane;
 CH₃OH:
 Methanol;
 H₂O:
 Water.

Common don cos	Access this article online
Correspondence:	Website: www.phcogres.com
Dr. Hoda Elbitar,	Quick Response Code:
AZM Centre for Research in Biotechnology	Quick Response obue.
and Its Applications, Laboratory of Applied	间清波对向
Biotechnology for Biomolecules, Biotherapy	加速的名称
and Bioprocess, Lebanese University, El Mitein	
Street, Tripoli, Lebanon.	
E-mail: elbitarhoda@yahoo.fr	145 A 4 5 5 5 5
DOI: 10.4103/pr.pr_171_18	国际经济部门

INTRODUCTION

Medicinal plants are of extreme value to the health of people and communities. Healing with medicinal plants has existed since ancient times, but in most cases, the pharmacological and chemical bases of these plants are not well known. Phytopreparations and medicinal plants are used for the prevention and therapy of diverse diseases, such as nervous, gastrointestinal, cardiovascular, and skin diseases.^[1] Plants are considered as primary sources of antioxidant compounds that play an important role as a health protecting factor. Studies suggest that antioxidants derived from medicinal plants reduce the risk of chronic diseases such as cancer and heart disease. In addition, the researches of antimicrobial components derived from plants have high importance because they lead to discover new antibiotics that face the rapid evolution of microbial resistance.^[2-5]

Placed on the East Coast of the Mediterranean region, Lebanon is considered one of the important centers of plant biodiversity and endemism.^[6] The Lebanese flora has 2607 species, distributed in 783 genera.^[7] Many of these species are used in Lebanese folk medicine, but

few of them are studied. Our research focuses on three Lebanese plants traditionally used for their healing properties.

Geranium robertianum

The *Geranium* genus is native from Central and Meridian Europe and Asia.^[8] It belongs to the *Geraniaceae* family and includes 380 species divided into at least ten sections.^[9] *Geranium* species are conventionally used as anti-asthmatic, antioxidant, anti-allergic,

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Alhage J, Elbitar H. *In vitro* screening for antioxidant and antimicrobial properties of three lebanese medicinal plants crude extracts. Phcog Res 2019;11:127-33.

antihepatotoxic, antidiarrheic, tonic, diuretic, hemostatic, antidiabetic, and stomachic. $^{\rm [10]}$

G. robertianum commonly known as Herb Robert or Red Robin is an annual or biennial herbaceous plant, which grows spontaneously in fresh and moist places in the shade of spruce-fir and beech forests located at altitudes above 1500 m.^[11] It has long petioles, stems branched in many directions from the base, and a fibrous rooting system. Its height may vary from 10 to 60 cm.^[12,13] The aqueous extract and decoction of the bark of this species are used in traditional Peruvian medicine for the relief of gastritis and arthritis pain and for the treatment of cancer.^[8] Tannins, phenolic acids, flavonoids, alkaloids, and saponins have been previously isolated from *G. robertianum* parts.^[13]

Asphodelus microcarpus

The *Asphodelus* genus is a Mediterranean genus. It belongs to the *Liliaceae* family and includes 16 species distributed in five sections.^[14] The species of *Asphodelus* genus have been used in traditional medicine to treat skin disorders, as well as psoriasis, ectodermal parasites, and microbial infection and for lightening freckles.^[15,16]

A. microcarpus is a perennial and robust herb with roots of several spindle-shaped tubers that can reach a height of 1 m. It is widely distributed over the coastal Mediterranean region. The bulbs and roots of *A. microcarpus* are used to treat jaundice, psoriasis, and ectodermal parasites.^[17] This species is also used by Bedouins as an antimicrobial agent.^[15] Previous studies revealed the presence of carbohydrates, triterpenes, sterols, lipids, anthraquinones, and arylcoumarins from *A. microcarpus*.^[18,19]

Alcea setosa

The *Alcea* genus is native from East Mediterranean. It belongs to the *Malvaceae* family and includes 60 species.^[20] *Alcea* species are used in folk medicine to treat inflammation, kidney stones, skin disorders, pulmonary disorders, urinary system disorders, and stomach disorders.^[21] *A. setosa* is a perennial herbaceous that grows mainly in mountains of the Mediterranean region. The purple-pink flowers are shaped as wide funnels, are 8–13 cm in diameter, are situated vertically on tall stalks of 1–2 m, and produce large amounts of nectar and pollen.^[22] The leaves of *A. setosa* are traditionally used to treat stomach and intestine pain, inflammation, and asthma.^[23] Studies showed that this species encloses mucilage in its parts.^[24] To the best of our knowledge, no study has been reported yet on the bioactivities of these Lebanese species. This work is, therefore, initiated to evaluate the antioxidant and antimicrobial efficacy of the dichloromethane, methanol, and aqueous extracts of *G. robertianum, A. microcarpus*, and *A. setosa* parts.

MATERIALS AND METHODS

Materials and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) (97%) and Vitamin C (99%) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). All other chemical reagents that are used in the research were purchased from reliable commercial sources.

Plant material

A. setosa was collected in Jounieh, North of Beirut, Lebanon, in April 2011. *G. robertianum* was collected in Koura, North of Lebanon, in March 2013. *A. microcarpus* was collected in Alma, North of Lebanon, in February 2014. The three species were identified by Professors Georges and Henriette Tohme (Professors in Natural Substances).

The plant material was dried in the shade at room temperature for 2 weeks and stored in a dry place.

Extraction and isolation

The air-dried parts of *A. setosa* (leaves, flowers, stems, and roots), *G. robertianum* (leaves, flowers, stems, and roots), and *A. microcarpus* (leaves, flowers, stems, roots, tubers, and buds) were macerated for 48 h at room temperature by successively dichloromethane, methanol, and water.

On extraction, the mixtures were filtered and evaporated to dryness under vacuum at temperatures not higher than 45°C. The extracts were stored at 4°C in the dark until use.

Antioxidant assay

The antioxidant activity of the plants' different extracts was determined by the evaluation of scavenging effect of these samples on the stable radical DPPH, according to the method described in the literature.^[25,26] Different concentrations of each sample were prepared in the solvent of extraction (0.3, 0.5, and 1.0 mg/mL). Briefly, 1.0 mL aliquot of each concentration of test sample was added to 1.0 mL of 0.16 mM DPPH methanolic solution. The mixture was vortexed and left at room temperature for 30 min in the dark, and then, absorbance was read at 517 nm at t = 30 min. The ability to scavenge the DPPH radical was calculated using the following equation:

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

where $A_{control}$ is the absorbance of the control (DPPH solution without sample) and the A_{sample} is the absorbance of the test sample (DPPH solution with test sample) at 517 nm. Triplicate measurements were carried out. Vitamin C was used as positive control.

Antimicrobial assay

The inhibition zones (IZs) were determined by whole diffusion using a cell suspension of about 1.5×10^6 CFU/mL obtained from a McFarland turbidity standard 0.5° N. The suspension was standardized by adjusting the optical density to 0.1 at 600 nm (SHIMADZU UV-120-01 spectrophotometer). A hole of 6-mm diameter was then made on the plate (8-mm thick). 50 µL of the different extracts solubilized in dimethyl sulfoxide (DMSO) (for the dichloromethane and methanol extracts) or water (for the aqueous extracts) of different concentrations (1.0, 2.0, and 10.0 mg/mL) was added to each well in the specific position. The negative control (DMSO) and the positive control (antibiotic or antifungal depending on the test) were added in two different wells. The inoculated plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of the zone of growth inhibition (IZ) around the hole. The assay was repeated twice, and the results recorded as mean of inhibition diameter.

Tests were performed against two Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacterial strains, one Gram-positive (*Staphylococcus aureus*) bacterial strain, and a fungal strain (*Candida albicans*). The micro-organisms were obtained from the Health and Environment Microbiology laboratory at Azm Center for Research on Biotechnology Sciences and its applications, Tripoli, Lebanon.

RESULTS AND DISCUSSION

G. robertianum, *A. microcarpus*, and *A. setosa* parts were harvested and then dried at room temperature under shade. The dried leaves, flowers, stems, roots, tubers, and buds were macerated with dichloromethane, methanol, and water for 48 h. The obtained mixtures were then filtered and evaporated to dryness under reduced pressure. The antioxidant and antimicrobial activities of all extracts were then investigated. For the nomenclature, GR, AM, and AS refers to the first initials of the plant species, followed by the first initial of the plant part (L for leaves, F for

flowers, S for stems, R for roots, T for tubers, and B for buds). This letter is followed by M for maceration as the type of extraction. Then, it is mentioned the solvent used for the extraction $(CH_2Cl_2, CH_3OH, or H_2O)$. The details of the extraction and nomenclature of the extract are presented in Table 1.

Antioxidant activity

The use of natural products as pharmaceutical or cosmetic additives to inactivate free radicals has attracted the researchers' attention due to their radical scavenging efficiency with lower toxicity than synthetic antioxidants.^[27,28] Medicinal plants contain a broad range of secondary metabolites with antioxidant properties.^[29]

Reactive species launch reactions that damage biologically important molecules causing the aging process, heart diseases, and cancer.^[30] Radical scavenging activity is considered to be involved in wound-healing, anti-inflammatory, anticancer, and antiaging processes.^[31]

DPPH method is a popular, easy, rapid, and sensitive way used to investigate the free radical scavenging activity of plant extracts. DPPH is a free radical, is stable at room temperature, and produces a violet solution in methanol.^[32,33] The advantage of this method is that DPPH

Table 1: Details of nomenclature use	ed for the extracts of the different species
--------------------------------------	----------------------------------------------

Plant species	Extract	Extraction material	Solvent
Alcea setosa	AS-L-M-DCM	Dry leaves	Dichloromethane
	AS-L-M-MeOH		Methanol
	AS-L-M-H ₂ O		Water
	AS-F-M-DCM	Dry flowers	Dichloromethane
	AS-F-M-MeOH		Methanol
	AS-F-M-H ₂ O		Water
	AS-S-M-DCM	Dry stems	Dichloromethane
	AS-S-M-MeOH		Methanol
	AS-S-M-H ₂ O		Water
	AS-R-M-DCM	Dry roots	Dichloromethane
	AS-R-M-MeOH		Methanol
	AS-R-M-H ₂ O		Water
Geranium	GR-L-M-DCM	Dry leaves	Dichloromethane
robertianum	GR-L-M-MeOH		Methanol
	GR-L-M-H ₂ O		Water
	GR-F-M-DCM	Dry flowers	Dichloromethane
	GR-F-M-MeOH		Methanol
	GR-F-M-H ₂ O		Water
	GR-S-M-DCM	Dry stems	Dichloromethane
	GR-S-M-MeOH		Methanol
	GR-S-M-H ₂ O		Water
	GR-R-M-DCM	Dry roots	Dichloromethane
	GR-R-M-MeOH		Methanol
	GR-R-M-H ₂ O		Water
Asphodelus	AM-L-M-DCM	Dry leaves	Dichloromethane
microcarpus	AM-L-M-MeOH		Methanol
-	AM-L-M-H ₂ O		Water
	AM-F-M-DCM	Dry flowers	Dichloromethane
	AM-F-M-MeOH		Methanol
	AM-F-M-H ₂ O		Water
	AM-S-M-DCM	Dry stems	Dichloromethane
	AM-S-M-MeOH		Methanol
	GR-S-M-H ₂ O		Water
	GR-R-M-DCM	Dry roots	Dichloromethane
	GR-R-M-MeOH		Methanol
	GR-R-M-H ₂ O		Water
	AM-T-M-DCM	Dry tubers	Dichloromethane
	AM-T-M-MeOH		Methanol
	GR-T-M-H ₂ O		Water
	GR-B-M-DCM	Dry buds	Dichloromethane
	GR-B-M-MeOH		Methanol
	GR-B-M-H ₂ O		Water

can react with the whole sample and adequate time given in the method allows DPPH to react with weak antioxidants. Further, this method may be applied to examine both lipophilic and hydrophilic antioxidants in nonpolar organic or aqueous solvents.^[34,35]

The DPPH test seeks to measure the hydrogen atom or electron donor capacity of the extracts to the stable radical DPPH formed in solution.^[36] The radical scavenging activity in the presence of an antioxidant can be observed as a decrease in the absorbance of the DPPH solution at 517 nm. The decrease in absorbance produced by reduced DPPH was monitored to evaluate the ability of crude extracts to act as free radical scavengers.^[37] There is no report on the antioxidant activity of the Lebanese *G. robertianum, A. microcarpus,* and *A. setosa* different parts extracts. Therefore, the dichloromethane, methanol, and aqueous extracts of these Lebanese species have been tested for their radical scavenging efficacy using DPPH. Vitamin C was used as the reference standard. The concentrations tested were 0.3, 0.5, and 1 mg/mL. The solution of DPPH alone in methanol was considered as the negative control with zero inhibition.

The DPPH radical scavenging effects of *G. robertianum*, *A. microcarpus*, and *A. setosa* extracts are presented, respectively, in Figures 1-3.

Methanol and aqueous extracts display stronger activities than that of the dichloromethane extracts. This may be due to the nature of the extracted secondary metabolites by the solvents and to their hydrogen-donating capacity. This is in agreement with previous studies which showed that the interaction between DPPH and antioxidants seems to be correlated with the number of available hydroxyl groups in their structures.^[29,37,38] In fact, methanol and water facilitate the extraction of these molecules such as flavonoids widely known for their antioxidant capacity.^[39,40] Furthermore, in comparison with other investigated plant extracts, *G. robertianum* reached the strongest antioxidant capacity.

The best activity of *G. robertianum* dichloromethane crude extracts was shown by the leaves at 1 mg/mL (inhibition of 83%). This species methanol and aqueous extracts revealed high scavenging capacity of the radical DPPH at 0.3 mg/mL. The more potent activity was for the roots methanol extract which possessed an inhibition of 97% of the radical at 0.3 mg/mL. This species antioxidant activity is mostly due to the presence of the nonvolatile components, mainly polyphenolic compounds such as ellagic acid, caffeic acid, gallic acid, hyperin, quercetin, 3',4'-dimethoxyflavone, homoeriodictyol, and kaempferol previously identified in this species water, methanol, and ethanol extracts.^[8,41-43]

A. microcarpus extracts displayed variable results depending on the plant's part and the solvent of extraction. Among *A. microcarpus* dichloromethane extracts, the buds extract showed a remarkable inhibition of the radical DPPH (93% at 1 mg/mL). This species extracts revealed a potent efficacy in a dose-dependent manner where the remarkable activity was shown at high concentration. These results are in accordance with a previous study, in which *A. microcarpus* leaves, flowers, and tubers methanol and aqueous extracts displayed weak antioxidant activities in comparison of the reference standard.^[44] This may be due to the lack of secondary metabolites having antioxidant properties or due to their presence in small amounts in the tested extracts.

Concerning *A. setosa*, our results revealed the studied extracts exhibit very weak antioxidant activity. For this species, the more potent activity was for leaf extracts which displayed an inhibition of 72% of the radical at 1 mg/mL. Our results are of very good agreement with a previous study where *A. setosa* species exhibited weak scavenging activity.^[45]

Antimicrobial activity

Bacteria have a genetic capability to acquire resistance to drugs utilized as therapeutic agents.

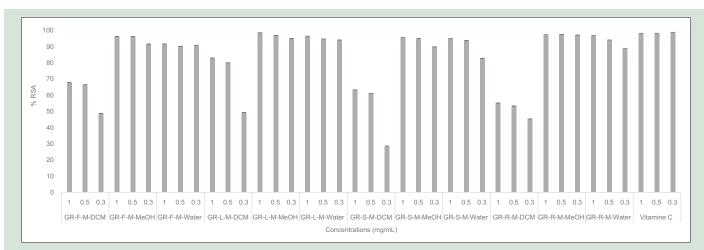


Figure 1: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of the extracts of *Geranium robertianum* and Vitamin C. All values are expressed as mean of triplicate ± standard deviation

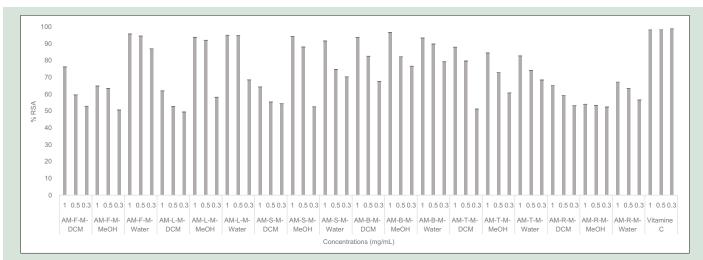


Figure 2: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of the extracts of *Asphodelus microcarpus* and Vitamin C. All values are expressed as mean of triplicate ± standard deviation

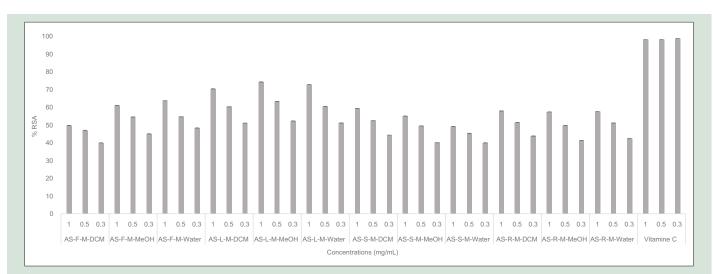


Figure 3: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of the extracts of *Alcea setosa* and Vitamin C. All values are expressed as mean of triplicate ± standard deviation

Even though pharmacological industries have synthesized a large number of new antibiotics in the last decades, the resistance of micro-organisms to these medicines has increased.^[46] Many scientific studies realized on the different parts of many plants have shown great antimicrobial potential of these parts extracts. Furthermore, many antibiotics are originally issued from plants. Moreover, the antimicrobial activity of plant extracts has formed the basis of many other applications, such as natural therapies, alternative medicine, pharmaceuticals, and food preservation.^[47,48]

Agar diffusion techniques are widely used to evaluate the antimicrobial activity of plant extracts, for which they are smooth and inexpensive ways for determining antimicrobial activity against pathogenic bacterial and fungal strains.^[29,49]

In our research, all the extracts were investigated for their antimicrobial activity by the plate hole diffusion technique against two Gram-negative (*P aeruginosa* and *E coli*) bacterial strains, one Gram-positive (*S aureus*) bacterial strain, and a fungal strain (*C albicans*). Extracts were tested

at 1, 2, and 10 mg/mL. Dichloromethane and methanol extracts were dissolved in DMSO, while aqueous ones were dissolved in water. The diameters of the IZs obtained are presented in Table 2.

The results displayed many of the tested extracts of *G. robertianum* and *A. microcarpus* were active against *S. aureus*. In addition, only *G. robertianum* extracts revealed an antimicrobial effect against the other strains.

G. robertianum dichloromethane extracts exhibited good inhibition diameters against *S. aureus*. At 1 mg/mL, leaves dichloromethane extract showed 8-mm inhibition diameter against *S. aureus* growth. Among the methanol extracts, only the leaves extract revealed an 8-mm inhibition of *S. aureus* growth at 10 mg/mL. Furthermore, the stems methanol extract was the only active extract against *C. albicans* at 10 mg/mL (9 mm). On the other hand, *G. robertianum* extracts revealed weak activities against *P. aeruginosa* and *E. coli*. There no previous data on the microbial activities of this species crude extracts while essential oils of

Plant species	Extract	Pseudo	monas aero CMUL 241			herichia :MUL 57			/lococcus CMUL 491			ida albio CC 1023	
		Inhibition diameter zone (mm)/concentration (mg/mL)											
		10	2	1	10	2	1	10	2	1	10	2	1
Alcea setosa	AS-L-M-DCM	-	-	-	-	-	-	-	-	-	-	-	-
	AS-L-M-MeOH	-	-	-	-	-	-	-	-	-	-	-	-
	AS-L-M-H ₂ O	-	-	-	-	-	-	-	-	-	-	-	-
	AS-F-M-DCM	-	-	-	-	-	-	-	-	-	-	-	-
	AS-F-M-MeOH	-	-	-	-	-	-	-	-	-	-	-	-
	AS-F-M-H ₂ O	-	-	-	-	-	-	-	-	-	-	-	-
	AS-S-M-DCM	-	-	-	-	-	-	-	-	-	-	-	-
	AS-S-M-MeOH	-	-	-	-	-	-	-	-	-	-	-	-
	AS-S-M-H ₂ O	-	-	-	-	-	-	-	-	-	-	-	-
	AS-R-M-DCM	-	-	-	-	-	-	-	-	-	-	-	-
	AS-R-M-MeOH	-	-	-	-	-	-	-	-	-	-	-	-
	AS-R-M-H ₂ O	-	-	-	-	-	-	-	-	-	-	-	-
Geranium robertianum	GR-L-M-DCM	+/-	+/-	+/-	+/-	+/-	-	10	9	8	+/-	-	-
	GR-L-M-MeOH	+/-	-	-	+/-	+/-	-	8	-	-	-	-	-
	GR-L-M-H,O	-	-	-	-	-	-	+/-	-	-	-	-	-
	GR-F-M-DCM	+/-	+/-	-	+/-	+/-	-	10	-	-	-	-	-
	GR-F-M-MeOH	-	-	-	-	-	-	-	-	-	-	-	-
	GR-F-M-H,O	-	-	-	+/-	-	-	-	-	-	-	-	-
	GR-S-M-DCM	-	-	-	-	-	-	8	8	-	-	-	-
	GR-S-M-MeOH	+/-	+/-	-	+/-	+/-	+/-	+/-	-	-	9	+/-	-
	GR-S-M-H ₂ O	-	-	-	-	-	-	-	-	-	-	-	-
	GR-R-M-DCM	-	-	-	+/-	-	-	10	-	-	-	-	-
	GR-R-M-MeOH	-	-	-	+/-	+/-	-	+/-	-	-	-	-	-
	GR-R-M-H,O	-	-	-	-	-	-	-	-	-	-	-	-
Asphodelus microcarpus	AM-L-M-DCM	-	-	-	-	-	-	-	-	-	-	-	-
	AM-L-M-MeOH	-	-	-	-	-	-	-	-	-	-	-	-
	AM-L-M-H ₂ O	-	-	-	-	-	-	-	-	-	-	-	-
	AM-F-M-DCM	-	-	-	-	-	-	-	-	-	-	-	-
	AM-F-M-MeOH	-	-	-	-	-	-	-	-	-	-	-	-
	AM-F-M-H ₂ O	-	-	-	-	-	-	-	-	-	-	-	-
	AM-S-M-DCM	-	-	_	_	-	-	-	-	_	-	-	-
	AM-S-M-MeOH	_	-	_	_	-	-	-	-	_	-	-	-
	GR-S-M-H ₂ O	-	-	_	_	-	-	-	-	_	-	-	-
	GR-R-M-DCM	-	-	_	_	-	-	-	-	_	-	-	-
	GR-R-M-MeOH	-	_	-	-	_	-	_	-	-	-	-	-
	GR-R-M-H ₂ O	_	-	_	_	-	-	-	_	_	_	-	_
	AM-T-M-DCM	_	_	_	-	_	-	_	_	_	-	-	_
	AM-T-M-MeOH	_	_	_	-	-	-	14	_	_	-	-	_
	GR-T-M-H ₂ O	_	_	_	-	-	-	-	_	_	-	-	_
	GR-B-M-DCM	_	_	_	-	_	-	_	_	_	-	-	_
	GR-B-M-MeOH	_	_	_	_	_	_	_	_	_	_	_	_
	GR-B-M-H ₂ O		_	_						_	_		

CMUL: Microbiological Collection of the Lebanese University; ATCC: American Type Culture Collection; -: Absence of antimicrobial activity; +/- : Inhibition Diameter < 6 mm

G. robertianum whole plant and aerial parts revealed strong antibacterial and antifungal activities.^[13]

Screening of the antimicrobial activity of *A. microcarpus* extracts revealed a moderate activity by only the tubers methanol extract against *S. aureus.* No activity was observed in the other *A. microcarpus* and *A. setosa* extracts. To our knowledge, this is the first study to evaluate the antimicrobial activity of *A. microcarpus* different parts of crude extracts. Concerning *A. setosa*, the obtained results are in accordance with a previous study, in which the tested methanol extracts of different parts showed no growth inhibition of similar bacterial strains.^[45] However, no studies were realized to assess the antimicrobial activities of the dichloromethane and aqueous extracts of this species.

CONCLUSION

In continuation of the research for the discovery of effective antioxidant and antimicrobial compounds, screening of plant extracts is indeed a practical procedure to discover new compounds that may be used in developing future medicines. In this study, we evaluated the antioxidant and antimicrobial potency of the dichloromethane, methanol, and aqueous crude extracts of three Lebanese medicinal plants, G. robertianum, A. microcarpus, and A. setosa. The results showed that G. robertianum methanol and aqueous crude extracts possess a good ability to scavenge the free radical DPPH. The best activity was observed by these species roots methanol extracts which possessed an inhibition of 97% of the radical at 0.3 mg/mL. A. microcarpus extracts possessed a dose-dependent efficacy where potent activities were shown at high concentration. Meanwhile, A. setosa extracts revealed weak antioxidant activity with a maximum of 72% inhibition of the radical shown by the leaf extracts at 1 mg/mL. On the other hand, a good antimicrobial effect was observed by G. robertianum dichloromethane extracts against S. aureus, meanwhile weak activity was noticed by this species extracts against P. aeruginosa, E. coli, and C. albicans. Moreover, A. microcarpus and A. setosa extracts did not display significant growth inhibition against the tested strains. Finally, this is the first report to assess in vitro antioxidant and antimicrobial effects of these three Lebanese species dichloromethane, methanol, and aqueous extracts. However, further studies should be carried on until the agent responsible for the activity of the active extracts has been identified. Furthermore, different kinds of screenings can be realized to assess these species biological properties.

Acknowledgement

We would like to thank the Lebanese University for the financial support of this project. We also thank Professors Georges and Henriette Tohme for the taxonomic identification.

We would like to thank Professor Monzer Hamzé (Director of the Health and Environment Microbiology laboratory at Azm Centre for Research on Biotechnology Sciences and its applications, Doctoral School for Sciences and Technology, Lebanese University, Tripoli, Lebanon) and his team for their assistance in carrying out antibacterial tests and antifungal agents.

Financial support and sponsorship

We would like to thank the Lebanese University for the financial support of this project.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

 Lovkova MY, Buzuk G, Sokolova S, Kliment'eva N. Chemical features of medicinal plants. Appl Biochem Microbiol 2001;37:229-37.

- Al-Amiery AA, Al-Temimi AA, Sulaiman GM, Aday HA, Kadhum AA, Mohamad AB. Synthesis, antimicrobial and antioxidant activities of 5-((2-oxo-2H-chromen-7-yloxy) methyl)-1, 3, 4-thiadiazol-2 (3H)-one derived from umbelliferone. Chem Nat Compd 2013;48:950-4.
- Al-Amiery AA, Kadhum AA, Obayes HR, Mohamad AB. Synthesis and antioxidant activities of novel 5-chlorocurcumin, complemented by semiempirical calculations. Bioinorg Chem Appl 2013;2013:354982.
- Kadhum AA, Al-Amiery AA, Musa AY, Mohamad AB. The antioxidant activity of new coumarin derivatives. Int J Mol Sci 2011;12:5747-61.
- Naama JH, Alwan GH, Obayes HR, Al-Amiery AA, Al-Temimi AA, Kadhum AH, et al. Curcuminoids as antioxidants and theoretical study of stability of curcumin isomers in Gaseous state. Res Chem Intermediates 2013;39:4047-59.
- Medail F, Quezel P. Hot-spots analysis for conservation of plant biodiversity in the Mediterranean Basin. Ann Mo Bot Gard 1997;84:112-27.
- Tohmé G, Tohmé H. Illustrated Flora of Lebanon. Beirut: CNRS Publications; 2007.
- Amaral S, Mira L, Nogueira JM, da Silva AP, Helena Florêncio M. Plant extracts with anti-inflammatory properties – A new approach for characterization of their bioactive compounds and establishment of structure-antioxidant activity relationships. Bioorg Med Chem 2009;17:1876-83.
- 9. Aedo C, editor The genus *Geranium* I (*Geraniaceae*) in North America. Annual species. An Jardín Bot Madr 2000;50:39-82.
- Baytop T. Therapy with Medicinal Plants in Turkey (past and present). Istanbul, Turkey: Publication of the Istanbul University; 1999. p. 312.
- Neagu E, Paun G, Moroeanu V, Radu GL. Evaluation of antioxidant capacity of Geranium robertianum extracts. Rev Roum Chim 2010;55:321-5.
- Frey FM, Bukoski M. Floral symmetry is associated with flower size and pollen production but not insect visitation rates in *Geranium robertianum* (Geraniaceae). Plant Species Biol 2014;29:272-80.
- Graça VC, Ferreira IC, Santos PF. Phytochemical composition and biological activities of *Geranium robertianum* L.: A review. Ind Crops Prod 2016;87:363-78.
- Peksel A, Altas-Kiymaz N, Imamoglu S. Evaluation of antioxidant and antifungal potential of *Asphodelus aestivus* brot. Growing in Turkey. J Med Plants Res 2012;6:253-65.
- Tackholm V. Student's Flora of Egypt. 2nd ed. Cairo: University of Cairo Press; 1974.
- Chiej R. The Macdonald Encyclopedia of Medicinal Plants. London: Macdonald and Co (Publishers) Ltd.; 1984.
- Ghoneim MM, Elokely KM, El-Hela AA, Mohammad AE, Jacob M, Radwan MM, et al. Asphodosides A-E, anti-MRSA metabolites from Asphodelus microcarpus. Phytochemistry 2014;105:79-84.
- El-Seedi HR. Antimicrobial arylcoumarins from Asphodelus microcarpus. J Nat Prod 2007;70:118-20.
- Bonsignore L, Cottiglia F, Loy G, Begala M, Sanna L, Scordo F, et al. Preliminary study on the chemical constituents and microbiologic activity of Asphodelus microcarpus (Salzm. et Viv.). Boll Chim Farm 1998;137:186-90.
- 20. Abedin S. Malvaceae. Pakistan: University of Karachi; 1979.
- Azab A. Alcea: Traditional medicine, current research and future opportunities. Eur Chem Bull 2017;5:505-14.
- Bar-Shai N, Keasar T, Shmida A. The use of numerical information by bees in foraging tasks. Behav Ecol 2011;22:317-25.
- Aburjai T, Hudaib M, Tayyem R, Yousef M, Qishawi M. Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights Region. J Ethnopharmacol 2007;110:294-304.
- Bruneton J. Pharmacognosy and Phytochemistry of Medicinal Plants. 3rd edition. Paris: Tec et Doc; 1999.
- Chandini SK, Ganesan P, Bhaskar N. *In vitro* antioxidant activities of three selected brown seaweeds of India. Food Chem 2008;107:707-13.
- Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 1995;43:27-32.
- Peschel W, Sánchez-Rabaneda F, Diekmann W, Plescher A, Gartzía I, Jiménez D, et al. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem 2006;97:137-50.
- Koleva II, Niederländer HA, van Beek TA. Application of ABTS radical cation for selective on-line detection of radical scavengers in HPLC eluates. Anal Chem 2001;73:3373-81.
- Alhage J, Elbitar H, Taha S, Benvegnu T. *In vitro* assessment of antioxidant, antimicrobial, cytotoxic, anti-inflammatory, and antidiabetic activities of *Campanula retrorsa* crude extracts. Pharmacognosy Res 2018;10:397-403.
- Gutteridge J, Halliwell B. Antioxidants in Nutrition, Health, and Disease. New York: Oxford University Press; 1994.
- Assimopoulou AN, Sinakos Z, Papageorgiou VP. Radical scavenging activity of *Crocus sativus* L. Extract and its bioactive constituents. Phytother Res 2005;19:997-1000.

- Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2004;26:211-9.
- Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal 2002;13:8-17.
- Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 2005;53:4290-302.
- Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 2011;48:412-22.
- Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem 2005;90:333-40.
- Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 1995;28:25-30.
- Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, *et al.* Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001;15:127-30.
- Sreeramulu D, Reddy CV, Chauhan A, Balakrishna N, Raghunath M. Natural antioxidant activity of commonly consumed plant foods in India: Effect of domestic processing. Oxid Med Cell Longev 2013;2013:369479.
- Alhage J, Elbitar H, Taha S, Guegan JP, Dassouki Z, Vives T, et al. Isolation of bioactive compounds from *Calicotome villosa* stems. Molecules 2018;23. pii: E851.

- Kobakhidze K, Alaniya M. Flavonoids from *Geranium robertianum*. Chem Nat Compd 2004;40:89-90.
- Paun G, Neagu E, Tache A, Radu G, Parvulescu V. Application of the nanofiltration process for concentration of polyphenolic compounds from *Geranium robertianum* and *Salvia officinalis* extracts. Chem Biochem Eng Q 2012;25:453-60.
- Ben Jemia M, Aidi Wannes W, Ouchikh O, Bruno M, Kchouk ME. Antioxidant activity of Tunisian *Geranium robertianum* L. (Geraniaceae). Nat Prod Res 2013;27:2076-83.
- Di Petrillo A, González-Paramás AM, Era B, Medda R, Pintus F, Santos-Buelga C, et al. Tyrosinase inhibition and antioxidant properties of Asphodelus microcarpus extracts. BMC Complement Altern Med 2016;16:453.
- Jamous R, Zaitoun S, Husein A, Qasem I, Ali-Shtayeh M. Screening for biological activities of medicinal plants used in traditional Arabic Palestinian herbal medicine. Eur J Med Plants 2015;9:1-13.
- Nascimento GG, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol 2000;31:247-56.
- Lis-Balchin M, Deans SG. Bioactivity of selected plant essential oils against Listeria monocytogenes. J Appl Microbiol 1997;82:759-62.
- Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 1999;86:985-90.
- Ojala T, Remes S, Haansuu P, Vuorela H, Hiltunen R, Haahtela K, et al. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. J Ethnopharmacol 2000;73:299-305.