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Antibacterial Activity of Two Lebanese Plants: *Eryngium creticum* and *Centranthus longiflorus*

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Abstract

Lebanon is one of many countries that are famous in their natural wealth and wide diversity. And due to the limited research regarding the traditional medicine worldwide, we aimed to conduct a study, for the first time, on two Lebanese plants: *Eryngium creticum* and *Centranthus longiflorus* L., in order to evaluate the antibacterial activity of the aqueous and ethanolic extracts of the different plant parts, during two harvesting periods, on five bacterial strains: Gram-positive and Gram-negative. It was observed that the aqueous extract showed a stronger antibacterial activity against *S. epidermidis* than the ethanolic extract in both plants and during the two harvesting periods. Regarding the Gram-negative bacteria, *E. coli* was highly resistant where it was not inhibited by *E. creticum* during the first period, however P. aeruginosa showed an alternating resistance and was stronger in the second period for *E. creticum* and in both periods for *C. longiflorus*. Further research studies need to be conducted in order to confirm the antibacterial effects of these two Lebanese plants.

Keywords: *Eryngium creticum; Centranthus longifloru*; Antibacterial activity; MIC; MBC

Introduction

Arab countries and more particularly "Lebanon" are distinguished from other countries by the great wealth and the wide diversity of plant species. In fact, about 2,600 wild species (92 of which are endemics), can be found only in Lebanon. For this reason, it is definitely interesting and necessary to conduct new scientific studies on these plants, especially those used locally as a traditional medicine.

One of the most known and beneficial Lebanese plants is the *Eryngium creticum*, a perennial plant belonging to the *Umbellifereae* family. It is found only in Lebanon, Palestine, Jordan, and Syria. This plant is cultivated for the consumption and the use as a leafy vegetable, mainly in the Lebanese salads. It is traditionally used as a diuretic or laxative. Roots and seeds that are immersed in water are drunk by people to treat kidney stones, infections, skin diseases, and tumors. This plant is an antidote, in which it's used for the treatment of the snakebite [1]. *E. creticum* is also known for its anti-inflammatory property, as well as for its anti-microbial activity [2]. Moreover, it is also used in the treatment of certain diseases such as: liver diseases, poisoning, anemia and infertility [3]. In addition to the various benefits mentioned above, this plant exhibited an antioxidant property through inhibiting the rat's lipid peroxidase in the liver [4]. Lately a study has confirmed both activities the antioxidant and the anti-tumor, of the Lebanese *E. creticum* [5-7].

On the other hand, *Centranthus longiflorus* L., which belongs to the *Caprifoliaceae* family, is another endemic plant used in the Mediterranean region (Lebanon, Syria, Turkey, Italy, and Palestine), and is known as the red valerian. Rammal et al have studied the scavenger activity of the aqueous and methanolic leaves extracts of this plant, using three different *in vitro* tests: the DPPH, H_2O_2, and the iron chelating test [8]. In another study, the antioxidant and anti-proliferative activity against cervical cancer cell line (HeLa), as well as the phytochemical screening concerning this plant, have been evaluated [9].

In the literature, there are no studies available, till now, that have detected the antibacterial activity of these two Lebanese plants. Due to this reason, our study aims, and for the first time, to evaluate the effect of the aqueous and the ethanolic extracts of the different parts, and during two harvesting periods, of these two Lebanese plants, on five bacterial strains: Gram-positive and Gram-negative.

Materials and Methods

Plant collection and preparation of powders

Fresh *E. creticum* was collected during 2013 in 2 different periods, the first one in March (the plant is edible and maturing) and the second one in May (the plant is mature and non-consumable) from South Lebanon.

Fresh *C. longiflorus* plant was collected from Mount Lebanon during 2013. The collection process covered two different periods, the first was on May (premature plant) and the second was on June (mature plant).

Before extraction, the plant's materials were well-cleaned and

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washed under the running tap water, then extended by ground in one layer, and left to dry in the shade inside the limit at room temperature, away from sun light. During the drying process, the plant was turned over to allow homogeneous drying. After this period, the dried plant should be grinded by a grinder to obtain a powder form and then preserved in a container away from light, heat, and moisture for later use.

After that, each part (leaves and stems) was placed in the selected solvent (100 g of each part of the plant in 500 ml of distilled water or ethanol). Then, the paste was macerated and stirred for 8 hours at room temperature and then at 37°C. After that, the macerate was filtered to remove insoluble residues. Subsequently, the filtrate was condensed by evaporating half of the solvent using a rotary evaporator. Finally, the filtrate was frozen before being lyophilized powder to be processed [10].

All of the chemicals used were of analytical grade. Methanol and ethanol were purchased from BDH, England.

Antibacterial activity assay

Bacterial strains: The strains used in this study were three Gram-positive bacteria (*Staphylococcus epidermidis* CIP 444, *S. aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 29212) and two Gram-negative strains (*Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853). The Gram-positive CIP 444 strain is a clinical strain that is isolated from an infected implanted device of a patient who is hospitalized in the Mignot Hospital of Versailles, France [11].

Ali Chokr has identified and characterized the properties of this strain and deposited it to be enclosed within the collection of microorganisms of Pasteur Institute in 2007 [11-13]. The other strains are ATCC. The latter were stored in glycerol stocks at -80°C and used as required. Brainheart infusion (BHI), Brain heart agar (BHA), and Mueller–Hinton broth (MHB) were purchased from HIMEDIA (Mumbai, India), in which they were prepared and then autoclaved as indicated by the manufacturer before their use.

MIC and MBC assays: Aqueous and ethanolic extracts of *Erynguim creticum* and *Centranthus longiflorus*, during two harvesting periods, were tested for their corresponding Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) by broth microdilution assay, as recommended by the Clinical Laboratory and Standard Institute (CLSI) [14]. From each collection, three groups (stems, leaves and roots) were isolated and an extract of 800 mg/ml of each was prepared. In a 96-well plate (200 µl/well) (Greiner Bio-One, Essen, Germany), serial two-fold dilutions in MHB of the different extracts were done. The wells were inoculated with 5 × 10^5 bacteria/ml. After incubating the plates at 37°C for 24 hours, the MIC (which is defined as the lowest concentration that yielded no growth) was determined. In addition, the wells with no visible growth, were plated on BHA in order to determine the MBC (which is defined as the lowest concentration which killed ≥99.9% of the initial inoculum). The Petri plates were incubated overnight at 37°C, and the MBC was determined.

Results and Discussion

Aqueous extracts from *E. creticum* show an antibacterial activity

	Bacterial type	Bacterial strain	Extracted part	MIC (mg/ml)	MBC (mg/ml)
Aqueous extract	Gram positive bacteria	*S. epidermidis* (CIP 444)	leaves	5	5
			stems	26	53
			roots	91	457
		E. faecalis (ATCC29212)	leaves	158	315
			stems	300	>800
			roots	>800	>800
		S. aureus (ATCC25923)	leaves	354	>800
			stems	430	>800
			roots	>800	>800
	Gram negative bacteria	*E. coli* (ATCC35218)	leaves	>800	>800
			stems	>800	>800
			roots	>800	>800
		P. aeruginosa (ATCC27853)	leaves	244	244
			stems	215	>800
			roots	450	450
Ethanolic extract	Gram positive bacteria	*S. epidermidis* (CIP 444)	leaves	5	10
			stems	>800	>800
			roots	>800	>800
		E. faecalis (ATCC29212)	leaves	180	362
			stems	184	184
			roots	>800	>800
		S. aureus (ATCC25923)	leaves	294	294
			stems	368	368
			roots	>800	>800
	Gram negative bacteria	*E. coli* (ATCC35218)	leaves	>800	>800
			stems	>800	>800
			roots	>800	>800
		P. aeruginosa (ATCC27853)	leaves	343	343
			stems	205	>800
			roots	>800	>800

Table 1: Antibacterial activity of the extracts of *Eryngium creticum* of the first period.

	Bacterial type	Bacterial strain	Extracted part	MIC (mg/ml)	MBC (mg/ml)
Aqueous extract	**Gram positive bacteria**	*S. epidermidis* (CIP 444)	leaves	27.9	27.9
			stems	42.3	>800
			roots	>800	>800
		E. faecalis (ATCC29212)	leaves	223	>800
			stems	338	>800
			roots	>800	>800
		S. aureus (ATCC25923)	leaves	354	>800
			stems	430	>800
			roots	>800	>800
	Gram negative bacteria	*E. coli* (ATCC35218)	leaves	2	28
			stems	86	>800
			roots	>800	>800
		P. aeruginosa (ATCC27853)	leaves	100	>800
			stems	44	>800
			roots	>800	>800
Ethanolic extract	**Gram positive bacteria**	*S. epidermidis* (CIP 444)	leaves	5.6	11.25
			stems	12.5	>800
			roots	103	103
		E. faecalis (ATCC29212)	leaves	>800	>800
			stems	400	>800
			roots	104	104
		S. aureus (ATCC25923)	leaves	294	294
			stems	368	368
			roots	>800	>800
	Gram negative bacteria	*E. coli* (ATCC35218)	leaves	300	300
			stems	25	25
			roots	>800	>800
		P. aeruginosa (ATCC27853)	leaves	400	400
			stems	>800	>800
			roots	>800	>800

Table 2: Antibacterial activity of the extracts of *Eryngium creticum* of the second period.

	Bacterial type	Bacterial strain	Extracted part	MIC (mg/ml)	MBC (mg/ml)
Aqueous extract	**Gram positive bacteria**	*S. epidermidis* (CIP 444)	leaves	9	75
			stems	300	300
		E. faecalis (ATCC29212)	leaves	>800	>800
			stems	>800	>800
		S. aureus (ATCC25923)	leaves	>800	>800
			stems	>800	>800
	Gram negative bacteria	*E. coli* (ATCC35218)	leaves	300	300
			stems	>800	>800
		P. aeruginosa (ATCC27853)	leaves	150	150
			stems	300	300
Ethanolic extract	**Gram positive bacteria**	*S. epidermidis* (CIP 444)	leaves	18	36
			stems	300	300
		E. faecalis (ATCC29212)	leaves	150	>800
			stems	300	>800
		S. aureus (ATCC25923)	leaves	300	300
			stems	300	>800
	Gram negative bacteria	*E. coli* (ATCC35218)	leaves	300	300
			stems	300	300
		P. aeruginosa (ATCC27853)	leaves	150	150
			stems	300	300

Table 3: Antibacterial activity of the extracts of *Centranthus longiflorus* of the first period.

which is stronger than the ethanolic extracts during the two periods against both Gram-positive and Gram-negative strains (Tables 1 and 2). Among these strains, Gram positive ones were more sensitive, with *S. epiderimidis* being the most inhibited with MIC=MBC=5 mg/ml for the leaves aqueous extract, in particular in the first harvest period. During the second period, however, the activity decreases, to show

	Bacterial type	Bacterial strain	Extracted part	MIC (mg/ml)	MBC (mg/ml)
Aqueous extract	**Gram positive bacteria**	*S. epidermidis* (CIP 444)	leaves	394	>800
			stems	428	>800
		E. faecalis (ATCC29212)	leaves	>800	>800
			stems	>800	>800
		S. aureus (ATCC25923)	leaves	>800	>800
			stems	>800	>800
	Gram negative bacteria	*E. coli* (ATCC35218)	leaves	>800	>800
			stems	>800	>800
		P. aeruginosa (ATCC27853)	leaves	200	200
			stems	450	450
Ethanolic extract	**Gram positive bacteria**	*S. epidermidis* (CIP 444)	leaves	30	30
			stems	160	>800
		E. faecalis (ATCC29212)	leaves	>800	>800
			stems	>800	>800
		S. aureus (ATCC25923)	leaves	400	>800
			stems	450	>800
	Gram negative bacteria	*E. coli* (ATCC35218)	leaves	198	198
			stems	450	>800
		P. aeruginosa (ATCC27853)	leaves	428	428
			stems	400	400

Table 4: Antibacterial activity of the extracts of *Centranthus longiflorus* of the second period.

equal concentrations (MIC=MBC=27.9 mg/ml), in the time where the MIC was 42.3 mg/ml for the stem extract. Whereas the stem aqueous extract, during the first harvest period, exhibited a considerable activity with MIC=26 mg/mL and MBC=53 mg/ml. For the leaves extract, the MBC increases to 10 mg/ml in the ethanolic group during the first period, in which the stems extract didn't record any activity in the highest concentrations tested. A comparable value, MIC=5.6 mg/ml, was found during the second period, for the ethanolic extract of leaves and increased to 11.25 mg/ml for the MBC. However, the stems extract displayed an MIC=12.5 mg/ml with the MBC being higher than the highest concentration (Tables 1 and 2).

The Gram-positive strain *E. faecalis* registered an MIC=158 mg/ml and a MBC=315 mg/ml for the leaves aqueous extract and MIC=300 mg/ml for the stems aqueous extract, during the first period. This strain showed more resistance against extracts during the second period to display 223 and 328 mg/ml as MIC for the leaves and stems aqueous extracts respectively, and 400 mg/ml as MIC for the stem ethanolic extract (Tables 1 and 2).

S. aureus showed the same sensitivity during both periods with MICs of 354 and 430 mg/ml for the leaves and stems aqueous extracts respectively, and MIC=MBC=294 and 368 mg/ml for the leaves ethanolic extract and for the stems ethanolic extract, during the two periods, respectively (Tables 1 and 2).

Among the Gram negative strains, *E. coli* was highly resistant and was not inhibited at the highest concentration during the first period for both groups, aqueous and ethanolic extracts. However, during the second period, the stem ethanolic extract showed MIC=MBC=25 mg/ml, where it increased to become 86 mg/ml in the aqueous group for the MIC; however the MBC was higher than the highest concentration. The leaves ethanolic extract recorded an MIC=MBC=300 mg/ml, where it decreased into a considerable value in the aqueous group to become 2 mg/ml for MIC and 28 mg/ml for MBC (Tables 1 and 2).

P. aeruginosa showed alternating resistance and was found to be stronger during the second period extracts. In the aqueous extract, the MIC was equal to the MBC in the leaves (MIC=MBC=244 mg/ml), while the MIC of the stems was of 215 mg/ml. On the other hand, the ethanolic extracts showed MIC=MBC=343 mg/ml in the leaves, and MIC=205 mg/ml in the stems during the first period. Whereas, the MICs of the leaves and stems aqueous extracts during the second period were 100 and 44 mg/ml, respectively (Tables 1 and 2).

Regarding the roots extracts, it was not effective (in general), and only rare activities were noticed during the first period against *P. aeruginosa*, (in the aqueous group, the MIC was similar to the MBC=450 mg/ml). It decreased to become 91 mg/ml as MIC and 457 mg/ml as MBC for *S. epidermidis*. The roots extracts showed MIC=MBC=103 mg/ml against this same strain during the second period, and an approximately equal value, MIC=MBC=104 mg/ml, was found against *E. faecalis* (Tables 1 and 2).

For the second plant, *C. longiflorus*, although Gram positive strains were more resistant, *S. epidermidis* stands also to be the most sensitive against the extracts during both periods (Tables 3 and 4). During the first period, the leaves showed MIC=9 mg/ml and MBC=75 mg/ml in the aqueous extract, and MIC=18 mg/ml and MBC=36 mg/ml in the ethanolic extract. Regarding the second period, the leaves aqueous extract was not strong, in which MIC was 394 mg/ml and MBC was larger than the highest concentration. However, it was strong for the leaves ethanolic extract where the MIC was equal to the MBC=30 mg/ml. Among Gram negative bacteria, *P. aeruginosa* was the most sensitive, having an MIC=MBC=150 mg/ml during the first period in both leaves groups, the aqueous and the ethanolic extracts. It increased slightly to become 200 mg/ml for both MIC and MBC of the leaves aqueous extract, and it continues increasing to reach 428 mg/ml in the ethanolic one (Tables 3 and 4). Regarding the second Gram negative strain, *E. coli*, MIC was equal to the MBC=300 mg/ml for both the leaves aqueous extract and the leaves and stem ethanolic extracts during the first period. Moreover, the MIC was equivalent to the MBC=198 mg/ml for the leaves ethanolic extract and increased to reach 450 mg/ml as MIC in the stems extract. *E. faecalis* showed resistance to all extracts during the two periods except for the leaves and the stems ethanolic extracts where MICs were 150 and 300 mg/ml, respectively. *S. aureus*

showed only certain sensitivity to the ethanolic extracts during the two periods with an MIC and MBC for the leaves of about 300 mg/mL, and MICs=400 mg/ml and 450 mg/ml for the stems extract during the first period and for the leaves and stems extracts during the second period, respectively (Tables 3 and 4).

Conclusion

Our obtained results demonstrated for the first time, that aqueous and ethanolic extracts of first and second harvest of Lebanese *E. creticum* and *C. longiflorus* have a promising antibacterial effect on different types of bacteria (Gram+ and Gram-).

Further investigations are needed to determine the compound responsible of this activity and on the other hand other interesting activities such as the antibiofilm property of these two plants will be evaluated.

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