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Antimicrobial activity of Tunisian *Euphorbia paralias* L.

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ABSTRACT

Objective: To examine the potential antimicrobial activity of *Euphorbia paralias* L. (Euphorbiaeae) leaves and stems extracts.

Methods: The antimicrobial activity was tested against six microbial strains: *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Salmonella enterica* CIP 8039, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 90028 by two different methods, the disk method and the dilution method.

Results: Our results showed the important antimicrobial activity of the chloroform extract of the stems towards the majority of the strains by using both methods. *Bacillus subtilis* was the most sensitive strain (MIC = MBC = 15 μg/mL).

Conclusion: Thus, some extracts of *Euphorbia paralias* can be used in the treatment of infectious diseases caused by microbes.

1. Introduction

Infectious diseases caused by bacteria have a large impact on public health [1]. The appearance of antibiotics had ameliorated humanity's health status and quality of life. Yet, misuse and overuse of antibiotics had resulted in occurrence of bacterial resistance to commercially available antibiotics [2,3]. Thus, the new alternative therapeutic agents of natural origins, such as plants, that are effective against antibiotic resistant bacteria, safe and cost-effective have been searched constantly [4–6].

Euphorbia paralias L. (*E. paralias*) is also called *Tithymalus paralias* L. [7]. This plant belongs to the family Euphorbiaeae. It is a glabrous, glaucous, long-branched, woody stalk plant that produces stems that are sturdy, upright or ascending, usually simple, 20–60 cm, bare at the bottom. Leaves imbricated, glaucous, thick, coriaceous, erect, concave, whole, linear-oblong. Umbelle with 3–5 dichotomous rays. Rooted-reniform floral bracts. Glands with short and divisive horns. Subglobular capsule of 4–6 mm, glabrous, with fairly deep furrows. Granular hulls on the back. Smooth ovoid seeds with a very small caruncle [8]. This species is found in the maritime sands. It is spread all over the coast. It is found in the Mediterranean basin and in Western Europe. *Euphorbia* species are widely used in traditional medicine to treat diseases of stomach, liver and uterine cancer. These species have also been used in the treatment of asthma diseases [9]. Several other species have been used for the treatment of skin diseases, gonorrhea, migraine, intestinal parasites, warts and calluses [10].

It is in this context that we were interested in the study of the antimicrobial activity of the acetone and chloroform extracts of the *E. paralias* stems and leaves.

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2. Materials and methods

2.1. Chemicals and reagents

The acetone, chloroform and dimethyl sulfoxide as well as the resazurin, the Mueller–Hinton broth, the filter paper and gentamicin used in the experiments have been purchased from Merck (Darmstadt, Germany).

2.2. Plant materials

E. paralias plant was harvested in the month of February in 2010 from the region of Monastir (Tunisia). The botanical identification was carried out by Professor Fethia Harzallah-Skhiri. A voucher specimen (*E. paralias*) was deposited in the herbarium of the Laboratory of Transmissible Diseases and Biological Active Substances, Faculty of Pharmacy, Monastir.

2.3. Preparation of extracts

The different organs of the plant: stems and leaves were separated, dried in the shade in a well ventilated place for two weeks. After drying, these various organs of the plant were ground to obtain fine powders which were used for the preparation of the extracts.

The stems and leaves of *E. paralias* were extracted by cold maceration with chloroform for 3 days at room temperature. The chloroform extracts of these two organs were obtained after filtration and evaporation of the solvent. A second maceration with acetone was carried out for 3 days at ambient temperature. After filtration and evaporation of the solvent, the acetone extracts of the leaves and the stems were obtained.

2.4. Antibacterial and antifungal activity

2.4.1. Microorganisms

Six microbial strains (5 bacteria and 1 *Candida*) were used: *Escherichia coli* (*E. coli*) ATCC 8739, *Bacillus subtilis* (*B. subtilis*) ATCC 6633, *Salmonella enterica (S. enterica)* CIP 8039, *Staphylococcus aureus* (*S. aureus*) ATCC 6538, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 9027 and *Candida albicans* (*C. albicans*) ATCC 90028. These strains were provided by the Laboratory of Transmissible Diseases and Biological Active Substances (Faculty of Pharmacy of Monastir).

2.4.2. Preparation of the culture medium

A total of 38 g of Mueller–Hinton medium powder were dissolved in 1 L of distilled water. After adjusting the pH to 7.3, the medium is placed in a boiling water bath until a homogeneous, liquid, transparent solution was obtained without any particle on the wall. The solutions were then autoclaved at 120 °C for 25 min and then cooled to 50 °C in order to be cast near a benzene nozzle in the Petri dishes at the rate of 15 mL/dish. Concerning the nutrient broth, 38 g of powder were dissolved in 1 L of distilled water. The flask was placed in a boiling water bath until a homogeneous, liquid, transparent solution was obtained. The solution was then poured into tubes, after which the latter had to be autoclaved at 120 °C for 25 min and remained closed until the time of use.

2.4.3. Measurement of antimicrobial activity by the disk method

The susceptibility of the strains to the acetone and chloroform extracts of *E. paralias* stems and leaves was tested by the disk method. The bacteria and *C. albicans* were prepared from colonies of less than 24 h in Petri dishes.

A single colony was mixed in 15 mL of the broth and then incubated for 3–5 h at 35 °C. The bacterial culture was spread uniformly on the surface of Muller–Hinton agar plates. The plates were dried for 15 min before placing paper disks that had been impregnated with solution of plant extract (10 µL). Standard commercial antibiotic (gentamicin) was used as positive control. After 30 min of diffusion at laboratory temperature, the Petri dishes were then incubated at 37 °C for 18–24 h. After the incubation, the plates were examined for the zone of inhibition and then the diameter of zone of inhibition was measured [11].

2.4.4. Measurement of antimicrobial activity by the liquid dilution method

2.4.4.1. Determination of MIC

The MIC is the lowest sample concentration capable of inhibiting any visible growth of the germ. It measures a bacteriostatic effect and does not provide information on the status of the bacterial population. It does not specify whether the bacteria were killed in whole or in part. MBC and MFC are the minimum sample concentrations capable of killing 99.9% of the bacterial and fungal population after incubation for 18–24 h at 37 °C [12]. In this study, the MIC, MBC and MFC were determined according to the protocol of Kitzberger *et al.*, [13]. All the tested bacteria were grown in Muller–Hinton broth for 18–24 h, followed by adjusting the bacterial suspension to the turbidity equivalent to 10^6 bacteria/mL with the addition of physiological water. Each sample was dissolved in 10% DMSO.

The MIC measurement was carried out using 96-well microplates according to the following steps: 200 µL of each extract were deposited in columns 3; a dilution series of factor 2 was carried out while taking 100 µL of column No. 3 and adding them in column No. 4 and so on to column No. 12. The last 100 µL of the wells of column No. 12 were discarded. Then 20 µL of the microbial suspension were deposited in the various wells to which 10 µL of resazurin (growth indicator which is initially blue and turns pink in case of cell growth) was added. In column 1, an antibiotic (gentamicin) replaced the extract (positive control). In column 2, only the culture medium, the microbial suspension and the resazurin were present (negative control). The plates were subsequently incubated at 37 °C for 24 h. After incubation, the plates were interpreted with the naked eye by the observation of color turning from blue to pink. The last well before the change of color to pink indicates the MIC.

2.4.4.2. MBC and MFC

The main steps in determining the MBC and MFC were as follows: 10 µL were taken from the well corresponding to the MIC and the one before. The specimens were then streaked (5 cm) onto the agar. The seeded Petri dish was subsequently incubated at 37 °C for 24 h. Finally, the absence of colonies of bacteria means that the corresponding concentration is that of the MBC.

3. Results

3.1. Study of the antimicrobial activity

The antibacterial and antifungal activities of acetone and chloroform extracts of stems and leaves of *E. paralias* were tested against five bacterial strains *E. coli*, *B. subtilis*, *S. enterica*, *S. aureus*, *P. aeruginosa*, and one fungus *C. albicans*. This antimicrobial activity was tested for the first time for *E. paralias*.

According to the results mentioned in Table 1, it was found that the chloroformic extract of stems showed the largest zone of inhibition (13 mm) against *P. aeruginosa* in comparison with the gentamicin (14 mm). This extract also showed a potential antibacterial activity against *B. subtilis* with an inhibition zone of 11 mm, which is close to that showed by the reference drug (12 mm). This same extract as well as the leaves acetonic extract showed an encouraging activity against *C. albicans* (10 mm) which is slightly less than that of gentamicin (11 mm).

3.2. Determination of MIC/MBC or MFC

After incubation of the 96-well microplate at 37 °C for 24 h, a blue-to-pink turn was observed with the naked eye. The pink color is due to the addition of the resazurin dye. Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) is a blue dye which can be irreversibly reduced to a pink and highly red fluorescent substance, resorufin by oxidoreductase within viable cells. The last well before turning the blue color to pink indicates the MIC.

The MIC and MBC values corresponding to the extracts deduced from the 96-well microplates are respectively showed in Table 2.

The chloroform extract of the stems of *E. paralias* showed the lowest MIC values (0.015 mg/mL) with respect to the three strains *C. albicans*, *B. subtilis* and *E. coli*.

Table 1
Zone of inhibition measured around the disks containing the extracts (mm).

Microbial strains	Antibiotic	Extracts (10 mg/mL)			
	G (60 µg/mL)	SAE	LAE	SCE	LCE
E. coli	15	5	4	7	–
S. enterica	13	8	–	8	–
P. aeruginosa	14	9	6	13	–
B. subtilis	12	–	–	11	–
S. aureus	16	–	–	–	–
C. albicans	11	–	10	10	–

SAE: stems acetonic extract; LAE: leaves acetonic extract; SCE: stems chloroformic extract; LCE: leaves chloroformic extract; G: gentamicin. –: no zone of inhibition is observed.

Likewise, this extract exerted a significant inhibition against the bacteria *S. enterica*, *S. aureus* and *P. aeruginosa* with MIC values of 0.31, 1.25 and 0.31 mg/mL, respectively.

MBC values of 0.015 and 0.31 mg/mL towards *B. subtilis* and *P. aeruginosa*, respectively, are the most important bactericidal concentration values observed in this extract.

For the chloroform extract of the *E. paralias* leaves, *C. albicans* was the most sensitive of the strains tested since it had the lowest MIC and MFC values of 0.015 and 0.31 mg/mL, respectively. It can also be noted that this extract exerted an important activity against *P. aeruginosa* with MIC and MBC values of 0.62 mg/mL.

For the other samples, MIC and MBC values were observed, which ranged from 1.25 to 10 mg/mL, with the exception of the acetone extract of the stems, which showed a MIC value of 0.015 mg/mL against *E. coli*.

4. Discussion

In the present study, the antibacterial and antifungal activities of acetone and chloroform extracts of stems and leaves of *E. paralias* were tested against five bacterial strains and one fungus. Many studies also showed that the chloroformic extract of medicinal plant inhibits the growth of *P. aeruginosa*, such as Benli *et al*., [14], who demonstrated that the chloroformic extract of *Artemisia dracunculus* L. has an potential antibacterial activity (11 mm) against this Gram-negative bacterium.

Sahoo *et al.* [15] also demonstrated that the *Barringtonia acutangula* chloroformic extract inhibited the growth of *P. aeruginosa* by the disc diffusion assay (11 mm). This antimicrobial activity can be associated with the presence of the phytochemical compounds in the stems chloroformic extract which may be of numerous modes of action, for example, the degradation of the bacteria cell wall, the interaction with its composition. These components cause leakage associated with cellular ingredients, coagulate cytoplasm, deplete proton motive force, alter fatty chemical and phospholipid constituents [16].

The use of plant extracts and phytochemicals, both with known antimicrobial activities, can be of importance in therapeutic remedy. In recent years, a number of investigations have been carried out in different areas to show the effectiveness of plant extracts [17–24]. A lot of plants have been used because of their antimicrobial properties, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the polyphenolic compounds [21–27]. In this work, the antimicrobial activity of *E. paralias* extracts was investigated for the first time. These results are in agreement with the findings of other researchers, such as Ashraf *et al.*, [26] who

Table 2
Antibacterial and antifungal activities of extracts of *E. paralias* (mg/mL).

Extracts and drug	*E. coli*		*S. enterica*		*P. aeruginosa*		*B. subtilis*		*S. aureus*		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
LAE	2.500	2.5000	2.50	2.500	5.00	5.00	5.000	5.000	5.00	5.000	2.500	2.5000
LCE	10.000	10.0000	10.00	10.000	0.62	0.62	2.500	2.500	10.00	10.000	0.015	0.3100
SCE	0.015	1.2500	0.31	1.250	0.31	0.31	0.015	0.015	1.25	1.250	0.015	2.5000
SAE	0.015	1.2500	5.00	5.000	10.00	10.00	10.000	10.000	10.00	10.000	5.000	5.0000
Gentamicin	–	0.0039	–	0.015	–	0.50	–	0.015	–	0.015	–	0.0005

SAE: stems acetonic extract; LAE: leaves acetonic extract; SCE: stems chloroformic extract; LCE: leaves chloroformic extract.

showed that the methanolic, hexanic and aqueous extracts of *Euphorbia rayleana* (Pakistan) showed zones of inhibition ranging from 5.67 mm to 7 mm against *E. coli* and 8.33 mm–12 mm against *B. subtilis*. These values are close to the results found in this paper.

Sudhakar *et al.*, [27] tested the antimicrobial activity by the disk method on extracts of *Euphorbia hirta*. Similarly, Natarajan *et al.*, [28] worked on the aerial parts and roots of *Euphorbia fusiformis* (India). These two research groups found different and variable results with those found in this work, which is due to the difference in species as well as the extraction solvents and the parts of the plant studied.

In the literature, two studies tested the antimicrobial activity of *Euphorbia* species using the dilution method. Therefore, Sudhakar *et al.*, [27] found that the ethanolic extract of *Euphorbia hirta* showed MIC values ranging from 0.166 to 0.296 mg/mL against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans*.

Another recent antibacterial study showed that ethanolic, hexanic, dichloromethane and the ethyl acetate extracts of *Euphorbia hirta* showed different MIC values from 0.125 to 1 mg/mL with respect to the same bacterial strains used [29].

The chloroform extract of *E. paralias* stems was the most effective extract against the three strains *C. albicans*, *B. subtilis* and *E. coli*. For the chloroform extract of the leaves of *E. paralias*, the fungus *C. albicans* was the most sensitive of the strains tested. These plant extracts could be new antimicrobial agents with great potential. Further studies which aimed at the isolation and structure elucidation of antimicrobial active constituents from the plant have been initiated.

Conflict of interest statement

We declare that we have no conflict of interest.

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