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In vitro antioxidant and antibacterial activity of the root extract of *Euphorbia resinifera*

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Abstract

Euphorbia resinifera Berg, a plant endemic to Morocco, has been reported to possess a number of medicinal properties and is one of the oldest drugs in the Western medicinal tradition. The present study was therefore undertaken to evaluate the effect of root extract of *Euphorbia resinifera* Berg., for its antioxidant and antimicrobial potential. The roots used in the study were dried and finely powdered. The powder was successively extracted by CH₂Cl₂, EtOAc, EtOH and acetone. After filtration, the filtrate was concentrated and dried. All obtained extracts were evaluated for their antioxidant and antibacterial activity properties.

Keywords: *Euphorbia resinifera*, Extraction, Antioxidant activity, Root extract, Antibacterial activity.

1. Introduction

Humans have always made use of their native flora, not just as a source of food, but also for fuel, medicines, clothing, home construction, and chemical production. Traditional knowledge of plants and their properties has always been transmitted from generation to generation through the natural course of everyday life [1]. Globally, millions of people in the developing world rely on medicinal plants for primary health care, income generation and livelihood improvement. Between 50.000 and 70.000 plant species are known to be used in traditional and modern medicinal systems throughout the world [2]. The genus *Euphorbia* is the largest in the plant family Euphorbiaceae, comprising about 2000 known species and ranging from annuals to trees. All contain latex and have unique flower structures. A significant percentage, mostly those originating in Africa and Madagascar. Samples of leaves, stems, flowers and roots from *Euphorbia hirta* were tested for total phenolic content, flavonoids content and *in vitro* antioxidant activity by diphenyl-1-picrylhydrazyl (DPPH) assay. Also reducing power was measured using cyanoferrate method. The leaves extract exhibited a maximum DPPH scavenging activity of 72.96% followed by the flowers, roots and stems whose scavenging activities were 52.45%; 48.59% and 44.42% respectively. Whereas the standard butylated hydroxytoluene (BHT) was 75.13% [3]. The alcoholic extract of *Euphorbia heyneana* produced dose dependent inhibition of superoxide radicals ranging from 43.17% to 91.22%. The mean (inhibition concentration) IC₅₀ values for superoxide radical by alcoholic extract of *E. heyneana* and ascorbic acid were found to be 68.11 and 62.27 µg, respectively. The alcoholic extract of *Euphorbia heyneana* produced dose dependent inhibition of hydroxyl radicals ranging from 32.54% to 78.34%. The alcoholic extract of *Euphorbia heyneana* produced dose dependent inhibition of DPPH radicals ranging from 46.12% to 91.03%. The mean IC₅₀ values for hydroxyl radical by alcoholic extract of *Euphorbia heyneana* and ascorbic acid were found to be 67.55 and 55.24 µg, respectively [4]. The aerial parts of *Euphorbia petiolata* were extracted successively with n-hexane, dichloromethane and methanol. The methanol extract had significant DPPH scavenging activity. Polar compounds all exhibited considerable levels of free radical scavenging activity in DPPH assay. Kaempferol, quercetin and myricetin derivatives were the most active principles in methanol extract [5]. As part of our ongoing valorisation of *Euphorbia resinifera* Berg., native to the anti-Atlas mountains of Morocco, we have investigated the antioxidant and antibacterial activity of various extracts from roots of *Euphorbia resinifera* Berg.

2. Material and Methods

2.1. Plant Material

Roots of *Euphorbia resinifera* were collected in the area of Damnat (Morocco), and identified by Dr. A. Echchahad (National Institute of Medicinal and Aromatic Plants, Morocco). The roots were shade dried for a period of 4 weeks after which they were finely powdered.

2.2. Extraction

400 g of the powder of roots of *Euphorbia resinifera* was extracted successively with CH₂Cl₂ (0.5 L), EtOAc (0.5 L), EtOH (0.5 L), and Acetone (0.5 L) at room temperature. All obtained extracts were evaporated and used for antioxidant and antibacterial properties.

2.3 Test on Antibacterial Activity

The following bacteria were tested : *Escherichia coli* (ATCC 35210), *Staphylococcus aureus* (ATCC 29213) *Salmonella typhimurium* (ATCC 13311), *Bacillus subtilis* (ATCC 10907), *Staphylococcus epidermidis* (ATCC 12228). The microdilution technique used as described [6]. The bacterial suspension was adjusted with sterile saline to a concentration of approximately 1.0 x 10⁷ cells/ml. Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique, using microtitre plates. The extracts tested were dissolved in DMSO (1.0 mg/ml) and added to broth medium with bacterial inocula. The microplates were incubated for 24 h at 37 °C. The minimum bactericidal concentrations (MBCs) were determined by serial

subcultivation of 2 µl in microtitre plates containing 100 µl of broth per well and further incubation for 24 h at 37 °C. The lowest concentration with no visible growth was defined as the MBC, indicating ≥ 99.5% killing of the original inoculum. DMSO was used as a negative control, while streptomycin was used as a positive control (1.0 µg/ml).

2.4 Test on Antioxidant Activity

Extracts were dissolved in appropriate solvents, mixed with 1 ml of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in MeOH, and final volume was adjusted to 5 ml. Mixtures were vigorously shaken and left for 30 min in the dark. Absorbance was measured at 517 nm using MeOH as blank. 1 ml of 0.5 mM DPPH diluted in 4 ml of MeOH was used as control. Neutralization of DPPH radical was calculated using the equation: $S(\%) = 100 \times (A_0 - A_s)/A_0$, where A_0 is the absorbance of the control (containing all reagents except the test compound), and A_s is the absorbance of the tested sample. The SC₅₀ value represented the concentration of the extract that caused 50% of neutralization [7]. Results were compared with the activity of L-ascorbic acid.

3. Results and Discussion

Scavenging of DPPH radical was concentration-dependent. EtOAc extract expressed the strongest activity (SC₅₀ = 18.20 ± 0.41 µg/ml), while Acetone and EtOH and dichloromethane extracts showed moderate activities (SC₅₀ = 98.44, 65.01 and 122.15 µg/ml, respectively (Table 1).

Table 1: Antioxidant activity of roots of *Euphorbia resinifera* extracts (µg/ml)

Extract	DPPH (SC ₅₀ , µg/ml)
CH ₂ Cl ₂	122.15 ± 0.52
EtOAc	18.20 ± 0.41
EtOH	65.01 ± 0.32
Acetone	98.44 ± 0.13
L- Ascorbic acid	5.09 ± 0.07

The results of testing of antibacterial activity of roots of *Euphorbia resinifera* extracts showed that the EtOAc extract was the most effective (with MICs of 1.0–1.5 mg/ml and MBCs 1.5 – 2.0 mg/ml), followed by the EtOH, CH₂Cl₂ and Acetone extracts. *S. typhimurium* was found to be the most resistant species, with

MICs of 1.5–2.0 mg/ml and MBCs of 2.0–2.5 mg/ml. *E. coli* was the most sensitive, with MICs of 1.0 – 1.5 mg/ml and MBCs of 1.5–2.0 mg/ml. commercial streptomycin showed higher antibacterial potency than did the extracts tested (Table 2).

Table 2: Minimum inhibitory and bactericidal concentrations (MICs and MBCs) of roots of *Euphorbia resinifera* extracts (mg/ml)

Bacteria		Extract				Streptomycin
		Acetone	EtOH	EtOAc	CH ₂ Cl ₂	
<i>S. typhimurium</i>	MIC	1.0 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	1.0 ± 0.2	0.0010±0.0002
	MBC	1.5 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	0.5 ± 0.3	0.0010±0.0002
<i>E. coli</i>	MIC	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.0005±0.0001
	MBC	0.5 ± 0.2	0.5 ± 0.0	0.3 ± 0.0	1.0 ± 0.2	0.0010±0.0002
<i>S. epidermidis</i>	MIC	0.5 ± 0.0	0.5 ± 0.0	0.2 ± 0.1	0.5 ± 0.0	0.0010±0.0000
	MBC	1.0 ± 0.2	1.0 ± 0.2	0.6 ± 0.2	1.0 ± 0.2	0.0010±0.0000
<i>S. aureus</i>	MIC	0.5 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.0010±0.0002
	MBC	1.0 ± 0.2	1.0 ± 0.2	0.3 ± 0.2	1.0 ± 0.2	0.0010±0.0003
<i>B. subtilis</i>	MIC	1.0 ± 0.2	1.0 ± 0.2	0.3 ± 0.0	0.5 ± 0.0	0.0005±0.0000
	MBC	1.5 ± 0.0	1.0 ± 0.0	0.1 ± 0.0	1.0 ± 0.2	0.0005±0.0002

Our experiments showed substantial antibacterial activities of roots extracts of *Euphorbia resinifera*, with MICs, MBCs of 0.50 – 1.50 mg/ml. The EtOAc extract was the most effective.

4. Conclusion

The results obtained herein on antioxidant and antibacterial

activity of different extract of roots of *Euphorbia resinifera* support the traditional use of this plant and provide grounds for further establishing its use in medicinal chemistry.

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