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Antioxydant and Antibacterial Activity of Alkaloids and Terpenes Extracts from *Euphorbia granulata*

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***Abstract*—**In order to enhance the knowledge of certain phytochemical Algerian plants that are widely used in traditional medicine and to exploit their therapeutic potential in modern medicine, we have done a specific extraction of terpenes and alkaloids from the leaves of Euphorbia granulata to evaluate the antioxidant and antibacterial activity of this extracts. After the extraction it was found that the terpene extract gave the highest yield 59.72% compared with alkaloids extracts.

The disc diffusion method was used to determine the antibacterial activity against different bacterial strains: Escherichia coli (ATCC25922), Pseudomonas aeruginosa (ATCC27853) and Staphylococcus aureus (ATCC25923). All extracts have shown inhibition of growth bacteria. The different zones of inhibition have varied from (7 -10 mm) according to the concentrations of extract used.

Testing the antiradical activity on DPPH-TLC plates indicated the presence of substances that have potent anti-free radical. As against, the BC-TLC revealed that only terpenes extract which was reacted positively. These results can validate the importance of Euphorbia granulata in traditional medicine.

Keywords*—Euphorbia granulata*, Euphorbiaceae, alkaloids, terpenoids, antioxidant activity, antibacterial activity.

I. Introduction

IN Africa and all over the word, herbs are important sources of drugs against chronic and acute diseases, more than 80% of rural and urban populations use medicinal plants as natural remedies [1].

Euphorbia granulata forssk annual Saharan herb toxic, almost glabrous, rich latex. One of the flowering plants of the Euphorbiaceae family [2], it is located in tropical Africa and in dry regions of Asia, in Algeria, it is found in the two areas of the northern and central Sahara and in Ahaggar. The main classes of secondary metabolites present in Euphorbia species are alkaloids, terpenes, tannins, steroids and cardiac glycosides [3]. It is used by Moroccans, Algerians and Egyptians in traditional medicine for treating various illnesses like skin diseases, cancer, gonorrhea, headaches, digestive disorders, kidney pain, nervous exhaustion, intestinal parasites and warts. This latex is used against snake bites and scorpion stings [4].

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Recent research is done to extract biologically active compounds from plants of Euphorbia, several compounds have been isolated that have the ability to reduce oxidation damage and replace traditional antibiotics to treat diseases of brain dysfunction and infectious diseases. In this context, we extracted alkaloids and terpenes compounds from Euphorbia granulata to evaluate the antioxidant and antibacterial activity of each extract.

II. Materials and Methods

A. Plant Material

Euphorbia granulata forssk was collected and identified in June 2010.The leaves were cleaned, washed, dried in the shade, grounded and stored in clean bags.

B. Alkaloids Extraction

The extraction of alkaloids was performed by method of Muzquiz [5], with minor modification. 300g of the ground aerial part of Euphorbia granulata were basified with 10ml of sodium carbonate Na2CO3 and soaked in 900 ml ethyl acetate for 72 h. After filtration, the marks have been eliminated and acetyl extract was evaporated to recover an organic phase containing alkaloids, lipids and pigments, which was placed in the separatory funnel and treated with100 ml of water acidified by HCL 1.5%. After settling, two phases were obtained, an organic phase contains neutral alkaloids and an aqueous phase which was basified again with 10 ml of sodium carbonate Na2CO3, the addition of 166 ml dichloroform to this phase allows the production of an aqueous phase containing quaternary alkaloids and an organic phase containing the basic alkaloids.

C. Terpenes Extraction

According to studies released by [6], we adapted a method for the extraction of terpenes, from the use of traditional solvents. The aerial crushed part of Euphorbia granulata (250 g) was soaked in 750ml petroleum ether for 3 days with gentle intermittent stirring. The ether-petrolic extract that was obtained was filtered and concentrated under vacuum at 40 ° C, then it was subjected to a liquid-liquid extraction (ethyl acetate 150 ml - 50 ml distilled water). After settling, the aqueous phase was recovered and the organic phase of ethyl acetate which includes terpenes was dried by MgSO4, filtered and evaporated by rota-vapor (rotatry steam).

The amount of extract was determined after evaporation of all extracts.

The extraction yields were calculated as a percentage.

D.Rapid Screening with TLC-DPPH and TLC-BC Method

To evaluated the antioxidant activity of our extracts we performed two tests on TLC plates, the first was based on the principle of reducing free radical provided by 1, 1'-diphenyl-2-picrylhydrazyle (DPPH) [7], the second test founded on the bleaching of beta carotene from the loss of their double bound by oxidation wich leading to a loss of their orange color.

E. Antibacterial Testing

The tests were conducted on the following reference strains: Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), and Escherichia coli (ATCC 25922).

For each organic extract, 1g was dissolved in 100ml of DMSO which is equivalent to 10mg/ml we made three dilutions, resulting in four concentrations $_C$ initial, C1/2, C1/4, C1/8. All extracts were filtered through a micro-filter 0.2µm.

Discs of 6mm diameter impregnated in 100ml of each extract, these discs were dried for 30 minutes to 1 hour.

Mueller-Hinton agar was cooled in Petri dishes (4mm), the seeding of the different strains was performed with a pure bacterial suspension, freshly prepared, we posed impregnated disks on the agar surface. Each dish contains a maximum of six discs, which have been left for 30 minutes at room temperature for pre-diffusion of extracts, before being incubated at 37 ° C for 18 to 24 hours. The antibacterial activity was determined by diameter measurements of inhibition zones.

F. Statistical Analyzes

The results from the antibacterial test were performed in triplicate, they were expressed as mean±SD and analyzed statistically by using the logiciel of Graph pad prism 5.

The use of univariate ANOVA allows the determination of the significance level between the different concentrations studied. Values of $p < 0.05$ were considered statistically significant.

III. Results and Discussion

A. Extraction

The result of the percentage and mass yield getting after extraction is shown in Table I.

The results of our study showed that the terpenes extract gives the highest yield than alkaloids extract neutral alkaloids extracted and basic alkaloids extract respectively which have low yields. This can be explained by the distribution of these compounds in the plant.

TABLE I
PERCENTAGE AND MASS YIELDS FROM THE AERIAL PARTS OF *EUPHORBIA GRANULATA*

Type of extract	Color	Mass yield (g)	Yield (%)
Terpenes extract	Dark green	34.98	59.72
neutral alkaloids extract	Black green	36.24	12.07
basic alkaloids extract	Green light	10.93	3.64

B. LTC-DPPH

The appearance of yellow-white spots on a purple background allowed us to demonstrate the presence of substances with anti-radical activity. The terpenes tasks are the first appears after 2 minute from the revelation. These substances were characterized by their Rf table II.

All the extracts of this test reacts positively. So they are composed of molecules that inhibit free radicals. This activity can be explained by the presence of terpenes and alkaloids, which have antioxidant properties, and / or the presence of tannins and flavonoïdes [8].

TABLE II
ANTIRADICAL ACTIVITY ON TLC IN HEXANE-CHLOROFORM (3:7) OF *EUPHORBIA GRANULATA* EXTRACTS

Different extracts	Number of spots	Rf
Terpene extract	4	0.65 ; 0.84 ; 0.9 :0.94
Neutral alkaloids extract	3	0.1 ; 0.49 ; 0.81
Basic alkaloid extract	2	0.55; 0.84

C. CCM-BC

After revelation of TLC by the solution of beta carotene we noted the appearance of a single yellow spot on white background.

Only the extract terpenes which shows inhibitory activity of beta carotene bleaching after 2 hours of revelation. The antioxidant activity terpene is due to the skeleton terpenes because of the presence of squalene, phenolic groups and functions quinines [9].

We note that alkaloids didn't reveal any inhibition of bleaching of beta carotene, according to [8].

D. Anti Bacterial Test

The observation of growth inhibition zones for 48 hours after application of different discs extract showed that all extracts were active against the three reference strains, but with different degrees.

The results about the inhibition zone diameters of different extracts applied on the reference strains are presented in the table III.

From the results of our study, the largest zone of growth inhibition was shown by the neutral alkaloids extract against S. aureus at a concentration of 10 mg / ml, however, the smallest zone of growth inhibition in the same concentration was observed by basic alkaloids extract against E. coli.

According to Perry [10], gram positive bacteria (S. aureus) are much more sensitive than gram negative bacteria (E. Coli), this sensibility can be attributed to the structure of bacteria. The results concerning the activity of extracts of *Euphorbia granulata* against *P. aeruginosa*, show their sensitivity to alkaloids extracts and their resistance to terpenes extract, the resistance was shown by the appearance of a particular

TABLE III
ANTIBACTERIAL ACTIVITY OF TERPENES AND ALKALOIDS EXTRACTS OF *EUPHORBIA GRANULATA*

Bacterial strain	Extrait type	Doses (mg/ml)/ Inhibition zones (mm)			
		10	5	2.5	1.25
S.aureus ATCC (25923)	Terpenes extract	8.33±0.58	7.50±2.18	0.0±0.0	0.0 ±0.0
	Basic alkaloids extract	8.67±0.58	7.0±1.73	7.33±1.15	0.0±0.0
	Neutral alkaloids extract	10.5±1.32	9.5±0.50	9±0.87	8.17±0.29
E.coli ATCC (25922)	Terpenes extract	8.5±0.5	7.17±0.29	0.0±0.0	0.0±0.0
	Basic alkaloids extract	7±0.87	0.0±0.0	0.0±0.0	0.0±0.0
	Neutral alkaloids extract	9.17±0.29	7.67±0.58	0.0±0.0	0.0±0.0
P.aeruginosa ATCC (27853)	Terpenes extract	24.07±1.61	22.40±2.51	19.67±1.53	21.33±3.51
	Basic alkaloids extract	9.67±1.15	9.67±1.15	8.83±1.04	8.83±1.04
	Neutral alkaloids extract	8.83±0.76	6.5±0.0	0.0 ±0.0	0.0±0.0

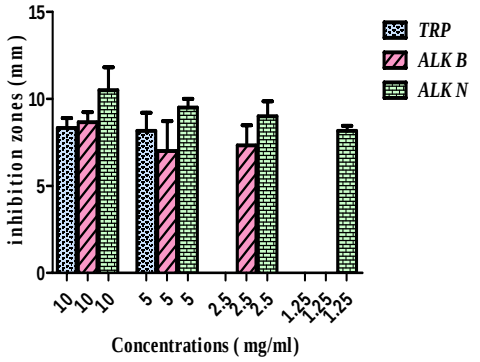

Fig. 1 Effect of the concentration of different extracts on the antibacterial activity against *S.aureus*

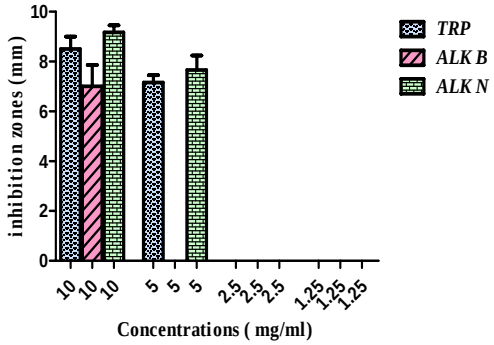

Fig. 2 Effect of the concentration of different extracts on the antibacterial activity against *E.coli*

phenomenon where colonies present around extract discs an area without culture (zone of inhibition) then cocarde growth and again a zone of inhibition. The statistical study of the antibacterial activity of different extracts of Euphorbia granulata against three reference strains are shown in fig. 3, 4 and 5. The results of statistical analysis showed no significant difference ($p = 0.051$) in growth inhibition of *S. aureus* by the effect of different concentrations of the neutral alkaloids extract, if we compared them with basic alkaloids extract and terpenes extract which representing a highly significant difference ($P < 0.001$) in their effect of different concentration on the antibacterial activity against *S. aureus*. In all cases, our extracts of *Euphorbia granulata* showed effective antibacterial activity at low concentrations used against *S. aureus*,

According to the statistical results of our study, there was a highly significant difference ($P < 0.0001$) in the effect of the extracts concentrations on the antibacterial activity against *E. coli*, so the concentrations of different extracts have a strong influence on the antibacterial activity, which is well presented by [11].

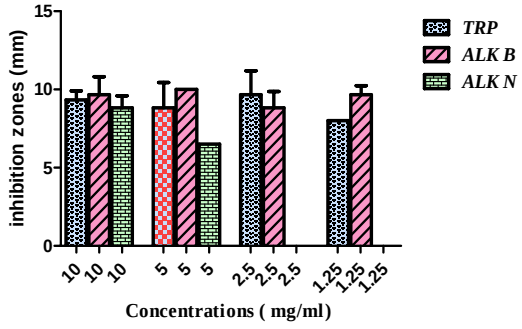

Fig. 3 Effect of the concentration of different extracts on the antibacterial activity against *P.aeroginosa*

The different concentrations of terpenes and basic alkaloids extracts indicated that there was no significant inhibition of

the growth of *P.aeroginosa* (P ≥0.05) respectively, in contrast to different concentrations of neutral alkaloids extract applied, which reveals a highly significant difference (P <0.001).

IV. Conclusion

Despite the importance of Euphorbia granulata Forssk in folk medicine, few scientific studies have been done on their phytochemistry and their biological activity. In this work we evaluated the antibacterial and antioxidant activity of aerial parts extracts of this plant.

Both specific extraction gave different yields, the highest yield was obtained from terpenes extract (50%) while the basic alkaloids extract showed the lowest yield (3.64%).

On one side terpenes and alkaloids extracts were shown a great antiradical activity by the characterization of several substances which inhibit free radical DPPH and on the other side the discoloration of β-carotene showed that only the terpenes extract that give the antioxidant activity.

The different extracts of Euphorbia granulata showed significant antibacterial activity against *S. aureus* at low concentrations (1.25mg/ml to neutral alkaloids extract, 2.5mg/ml basic alkaloids extract and 5mg/ml terpenes extract). However, the antibacterial activity of terpenes and alkaloids extracts against E. coli showed highly significant differences. Moreover, *P.aeruginosa* shows resistance to terpenes extract and sensitivity to alkaloids extracts with a highly significant difference in their antibacterial activity.

Our extracts included substances that are very promising for the discovery and development of new pharmaceutical products this shows the richness of the flora of Algeria, a cause for which further research should be conducted to identify and separate these substances.

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