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Phytochemical screening of five Algerian plants and the assessment of the antibacterial activity of two *Euphorbia guyoniana* extracts

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ABSTRACT

Medicinal plants may offer a natural and new source of antibacterial agents. Many species growing in Algeria such as Euphorbia guyoniana have been used as medicinal plants in the treatment for skin diseases. The present work focuses essentially on the phytochemical screening of 5 species namely: Parentucellia viscosa, Verbascum signatum, Ecbalium elaterium, Scabiosa atropurpura, and Euphorbia guyoniana. 250 phytochemical tests have been carried out among which 67.20 % were positive. It is worth to note the presence of many chemical classes such as: flavonoids, sterols or triterpenes, saponins, tannins, carotenoids and alkaloids. In addition the evaluation of the antibacterial activity of two extracts of the endemic Euphorbia guyoniana Boiss. et Reut by the disc diffusion assay was performed against six bacteria strains. The sensitivity order of the Methylene chloride-Methanol crude extracts was illustrated by the corresponding inhibition zone diameter to be: Pseudomonas aeruginosa> Proteus vulgaris > Klebsiella pneumonia> Enterobacter> Escherichia coli> Staphylococcus aureus. On the other hand the n-butanol extract sensitivity order was as follows: Proteus vulgaris > Klebsiella pneumonia> Pseudomonas aeruginosa> Staphylococcus aureus >Enterobacter> Escherichia coli.

Keywords: phytochemical Screening, antibacterial activity, medicinal plants.

INTRODUCTION

Herbs and spices have been used since ancient times for their perfume, flavour, and preservative properties in a variety of products and applications with medicinal and cosmetic uses. However, nowadays there is a renewal of scientific interest in the use of these natural antimicrobials for food preservation [1]. Recently, there has been a considerable interest in extracts and essential oils (EOs) from common culinary herbs, spices and aromatic plants characterized by a notable antimicrobial activity [2-6]. Such substances can be used to delay or inhibit the growth of pathogenic and/or toxin producing microorganisms in foods [7]. In such context, plant-based essential oils or organic extracts are well known to exhibit a wide range of biological activities [8]. To assess and valorise our natural patrimony which is still unexplored we have undertaken the study of some plants widely used in the Algerian folk medicine.

The genus *Parentucellia* (Scrofulariaceae) is represented in Algeria by two species, *P. latifolia* (L.) Caruel and *P.viscosa* L, but *Verbascum* (Scrofulariaceae) is represented by *V. Blattaria* L., *V. simplex* Hoffm. et Link ampl. Murb., *V. maurum* Maire et Murb., *V. atlanticum* Batt., *V. dentifolium* Del., , *V. rotundifolium* Ten. ampl. and *V. sinuatum* L.

Other genus ,*Ecballium* represented by one species named *E. elaterium* Rich.

In adition *Genus Scabiosa* represented by twelve species *Sc. rutifolia* Vah., *Sc. camelorum* Coss. et Dr., *Sc. Succisa* L., *Sc. Cartenniana* Pons et Quézel, *Sc. semipapposa* Salzm., *Sc. daucoides* Desf., *Sc. arenaria* Forsk., , *Sc. stellata* L., *Sc. crenata* Cyr, *Sc. Ucranica* L., *Sc. Columba ria* L.and *Sc. atropurpurea* L. [9]

Euphorbia guyoniana Boiss. and Reut. is an endemic species in Algeria belonging to the Euphorbiaceae family [9,10]. It is a shrub 30–100 cm high that grows in sandy and desert habitats [9]. This species is used in folk medicine against the venomous bites of scorpions and is known as a wart remover [11]. Like the plants of the genus *Euphorbia*, it is characterized by the presence of latex which possesses an irritant effect on the eyes and skin [6]. Previously, We have investigated the chemical constituents of *E. guyoniana* and reported the isolation of guyonianins A and B [12].

Many species of the genus *Euphorbia* have been used as medicinal plants in the treatment for skin diseases, gonorrhea, migraine, intestinal parasites and warts [13-15]. Phytochemical investigation of this genus revealed that many its components are highly bioactive [16,17]. Roots, seeds, latex, stem, stem barks, leaves, and whole plants of the Euphorbia species have been researched. Plants in the Euphorbiaceae family are well known for the chemical diversity of their isoprenoid constituents [18]. Diterpenoids are found in the majority of the genus with many different coreframeworks such as jatrophanes, lathyranes, tiglianes, ingenanes, myrsinols, *etc*. [19–26].

Our investigation carried out on chemical constituents of some medicinal plants [8] and especially on the genus *Euphorbia.* [14], led to the detection of 17 chemical groups. Moreover the evaluation of antibacterial activity of two different crude extracts revealed a moderate effect against some pathogenic bacteria strains.

MATERIALS AND METHODS

Plant Material

The roots and leaves of *Euphorbia guyoniana* were collected in march 2011 (flowering stage) in the El Oued area, south east of Algeria, but all organs of : *Parentucellia viscosa, Verbascum signatum, Ecbalium elaterium, Scabiosa atropurpura,* were collected in April 2011 (flowering stage) in the Grarem area – Mila, north east of Algeria. The five species were identified by Pr. Kaabache Mohamed, Department of biology, Setif University, Algeria. voucher specimens (ZA/71, ZA/34, ZA/18, ZA/29, ZA/09) succesively, were deposited at the Chemistry Department, University of Mentouri-Constantine.

Extraction and detection of chemical groups [27]

25g of powdered dried plant was extracted with petroleum-ether in a continuous extraction apparatus soxlet. The ether extracts were combined, filtered and concentrated up to 40-50 mL. The remaining dry material was extracted under reflux three times with methanol for 20-40 mn. The residue was then extracted with warm water for 20 minutes. The constituents were identified as follows:

- ***Identification of volatile oils***

The ether extract was evaporated to dryness. The residue had a characteristic pleasant odour, thus the plant product contains volatile oils. The vegetable product was distilled with water in a Neo-Clevenger apparatus to extract the volatile oils.

- ***Identification of sterols and triterpenes***

The residue of ether extract was dissolved in 0.5 mL acetic anhydride and then in 0.5 mL of chloroform. Then 1 mL of concentrated sulphuric acid is added (Libermann- Burchards reaction). At the contact zone of the two liquids a brownish red ring was formed denoting the presence of sterols and triterpenes.

- ***Identification of carotenoids***

The ether extract was evaporated to dryness and 3 drops of saturated solution of antimony trichloride in chloroform were added (Carrprice's reaction). The pigments are firstly blue and later became red, denoting the presence of carotenoids.

- ***Identification of fatty acids***

An alkaline aqueous solution cont exhaustively extracted with ether and acidified by HCl (pH=3-4). The acidic aqueous solution becomes opalescent. The fatty acids are extracted by ethyl ether and evaporated. If the residue is oily, fatty acids are present.

● ***Identification of flavone aglycones***
The residue of ether extract was dissolved in 2 mL of methanol at 500 °C. Metallic magnesium and 5 drops of concentrated HCl were added. A red or orange colour indicates the presence of flavones aglycones (Shibata's reaction).

● ***Identification of anthracenoside aglycone (emodols)***
1 mL of 25 % of NH_4OH was added to the ether extract and shaken (Bortrager reaction). A red colour indicates the presence of emodols.

● ***Identification of coumarins***
The residue of ether extract or alcohol extract is dissolved after dryness in hot water. The solution is divided into two equal volumes: one of which contains the reference, and the second is made alkaline with 0.5 mL of 10 % ammonia solution. The appearance of an intense fluorescence under UV light indicates the presence of coumarins and derivatives.

●***Identification of tannins:***
The water extract (1mL) was diluted with water (2 mL) and a diluted solution of ferric chloride (3 drops) was added. The appearance of a blackish blue or blackish green colour indicates the presence of tannins.

● ***Identification of reducing compounds***
1 mL of Fehling solution was added to the alcohol extract then the mixture was heated. A brick red precipitate denotes the presence of reducing compounds.

● ***Identification of anthracenosides***
In alcohol extract (see the method of Bortrager reaction, ether extract)

● ***Identification of anthocyanosides:***
The alcohol extract was acidified. the acidic solution turns red at pH=7 and did not change to green or violet at alkaline medium indicates the presence of anthocyanins.

● ***Identification of polyuronides (pectins ,mucilage and gums)***
2 mL of the extract were added drop-wise in a test tube, where 10 mL of acetone had already been placed. A thick precipitate was formed denoting the presence of polyuronides.

● ***Identification of carbohydrates***
3-4 drops of the alcoholic solution saturated with thymol (Molish's reagent) were added. The appearance of a red colour denotes the presence of carbohydrates (oses, polyoses)

● ***Extraction procedure for (methylene chloride: Methanol) (1 :1)***
After drying and powdering, the crude material was extracted with ***(methylene chloride : Methanol) (1 :1)*** in soxhlet apparatus for 48 h. The solvent was evaporated to dryness.

● ***Extraction procedure for flavonoids:***
Air dried aerial parts were extracted in soxhlet apparatus with MeOH (70%). The concentrated MeOH extract was suspended in H_2O and extracted with Ethyl acetate (two times). The aqueous solution is extracted for three times by *n*-butanol to give the crude flavonoids.

Antibacterial Activity
The antibacterial activity test was carried out on the extracts of *Euphorbia guyoniana* using disk diffusion method [28] against six human pathogenic bacteria, including Gram positive (Staphylococcus aureus) and Gram-negative *Proteus vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa , Enterobacter sp. and Escherichia coli.*

The bacterial strains were first grown on Muller Hinton medium (MHI) at 37°C for 24 hour prior to seeding on to the nutrient agar. A sterile 6-mm-diameter filter disk (Whatman paper n° 3) was placed on the infusion agar seeded with bacteria, and each extract suspended in water was dropped onto each paper disk (40 µL per disk) for all prepared concentrations (8mg/mL, 4mg/mL, 2mg/mL, 1mg/mL, 0.5mg/mL, and 0.25mg/mL). The treated Petri disks were kept at 4◦C for 1 hour, and incubated at 37◦C for 24 hour. The antibacterial activity was assessed by measuring the zone diameter of growth inhibition surrounding the disks. Each experiment was carried out in triplicate.

Table 1: Phytochemical screening of *Parentucellia viscosa* L. *and Verbascum signatum L.*

extract	Chemical Groups	*Parentucellia viscosa*						*Verbascum signatum*				
		R	L	St	Fl	F&S		R	L	St	Fl	F&S
	Volatile oils	-	-	-	-	-		-	-	-	-	-
	Sterols and triterpenes	+	+	+	+	+		-	++	-	+	++
	Carotenoids	+	+	+	+	+		-	+	+	-	-
	Fatty acids	+	+	-	+	++		+	+	+	+	+
	Alkaloids	-	-	-	-	-		-	-	-	-	-
	Flavone Aglycones	-	-	-	+	+		-	-		-	-
	Emodols	-	-	-	-	-		-	-	-	-	-
A	Coumarins	-	-	-	-	-		-	-	-	-	-
	Sterols or triterpenes agl.	++	+	++	++	++		+	++	-	++	++
	Carotenoids	+	++	+	++	++		-	+	-	-	+
	Tannins	+	+	+	+	+		-	+	+	+	-
	Reducing compounds	-	-	++	-	-		+	-	+	-	-
	Alkaloids	+	++	++	++	+		-	-	-	-	-
	Anthracene glycoside	-	-	-	-	-		-	-	-	-	-
	Coumarins	-	-	-	-	-		-	-	-	-	-
	Steroides or triterpenes	-	-	-	-	-		-	-	-	-	-
	Triterpene glycosides	+	+	+	+	+		-	+	-	-	-
B	Flavone glycosides	-	-	-	-	-		-	-	-	-	-
	Anthocianosides	-	-	-	-	-		-	-	-	-	-
	Polyuronides	+	+	++	+	+		-	+	-	-	+
	Reducing compounds	+	-	+	-	-		+	+	+	+	+
	Oses polyoses	-	-	-	-			+	+	+	+	+
	Saponins	+	-	-	++	+		+	+	+	-	-
	Tannins	+	+	+	+	+		+	+	+	+	-
	Alkaloids	+	+	++	++	++		-	-	-	+	++
	Anthracene glycoside	-	-	-	-	-		-	-	-	+	-
	Coumarins	-	-	-	-	-		-	-	-	-	-
C	Steroid glycoside	-	+	-	-	+		-	-	-	-	-
	Triterpene glycosides	-	-	-	-	-		-	+	-	-	-
	Flavone glycosides	-	-	-	-	-		-	-	-	-	+
	Anthocianosides	-	-	-	+	+		-	-	-	-	-

(+) : positive (-): negative (+ -) : traces

R : roots , L : leaves , St : stems Fl : flowers F&S : fruits and seeds

A : ether extract , B : methanol extract , C : water extract

Table 2: Phytochemical screening of *Scabiosa atropurpura* L. *and Ecbalium elaterium* Rich.

extract	Chemical Groups	*Scabiosa atropurpura*						*Ecbalium elaterium*				
		R	L	St	Fl	F&S		R	L	St	Fl	F&S
	Volatil oils	-	+	-	+	-		-	-	-	-	-
	Sterols and triterpenes	-	+	-	-	+		+	+	+	+	+
	Carotenoids	-	+	-	-	-		+	+	+	+	+
	Fatty acids	+	+	+	+	+		+	+	+	+	+
	Alkaloids	-	-	-	-	-		-	-	-	-	-
	Flavone aglycones	-	-	-	-	-		-	-	-	-	-
	Emodols	-	-	-	-	-		-	-	-	-	-
A	Coumarins	-	-	-	-	-		-	-	-	-	-
	Sterols or triterpenes agl.	-	-	-	-	+		±	+	+	+	+
	Carotenoids	-	+	-	-	-		+	+	+	+	+
	Tannins	-	-	+	+	+		±	++	-	++	-
	Reducing compounds	++	-	++	-	-		-	-	+	++	++
	Alkaloids	-	-	-	-	-		+	+	+	+	+
	Anthracene glycoside	-	-	-	-	-		-	-	-	-	-
	Coumarins	-	-	-	-	-		-	-	-	-	-
	Steroid glycosides	-	-	-	-	-		-	-	-	-	-
	Triterpene glycosides	-	-	-	-	-		±	±	±	+	+
B	Flavone glycosides	-	-	-	-	-		-	+	+	+	+
	Anthocianosides	-	-	-	-	-		-	-	-	-	-

	Polyuronides	-	+	-	-	-		-	+	-	+	-
	Reducing compounds	++	-	++	+	+		++	-	+	+	+
	Oses polyoses	++	++	++	++	-		+	+	+	+	+
	Saponins	-	-	-	++	+		-	-	-	-	+
	Tannins	-	+	+	+	+		++	-	-	++	-
	Alkaloids	-	±	-	±	-		+	-	-	+	+
	Anthracene glycoside	-	-	-	-	-		-	-	-	-	-
	Coumarins	-	-	-	-	-		-	-	-	-	-
C	Steroides glycosides	-	-	+	+	+		-	-	-	-	-
	Triterpene glycosides	-	-	-	-	-		+	+	+	+	+
	Flavone glycosides	-	-	+	+	-		-	+	+	+	+
	Anthocianosides	-	-	-	-	-		-	-	-	-	-

(+) : positive (-): negative (+ -) : traces
R : roots , L : leaves , St : stems Fl : flowers F&S : fruits and seeds
A : ether extract , B : methanol extract , C : water extract

Table 3: Phytochemical screening of *Euphorbia guyoniana*

	Chemical Groups	***Euphorbia guyoniana***	
extract		**R**	**L**
	Volatil oils	-	-
	Sterols and triterpenes	++	++
	Carotenoids	-	++
	Fatty acids	+	+
	Alkaloids	-	-
	Flavone Algycones	-	-
	Emodols	-	-
A	Coumarins	-	-
	Sterols or triterpenes agl.	++	++
	Carotenoids	-	++
	tannins	+	+
	Reducing compounds	+	++
	Alkaloids	-	-
	Anthracene glycoside	-	-
	Coumarins	-	-
	Steroid glycosides	-	-
	Triterpene glycosides	+	+
B	Flavone glycosides	-	+
	Anthocianosides	+	-
	Polyuronides	-	+
	Reducing compounds	++	-
	Oses polyoses	±	++
	Saponins	±	±
	tannins	±	±
	Alkaloids	-	-
	Anthracene glycoside	-	-
	Coumarins	-	-
C	Steroid glycosides	-	-
	Triterpene glycosides	-	-
	Flavone glycosides	+	+
	Anthocianosides	+	+

(+) : positive (-): negative (+ -) : traces
R : roots , L : leaves
A : ether extract , B : methanol extract , C : water extract

RESULTS

The present work is focused essentially on the phytochemical and antimicrobial screening of five medicinal plants. All the species have been screened for several chemical groups table 1, table 2 and table 3. It is worth noting the absence of coumarins and emodols in all species and the absence of Volatile oils in all species except in *S.*

atropurpura. On the contrary the alkaloids are present in all species except *E. guyoniana*. Nevertheless, the flavonoids, sterols or triterpenes, saponins, tannins, carotenoids are present in all species.

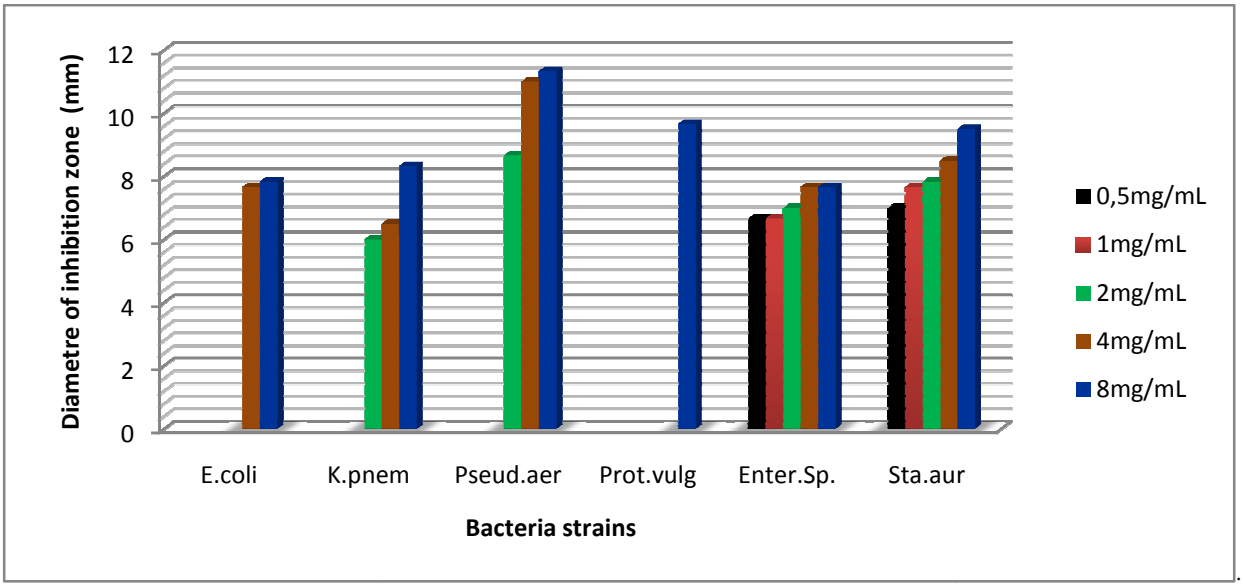

Fig. 2: inhibition effect of (CH_2Cl_2 / MeOH (1: 1) extract of *Euphorbia guyoniana* on six bacteria strains

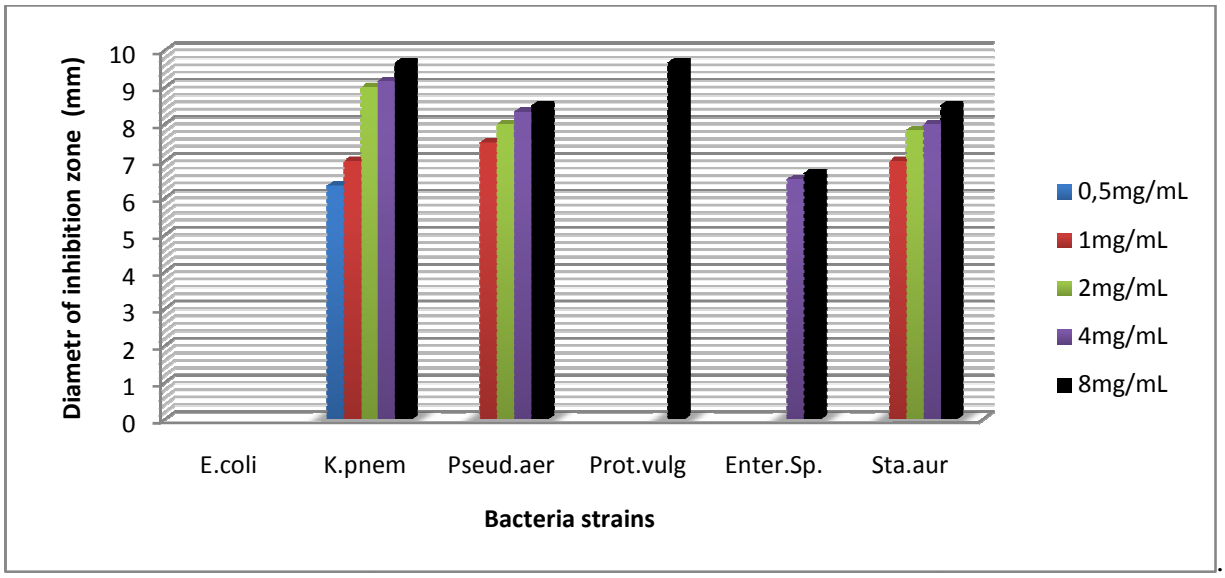

Fig. 3: effect inhibition from *n*-butanolic extract of *Euphorbia guyoniana* on six bacteria strains

Herbs have many potential clinical and therapeutic applications in the modern medical setting, as numerous studies have revealed that they contain bioactive components, and have resulted in a better understanding of their physiological, therapeutic and clinical actions. Antimicrobial agents can also be derived from herbs, and over 1000 plants exhibit antimicrobial effects [24]. In this study *E. guyoniana* extracts have been subjected to antibacterial tests.

The tested bacteria strains have shown a moderate sensitivity to the various extracts **Methylene chloride-Methanol** and n-**butanol** extract at different concentrations and have revealed that the medium diameter of inhibition zone increases with increasing extract concentrations.

The results summarized in **fig.1 and fig.2** showed that the Methylene chloride-Methanol extract and *n*-butanol extract from *E. guyoniana* prevented the growth of all the tested microorganisms with an inhibition zone medium diameter varied depending on the extract and concentrations and the type of the bacterium.

It should be mentioned that there are no background antibacterial studies on *E. guyoniana* (Cass. Hook. F. While in genus *Euphorbia* some studies have been reported such as the ethanol extracts from aerial parts of *E. hirta* which exhibited a broad spectrum of antimicrobial activity against *E. coli*, *P. vulgaris*, *P. aeruginosa*, and *S. aureus* [25].

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