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Phytochemical Screening and Evaluation of Antibacterial Activity of Alkaloids Extract of *Senecio delphinifolius* Vahl.

ZELLAGUI Amar[1,2*], TIJANI Soukayna[2], GHERRAF Noureddine and RHOUATI Salah[2]

[1]*Laboratory of Biomolecules and Plant Breeding, Life Science and Nature Department, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria*
[2]*Laboratory of Natural Products and Organic Synthesis, Department of Chemistry, Faculty of Exact Sciences, University Mentouri–Constantine, Algeria*.

ABSTRACT

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. The present work focuses essentially on the phytochemical and antibacterial screening of Senecio delphinifolious. The results revealed the presence of some chemical groups such as flavonoids, terpenes, alkaloids and saponins. The crude alkaloid extract was evaluated for their antimicrobial activity against four bacteria by the disc diffusion assay. The findings showed a broad spectrum of activity according to the following order in the sensitivity as indicated by the corresponding inhibition zone diameters: Escherichia coli > Salmonella typhimurium> Staphylococcus aureus > Pseudomonas aeruginosa .

Keywords : *Senecio delphinifolius*, phytochemical Screening, antibacterial activity

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INTRODUCTION

Because of the side effects and resistance that pathogenic micro-organisms build against the antibiotics, much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. Medicinal plants may offer a natural and new source of antibacterial agents for use [1]. *Senecio* species are used in folk medicine for the treatment of wounds and as antiemetic, anti-inflammatory, antimicrobial and vasodilator. The parts mostly used are leaves, stems, flowers,–[2-4] The pyrrolizidine alkaloids (Pas) and the furanoeremophilane sesquiterpenoids are the most important constituents of this genus and thought to be responsible for all of pharmacological activities [5]

. The genus *Senecio*, which belongs to the tribe Senecioneae, is the largest and most complex genus in the family of the Asteraceae (Compositae) and includes more than 1500 species with a worldwide distribution [6].

The chemical constituents of the genus *Senecio* include notably sesquiterpenoids, monoterpenoids [7,8], diterpenoids [9], triterpenoids [10], phenolic and flavonoid compounds [11-16], essential oils [17] and pyrrolizidine alkaloids [5]

The genus *Senecio* is represented in Algeria by eighteen species including *S. delphinifolius* Vahl (Figure 1) which grows endemically in Sicile (Italy) and in North Africa [18]

In continuation of our phytochemical and antibacterial studies of the Algerian medicinal plants [19–21], we report here the findings of our studies on the characterization of secondary metabolites and evaluation of antimicrobial activity of *S. delphinifolious*. To the best of our knowledge, there are no reports about the chemical content and biological activity of this species

MATERIALS AND METHODS

Plant Material

The aerial parts of *S. delphinifolious* were collected in April 2010 (flowering stage) in Grareme Mila, Algeria. The plant was identified by Dr. Kaabache Mohamed, Department of biology, Setif University. A voucher specimen was deposited at the Chemistry Department, University of Mentouri-Constantine under the code number ZA 117.

***Extraction and detection of chemical groups* [22]**

25 of powdered dried plant was extracted with petroleum-ether in a continuous extraction apparatus soxhlet. The ether extracts were combined, filtered and concentrated up to 40-50 mL. The remaining dry vegetable product was extracted by refluxing three times with methanol for 20-40 minutes. The vegetable product 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 has a characteristic pleasant odor, thus the plant product contains volatile oils. The vegetable product is distilled with water in a Neo-Clevenger apparatus to extract the volatile oils.

● ***Identification of sterols and triterpenes***

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

● ***Identification of carotenoids***

The ether extract is 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 become red, denoting the presence of carotenoids.

● ***Identification of fatty acids***

An alkaline aqueous solution 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 is dissolved in 2 mL of methanol at 500 C. Metallic magnesium and 5 drops of concentrated HCl were added. A red or orange color indicates the presence of flavones aglycones (Shibata's reaction).

● ***Identification of anthracenoside aglycone (emodols)***

1 mL of 25 % of NH_4OH were added to ether extract and shaken (Bortrager reaction). A red color shows 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 occurrence 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 the diluted solution of ferric chloride (3 drops) was added. The occurrence of a blackish blue or blackish green color 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 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 *anthocyanosides*.

● ***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 have already been placed. A thick precipitate was formed indicating the presence of polyuronides.

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

● ***Extraction procedure for alkaloids*** **[23]**
After drying and powdering, the crude material was extracted with MeOH in soxhlet apparatus. The solvent was evaporated to dryness. the crude residue was taken up in a 2% aqueous HCl. The aqueous acidic phase was extracted with dichloromethane. The CH_2Cl_2 extract was evaporated to give a crude alkaloid mixture.

Antibacterial Activity
The antibacterial activity test was carried out on crude extract alkaloid of *S. delphinifolius* using disk diffusion method [24] against four human pathogenic bacteria *(Staphylococcus aureus, Escherichia coli, Pseudomonas aereginosa, Salmonella sp).*The bacterial strains were first grown on Muller Hinton medium (MHI) at 37◦C for 24 h 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 h, and incubated at 37◦C for 24 h. The antibacterial activity was assessed by measuring the diameter of the growth inhibition zone surrounding the disk. Each experiment was carried out in triplicate.

RESULTS AND DISCUSSION

The present work is focused essentially on the phytochemical and antimicrobial screening of *S. delphinifolious* which has been screened for 17 chemical groups. It is worth noting the absence of flavone aglycones, anthocianosides, emodols , coumarins and saponins. Nevertheless, the flavone glycosides, sterols or triterpenes, tannins, carotenoids and alkaloids are present in all organs and have not previously been reported in the literature (Table 1).

The genus Senecio is known to contain pyrrolizidine alkaloids. sesquiterpenes with a furanoeremophilane skeleton are reported as the major components of the genus Senecio. the Macrocyclic senecionine type are secondary metabolites characteristic for most species of the genus *Senecio* (Asteraceae). These compunds are deterrent and toxic to most vertebrates and insects and provide plants with a chemical defense against herbivores[□□]. Moreover, this study involves the antibacterial activity of crude alkaloid extracted *from S. delphinifolious* aerial parts. the results are summarized in Table **2** and showed that the extract prevented the growth of all the tested microorganisms with an inhibition zone medium diameter increasing proportionally with the concentrations of the tested samples. The obtained inhibition on bacteria strains varied from 7 to 24 mm with a highest inhibition zone recorded with *E. coli* at 8mg/ml, and a moderate inhibition effect with the same concentration on *Staphylococcus aureus, and Salmonella sp*.

It should be mentioned that there are no background antibacterial study on *S. delphinifolious,* while in genus *senecio* some studies have been reported [26-32].

Table 1: Phytochemical screening of *Senecio delphinifolious*

EXT.	Chemical Groups	*Senecio delphinifolius*					
		R	**L**	**St**	**Fl**	**F&S**	
	Volatil oils	-	+	+	+	+	
	Sterols and triterpenes	+	+	+	+	+	
	Carotenoids	-	+	-	±	-	
	Fatty acids	±	±	±	±	±	
A	Alkaloids	-	-	-	-	-	
	Flavone Algycones	-	-	-	-	-	
	Emodols	-	-	-	-	-	
	Coumarins	-	-	-	-	-	
	Sterols or triterpenes agl.	+	+	+	±	+	
	Carotenoids	-	+	-	+	-	
	tannins	±	+	+	+	+	
	Reducing compounds	-	+	+	+	+	
B	Alkaloids	-	-	-	-	-	
	Anthracene glycoside	-	-	-	-	-	
	Coumarins	-	-	-	-	-	
	Steroid glycosides	-	-	-	-	-	
	Triterpene glycosides	+	+	-	-	+	
	Flavone glycosides	-	-	++	-	-	
	Anthocianosides	-	-	-	-	-	
	Polyuronides	-	+	-	+	++	
	Reducing compounds	-	++	-	++	++	
	Oses polyoses	++	++	++	-	-	
	Saponins	-	-	-	-	-	
	tannins	+	+	+	+	+	
C	Alkaloids	+	++	+	+	+	
	Anthracene glycoside	-	-	-	+	-	
	Coumarins	-	-	-	-	-	
	Steroid glycosides	-	-	-	+	+	
	Triterpene glycosides	-	+	-	-	-	
	Flavone glycosides	+	+	+	+	+	
	Anthocianosides	-	-	-	-	-	

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

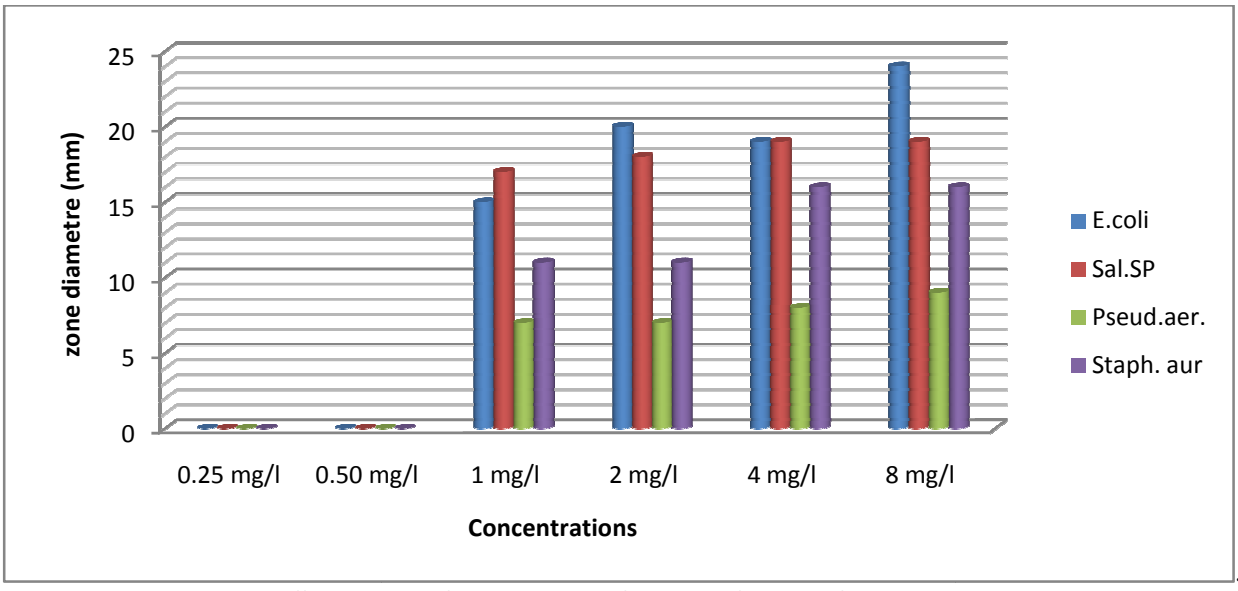

Fig 1: effect inhibition of alkaloid extract of *S. delphinifolious* on four bacteria strains

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