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Research Article

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Evaluation of antioxidant and antimicrobial activities of tannins extracted from *Sedum pubescens Vahl.*

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ABSTRACT

The aim of this study was to assess the antioxidant and antimicrobial activities of tannins extracted from North African endemic species, which constitute an enigma toward their capacities. Antioxidant properties were evaluated through the ability of the extract to scavenge DPPH radicals and the reducing power essay, however, the antimicrobial activity was tested with three bacterial strain and three fungi including yeast (Escherichia coli ATCC 25922, Salmonella typhimurium ATCC 13311, Staphylococcus aureus ATCC25923, Aspergillus niger 2CA936, Aspergillus flavius NRRL3357 and Candida albicans ATCC1024). The result shows a very interesting antimicrobial and antioxidant activities and this plant seems to be a good source of natural compounds for different use.

Key words: *Sedum pubescens*, antioxidant, antimicrobial, tannins.

INTRODUCTION

Sedum, a large genus of family *Crassulaceae*, encompasses a great number of species used in folk medicine against different disease symptoms [1]. The genus *Sedum* is included within subfamily *Sedoideae*. *Sedum* is the largest genus in the family, consisting of approximately 600 species [2]. *Sedum* is generally considered to contain the most primitive and ancient *Crassulaceae*. This view was first put forward by Schônland (1891) who regarded *Sedum* as the core genus of the *Crassulaceae* and derived all other genera from this central one [3].

Sedum pubescens Vahl is characterised by yellow flowers, arranged on top of a small stalk, tortuous and, generally at least as long as the flower. Plant hispid, rower, 10-20 cm. growth in rockeries, clear brush [4].

Species of the genus *Sedum* were used for their anti-inflammatory, antinociceptive, antioxidant, hepatoprotective and antitumoral effects [5-9]. Secondary metabolites of plants belonging to this genus are phenolic acids [10-11], flavonoids [12-14], coumarins [12] [15] [16], terpenes [17-18] and alkaloids [19-20].

Tannins are secondary plant metabolites subdivided into condensed and hydrolysable compounds. Condensed tannins are also known as proanthocyanidins (PAs), the oligomeric and polymeric flavan-3-ols. The size of PA molecules can be described by their degree of polymerization (DP). Proanthocyanidins are of great interest in nutrition and medicine because of their potent antioxidant capacity and possible protective effects on human health [21]. Hydrolysable tannins contain a central core of a polyhydric moiety, such as glucose and hydroxyl groups, which are esterified, either partially or wholly, by gallic acid (gallotannins) or ellagic acid (ellagotannins) [22]

This study aimed for the first time the identification of the antioxidant and the antimicrobial activities of the total tannins extracted from aerial part of *Sedum pubescens Vahl.*

EXPERIMENTAL SECTION

Plant material

The areal part of *Sedum pubescens Vahl.,* were collected from the mountain of Megriss Setif -Algeria in June 2015 and determined by Nouioua Wafa in Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Natural Life and Sciences - Ferhat Abbas University -Setif –Algeria..

Tannins extraction

Powdered materials (10 g) was macerated in 100 mL of acetone for 24 hours; The supernatant was then separated from the residue by filtration using Whatman no.1 filter paper, the fraction was concentrated and dried to a constant weight in a vacuum oven at 45°C and the residues obtained was stored in a refrigerator [23].

Determination of total tannin content :

Tannin content was evaluated using the haemoglobin precipitation assay. An aliquot of 0.5 mL of each extracts is mixed with 0,5 mL of haemolysis bovine blood to reach a final concentration of 1mg/mL, then the mixture was centrifuged at 480g for 20 minutes and the absorbance was measured at 578 nm [24]. Tannins was expressed as mg equivalent tannic acid per gram of extract (mg ETA /g E)

Evaluation of DPPH scavenging activity

The donation capacity of extracts were measured by bleaching of the purple-colored solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al*., (1998) [21]. One milliliter of the extracts at different concentrations was added to 0.5 mL of a DPPH-methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The antiradical activity was expressed as IC_{50} (micrograms per milliliter), the antiradical dose required to cause a 50 % inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = [(A_0 - A_1)/ A_0] \times 100$$

Where A_0 is the absorbance of the control at 30 minutes and A_1 is the absorbance of the sample at 30 minutes. BHT was used as a positive control. Samples were analyzed in triplicate [22].

Evaluation of reducing power

The reducing power was determined according to the method of Oyaizu [27]. The extract at different concentration (2.5 mL) was mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 10 mg/mL potassium ferricyanide, and the mixture was incubated at 50 ° C for 20 minutes. After, 2.5 mL of trichloroacetic acid (100 mg/mL) were added, the mixture was centrifuged at 200g for 10 minutes. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1mL of ferric chloride (1 mg/mL). Then the absorbance was measured at 700 nm against a blank.

Antibacterial activity

Agar disc diffusion method was employed for the determination of antibacterial activities of tannins of *Sedum pubescens Vahl* [28] [29]. Briefly, a suspension of the tested microorganism (0.1 mL 10^8 cells per mL) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 10 µL of 100 mg/mL of the tannins and placed on the inoculated plates.

These plates were incubated at 37 °C for 24 hours. Gentamicin (10 µg/disc) was used as a standard and dimethylsulfoxide DMSO as a control.

The antibacterial activity was determined by measuring of inhibition zone diameters (mm) and was evaluated according the parameters suggested by Alves *et al*. (2000) [30]:

- <9 mm, inactive ;
- 9–12 mm, less active ;
- 13–18 mm, active;
- >18 mm, very active

Antifungal Activity:

The antifungal activity was tested by disc diffusion method [31]. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper discs (6 mm in diameter) impregnated

with 100 mg/ mL concentrations of the extract were placed on test organism-seeded plates. The activity was determined after 72 hours of incubation at 28 °C. The diameters of the inhibition zones were measured in mm.

Statistical analysis

Results were expressed as the mean ± standard deviation. Data was statistically analysed using t test of *Student* as primary test followed by Fisher test with the criterion of P values < 0.05 to determine whether there were any significant differences between tannins and standards, using Graphpad prism 5 Demo Software.

RESULTS AND DISCUSSION

Total tannin content

The yield of total tannins was 15 % with a quantity of total tannins estimated of 85,85 ±2,67 mg ETA /g E

Antioxidant activity

The results indicated that the total tannin was able to act as free radical inhibitor (figure1), and the IC_{50} of extract which attaint 5,68±0,516 µg/mL indicate a very strong activity, better than BHT 8, 76±0,69 µg/mL.

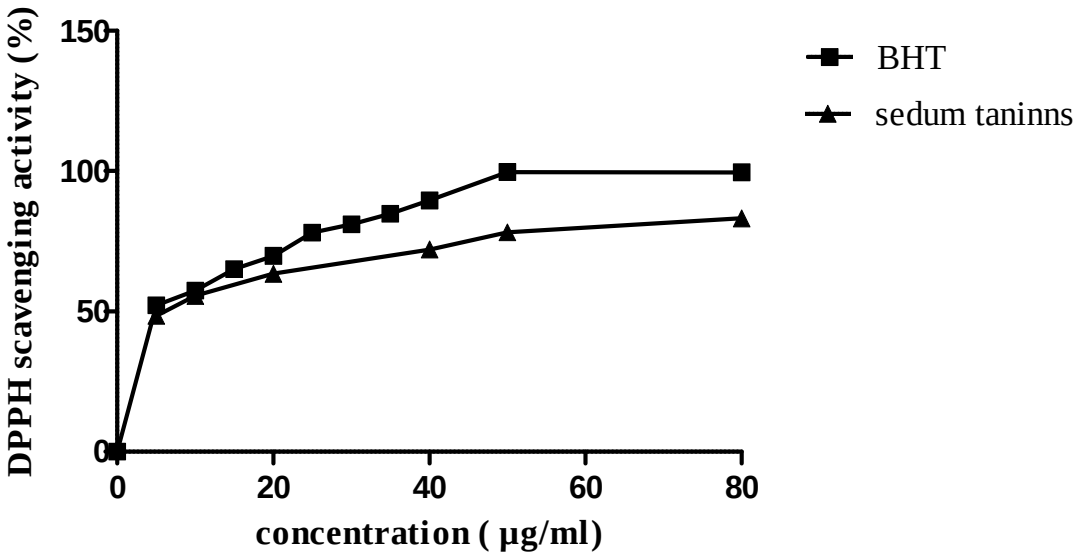

Figure 1: scavenging effect of tannins extracted from *sedum pubescens* Vahl.

Statistic treatment prove that there is no significant difference between BHT and tannins. The purple-colured DPPH is a stable free radical, which is reduced to α, α-diphenyl-β- picrylhydrazine (yellow coloured) by reacting with an antioxidant. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation [32].

Antioxidant interrupt free radical chain oxidation by donating hydrogen from hydroxyl groups to form a stable end product, which does not initiate or propagate further oxidation of lipid [33]. However, research has shown tannins to be natural antioxidant [34]. Hagerman *et al*. [35] provided insights into the mechanism of procyanidin as the potential antioxidants, which showed that hydroxyl groups were important factors for free radical scavenging by tannins. Thus explain the high scavenging effect of tannins extracted from *sedum pubescens* Vahl.

The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity [36]. Antioxidant potential of tannins from *sedum pubescens* Vahl was estimated from their ability to reduce the Fe^{3+}/ferricyanide complex to the ferrous form. (Figure 2):

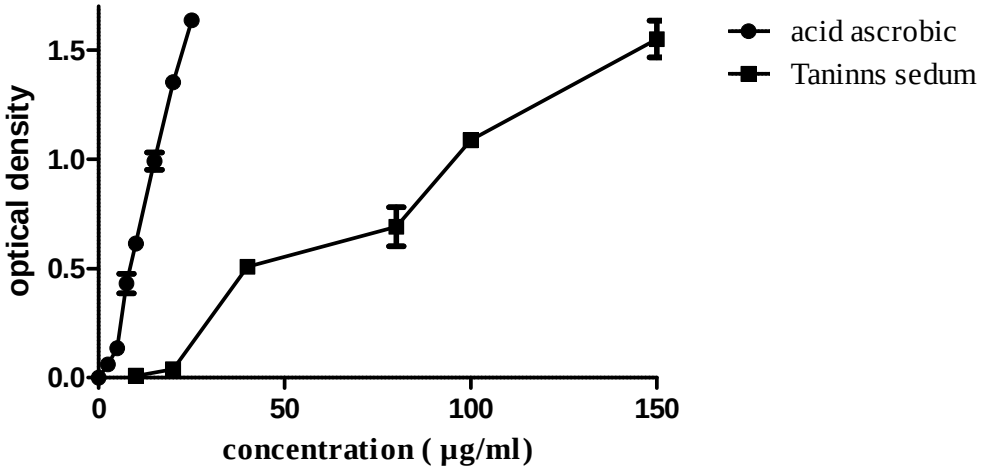

Figure 2: Reducing power of tannins extracted from the branch of sedum pubescens Vahl.

A higher absorbance corresponds to a higher ferric reducing power. Tannins showed increased ferric reducing power with the increasing concentration with EC_{50} correspond to 52±1.25 µg/mL against 8.46±0.09 µg/mL of ascorbic acid and the statistic comparison show a very significant difference. The extract expressed electron donating activity, but their power was inferior to ascorbic acid, which is known to be a strong reducing agent.

Because iron is a primary cause of ROS generation in vivo and because it plays such a pivotal role in contributing to oxidative stress, DNA damage, and cell death, iron has been the target of many antioxidant therapies [37]. When chemical structure of tannins is considered, it could be presumed that condensed tannins, which are catechin polymers, bind metal ions mainly to catechol groups, whereas hydrosylable tannins (derivatives of gallic acid) to galloyl groups [38]. When deprotonated, as is required for metal binding, catechol and gallol functionalities are referred to as catecholate and gallate groups, respectively. Metal ions that prefer octahedral geometry, such as Fe^{2+} and Fe^{3+}, can coordinate up to three catecholate or gallate groups [37]. However, since polyphenol compounds are so structurally varied and the complexes formed are pH dependent, they often exhibit variable coordination modes [39]. The weakness in accordance with DPPH scavenging activity could be explained by the diversity of their structure and /or pH dependence.

Antimicrobial activity

Since multidrug resistance of microorganisms is a major medical concern, screening of natural products in a search for new antimicrobial agents that would be active against these microorganisms is the need of the hour [40]. The antimicrobial activity of the extract was less effective and present low capacity against bacteria and fungi and this finding represent the first report concerning the antimicrobial action of tannin of *sedum pubescens Vahl*.
The results were demonstrated in tables below:

Table 3: Inhibition zones in millimetre of the antibacterial activity of tannins, standard and control

	Escherichia coli ATCC 25922	*Salmonella typhimurium ATCC 13311*	*Staphylococcus aureus ATCC25923*
standard	*18,50±0,41*	*19,17 ± 0,24*	*27,67±0,47*
Tannins	*7±0,47*	*No inhibition*	*9.25±0,05*
Control	*No inhibition*	*No inhibition*	*No inhibition*

Table 4: Inhibition zones in millimetre of the antifungal activity of tannins, standard and control

	Aspergillus flavius NRRL3357	*Aspergillus niger 2CA936*	*Candida albicans ATCC1024*
Nystatin	15,53±0,79	9,40±0,22	9,29±0,19
Clotrimazon	23,86±1,15	15,85±0,32	44,28±0,49
Amphotericin	16,20±1,19	17,55±0,14	15,58±0,12
Tannins	*13±0,85*	*10±0,48*	*9±0,58*
Control	*No inhibition*	*No inhibition*	*No inhibition*

CONCLUSION

Sedum a large genus of family *Crassulaceae*, which is widely used in traditional medicine for treating ulcers, infected wounds and hypotension, but *sedum pubescens Vahl* an endemic species of Algeria, constitute an enigma toward their phytochemical composition and phytotherapeutic use. Tannins extracted from this plant, are so important molecules, constitute a harmonium natural group of therapeutic molecule were very strong in DPPH scavenging and have a very interesting iron chelating capacities, but a low antimicrobial activity.

Further study are need to purify and investigate the molecular composition this natural product with preservation of natural diversity of molecules which constitute the alternative of the synthetic medicines.

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