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Antioxidant Activity Phenolics Flavonoids and Proanthocyanidins Content of *Senecio anteuphorbium*

Fatima Azzahra Lahlou[1*], Fouzia Hmimid[1], Mohammed Loutfi[1] and Noureddine Bourhim[1]

[1]Department of Biology, Laboratory of Biochemistry and Molecular Biology, Faculty of Sciences, University Hassan II-Casablanca, km 8 route d'El Jadida BP. 5366 Casablanca, Morocco.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The present study aimed to evaluate the antioxidant activity of methanolic and aqueous extracts of *Senecio anteuphorbium* and to determine total phenolics, flavonoids and proanthocyanidins content of this plant from Southern Anti-Atlas of Morocco. Moreover the effect of solvent and heat was also investigated.
Place and Duration of Study: Department of biology, laboratory of Biochemistry and Molecular Biology Faculty of Sciences, University Hassan II, between August 2013 and November 2013.
Methodology: *Senecio anteuphorbium* were subjected to a decoction, maceration (with both water and methanol) and soxhlet extraction. Ferric reducing/antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging tests were used to evaluate antioxidant activity. Folin- Ciocalteau reagent serves to determine total phenolic content, aluminium trichloride method for flavonoids and a mixture of vanillin and hydrochloric acid for proanthocyanidins; employing colorimetric method.
Results: Results obtained revealed that *Senecio anteuphorbium* possesses a high antioxidant capacity that has a positive correlation with quantified phytochemicals (R^2=0.96/0.9/0.81). Polyphenols ranged from 21.53±1.03mg GAE/ gE or 20.38±0.76 mg

**Corresponding author: Email: lahloufz@hotmail.fr;*

TAE/gE to 2.46±0.11 GAE/ gE or 2.33±0.09mg TAE/gE, flavonoids registered the highest amount: 39.47±1.01mg RE/gE or 26.59±0.24mg QE/gE, followed by proanthocyanidins: 36.66 ±1.26 mgCE/gE. In addition when methanol is combined with temperature (80ºC), it seems to be the appropriate method to extract antioxidant compounds.
Conclusion: The present results reveal that *Senecio anteuphorbium* is a potent source of natural antioxidants, containing a mixture of phenolic, flavonoid and proanthocyanidin compounds that could have a great importance as therapeutic agents in preventing oxidative damages.

Keywords: Senecio anteuphorbium; antioxidant; phenolics; flavonoids; proanthocyanidis; DPPH, FRAP.

1. INTRODUCTION

The antioxidant hypothesis says that "as antioxidants can prevent oxidative damages, increased intakes from the diet will also reduce the risks of chronic diseases" [1]. This explains the huge volume of research work and the efforts of many researchers to link diets rich in natural antioxidants with degenerative disease.

Apart from their role of health benefactors, antioxidants are added in foods to prevent or delay oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature [2].

Most of phytochemicals from plant extracts have been identified to exhibit antioxidant activities [3]. Thus many of the biological functions, such as antimutagenicity, anticarcinogenicity, and antiaging, among others, originate from this property [4,5]. Some of the better-known phytochemicals include: Isoflavones, Anthocyanins, Carotenoids such as lycopene, beta-carotenes [6,7]

Senecio anteuphorbium, native to Morocco, forms a shrubby plant with many grayish-green upright branches with faint vertical striping. Tapered, fleshy grayish-green leaves are present along the upper half of the stems with whitish-yellow flowers typical of Compositae. The genus *Senecio* (*Asteraceae*) is widely distributed throughout the World, and is known to be a source of pyrrolizidine alkaloids, eremophilanolides, and furanoeremophilanes [8,9]. Many *Senecio.* species are used in folk medicine as emmenagogues, anti-inflammatory agents and vasodilators [10]. Some other studies reported the antioxidant activity of some *Senecio* species for instance: *Senecio inaequidens* [11], *Senecio gibbosus* [12] and *Senecio stabianus* [13]. Therefore, given the interesting biological properties reported for *Senecio species;* the aim of this study is to investigate for the first time the antioxidant properties by DPPH and FRAP tests of *Senecio anteuphorbium* and to explore relationship between this activity and phenolic, flavonoid and proanthocyanidin content. In addition the effect of two solvents plus heat was evaluated.

2. MATERIALS AND METHODS

2.1 Plant Material

The whole plant of *Senecio anteuphorbium* was collected from Sidi Ifni, Southern Anti-Atlas of Morocco and was authenticated by Prof. Laila RHAZI of the Department of Biology, Faculty of Sciences, University Hassan II Casablanca.

To avoid any contamination or dust, the plant's aerial parts (leaves and stems) were cleaned and spread to dry at room temperature in a clean room.

2.2 Preparation of Plant Extracts

2.1.1 Soxhlet extraction

Powdered sample of *Senecio anteuphorbium* was extracted with methanol using soxhlet system. Extraction was carried out for 16 hours at 80ºC. The extract was filtered, then concentrated by Rotavapor-R20 (Heidolph Bioblock Scientific) at 40ºC.

2.1.2 Decoction

Powdered sample was mixed with distilled water in a round-bottom flask, linked to a column connected to a refrigerant. Then it was placed at 60ºC for 1 hour. The decoction extract was filtered using gauze and Whatman No. 1 filter paper and then concentrated by Rotavapor-R20 (Heidolph Bioblock Scientific) at 40ºC.

2.1.3 Maceration

Plant material was allowed to stand for 24 hours at room temperature, under shaking in methanol and water separately. After filtration and concentration as described above, methanol and aqueous filtrates were obtained.

2.3 Total Phenolic Content

Total phenolics of various samples were determined by the Folin-Ciocalteau method [14]. 0.1ml of sample was combined with 2.8ml of 10% Na_2CO_3 and 0.1 ml of 2N Folin-ciocalteu reagent. After 40min absorbance at 725nm was measured by UV-visible spectrophotometer (Thermo electron corporation, Biomate 3). Total phenolics were determined as milligrams of gallic acid and tannic acid equivalents per gram of sample (GAE mg/gE or TAE/gE) using a standard calibration curve between (0 to 100 µg/ml).

2.4 Total Flavonoid Content

Total flavonoid content was determined using the method aluminium trichloride [15]. 1 ml of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution. After 10 minutes, absorption readings at 415 nm against a blank sample consisting of a 1ml extract solution with 1 ml methanol or distilled water without $AlCl_3$ were assessed (using UV-visible spectrophotometer: Thermo electron corporation, Biomate 3). Total flavonoid content was determined using a standard curve with quercetin or rutine (0 - 80µg/ml), then expressed as mg of quercetin or rutin equivalents (QE or RE) / g of extract.

2.5 Proanthocyanidin Content

Proanthocyanidin content was estimated according to the procedure reported by [16]. A volume of 1ml solution was mixed with 3 ml of 4% vanillin/methanol solution and 1.5ml hydrochloric acid and the mixture was allowed to stand for 15min at room temperature. The absorbance at 500 nm was measured (using UV-visible spectrophotometer: Thermo electron

corporation, Biomate 3) and proanthocyanidins content was expressed as mg catechin equivalents (mg CE/1g dry mass) using a catechin (0-80 µg/ml) standard curve.

2.6 Reducing Power Activity Assay

The ability of extracts to reduce Fe^{3+} was assayed by the method of [17]. Briefly, 1ml of each extract were mixed with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% $K_3Fe(CN)_6$. After incubation at 50ºC for 25 min, 2.5ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 g for 10 min. Finally, 2.5ml of the upper layer were mixed with 2.5ml of distilled water and 0.5 ml of 0.1% aqueous $FeCl_3$. The absorbance was measured at 700nm (using UV-visible spectrophotometer: Thermo electron corporation, Biomate 3). The mean of absorbance values were plotted against concentration and a linear regression analysis was carried out. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid and trolox were used as positive controls.

2.7 DPPH Radical Scavenging Activity

The free radical scavenging activities of the samples on the DPPH radical were measured using the method described by [18]. 0.1ml of various concentrations of each extracts at different concentrations was added to 3.9ml of DPPH solution (25mg/l in methanolic solution). After the mixture was shaken and left at room temperature for 30min, the absorbance at 517nm was measured (using UV-visible spectrophotometer: Thermo electron corporation, Biomate 3). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The IC50 value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results and used for comparison. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A1 - A2)/A1] \times 100$$

Where A1 = the absorbance of the control reaction, A2 = the absorbance in the presence of the sample. Ascorbic acid and Trolox were used as standards.

3. RESULTS AND DISCUSSION

Total phenolic content ranged between 21.53±1.03mg GAE/ gE or 20.38±0.76mg TAE/gE and 2.46±0.11 GAE/ gE or 2.33±0.09mg TAE/gE (Table 1). The highest amount was obtained with methanol when combined with temperature (80ºC) followed by decoction; while the lowest value was obtained with aqueous maceration. Plant's polyphenols are very important secondary defense metabolites and protect the plant from infections and give oxidative stabilities in case of injuries [19,20]. Gallic (a trihydroxybenzoic acid) and tannic acids (a mixture of polyphenols) have different chemical structures, thus most plants are complex mixtures with a large group of compounds (other than polyphenols) such as beer; this may explain the differences in total polyphenol content when using GAE and TAE as reported in some studies [21]. Values obtained with both standards were approximately similar which suggests that gallic acid and tannic acid are both appropriate for this method. It also suggests that *Senecio anteuphorbium* contains rihydroxybenzoic acid as much as polygalloyl glucoses.

Table 1. Total phenolic content of *Senecio anteuphorbium extracts*

Extracts/test	Total phenolic content	
	mg GAE/ gE	mg TAE/gE
MS	21.53±1.03	20.38±0.76
MM	6.92±0.26	6.55±0.45
D	14.56±0.95	13.78±0.71
AM	2.46±0.11	2.33±0.09

GAE: gallic acid equivalents, TAE: tannic acid equivalents, gE: g of extract, MS: methanolic Hot Continuous Extraction (Soxhlet). MM: methanol maceration, D: decoction, AM: aqueous maceration. All results are presented as mean±standard mean error of three assays

Methanolic extract obtained with soxhlet extraction expressed the highest total flavonoid content: 26.59±0.24mg QE/gE or 39.47±1.01mg RE/gE. However aqueous maceration showed the lowest amount: 6.52±0.09 mg QE/gE or 9.68±0.22mg RE/gE (Table 2). Flavonoids have been found to exert a variety of biological effects, they have been shown to accelerate wound healing and protect cultured skin cells and tissues from oxidative damage [22].

Table 2. Total flavonoid content of *Senecio anteuphorbium extracts*

Extracts/test	Total flavonoid content	
	mg QE/gE	mg RE/gE
MS	26.59±0.24	39.47±1.01
MM	13.51±0.37	20.05 ± 0.5
D	13.51±0.55	29.05 ±0.78
AM	6.52±0.09	9.68±0.22

QE: quercetin equivalents, RE: rutine equivalents, g E: g of extract, MS: methanolic hot Continuous Extraction (Soxhlet). MM: methanol maceration, D: decoction, AM: aqueous maceration. All results are presented as mean±standard mean error of three assays

Proanthocyanidins or condensed tannins are polymeric flavonoids. They have received considerable attention owing to their various biological activities, in particular their oxygen free radical scavenger ability [23]. Results in Table 3, show that the maximum amount of proanthocyanidins is obtained with methanolic hot continuous extraction (36.66±1.26 mgCE/gE).

Methanolic extract combined with temperature (80ºC) exhibited the highest radical scavenging activity with the lowest IC50 (3.38), compared to standards (Table 4); which means that it can scavenge more free radicals than trolox and vitamin C. The scavenging activity of methanolic and aqueous maceration was not effective than standars.

Table 3. Total proanthocyanidin of *Senecio anteuphorbium* extratcs

Extracts	MS	MM	D	AM
Proanthocyanidin (mgCE/gE)	36.66±1.26	13.71±0.05	15.42±0.47	5.8±0.19

CE: cathechins equivalents, gE: g of extract, MS: methanolic Hot Continuous Extraction (Soxhlet). MM: methanol maceration, D: decoction, AM: aqueous maceration. All results are presented as mean±standard mean error of three assays

Table 4. Antioxidant activity of *Senecio anteuphorbium* extratcs (DPPH reagent)

Extracts and standards	MS	MM	D	AM	Trolox	Vitamin C
IC50 (mg/ml)	3,38	30,7	5,6	39,98	5	3.6
AA	0,287	0,032	0,178	0,025	0,2	0,277

MS: methanolic Hot Continuous Extraction (Soxhlet). MM: methanol maceration, D: decoction, AM: aqueous maceration, AA: antioxidant activity. All results are presented as mean±standard mean error of three assays

The highest capacity in reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) was observed in methanolic extract (soxhlet) followed by decoction. However the weakest abilities to reduce ferric ion were exhibited by aqueous and methanolic maceration as it's shown in Fig. 1.

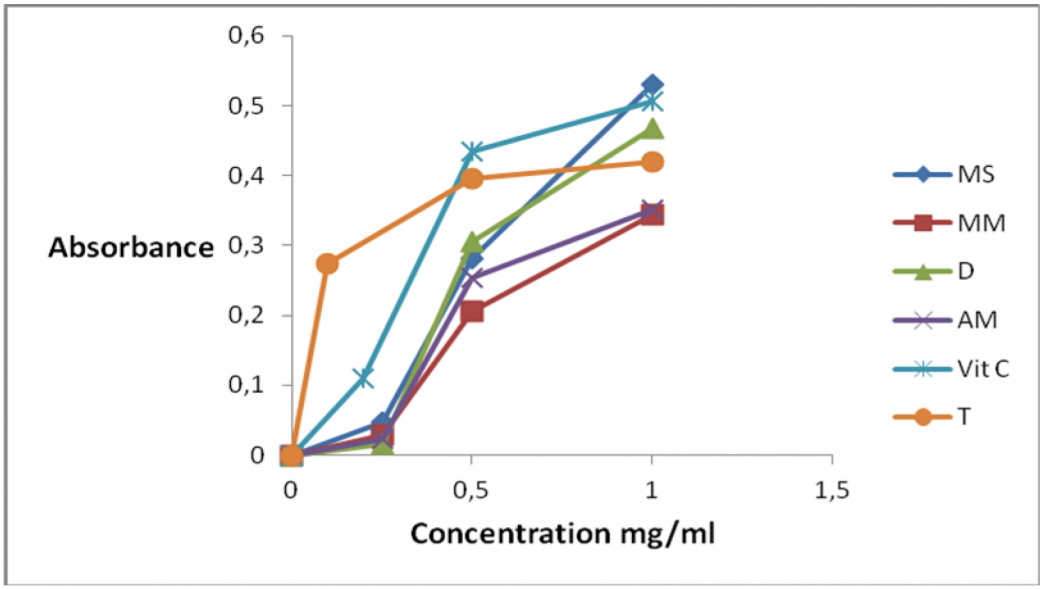

Fig. 1. Reducing power of *Senecio anteuphorbium extratcs*

MS: methanolic Hot Continuous Extraction (Soxhlet). MM: methanol maceration, D: decoction, AM: aqueous maceration, T: trolox, Vit C: Vitamin C

The correlation between phenolic, flavonoid, proanthocyanidin compounds and antioxidant activity showed a positive correlation for the two first chemicals showing respectively a R^2= 0.96/0.90/0.81 (Fig. 2). This indicates the close relationship between total phenolics compounds and antioxidant activity as reported by Prasad et al [24] and Lei et al [25]. The conception of antioxidant action of phenolic compounds is not novel. Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups, able to bind particularly iron and copper [26].

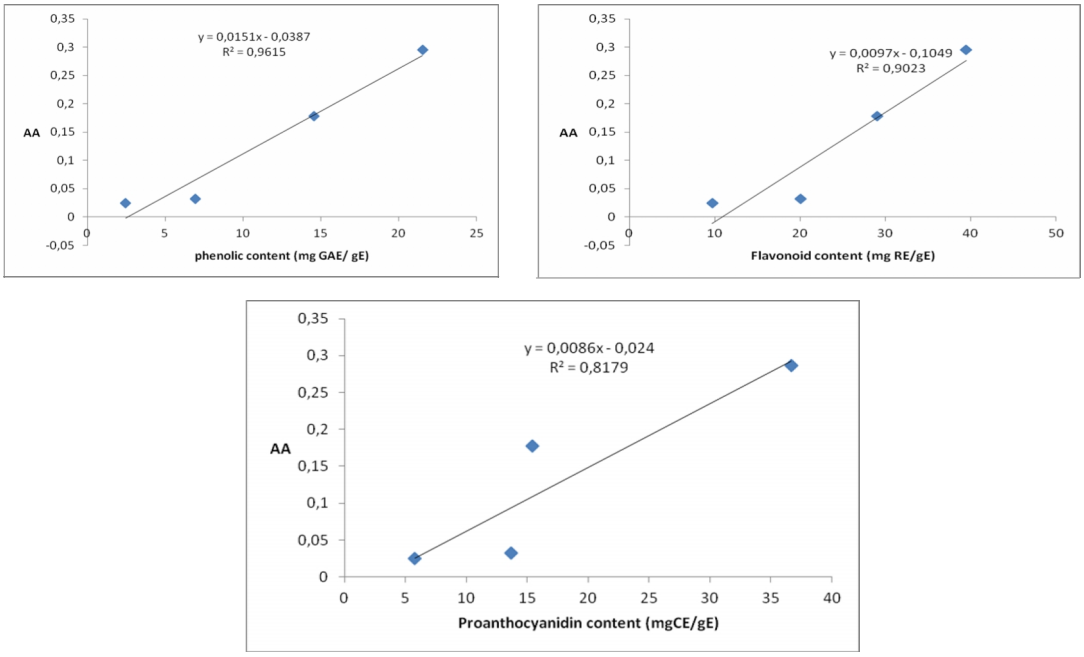

Fig. 2. Correlation between phenolic, flavonoid, proanthocyanidin and AA (antioxidant activity)

4. CONCLUSION

In conclusion, these results show that antioxidant phytochemicals from aerial parts of *Senecio anteuphorbium* were more extractible by methanol combined with temperature (80ºC) which might be due in part to the high polarity and efficacy of methanol. In addition, another important parameter was the increase of temperature and time that was demonstrated when comparing the amounts obtained with decoction (60ºC/1 h) and Soxhlet (80ºC/16 h). The present study also suggested that *Senecio anteuphorbium* could be a potential source of natural antioxidants and thus could be useful as therapeutic agents as it showed a high reducing and radical scavenging activity. Further works should include the separation and identification of active components on one hand, and *in vivo* studies of their effect in the other hand.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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