

Original Research Article

Antioxidant activity phenolics, flavonoids and proanthocyanidins content of *Senecio anteuphorbium*

ABSTRACT

Aims: The present study aimed to evaluate the antioxidant activity 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 ~~temperature~~ 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: ~~we used~~ *Senecio anteuphorbium* as a plant material ~~which~~ was 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- Ciocalteu ~~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 ± 1.03 mg GAE/ gE or 20.38 ± 0.76 mg ATE/gE to 2.46 ± 0.11 GAE/ gE or 2.33 ± 0.09 mg ATE/gE, flavonoids registered the highest amount: 39.47 ± 1.01 mg RE/gE or 26.59 ± 0.24 mg QE/gE, followed by proanthocyanidins: 36.66 ± 1.26 mgCE/gE. In addition ~~when methanol is combined with temperature, 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, proanthocyanidins, 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.

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

24
25 Most of phytochemicals from plant extracts have been identified to exhibit antioxidant
26 activities [3]. Thus many of the biological functions, such as antimutagenicity,
27 anticarcinogenicity, and antiaging, among others, originate from this property [4, 5].

28
29 *Senecio anteuphorbium*, native to Morocco, ^{is} forms a shrubby plant with many grayish-green
30 upright branches with faint vertical striping. Tapered, fleshy grayish-green leaves are present
31 along the upper half of the stems with whitish-yellow flowers typical of Compositae. The
32 genus *Senecio* (Asteraceae) is widely distributed throughout the World, and is known to be a
33 source of pyrrolizidine alkaloids, eremophilanolides, and furanoeremophilanes [6, 7]. Many
34 *Senecio* species are used in folk medicine as emmenagogues,
35 anti-inflammatory agents and vasodilators [8]. Some other studies reported the antioxidant
36 activity of some *Senecio* species for instance; *Senecio inaequidens* [9], *Senecio gibbosus*
37 [10] and *Senecio stebianus* [11]. Therefore, given the interesting biological properties
38 reported for *Senecio* species, ~~the aim of this study is to~~ investigate for the first time the
39 antioxidant properties by DPPH and FRAP tests, of *Senecio anteuphorbium* and to explore
40 the relationship between this activity and phenolic, flavonoid and proanthocyanidin content. In
41 addition the effect of two solvents plus temperature was evaluated.

42 2. MATERIALS AND METHOD

43 2.1 Plant material

44
45 The whole plant of *Senecio anteuphorbium* was collected from Sidi Ifni, Southern
46 Anti-Atlas of Morocco and was authenticated by Prof. Leila EL GHAZI of the Department of
47 Biology, Faculty of Sciences, University of Hassan II, Casablanca.

48
49 To avoid any contamination or dust, the plant's aerial parts were cleaned and spread to dry
50 at room temperature in a clean room.

51 2.2 Preparation of plant extracts

52 2.2.1 Soxhlet extraction

53
54 Powdered sample of *Senecio anteuphorbium* was extracted with methanol using soxhlet
55 system. Extraction was carried out for 16 h at 80°C. The extract was filtered, then
56 concentrated by Rotavapor-R20 (Heidolph Bioblock Scientific) at 40°C.

57 2.2.2 Decoction

58
59 Powdered sample was mixed with distilled water in a round-bottom flask, linked to a column
60 connected to a refrigerant. Then it was placed at 60°C for 1 hour. The decoction extract was
61 filtered using gauze and Whatman No. 1 filter paper and then concentrated by
62 Rotavapor-R20 (Heidolph Bioblock Scientific) at 40°C.

63 2.2.3 Maceration

64
65 Plant material was allowed to stand for 24 hours at room temperature, ^{while} ~~under shaking~~ ^{en} in
66 methanol and water separately. After filtration and concentration as described above,
67 methanol and aqueous filtrates were obtained.

2.3 Total phenolic content

Total phenolics of various samples were determined by the Folin-Ciocalteu method [12]. 0.1 ml of sample was combined with 2.8 ml of 10% Na_2CO_3 and 0.1 ml of 2N Folin-ciocalteu reagent. After 40 min, absorbance at 725 nm 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 $\mu\text{g/ml}$).

2.4 Total flavonoid content

Total flavonoid content was determined using the ~~method~~ aluminium trichloride [13]. 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 1 ml extract solution with 1 ml methanol or distilled water without AlCl_3 were assessed. The total flavonoid content was determined using a standard curve with quercetin or rutin (0 - 80 $\mu\text{g/ml}$), 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~~ [14]. A volume of 1 ml solution was mixed with 3 ml of 4% vanillin/methanol solution and 1.5 ml hydrochloric acid and the mixture was allowed to stand for 15 min at room temperature. The absorbance at 500 nm was measured and the Proanthocyanidins was expressed as mg catechin equivalents (mg CE/1g dry mass) using a catechin (0-80 $\mu\text{g/ml}$) standard curve.

2.6 Reducing power (FRAP)

The ability of the extracts to reduce Fe^{3+} was assayed by the method of [15]. Briefly, 1 ml of each extract were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% $\text{K}_3\text{Fe}(\text{CN})_6$. After incubation at 50°C for 25 mn, 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 g for 10 min. Finally, 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% aqueous FeCl_3 . The absorbance was measured at 700 nm. 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 control.

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 [16]. A 0.1 ml of various concentrations of each extracts at different concentrations was added to 3.9 ml of DPPH solution (25 mg/l in methanolic solution). After the mixture was shaken and left at room temperature for 30 min, the absorbance at 517 nm was measured. 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±1.03 mg GAE/ gE or 20.38±0.76 mg ATE/gE and 2.46±0.11 GAE/ gE or 2.33±0.09 mg ATE/gE (Table 1). The highest amount was obtained with methanol when combined with temperature 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 [17, 18].

Table 1. Total phenolic content of Senecio anteuphorbium

Extracts/test	Total phenolic content	
	mg GAE/ gE	mg ATE/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

EAG: gallic acid equivalents, ATE: 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±0.24 mg QE/gE or 39.47±1.01 mg RE/gE. However aqueous maceration showed the lowest amount: 6.52±0.09 mg QE/gE or 9.68±0.22 mg 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 [19].

Table 2. Total flavonoid content of Senecio anteuphorbium

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

EQ: 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 [20].

Table 3. Total proanthocyanidin of Senecio anteuphorbium

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: catechins 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 $\pm$ standard mean error of three assays.

Methanolic extract combined with ^{heat} temperature 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 ^{were lower} was not effective than standards.

Table 4. Antioxidant activity of Senecio anteuphorbium DPPH reagent

Extracts and standards	MS	MM	D	AM	Trolox	Vitamin C
IC ₅₀ (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 $\pm$ standard mean error of three assays.

The highest ^{reducing capacity} capacity in reducing ferric ion (Fe³⁺) to ferrous ion (Fe²⁺) 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 Figure 1.

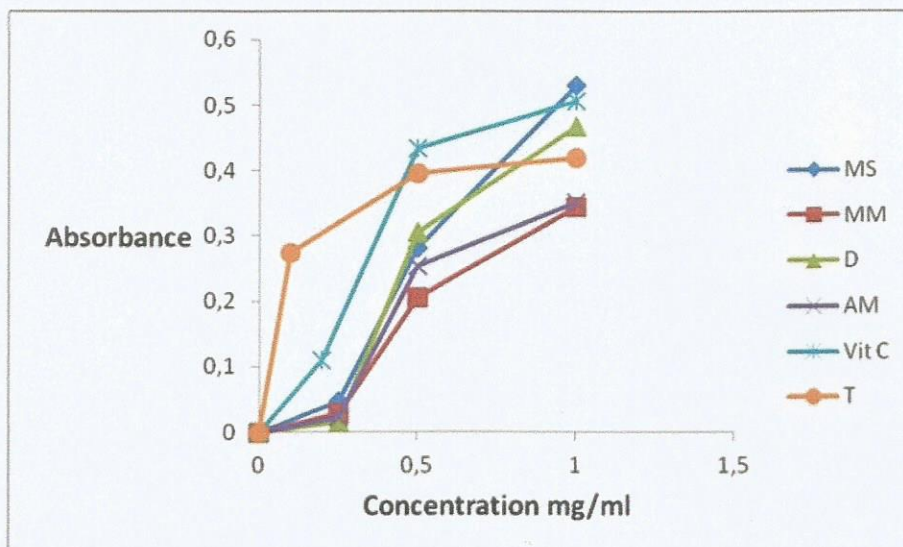


Fig. 1. Reducing power of different extracts of Senecio anteuphorbium

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 $R^2 = 0.96/0.90/0.81$ (figure 2). This indicates the close relationship between total phenolics compounds and antioxidant activity as reported by Prasad *et al* [21] and Lei *et al* [22].

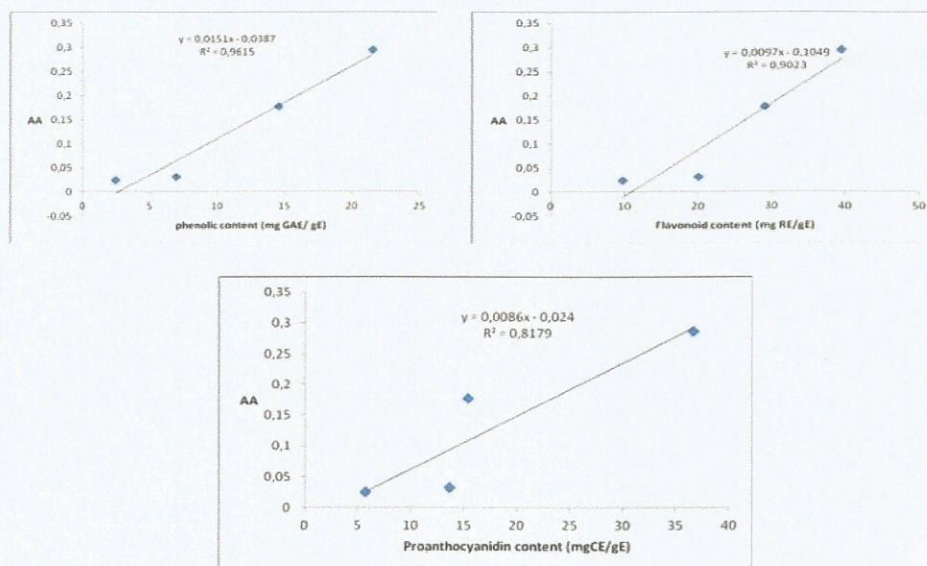


Fig. 2. Correlation between phenolic, flavonoid, proanthocyanidin and AA (antioxidant activity)

4. CONCLUSION

In conclusion, these results show that antioxidant phytochemicals are more extractable by methanol combined with temperature which might be due in part to the high polarity and efficacy of methanol. The present study also suggested that Senecio anteuphorbium could be a potential source of natural antioxidant 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.

COMPETING INTERESTS

"Authors have declared that no competing interests exist."

CONSENT (WHERE EVER APPLICABLE)

Not applicable.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Not applicable.

REFERENCES

[1] Stanner SA, Hughes J, Kelly CN, Buttriss JA. review of the epidemiological evidence for the 'antioxidant hypothesis'. Public Health Nutr. 2004; 7: 407-22.

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