



Study of the antioxidant activity of phenolic extracts of *A. halimus* L and *Haloxylon scoparium* Pomel northern Sahara

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ABSTRACT

The antioxidants are the subject of many studies, because in addition to their use as preservatives in food products by replacing the synthetic antioxidants, they intervene in the treatment of many diseases. In this study, we evaluated the antioxidant properties of leaves of *atriplex halimus* L as well as flowers and stems of *haloxylon scoparium* pomel. Butanolic Fractions of flowers of the two plants have a higher total phenols content; the fraction of ethyl acetate and butanol, for these two plants, presents a better total antioxidant capacity, and higher activity to trap the DPPH radical, radical OH and the ABTS radical cation.

Keywords: medicinal plants, secondary metabolites, antioxidant activity, northern Sahara.

INTRODUCTION

The natural substances from plants have multiple interests leveraged industry : for food, cosmetics and dermopharmacy. Among these compound found in a great extent that secondary metabolites are mostly illustrated in therapy. Pharmacy still uses a high proportion of drug of plant origin and is in the search of new active molecules plants , or raw material for the semi- synthesis [1].

In recent years, the interest in natural antioxidants, in relation to their therapeutic properties, has increased dramatically. Scientific research in various specialties have been developed for the extraction, identification and quantification of these compounds from several natural substances namely, medicinal plants and food products [2] [3] [4]. The increased interest in natural antioxidants in order to increase food preservation is explained by the fact that some synthetic antioxidants have carcinogenic risks. [5] Many plants, or medicinal food, contain antioxidant constituents. Regular intake of phytonutrients with antioxidant capacity significant is associated with a low prevalence of diseases related to oxidative stress (cancer, cardiovascular disease and atherosclerosis) and low mortality rate. [6]

The characterization of secondary metabolites in the plant kingdom, is still relevant in view of their antioxidant effect (among others) they provide to ward off the onset of many diseases. Polyphenolic compounds such as flavonoids are part of this arsenal of natural products that researchers working in the elucidation of their structure and the setting Obviously different active principles is of great number of said medicinal plants. In this work, we proposed to study the antioxidant activity of polyphenolic extracts from two plants: *A. halimus* L and *Haloxylon scoparium* Pomel (chenopodiaceae family) growing in a wild state in the Laghouat region. These plants are endemic and traditionally used as medicinal plants in the Algerian Sahara. The species *A. halimus* L., is known by these hypoglycemic and lipid-lowering properties [7] [8]. Some studies have shown that the species *Haloxylon scoparium* Pomel, has anti-cancer properties as well as antiplasmodial and larvicidal activity [9] [10].

EXPERIMENTAL SECTION

2.1 . plant material

Plants "Atriplex and Haloxylon scoparium halimus L Pomel" were collected during the months of November and March, in two regions of Laghouat, Algeria (El Kasr Hiran and Sidi Mekhlouf, respectively). The plant material was identified [11] and washed in the laboratory and dried in the dark in a well ventilated area at room temperature.

2. 2. Extraction of phenolic compounds

The plant material dried in air was extracted using methanol-water solvent system (8/2). The crude extract was collected and concentrated in a rotary evaporator at 40 ° C and partitioned successively with petroleum ether, dichloromethane, ethyl acetate and butanol, so as to fractionate the compounds in the raw materials according to their polarity.

2. 3. Determiation of total phenols

The total phenols content of plant extracts was determined using the Folin-Ciocalteu reagent [12] [13]. A volume of 200 . μ l for each extract is introduced into the test tubes, the mixture 1 ml of Folin Ciocalteu, diluted 10 times and 0.8 ml of sodium carbonate 7.5%, is added. The tubes were shaken and kept for 30 minutes. The absorbance is measured at 765 nm using the spectrophotometer Jenway 6405 UV / Vis. The contents of total phenols in the extracts are expressed in milligrams gallic acid equivalent per gram weight of the dry matter (EAG mg / g DM).

2. 4. Measurement of flavonoids

Quantification of flavonoids was carried out by a suitable colorimetric method [14] [15]. An amount of 500 . μ l of methanol solution of quercetin in different concentrations of the methanol extract or diluted, is added to 500 . μ l of aluminum trichloride ($AlCl_3$) to 2%. After incubation for 15 min at room temperature, the absorbance of the solution pinkish color is measured at 430 nm against the blank. The total flavonoid content of the plant extracts is expressed in milligrams of quercetin equivalents per gram of the weight of the dry matter (EQ) / g). Each test is repeated three times.

2. 5. Determiation of condensed tannins

The amounts of condensed tannins are estimated using the method with vanillin under acidic conditions [16]. A volume of 500 . μ l of the crude extract is added to 2500 . μ l of vanillin solution (1%) hydrochloric acid (8%) and then mixed using a vortex mixer and allowed to react at 30 ° C in a water bath for 20 min. The absorbance at 500 nm is measured against a blank. The concentration of tannins is estimated in milligrams of catechin equivalents per gram of the weight of the dry matter (EC) / g) from the calibration curve .

3-antioxidant test

3-1-total antioxidant activity (TAC)

The total antioxidant capacity (TAC) extracts was evaluated by the method of phosphomolybdenum [17]. This technique is based on the reduction molybdenum Mo (VI) present in the form of molybdate ions MoO_4^{2-} molybdenum Mo (V) MoO_4^{2+} in the presence of the extract to form a complex phosphate green / Mo (V) at pH acid .

A volume of 0.2 ml each of methanol extract is mixed with 2 ml of solution reagent (0.6 M sulfuric acid , 28 mM sodium phosphate and 4 mM molybdate ammonium) . The tubes are screwed and incubated at 95 ° C for 90 min. after cooling, the absorbance of the solutions was measured at 695 nm against the blank containing 2 ml of the reagent solution and 0.2 ml methanol and it is incubated under the same conditions the sample. Total antioxidant capacity is expressed in milligram equivalents ascorbic per gram of dry matter (mg EAA / g MS) acid. The experiments are repeated 3 times.

3-2-DPPH scavenging assay

The antioxidant test was carried out by the DPPH method with some modifications [18] [19]. This free radical (2,2'-diphenylpicrylhydrazyl, $C_{18}H_{12}N_5O_6$, M: 394.33) has a dark purple color when reduced, the color becomes pale yellow. DPPH was dissolved in methanol to a solution having 250 μ M. One milliliter (1ml) of this solution is added to 1 ml of the extract solution in methanol à différentes concentrations. After stirring, the tubes are placed in the dark at room temperature for 30 minutes. For each concentration, the test is repeated three times. Reading is performed by measuring the absorbance at 517 nm by a spectrophotometer (6405UV/Vis. Spectrophotometer).

For each dilution, white is prepared. The negative control consists of 1 ml of the DPPH solution (250 μ M) and 1 ml of methanol. The positive control is represented by a standard solution of an antioxidant; and ascorbic acid Trolox (6-Hydroxy -2 acid, 5, 7, 8 - Tetramethylchroman -2- Carboxylic " C₁₄H₁₈O₄"') whose absorbance is measured

under the same conditions as the test sample. The results were expressed in antioxidant activity (AA % = 100 - $\{[(\text{Abs test} - \text{Abs White}) \times 100] / \text{Abs control}\}$) and EC50 values were determined graphically.

3-3-ABTS test

The antioxidant test was performed according to the ABTS method [20]. The radical cation ABTS • + is produced by reacting 10 ml of ABTS [2,2'-azino-bis (3 éthylebenzothiazoline-6-sulfonic acid)] (20 mM) with 100 µl of potassium persulfate (K₂S₂O₈) (70 mM), the mixture was left in the dark at room temperature for 24 hours. 1 ml of the latter, is added to 25 ml of phosphate buffered saline (0.2 M); (pH = 7.4).

a sample of 10µl of each extract was placed in the presence of 1ml of radical cation ABTS • + . The absorbance was recorded at 734 nm for 6 min against a blank. the results obtained allows us to calculate the inhibitory power (PI) based on the concentration of extract (antioxidant) , with :

(PI % = 100 - $\{[(\text{Abs test} - \text{AbsBlanc}) \times 100] / \text{Abs control}\}$) and EC50 values were determined graphically.

A cont : Absorbance da the ABTS solution.

Ext A : Absorbance of the ABTS solution in the presence of the extract.

3-4-Hydroxyl radical scavenging (deoxyribose assay)

Deoxyribose method adopted in this study is that of [21]. The reaction mixture contains the following reagents: 0.4 ml of phosphate buffered saline (50 mmol / l, pH = 7.4), 0.1 ml of the extract at different concentrations, 0.1 ml EDTA (1.04 mmol / l), 0.1 ml of FeCl₃ (1 mmol / l) and 0.1 ml of 2-deoxyribose (60 mmol / l). There reaction is started by adding 0.1 ml of ascorbic acid (2 mmol / l) and 0.1 ml of H₂O₂ (10 mmol / l). After incubation at 37 ° C for 1 hour, 1 ml of the acid thiobarbutirique (TBA) (10 g / l) is added to the reaction mixture followed by 1 ml of hydrochloric acid (HCl) (25%). The mixtures were placed in a water bath at 100 ° C for 15 min and then are cooled with water. The absorbance of the solutions is measured at 532 nm with spectrophotometer against white. The ability of trapping the hydroxyl radical is evaluated with the percentage inhibition of the oxidation of 4-deoxyribose with the radicals hydroxyls.

The positive control used is that of BHA. Experiments are repeated 3 times.

RESULTS AND DISCUSSION

4-1-Yields of crude extracts :

The extraction of the phenolic compounds of methanol / water (8/2) of the plants tested, we have used to determined the yields of extracts (Table 1).

Table1 Yield of bioactive compounds

	Bioactive compounds	yeld (%)
Atriplex halimus(top)	Ethyl acetate fraction	0,40 ± 0,01
	Dichloromethane fraction	0,60 ± 0,02
	Butanolic fraction	5,96 ± 0,23
Haloxylon scoparium (stems, leaves)	Ethyl acetate fraction	0,80 ± 0,01
	Dichloromethane fraction	0,40 ± 0,03
	Butanolic fraction	6,20 ± 0,34
Haloxylon scoparium (Flowers)	Ethyl acetate fraction	1,12 ± 0,03
	Dichloromethane fraction	0,60 ± 0,01
	Butanolic fraction	15,38 ± 1,12

Extracts of butanol fractions of our plants are the highest in terms of returns, as shown in table1: starting with the fruits of Haloxylon scoparium (15.38%), followed by the leaves of the same plant (6, 2%) and the aerial part of A. halimus (5.96%). Other more or less considerable yields were observed, the most important being to the extracts from the ethyl acetate fraction of flowers Haloxylon scoparium Pomel (1.12%).

As an indication, some authors have shown that methanol remains the most suitable solvent to extract the antioxidants from a plant [22] [23].

4.2. Contents of total phenols, flavonoids and tannins:**Table 2 contents of total phenols, flavonoids and tannins**

Bioactive compounds	Total phenols (mg EAG/g MS)	Flavonoids (mg EQ/g MS)	Tannin (mg EC/g MS)
Ethyl acetate fraction	1,616 ± 0,005	0,058 ± 0,001	0,122 ± 0,002
Dichloromethane fraction	1,582 ± 0,034	0,026 ± 0,001	0,039 ± 0,001
Butanolic fraction	3,648 ± 0,001	0,120 ± 0,003	1,036 ± 0,002
Ethyl acetate fraction	2,416 ± 0,008	0,128 ± 0,001	0,315 ± 0,003
Dichloromethane fraction	2,104 ± 0,023	0,090 ± 0,002	0,019 ± 0,001
Butanolic fraction	18,666 ± 0,176	0,305 ± 0,001	2,862 ± 0,012
Ethyl acetate fraction	4,163 ± 0,028	0,531 ± 0,003	1,641 ± 0,017
Dichloromethane fraction	2,456 ± 0,003	0,058 ± 0,001	1,468 ± 0,033
Butanolic fraction	62,590 ± 2,051	0,139 ± 0,003	10,501 ± 1,435

The extraction of phenolic compounds studied plants, allowed us to determine the contents of total phenols, flavonoids and tannins (Table 2).

The results indicate that the butanol fraction of the extract of *A. halimus* has a high content of total phenols (3.648 ± 0.001 mg EAG / g MS), the flavonoids 0.120 ± 0.003 mg EQ / g DW and tannins 1.036 mg CE / g DW. In second place comes the ethyl acetate fraction from the same plant with total polyphenol content of 1.616 ± 0.005 mg EAG / g DW, flavonoids and tannins: 0.058 ± 0.001 mg EQ / g MS and 0.122 ± 0.002 mg EC / g DW, respectively. The results observed in the literature [24] show the contents of total phenols in the extract of *Atriplex rods halimus* equal to 3.77 mg EAG / g DW and 10.12 mg EAG / g DW), whereas in the extracted from the leaves of the same plant results are much higher than ours.

In *Haloxylon scoparium*, the results indicate that the butanol fractions flowers have the highest content of total phenols and tannins that. flavonoids contents are lower (0.139 ± 0.003 mg EQ / g DW). Followed by the ethyl acetate fraction of the same plant with total polyphenol contents equal to 4.163 ± 0.028 mg EAG / g DM, flavonoids and tannins to 0.531 ± 0.003 mg EQ / g DW and 1.641 ± 0.017 mg CE / g DW respectively. These values are large compared to those found in the stems. These results indicate that the distribution of secondary metabolites may fluctuate between different plant organs [25] [26] [27]. We find that the plants of the Sahara have high levels of tannins relative to flavonoids.

Variability polyphenol concentrations in these plant species is probably due to the phenolic composition of the extracts [28], for genotyping factors [29], the biotic (case member and the physiological stage) and abiotic (edaphic) [27], the soil type and microclimate, [30] and also bioclimatic zones where these plants grow.

4-3 Antioxidant power of phenolic compound

The diversity of nature and the complexity of phytochemical compound of plant extracts impose the development of many methods to evaluate the antioxidant activity and to estimate the effectiveness of these substances. The majority of these methods are based on the colouring or the discolouration of a reagent in the reactional medium. They can be classified into two groups: those assays used in food and biological system to evaluate lipid peroxidation while measuring the degree of oxidation inhibition [31] and those assays used to measure free radical scavenging ability [32].

Table 3 Antioxydant activities

Bioactive compounds	Total antioxidant capacity (mgAA/gMS)	EC ₅₀ (mg/ml) DPPH [•]	IC ₅₀ (mg /ml) ABTS ^{•+}	EC ₅₀ (mg /ml) OH [•]
Ethyl acetate fraction	0,241	0,23	0,233	0,433
Dichloromethane fraction	0,11	0,094	0,283	0,546
Butanolic fraction	0,112	0,4059	0,202	0,503
Ethyl acetate fraction	0,224	0,088	0,687	0,136
Dichloromethane fraction	0,035	0,326	0,218	0,195
Butanolic fraction	0,046	0,233	0,0069	0,163
Ethyl acetate fraction	0,023	0,971	0,0268	0,111
Dichloromethane fraction	0,034	0,343	0,174	0,144
Butanolic fraction	0,414	0,329	0,0032	0,15
Ascorbic acid	-	0,0062	0,0002	0,095
Vit E	-	0,01147	0,0052	-
BHA	-	0,00591	0,0016	0,139

Some can be based on metal reducing power (ferric reducing antioxidant power, FRAP), peroxy radical scavenging (oxygen radical absorbance capacity, ORAC; total radical trapping antioxidant parameter, TRAP), hydroxyl radical scavenging (deoxyribose assay), organic radical scavenging like ABTS and DPPH, ABTS, Trapping the hydroxyl radical, DPPH and total antioxidant activity (TAC) have been used in the present investigation.

The results shown in Table 3 show that all samples have different antioxidant activities.

The best natural antioxidants are those of butanol fractions and / or ethyl acetate, not only for testing but also for other methods applied in this work. The extract of the flowers of butanol fraction *Haloxylon scoparium* Pomel has the best total antioxidant capacity of the order of 0.414 mgAA / g DW) compared to that found by the rods of the same plant; against by the ethyl acetate fraction of the stems revealed a higher reducing activity over flowers (of the order of 0.224 mgAA / g DW) and dichloromethane fraction from the same plant, we note that the results are almost the same (0.035 mgAA / g DW for rods and 0.034 mgAA / g DW for flowers).

The values of total antioxidant capacity of the ethyl acetate fraction of the *Atriplex halimus* reveal the best activity (about 0.241 mgAA / g DW), then comes the butanol fraction (of the order of 0.112 mgAA / g DW) and finally 0.110 mgAA / g DW for the fraction of dichloromethane the same plant. Other reducing activities remain low (about 0.023 mgAA / gDW / 0.046 mgAA / g DW).

Various studies have experimentally determined the capacity of natural extracts to scavenge free radicals. This activity depends on a number of parameters: the dose, the structure, the substituents and the degree of polymerization of the molecule.

The most effective scavengers of free radical DPPH, ABTS and OH are those with EC₅₀ values lower. All our plants extracts showed activity of DPPH free radical scavenging. The lowest concentrations are reported in the acetate moiety of the leaves of the plant *Haloxylon scoparium* and the dichloromethane fraction of the plant *Atriplex halimus* L. Similarly, significant EC₅₀ values are 0.230 mg / ml for the ethyl acetate fraction of *A. halimus* to 0.233 mg / ml for the butanol fraction of the leaves of the plant *Haloxylon scoparium*.

These results clearly show that the extracts having the same activity can be composed of the same molecules. Indeed, the highest concentration is recorded for the ethyl acetate fraction of flowers of the plant *Haloxylon scoparium*.

These results allowed us to conclude that the ethyl acetate fraction of the leaves, has better antioxidant activity. Very little work has been done on studying the antioxidant properties of selected plants. EC₅₀ values found in the literature [24] for *A. halimus* L are superior to our results while others values [33] to *Haloxylon scoparium* are almost the same.

The ethyl acetate fraction of the *A. halimus* L has a good ability to scavenge DPPH radical. This activity is probably due to the abundance of flavonols (kaempferol, quercetin ...) which represent the major class of *Atriplex* species [34] [35].

From Table 2, it is noted that IC₅₀ values ABTS phenolic extracts generally vary from 0.003 mg / ml to 0.687 mg / ml. The lowest concentrations are noted in the extract from the butanol fraction of the flowers of the plant *Haloxylon scoparium*; extract the butanol fraction of the leaves of the plant also presents an important IC₅₀ value of about 0.007 mg / ml. For other extracts, the IC₅₀ values ranged from 0.174 mg / ml and 0.283 mg / ml against by the highest value is recorded for the ethyl acetate fraction leaves the *Haloxylon scoparium* Pomel.

From these results, it appears that the extract of the butanol fraction of the flowers of the plant *Haloxylon scoparium* has the best antioxidant activity compared to vitamin E.

The activity of our extracts can be attributed to phenolic compounds including flavonoids that are reported in several studies as the best antioxidants. Phenolic acids and phenolic diterpenes are also involved in this activity. [36] Nevertheless, the structural variability of these same flavonoids affect in no small measure this activity [37] and an indication, the most active compounds are those that combine the following three criteria:

- * The ortho-dihydroxy structure in the B ring (catechol), which imparts stability flavonoxy radical and participates in the delocalization of the electrons.
- * The double C2-C3 bond in conjugation with the function 4-oxo.
- * The presence of the 3-OH group in combination with the double bond C2-C3.

The best natural antioxidants are those fractions of ethyl acetate and butanol, not only for testing but also for other methods applied in this work. The extract of the ethyl acetate fraction of flowers *Haloxylon scoparium* Pomel, the ethyl acetate fraction of the stems of the same plant have the best activity to trap the radical \bullet OH compared auBHA ($EC_{50} = 0.139 \pm 0.053$ mg / ml). EC_{50} other pellets are:

The values of the activity to trap the radical OH \bullet the ethyl acetate fraction of the *atriplex halimus* reveal the best activity (of the order of 0.433 mg / ml), then comes the butanol fraction (around of 0.503 mg / ml) and finally 0.546 mg / ml for the fraction of dichloromethane the same plant.

Recently, there is great interest in the therapeutic potential of medicinal plants such as antioxidants with the reduction potential of the phenolic compounds. The antioxidant activity of our extracts was compared with that of synthetic antioxidants and pure phenolic compounds .

The overall results show that our extracts can replace antioxidants Synthesis.

CONCLUSION

The results obtained in this work , we have shown that our plants extracts a high content of total phenols and particularly in the butanol fractions and they are endowed with antioxidant capacity and therefore interesting that capture free radicals , The synthesis of the different results for the enzymatic activity of our extracts clearly shows that the power vis-à-vis the α -amylase inhibition is a specific character for each extract which can varies according to its composition and rich in molecules responsible for the activity we incentive to insulate their components and characterize their structures to eventually lead to the identification of the active principles responsible for this activity. Cet will be the subject of our second part of the thesis. the antioxidant activity of our extracts was compared with that of synthetic antioxidants and pure phenolic compounds. It shows that our extracts can replace some synthetic antioxidants.

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