COMPARATIVE STUDY OF THE ANTIOXIDANT ACTIVITY AND PHENOLS AND FLAVONOIDS CONTENTS OF THE ETHYL ACETATE EXTRACTS FROM TWO SAHARAN CHENOPODACEA: *Haloxylon scoparium* AND *Traganum nudatum*

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Abstract.- Haloxylon scoparium *and* Traganum nudatum *are two medicinal plants widely used in Algerian traditional medicine. In the present work, a comparative study was conducted on the Antioxydant evaluation and the quantification of total phenols and flavonoids contents (TPC and TFC) in the ethyl acetate extracts from the two species. The quantitative estimation showed that the extracts are rich in these compounds. Evaluation of antioxidant activity performed by DPPH free radical trapping, indicated that the extracts present a good antioxidant efficiency. The result obtained showed that the highest antioxidant activity, total phenolic and flavonoid content were exhibited by the extract of ethyl acetate of Haloxylon scoparium compared to the* Traganum nudatum *ethyl acetate extract.*

Key words: Haloxylon scoparium, Traganum nudatum, *antioxidant activity, phenolic compounds, flavonoids.*

ETUDE COMPARATIVE DE L'ACTIVITE ANTIOXYDANTE ET DU TAUX DES PHENOLS ET DES FLAVONOÏDES DES EXTRAITS D'ACETATE D'ETHYLE DE DEUX CHENOPODIACEAE DU SAHARA: *Haloxylon scoparium* AND *Traganum nudatum*

Résumé.- Haloxylon scoparium *et* Traganum nudatum *deux plantes médicinales, largement utilisée en médecine traditionnelle algérienne. Dans le présent travail, il est présenté une étude comparative sur l'activité antioxydante et la quantification des phénols et des flavonoides totaux dans les extraits acétate d'éthyle des deux plantes. L'estimation quantitative a montré que les extraits sont riches en ces composés. L'évaluation du pouvoir antioxydant réalisée par le piégeage du radical libre DPPH a indiqué que les extraits ont montré une bonne efficacité antioxydante. Les résultats obtenus montrent que par l'extrait d'acétate d'éthyle de* Haloxylon scoparium *présente une plus forte activité antioxydante, et des taux élevés en composés phénoliques et en flavonoïdes comparé à l'extrait d'acétate d'éthyle de* Traganum nudatum.

Mots clés: *Haloxylon scoparium, Traganum nudatum, activité antioxydante, composés phénoliques, flavonoides.*

Introduction

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress, Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process, Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [1,2,3,4].

Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants. Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols etc [5].

Several methods are used to evaluate, in vitro and in vivo antioxidant activity by trapping different radicals such as ROO peroxides by the methods ORAC (Oxygen Radical Absorbance Capacity) and TRAP (Total Radical-Trapping Antioxidant Parameter); ferric ions by the FRAP method (Ferric ion Reducing Antioxidant Parameter); or the radical ABTS $^{\bullet}$ (ammonium salt of the acid 2,2 '-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) and the method using free radical DPPH $^{\bullet}$ (diphenyl-picrylhydrazyl) [6].

The objectives of the present study were compared the antioxidant activity, total phenolic content and total flavonoid content of the ethyl acetate extracts from two chenopodiceae : *Haloxylon scoparium* and *Traganum nudatum*

Haloxylon scopariun known locally as '*Remth*' is used in local folk medicine to cure stomachache, scorpion bites, wounds infertility and bone pain. In Tunisia and Morocco it is used to treat eye disorders. Aqueous extracts of this plant have also been reported to show anti-cancer, antiplasmodial and larvicidal activity. Infusion and powder infusion of aerial part of *H. scoparium* are sometimes used for their antidiabetic effects [7,8,9,10].

Traganum nudatum known locally as 'Damran' is used in traditional medicine to cure some diseases such as Diarrhea, wounds, rheumatism, dermatosis , and others [11,12].

1.- Materials and Methods

1.1.- Preparation of Extract

The aerial parts of *H. scoparium* were collected from Ghardaia (Barienne region) in November 2012. The aerial parts of *T. nudatum* were collected from Touggourt (gamaa region) in April 2013. The plants were identified by Pr. Abdelmadjid Chehma from Ouargla University and voucher specimens (MA4 and MA5), were deposited at the Chemistry Department, University of Ouargla. The plant materials were dried under shade and then ground and stored in closed container away from light and moisture.

The extracts were prepared by soaking 500 g of the plant powder in a solution of EtOH/H_2O [70/30] for 24H. The procedure was repeated three times and the filtrates were combined before being evaporated under reduced pressure. The resulting extracts were diluted with distilled water and left for a whole night. The filtrates were then subjected to

extraction by various solvents with increasing polarity (petroleum ether, dichloromethane, ethyl acetate, and butanol). The organic phases were separated and evaporated.

1.2.- Determination of total Phenolic

Total phenolic compound contents were determined by the Folin-Ciocalteau method (EBRAHIMZADED *et al.,* 2008a, b; NABAVI *et al.,* 2008).The extract samples (0.1 ml of different dilutions) were mixed with Folin Ciocalteu reagent (1.5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (1.5 ml, 6٪) were then added. The mixture was allowed to stand for 90 min and the phenols were determined by colorimetry at 725 nm. The standard curve was prepared (0.03-0.3 mg/ ml) solutions of gallic acid in methanol. The total content of phenolic compounds in the extract in gallic acid equivalents (GAE) was calculated by the following formula:

$$T = \frac{C.V}{M}$$

Where, T = total content of phenolic compounds, milligram per gram extract, in GAE;C = the concentration of gallic acid established from the calibration curve, milligram per milliliter; V = the volume of extract, milliliter; M = the weight of extract, Gram [1,4,13,14,15,16].

1.3.- Determination of total Flavonoids

Estimation of the total flavonoids in the plant extracts was carried out using the method of ORDONEZ *et al*.. To 1.5 ml of sample, 1.5 ml of 2% $AlCl_3$ methanol solution was added. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: y = 30,493x + 0,0914 , R^2 = 0,999 , where x was the absorbance and was the quercetin equivalent (mg/g) [17,18,19,20].

1.4.- DPPH radical scavenging activity method:

Quantitative measurement of radical scavenging properties was carried out in a universal bottle. A solution of 0.4 mM DPPH in methanol was prepared and 1.5 ml of this solution was mixed with 1.5 ml of extract in methanol containing 0.05 mg of extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min.. Different known antioxidants, Ascorbic acid was used as standard in (0.04-0.4 mg/ml) solution, and butylated hydroxytoluene (BHT, Sigma) were used for comparison or as a positive control. All samples were diluted in solution tampon TRIS-HCl (100mM, Ph = 7,4). Discoloration was measured at 517 nm after incubation for 30 min. Measurements were taken at least in triplicate. DPPH radical's concentration was calculated using the following equation:

Percentage (%) of DPPH radical scavenging = $\left(1-\frac{As}{Ac}\right) x100$

Where; AC =absorbance of control and AS =absorbance of sample solution,
IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical

[21,22,23,24,25].

1.5.- Reducing power assay

The reducing power of different extracts or fractions were measured according the method used by Hinneburg *et al*. (2006). One milliliter of extracts or fractions with different concentrations was mixed with 2.5 ml of phosphate buffer (200 mM; pH 6.6) and 2.5ml of potassium ferricyanide1% and incubated at 50°c for 20 min. The mixture was added with 2.5 ml of 10% TCA and centrifuged at 3000 rpm for 10 min. A-2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl3 (0.1%) and the absorbance was measured spectrophotometrically at 700 nm. Increase in absorbance of the reaction mixture was interpreted as increase in reducing activity of the extract and the results were compared with ascorbic acid which was used as a positive control. The percentage of reduction of the sample as compared to standard (ascorbic acid) was calculated using the formula:

Percentage (%) of reduction power = $[1-(1-\frac{As}{Ac})]\,x\,100$

AC = absorbance of standard at maximum concentration tested and AS = absorbance of sample [4,26,27,28,29].

2.-Results and discussion

2.1.- Determination of total phenolic contents

Total phenol compounds, as determined by folin Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve (y = 4,0914x + 0,0719, R^2 = 0,995). The total phenolic contents were higher in ethyl acetate of *Haloxylon scoparium* then of ethyl acetate extract of *Traganum nudatum* that is in table I.

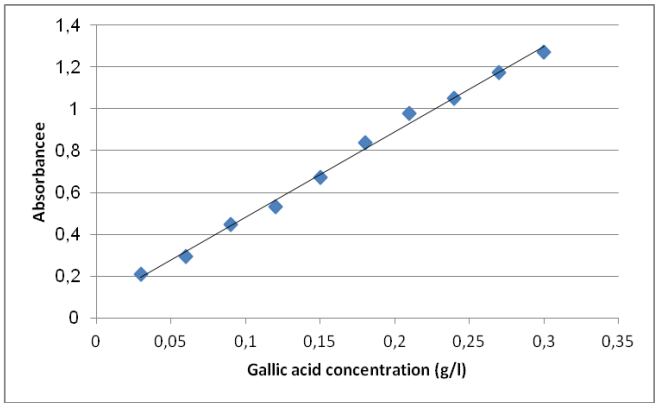

Figure 1.- Calibration curve of standard gallic acid for determination of total phenolics

The total flavonoid content was expressed as quercetin equivalents (RE) in milligram per gram dry material of extracts and fractions. The calibration curve of quercetin to determine flavonoid content was shown in figure 2 (y = 30,493x + 0,0914, R^2 = 0,999). Total flavonoid content of ethyl acetate extracts was compiled in table I. The result showed that ethyl acetate of *Haloxylon scoparium* had the highest flavonoid content compared than that of ethyl acetate of *Traganum nudatum*.

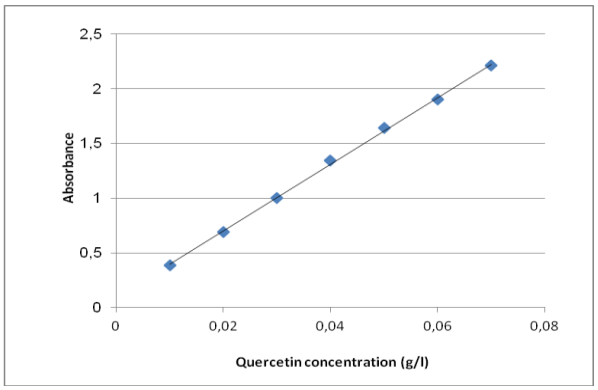

Figure 2.- Calibration curve of standard Quercetin for determination of total flavonoid content

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) method was used to evaluate the free radical scavenging ability of ethyl acetate from two extracts. The percent inhibition of the DPPH radical as a function of the antioxidant concentrations is shown in figure 3.

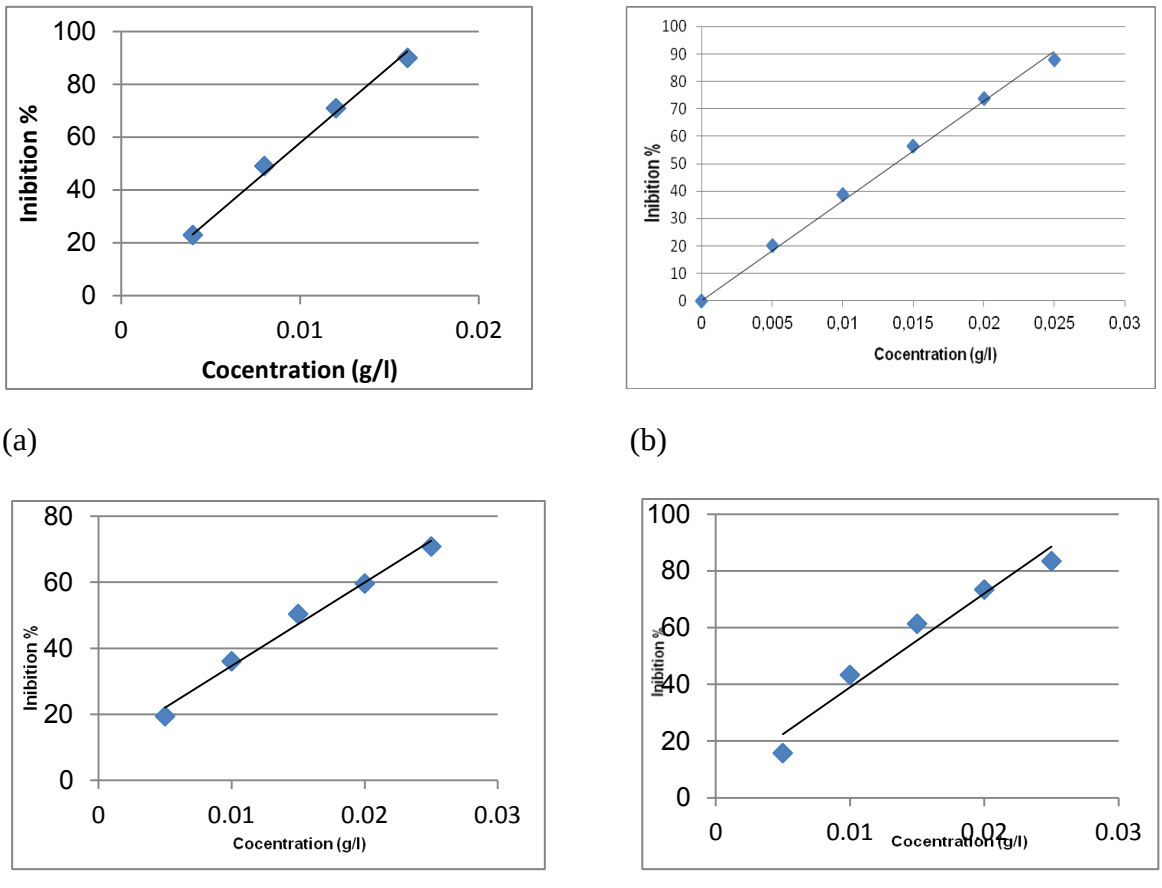

Figure 3.- (a) Antioxidant activity of DPPH, (b) Antioxidant activity of BHT (c) Antioxidant activity of Ethyl Acetate of *Haloxylon scoparium* and (d) Antioxidant activity of Ethyl Acetate of *Traganum nudatum*

The parameter used to measure the radical scavenging activity of extracts evaluated is IC_{50} value, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period. The smaller IC_{50} value explained the higher antioxidant activity of the plant extract (MAISUTHISAKU *et al*., 2007). The IC_{50} value of extracts was shown in table I. It is found that of ethyl acetate extract of *Haloxylon scoparium* revealed the higher activity compared than that of ethyl acetate extract of *Traganum nudatum*

Table I .- Radical scavenging activity and total phenol and flavonoids contents in Ethyl Acetate of *Haloxylon scoparium* and in Ethyl Acetate of *Traganum nudatum* (* mg gallic acid equivalent/g of extract powder, ** mg quercetin equivalent/g of extract powder, ***mg/ ml. The IC50 values for ascorbic acid and BHA were 0.00864 and 0.01372 mg /ml respectively

Sample	**Phenol content ***	**Flavonoids content ****	**DPPH IC50*****(mg/ml)***
Ethyl Acetate of *Haloxylon scoparium*	397.7426	82.8355	0.01102
Ethyl Acetate of *Traganum nudatum*	311.051	44.5284	0.01315

2.2.- Reducing power assay

The reducing power (RP) of the extracts and ethyl acetate fractions was determined by direct electron donation in the reduction of ferri cyanide $[Fe(CN)_6]^{-3}$ to ferro cyanide $[Fe(CN)_6]^{-4}$. The product was visualized by addition of free Fe^{+3} ions after the reduction reaction, by forming the intense Prussian blue color complex, $(Fe^{+3})4[Fe^{+2}(CN^{-})_6]^{-3}$, and quantified by absorbance measurement at 700 nm (RIBERIO *et al*., 2008). The reductive capabilities of the two extracts compared to ascorbic acid shows in figure 4.

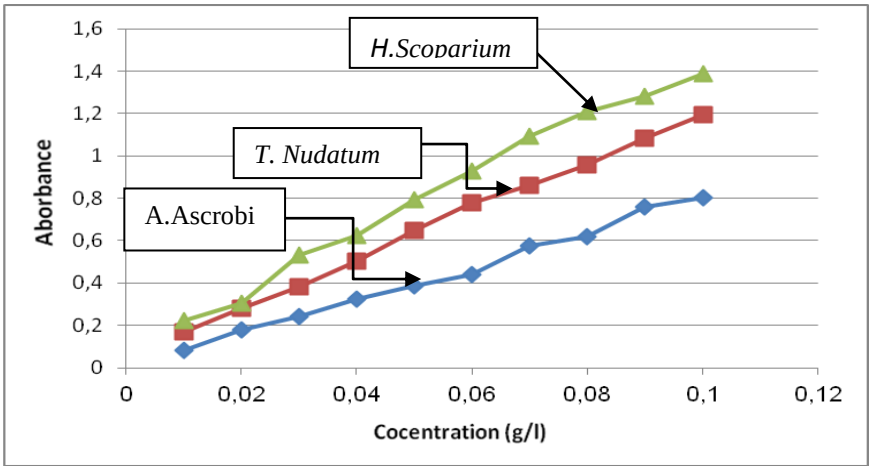

Figure 4.- Curve of reducing power assay

The Curves described in figure 4 show Increased absorbance with the increased concentration of extracts as standard antioxidants, The extract could reduce the most Fe^{+3} ions, which had a lesser reductive activity than the standard of ascorbic acid. Increased

absorbance of the reaction indicated increased reducing power.

The reducing power of ethyl acetate extracts was expressed as mg ascorbic acid equivalent per gram extract and its results were shown in table II. So the reducing power of ethyl acetate extract of *Haloxylon scoparium* revealed more than of ethyl acetate extract of *Traganum nudatum*, which was in agreement with the total phenolics content and total flavonoid content.

Table II.- The reducing power of ethyl acetate extract of *Haloxylon scoparium* and of *Traganum nudatum*

Sample	Reducing power (µg ascorbic acid equivalent per gram extract)
Ethyl Acetate of *Haloxylon scoparium*	172.1525
Ethyl Acetate of *Traganum nudatum*	140.2585

Conclusion

The results of this study showed that a higher antioxidant activity, total phenolic content and total flavonoid content were exhibited by the ethyl acetate extract of *Haloxylon scoparium.* The phytochemical study aiming to separate the active principles and to elucidate the mechanism of action of this extract is the subject of ongoing investigation in our group.

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