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Evaluation of the antioxidant activity and the physicochemical composition of methanolic and aqueous extracts of Spergularia rubra L. from Algeria

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ABSTRACT/RESUME

Abstract: *Spergularia rubra L. known by the vernacular name "fatete alhjar" is a medicinal plant of the Caryophyllaceae family, widely used in traditional Algerian medicine and as a food condiment. In the present work two extracts were prepared from the leaves of this plant: one organic methanolic and the other aqueous. The yields of crude solids are in the order of 52% and 62% respectively. The evaluation of the antioxidant power which was carried out using the method of scavenging the diphenyl-picrylhydrazyl free radical (DPPH), indicated that the methanolic extract showed a good high antioxidant activity than that of the aqueous extract.*

I. Introduction

The use of synthetic antioxidant molecules is currently being questioned due to potential toxicological risks, phenolic acids, stilbenes, flavonoids, tannins and lignans are predominantly present in the leaves, flowers and bark. These molecules play a major role in the growth of plants and in the fight against pathogens and infections. The role of natural antioxidants arouses more and more interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases [23].

Spergularia rubra (L.), Is from the Caryophyllaceae family. [2], she is considered a cosmopolitan race, ranging from temperate regions from all continents to subtropical climatic regions, except the Antarctic continent. Many of these strains have been studied chemically and pharmacologically, such as hypoglycemic, diuretic, antihypertensive and cholesterol-lowering agents, antidiabetics, antidiabetic anti-cholinesterases. **[21]**

The present work is part of the research and development of bioactive substances such as natural substances endowed with antioxidant activity which are of interest in the field of bio pharmacology. The main objective of this work is to evaluate in vitro the antioxidant activity of methanolic and aqueous extracts of Spergularia rubra L using the DPPH free radical scavenging method.

II. Material used

II.1. Origin of the plant: The plant was collected in 2018, in the region of southern Algeria (Fig. 1)

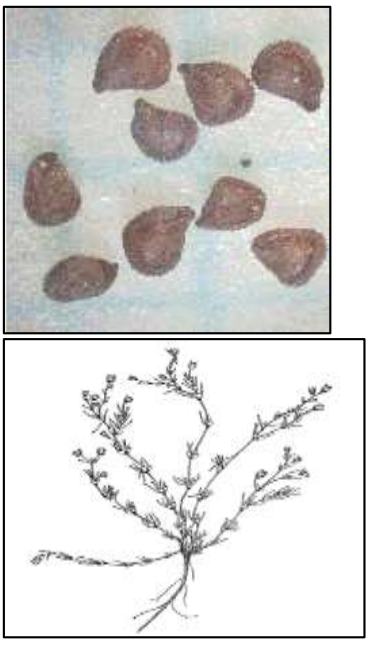

Figure 1. *Saharian Spergularia rubra (Adrar, Algeria)*

II.2. Chemical reagents: The reagents used are: DPPH (1,1-diphenyl-2-picryl-hydrazyl), Folin-Ciocalteu reagent, ascorbic acid, gallic acid, AlCl3 (aluminum trichloride), quercetin, these products all come from Sigma, the solvent used being methanol

III.Methods

III.1. Preparation of the methanolic extract: by the method of [12], the leaves of Spergularia rubra cleaned and ground beforehand are macerated in a methanol/water mixture (7: 3 V / V) at a ratio of 1/10 (P / V), with gentle stirring overnight at room temperature. The hydro-alcoholic extract is recovered after filtration of the mixture using a filter paper, the methanol is removed from the filtrate by evaporation under reduced pressure in a rotary evaporator (BÜCHI). Thus obtaining an extract characterized by a dark brown color, which is considered to be the raw extract.

III.2. Preparation of the aqueous extract: A quantity of 5 mg of the powder The leaves of Spergularia rubra is macerated in 10 ml of D.E. at a ratio of 1/2 (W / V), for 10 min at room temperature. The aqueous extract is recovered first after filtering the mixture using filter paper. Thus obtaining an extract characterized by a grayish color, which is considered to be the raw extract.

III.3. Phytochemical screening: The revelation of certain chemical families present in the methanolic extract of Spergularia rubra leaves has been carried out using chemical detection tests described in the work of certain researchers **[3], [15]** and **[18].**

III.4. Determination of total polyphenols: The polyphenols are determined by the Folin-Ciocalteu method. This method, initially described by [19], makes it possible to know the total polyphenolic content of a given sample. The sample of the methanolic extract of the leaves (0.5 ml) and 2 ml of sodium carbonate (75 g / L) were added to 2.5 ml of 10% (v / v) of the Folin- reagent. Ciocalteau with gallic acid as standard. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm. Tests were performed three times to ensure reproducibility of results. The total phenolic content was expressed in mg Gallic Acid Equivalent per gram of sample.

III.5. Determination of total flavonoids: The determination of the level of total flavonoids in the methanolic extract of the leaves is carried out by the method described by [1]. 0.5 ml of a 2% AlCl3-ethanol solution was added to 0.5 ml of sample or standard. After 1 h at room temperature, the absorbance was measured at 420 nm. Quercetin was used as a standard to plot the calibration curve. Tests were performed three times to ensure reproducibility of results. Results were expressed as milligrams of Quercetin equivalent per gram of sample.

III.6. Anti-free radical activity by the DPPH test The antioxidant activity in vitro was evaluated by measuring the scavenging power of the DPPH radical (1,1-Diphenyl-2-picryhydrazyl) according to the method described by [7], where 3 ml of the methanolic extract solution tested at different concentrations (5, 10, 15, 25.50, and 60 µg / ml) are mixed with 75 µl of a methanolic solution of DPPH (1.3 mg/ml). After an incubation period of 30 minutes at room temperature, the absorbance is measured at the wavelength of 517 nm. The inhibition of the free radical DPPH by ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) were also analyzed at the same concentration for comparison. The reaction kinetics and the parameters for calculating the antioxidant activity are determined for alpha-tocopherol, ascorbic acid and for the methanolic extract (Percent inhibition, IC50 index). All the tests were carried out three times in order to check the reproducibility.

IV. Results and discussion:

IV.1. Phytochemical screening:

The preliminary evaluation of the phytochemical composition of our plant selected for this study made it possible to highlight the presence of some chemical groups presented in Table 01 below

Tableau 1. *Results of criblage photochimique*

	Observation	Maceration	Infusion
Alkaloids	No rush	-	-
Tannins	Blue-blackish coloration	+++	+++
Flavonoids	Pink coloring	+++	+++
Coumarins	Fluorescence	++	++
Sterols and terpenes	Purple color of the supernatant and formation of a red ring at the contact area of the two liquids	-	+

Reducing compounds	Red precipitant	+++	+++
Free quinones	Yellow coloring	+	++

frankly positive: + + + / moderately positive: + + / weakly positive: + / negative: -

IV. 2. Quantitative analysis

Colorimetric methods, based on the use of the UV-visible spectrophotometer, were used to assess the amount of phenolic compounds, flavonoids and tannins in plant material.

Dosage of polyphenols

The quantitative study of the hydromethanolic extracts of our plant is carried out by colorimetric assays by the spectrophotometer. In order to characterize this extract, an assay of total polyphenols was carried out. The main reason for choosing these substances is that the majority of the antioxidant properties of plants are attributed to them. The method for the determination of total polyphenols is that of Folin –Ciocalteu [14], a calibration curve for gallic acid has been drawn for this objective.

Density measurements for each extract were carried out at 760nm for the phenols. The quantities of the corresponding polyphenols were reported in gram equivalent of the standard used and determined by the type equation: y = 0.0034x-0.0081

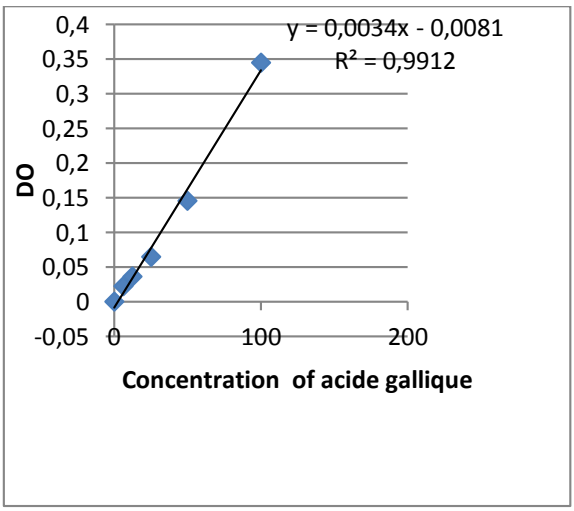

Figure 2.*Gallic acid calibration curve*

From the gallic acid calibration curve we calculated the levels of phenolic compounds, the results are shown in the following (figure n ° 2):

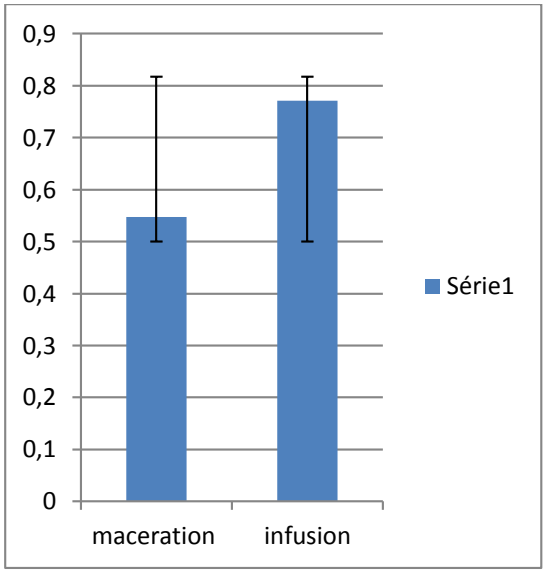

Figure 3. *Appearing concentration in (mol / l) of total polyphenols in both extract.*

The polyphenol content was estimated by the colorimetric method of Folin-Ciocalteu. Spergularia was found to be rich in polyphenols, the results show that phenolic compounds are abundant in the plants studied. The high content of polyphenols in the hydromethanolic extract is linked to the high solubility of phenols in polar solvents [9]. In view of the results presented below, it emerges that the extraction of phenolic compounds is a crucial step for the recovery of active ingredients. It depends both on the extraction solvent and on the nature of the organ studied. Therefore, selecting an appropriate solvent system remains one of the most important steps in optimizing the extraction of polyphenols, flavonoids and other antioxidant compounds. [24]

. Flavonoids content

The main property initially recognized by flavonoids is to be "veino-active", that is to say capable of reducing the permeability of blood capillaries and of strengthening their resistance [6] The aluminum trichloride method [8] is used to quantify the flavonoids in the crude extracts of the plants studied

A Quercetin calibration curve. Density measurements for each extract were plotted for this purpose were carried out at 510 nm for the flavonoids. The amounts of the corresponding flavonoids have been reported in equivalent

gram of the standard used and determined by the following equation: y = 0.0016x + 0 .0046Tannin content

The analysis of condensed tannins was carried out by vanillin in an acidic medium described by [4] [22] and [17] Flavonoids prevent diabetes by inhibiting alkalosis reductase. In addition, several studies have shown that the consumption of foods rich in flavonoids is inversely related to the risk of developing cardiovascular disease [16] and [11]. On the other hand, the anti-diabetic action. using catechin as standard.

A catechin calibration curve was plotted for this objective. Density measurements for each extract were carried out at 550 nm for the tannins. The amounts of the corresponding tannins by the type equation: y = 0.0012 X +0.0028

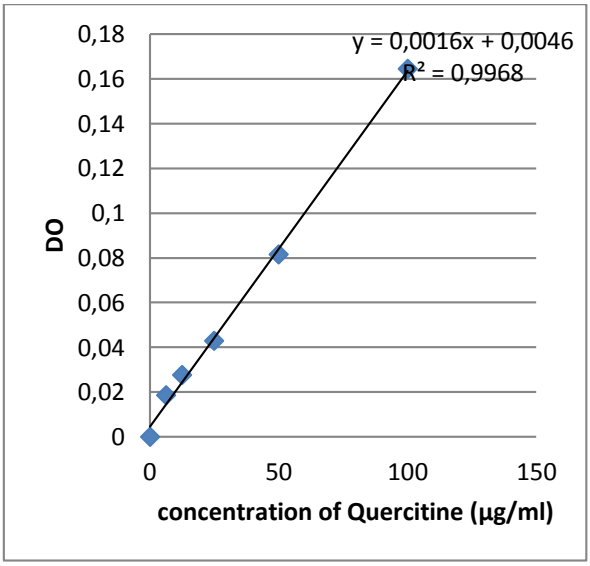

Figure 4. Quercetin calibration curve

From the quercetin calibration curve we calculated the flavonoid content of our studied plant, the results are shown in the following figure:

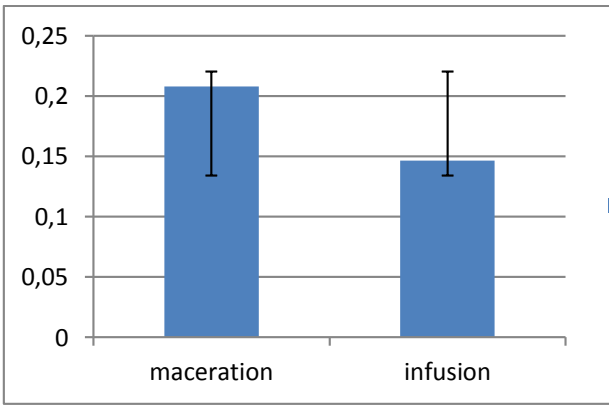

Figure 5. *appearing concentration in (mol / l) of flavonoids in the two extracts*

Flavonoids are endowed with hypoglycemic and antidiabetic properties according to the results of several studies carried out [10] and [13]. Several mechanisms are attributed to flavonoids for this activity. According to these authors

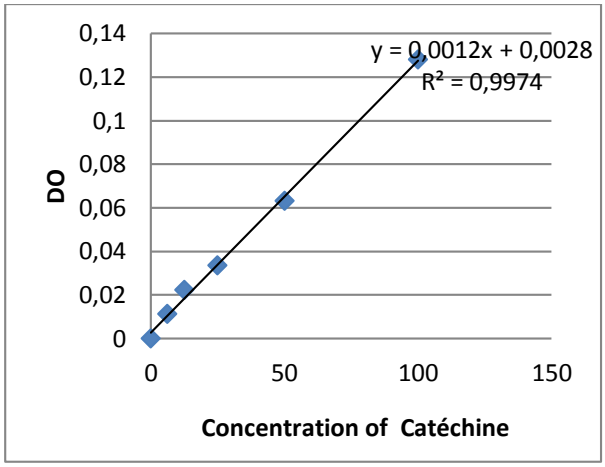

***Figure* 6.** *Catechin calibration curve*

From the catechin calibration curve we calculated the tannin contents of the plants studied, the results are shown in the following figures:

a) tanins condensés

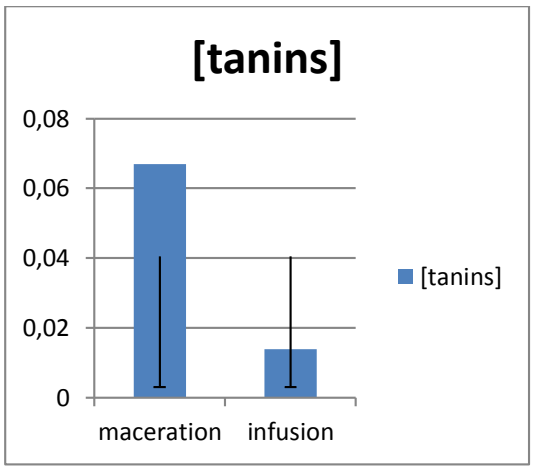

***Figure 7*.** Appearing concentration in (mol / l) of the tannins condensed in the two extracts

Tannins are indicated by its action on diabetes itself at the cellular level, by promoting the action of insulin (by reducing insulin resistance) and on the complications of diabetes by their antioxidant and anti-enzymatic power, neutralizing the effect of free radicals and limiting the inflammatory reaction in different tissues.

Antioxidant activity

.DPPH (2.2'-diphenyl-1-picrylhydrazyl)

Numerous methods are used for the evaluation of the antioxidant activity of pure phenolic compounds or extracts. Most of these methods are based on staining or decoloring a reagent in the reaction medium, we used the DPPH test for the evaluation of anti-free radical activity and the results are as follows:

. DPPH results

.DPPH (2.2'-diphényl-1-picrylhydrazyl)

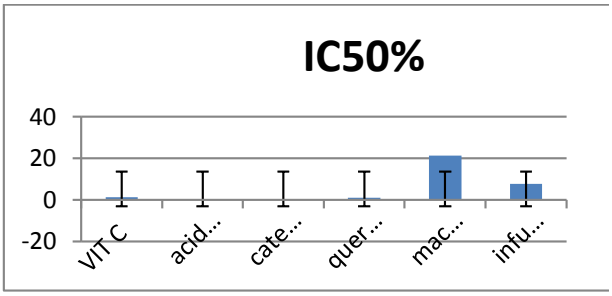

Figure 8. *Concentration of deferent antioxidant DPPH and plant extract*

The antioxidant activity of our extracts was evaluated by DPPH test; DPPH is a free radical, stable, with an absorbance band of 517 nm, used to assess the antioxidant activity of phenolic compounds. In this test, ascorbic acid is used as standard, the results obtained (percentage of inhibtions I%) are represented in the calibration curve (figure n ° 42), having the equation: y = 0.488 + 49 .29

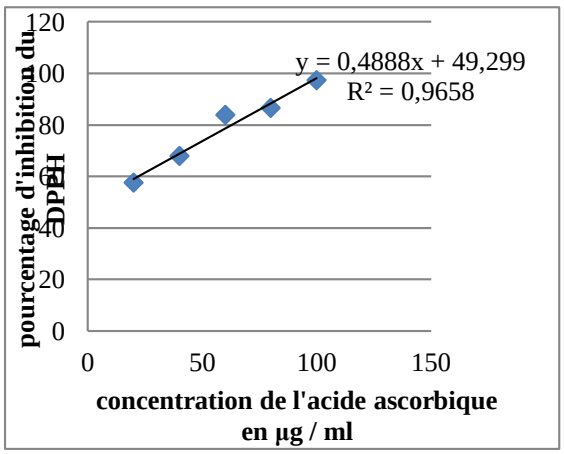

Figure 9. *anti-free radical activity of ascorbic acid by the DPPH test*

The percentage of anti-free radical activity is calculated according to the following equation: anti-free radical activity (%) = (Ac-At) / Ac * 100

The results of the anti-free radical activity by the DPPH test are presented in the following figures: From these results, it can be seen that the percentage inhibition of the free radical increases with increasing concentration. The inhibition rates of DPPH recorded in the presence of the various extracts are lower than those of ascorbic acid. The anti-radical activity is carried out by the 2,2-diphenyl-1 picrylhydrazyl radical method (DPPH) which is a method frequently used for its simplicity. This method is based on the reduction of an alcoholic solution of DPPH in the presence of an antioxidant which gives an hydrogen or an electron, the non-radical form DPPH-H is formed [7]. The inhibition of the discoloration of the DPPH radical depends on the concentration of the various extracts used and the control (reference antioxidant) (µg / ml).

The antioxidant activity of the extracts is expressed in IC50, this parameter has been used by several groups of researchers to present their results, it defines the effective concentration of the substrate which causes the loss of 50% of the activity of the DPPH radical. (Color). These IC50s are determined from graphs where the abscissa represents the concentration of the crude extract and ordinate the antioxidant activity as a percentage.

Anti-radical effect

The concentrations which trap 50% of free radicals or effector concentrations (EC50) are calculated and represented in table n ° 09

Figure 9. Anti-free radical activity of ascorbic acid by the DPPH test

The DPPH radical method, used in this study, is a common procedure in which the antioxidant activity of the test sample is estimated by the degree of discoloration of the DPPH solution. This violet chromogen is easy to use, has high sensitivity, allows rapid analysis of the antioxidant activity of large numbers of samples and gives reproducible results. [10] The anti-free radical activity profiles obtained reveal that the extracts tested have dose-dependent activity. The results obtained with this test show that the hydromethanolic extracts of Spergularia. These results suggest that the extracts contain free radical scavengers acting as primary antioxidants. The action of these antioxidants is believed to be due to their ability to donate hydrogen atoms or electrons mainly derived from the A-ring hydroxyl of flavonoids. [14]

V. Conclusion

The study of the antioxidant activity of extracts from the species Spergularia rubra using the DPPH free radical scavenging method showed that both methanolic and aqueous extracts have moderate antioxidant activity. These extracts could therefore constitute an alternative to certain synthetic additives. However, this activity remains significantly lower than that of ascorbic acid, but these are crude extracts containing a large number of different compounds. It is therefore very likely that they contain compounds which, when purified, may exhibit activity comparable to that of ascorbic acid. Further research is needed to identify, isolate and purify these constituents.

Thanks

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VI. References

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