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The Flavonoids for a plant grows in the arid and semi-arid zone of the northern Sahara of Algeria - *Atriplex Halimus* L.

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ABSTRACT

Atriplex halimus L. is particularly well adapted to arid and salt-affected areas. In this species, salinity resistance is often attributed to the presence of vesiculated hairs covering leaf surface and containing a large amount of salt. Atriplex halimus L. (Chenopodiaceae) is a perennial shrub native to the Mediterranean basin with excellent tolerance to drought and salinity. The species is present in semiarid to sub-humid areas of the north Mediterranean and in arid zones from North Africa and the eastern Mediterranean. The main aim of this study was to identify a medicinal plant used in the Ouargla (Est-southern Algeria) for the treatment of several human pathologies. This plant is an important source for livestock in nitrogenous matter, it is an effective and relatively inexpensive tool in the fight against erosion and desertification and rehabilitation of degraded lands. Phytochemical investigation is applied to the majority of extracts of the powder of the aerial parts of Atriplex halimus L. Different chromatographic methods after liquid-liquid extraction are used; it is the thin layer chromatography (TLC) and paper using multiple systems and chemical revelations. This study followed by an evaluation by the phenol assay the Folin-Ciocalteu method, using gallic acid as a reference for phenols and quercetin for flavonols. Some polar extracts showed an interesting result better than the less polar extracts.

Keywords: *Atriples halimus* L., Chenopodiaceae, Flavonoids, Phenols evaluation, Extraction

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INTRODUCTION

ATRIPLEX species (saltbushes) are dominant in many arid and semi-arid regions of the world, particularly in habitats that combine relatively high soil has been identified on all continents [1, 2]. The Mediterranean Basin, with 40-50 Atriplex species, mostly in its southern and eastern bordering areas, is a region where saltbushes have been extensively used as fodder reserves during periods of scarcity (e.g. drought and cold periods), and as a supplementary forage resource in arid and semi-arid countries [3]. *Atriplex halimus* L. (Chenopodiaceae) (Mediterranean saltbush) is a halophytic shrub that is widely distributed in arid and semi-arid regions around the Mediterranean basin. It grows on a variety of soils, from fine to coarse texture, with varying degrees of salinity [4]. *Atriplex Halimus* L. has been used as traditional cures for many conditions for thousands of years. This plant is very effective to remove the ovaries cysts (from experience) as a testimony. Although in some cases ineffective, it used to

treat syphilis and Arabic indigenous herbal practitioners employ the leaves to treat heart diseases and diabetes and rheumatism. The antidiabetic effect has been developed further, in a product combining leaf extracts of *Atriplex Halimus* L., Juglans regia L., Olea europea L. and Urtica dioica L. [5, 6]. Extracts of the aerial parts of *Atriplex halimus* L. obtained with different solvents (alkaloids, steroids, flavonoids and glycosides) showed antibacterial activity against various Gram-positive and negative pathogenic bacteria [6]. Evaluation by the phenol assays the Folin-Ciocalteu method, using gallic acid as a reference for phenols and quercetin for flavonols. The butanol extract showed some interesting results better than the less polar extracts.

MATERIALS AND METHODS

A. Vegetal Material

The aerial parts of *Atriplex halimus* L. this plant was used for her therapeutic use. It was collected in February 2014 in the south-eastern of Algeria, Ouargla region. The parts of the plant were dried in the open air and protected from light and for few days and then sprayed with a fine powder mill.

B. Extraction

The powder of the areal parts of *Atriplex halimus* L. were macerate ether petroleum thrice and then filtered. The powder dried for 5 hours at 30 °C. This step flowed by cold maceration with 80 % for 24 hours at room temperature three times. All the solutions were filtrated and extracted by solvents with different polarity (dichloromethane, ethyl acetate and n-butanol). The crude extracts were filtered and dried using rotavapor buchi r-200. Crude extracts filtered using a whattman filter paper (110 mm Φ). The extraction efficiency was quantified by determining the weight of the extract and the percentage yield was calculated to be 15 %. This study followed by chromatographic study TLC and paper.

C. Determination of Total Phenolic Content

Although quantitative determination of polyphenols is hampered by their structural complexity and diversity, several methods have used to determine polyphenols in plant extracts. The Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry. A physicochemical analysis included the evaluation Polyphenols were analyzed using Folin-Ciocalteau reagent method. Total phenolic content of the extract in our work was determined by the Folin- Ciocalteu reagent method [7]. The Folin-Ciocalteu method is described in several pharmacopoeias. The reaction forms a blue chromophore constituted by a phosphotungstic phosphomolybdenum complex, where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds. However, this reagent rapidly decomposes in alkaline solutions, which makes it necessary to use an enormous excess of the reagent to obtain a complete reaction. This excess can result in precipitates and high turbidity, making spectrophotometric analysis impossible. To solve this problem, Folin and Ciocalteu included lithium salts in the reagent, which prevented the turbidity. The reaction generally provides accurate and specific data for several groups of phenolic compounds, because many compounds change color differently due to differences in unit mass and reaction kinetics [8]. 10µl of the plant extracts with different concentrations/standard was mixed with 0,5 mL of Folin-Ciocalteu reagent [previously diluted with water 1:10 v/v] and 3 mL of sodium carbonate (20%). The mixtures were vortexed for a few seconds and allowed to stand for 30 min at 30° C for color development. Absorbance of samples and standard was measured at 760 nm using a spectrophotometer (JASCO-V-530) against blank, with a quartz cell (1 cm path length). The total phenolic content of the plant extract was calculated as the gallic acid equivalent.

D.Determination of Total Flavonoid Content

Total flavonoid content was determined by aluminum chloride method [9]. One milliliter of the plant extract was mixed with 3 mL of methanol, 0.2 mL of aluminum chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water. The mixture was placed at room temperature for 30 min, and the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer against blank. The total flavonoid content of the plant extract was calculated as the quercetin equivalent.

RESULTS AND DISCUSSION

Use The revelation with UV lamp, the color and RF comparison accorded to Wagner (1999) in chromatography study of extracts with TLC and paper showed us that extracts rich in phenolic compounds. The chromatography with wattman paper N° 3 doesn’t show good results for all extracts leaves and stems of the plant.

The total phenolic content of the aqueous extract of *Atriplex halimus* L. was determined using the Folin–Ciocalteu reagent and expressed as gallic acid equivalent per gram of plant extract. To establish the linearity of the proposed method, five stock solutions were prepared, in two replicates. The total phenolic content of the test fractions was calculated using the standard curve of gallic acid (y = 3.4208 x; R^2 =0.9993) fig. 1: The determination of phenolics using Folin-Ciocalteu reagent, for all extracts of *Atriplex halimus* L. showed adequate results in phenolic compound, but the high result in this plant was found in butanolic extract in leaves and stems 1.2 mg/g phenolic content (fig.2).

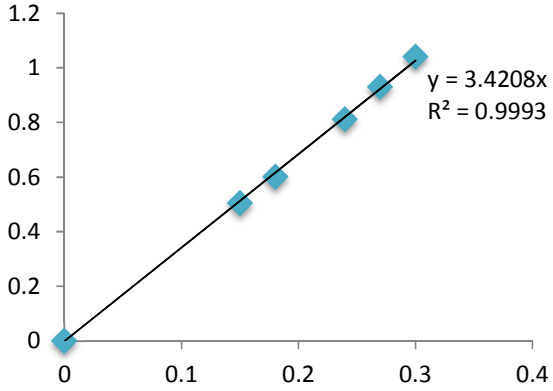

Fig.1: Linearity curve of gallic acid calibration

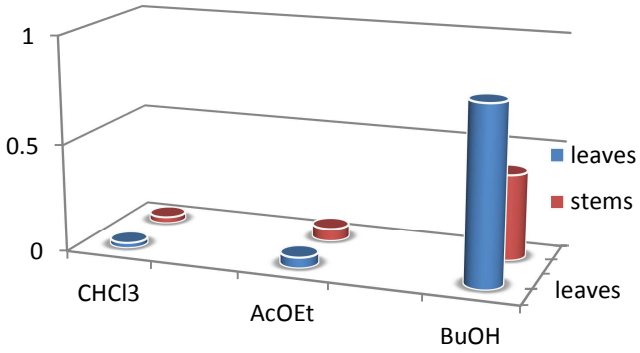

Fig. 2: Extracts concentration of *Atriplex halimus* L. in phenolic compounds

Aluminum chloride colorimetric methods were used to determine the total flavonoid content of the aqueous extract of *Atriplex halimus* L. Plant extract samples were mixed with 1.5 ml of methanol, 0.1 ml 10% $AlCl_3$, 0.1 ml of 1M. Potassium Acetate and 2.8 ml distilled water. It remained at room temperature for 30 minutes the absorbance of the reaction mixture was measured at 415 nm with double beam U.V spectrometer. The calibration curve was prepared by preparing quercetin solution at concentration 20 to 100 µg/ml in methanol total flavonoid content was calculated using the standard curve of quercetin (y = 11,499 x + 0,02 ; R^2 = 0.9988) and expressed as quercetin equivalent per gram of the plant extract. Aqueous extract of *Atriplex halimus* L. was found to have 1,385 mg/g flavonoid (fig .1).

$$TFC\ (\%) = \frac{(\text{absorbance x dilution factor})}{(E^{1\%,1cm} \text{ x weight of extract (gm)}} \text{ x } 100, \quad E^{1\%},$$

1^{cm} : Specific absorbance of the quercetin and $AlCl_3$ complex (500).

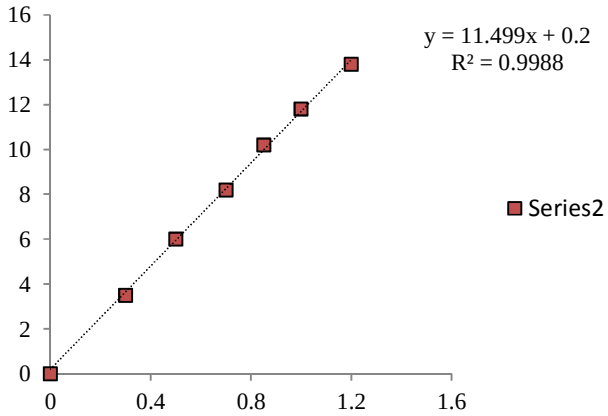

Fig. 3: Linearity curve of Quercetin concentration g/L

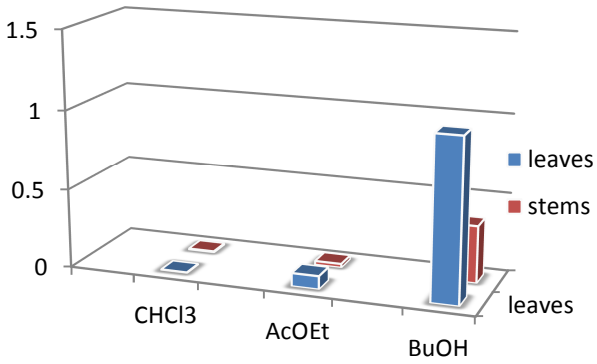

Fig.4: Extracts concentrations of *Atriplex halimus* L. in flavonoids

CONCLUSION

The Results of our study suggest the great value of the specie *Atriplex halimus* L. for use in phytotherapy. Based on this information, it could be concluded that this plant is natural sources of Flavonoid. It is noticed that the highest concentration of phenolic compounds in the extracts were obtained using solvents of high polarity; the butanolic extract of stems and leaves but the extract of leaves was butter then stems and folin – ciocalteu method for total phenolic contain clears that *Atriplex halimus* L. contain large amount of phenolic compound Other outstanding studies on this plant to isolate the compounds natural active for use it in pharmacies.

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