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Solvent Effects on Phenolic Contents and Antioxidant Activities of the *Echinops Spinosus* and the *Limoniastrum Monopetalum.*

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ABSTRACT

Limoniastrum monopetalum is a traditional medicinal species whose leaves exhibit antidysenteric properties against infectious diseases. Even the *Echinops spinosus* is used as a vasoconstricteur in varix and hemorrhoid. In this study, four kinds of extracts were used to examine the effect of extraction solvent system on polyphenol contents and DPPH scavenging activity. Results showed that polyphenol contents and antioxidant activities varied considerably as function of solvent polarity. Plant extract using ethanol showed the highest polyphenol content with the two plants. Moreover, antiradical capacities against DPPH are maxima in an ethanol extract of two plants. Our findings identified the appropriate solvent for extracting phenolics which might provide a rich and novel source of natural antioxidants.

Keywords: Phenolic compounds, *Limoniastrum monopetalum*, *Echinops spinosus*, Antioxidant activity.

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INTRODUCTION

The use of plants for healing dates from prehistoric times and all peoples on all continents this ancient tradition. Despite the efforts of chemists synthesize new molecules; over 25% of drugs in developed countries derive directly or indirectly from plant [1-3]. However, as sources of drugs, plants are still under exploited especially in the field of medical [3-7]. Natural products and naturally derived components from plants have their place in aromatherapy, pharmacy, perfumery, cosmetics and food preservation. Their use is linked to their broad spectrum of biological activities recognized [8-11]. The use of plants as antioxidants in processed food is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants [12-15]. A great number of plants contain chemical compounds exhibiting antioxidant properties. Antioxidants are useful for providing protection against oxidative damage. Hence, adequate amounts of antioxidants are important to prevent build of free radicals and oxidative damage in the body [16]. Polyphenols are the most abundant antioxidants in our diet and are widespread constituents of fruits, vegetables, cereals, olive, dry legumes and beverages [17-19]. Consequently, it is common practice to measure both phenolic content and antioxidant activities when investigating the antioxidant potential of plants as various studies have shown that plants rich in phenolics are also potent antioxidants [20]. Different solvent systems have been used for extraction of polyphenols from plant material [21].

Extraction yield is dependent on the solvent and method of extraction [12,22-24]. In view of the potential of plants to provide a natural source of antioxidants, studies are on going in search of plants with extracts of high phenolic content and antioxidant activities. Saharian plants are known by their resistance to several stress factors, their high content of natural antioxidants such as phenolic compounds, have attracted a great deal of public and scientific interest because of their health promoting effects as antioxidants [19,25]. *Limoniastrum monopetalum* and *Echinops spinosus* are plants that thrive in desert conditions and have a wide ecological range for soil [26,27]. *Limoniastrum monopetalum* is a plant belongs to the family Plumbaginaceae. It is a traditional medicinal species which leaf infusion exhibits antidysenteric properties against infections diseases [24,28]. *Echinops spinosus* is a perennial herb growing 1 meter, and more, with erect brownish to reddish stems. As a medicinal plant, it was frequently employed in folk medicine as an abortifacient and a diuretic and for blood circulation, diabetes, gastric pain, indigestion and sposmolytic problems. The aim of the present study was to as certain the potential effects of extracting solvents on *Limoniastrum monopetalum* and *Echinops spinosus* total phenolics, flavonoids and condensed tannins contents. In addition, antioxidant activity was carried out by means to quench DPPH radical.

MATERIALS AND METHODS

Chemical and reagents

Folin–Ciocalteu reagent, sodium carbonate anhydrous (Na_2CO_3), gallic acid, aluminium chloride hexahydrate solution ($AlCl_3$, $6H_2O$), vanillin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), quercetin, Catechin and chlorhydric acid (HCl).

Preparation of plant extracts

Plants were dried in the shade to ambient temperature until total dehydration. Dried aerial part of *Limoniastrum monopetalum* and root of *Echinops spinosus* were blended into fine powder and stored in a dark at a dry place.

The powdered part of plants was extracted by maceration in a solvent for 36 hours at room temperature. The extracts were concentrated by rotary evaporation under vacuum and the yield of extraction was determined. All the dried extracts were preserved in the refrigerator until further use.

Determination of total phenolics

Phenolic content was determined according to the Folin–Ciocalteu method [12,24,29,30]. 300 µl of diluted sample extract were added to 1500 µL of Folin–Ciocalteu reagent (10/100). After 1 min, 1200 µL of aqueous sodium carbonate (7.5 g/100 mL) was added. The mixture was vortexed and allowed to stand at room temperature with exclusion of light for120 min. The absorbance was read 760 nm, using a UV-Visible spectrometer (BECKMAN DU 800) in a 10 mm quartz cuvette. The total phenolic content in the extract was calculated from the calibration curve, using gallic acid as a standard, and the results were expressed as mg of gallic acid equivalents (mg GAE) per 100 g plant. Three determinations were performed on each sample. For gallic acid, the curve of absorbance versus concentration is described by the equation: $Y= 8.9321 X + 0.0102$ ($R^2 = 0.9987$).

Determination of flavonoid content

The content of flavonoid was determined colorimetric assay using quercetin as reference [5, 31]. 1 mL of plant extract in methanol was mixed with 1 mL aluminium trichloride in methanol (2 g/100 mL). The absorption at 430 nm was read after 10 min. Blank samples were prepared from 1mL plant extract and l mL of methanol.

The content of flavonoids, as quercetin equivalents (mg Queq/100g plant) was calculated from the calibration curve, using quercetin as standard. The quercetin calibration curve was prepared by using (2.5 to 40 µg/mL) quercetin methanolic solution.

The curve of absorbance versus concentration is described by the equation: $Y = 0.0282 X – 0.0114$ ($R^2 = 0.9989$).

Determination of condensed tannins content

Condensed tannin content was measured using the modified vanillin assay [32-35]. To 1 mL of diluted sample were added 2.5 mL of methanol vanillin solution (1 g/100 mL) and 2.5 mL of methanol HCl solution (8 mL/100 mL) respectively. The mixture was allowed to stand for 20 min at temperature (30°C), and the absorption was measured at 500 nm against 1 mL of diluted sample with 5 mL methanol HCl solution (4 mL/100 mL) as a blank. The amount of total condensed tannins is expressed as mg catechin /100 g plant. The calibration curve range was 0-300 µg /mL and the equation was: $Y= 0.3472X + 0.0019$

(R^2 = 0.9989). Catechin was used as standard. All samples were analyzed in three replications.

DPPH radical–scavenging activity

The antioxidant activity of different solvent extracts of *Limoniastrum monopetalum* and *Echinops spinosus* was measured in term of hydrogen donating or radical-scavenging ability using the stable DPPH method [24,36,37].

The extract was diluted in methanol at different concentrations (0; 50; 100; 200; 300; 400; 500; 600; 800; 1000 µg /mL), then 1 mL of each diluted plant extract was added to 0.5 mL of a 20 mg/L DPPH methanolic solution.

The mixture of different extract concentration and DPPH were placed in the dark at room temperature for 30 min. The absorbance of the resulting solution was then read at 517 nm. The antiradical activity was expressed as IC50 (µg/mL). The ability to scavenge the DPPH radical was calculated using the following equation:
DPPH scavenging effect % inhibition = $(A_0 - A_1/A_0)*100$.

Where A_0 is the absorbance of the control at 30 min and A_1 is the absorbance of the sample at 30 min. All samples were analyzed in three replications.

RESULTS AND DISCUSSION

Extraction yields

The difference between extractions yields obtained depended on the raw material analyzed (Table 1). The highest yield in extractions was achieved by the polar solvents. Variation in the yields of various extracts is attributed to polarities of different compounds present in the plant and such differences have been reported in literature [38].

Table-1: Extraction yield.

Solvent	Yield (%)	
	Limoniastrum monopetalum	*Echinops spinosus*
Chloroform	0.34	0.27
Ethanol	0.87	0.27
Ethyl acetate	0.23	0.31
Hexane	0.19	0.20

Yield: is expressed as % of dry plant powder.

Polyphenols contents

The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Furthermore, solvent polarity will play a key role in increasing phenolic solubility [39]. Therefore, it is hard to develop a standard extraction procedure suitable for the extraction of all plant phenols.

The amount of total phenolics varied in the different extracts and ranged from 6.7 to 85.6 mg GAE/100 g of aerial parts of dry *Limoniastrum monopetalum* and from 7.3 to 19.3 mg GAE/100 g of roots of dry *Echinops spinosus* (Table 2). Among the four extraction systems used, the ethanol extract showed the highest amount of phenolic compounds. Similar results were noted when the lowest amount of phenolics was recorded in non-polar extracts from aerial parts of *Limoniastrum monopetalum* and roots of *Echinops spinosus* [40].

The results showed that (table2), for both plants, polyphenol content was strongly dependent on the solvents. Polar fractions had more phenolics than had non-polar fractions. As mentioned above, our results clearly showed that a higher content of polyphenols was obtained with an increase in the polarity of the solvent used.

From this study, ethanol extract of aerial parts of *Limoniastrum monopetalum* and roots of *Echinops spinosus* has a higher flavonoids content compared to the other extracts (Table 2). Hexane exhibiting the lowest capacity to extract flavonoids from the two plants and this result reported in literature for *Limoniastrum monopetalum* [24]. The ethanol provides the maximum of condensed tannins (Table 2).

Table-2: Total phenols content, total flavonoids content and tannins condensed content of extracts.

Solvant	Total phenolics mg GAE/100g	Total flavonoids µg QuE/100g	Condensed Tannins mg CE/100g
Limoniastrum monopetalum			
Ethanol	85.6	4825.6	37.1
Chloroform	6.7	706.0	3.0
Hexane	7.0	0	3.5
Ethyl acetate	10.3	0	9.3
Echinops spinosus			
Ethanol	19.3	680.1	10.5
Chloroform	14.4	179.5	7.5
Hexane	7.3	144.1	2.6
Ethyl acetate	14.6	443.2	8.1

Antioxidant activities by DPPH radical-scavenging activity

In the objective to choose the adequate solvent for antioxidant capacity, the antiradical activity (DPPH test) was also evaluated using the same pure solvents. Results showed that different extracts possess a significant variability in theirs inhibitory activity against this radical (Figure 1) and (Figure 2). Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers [41,42].

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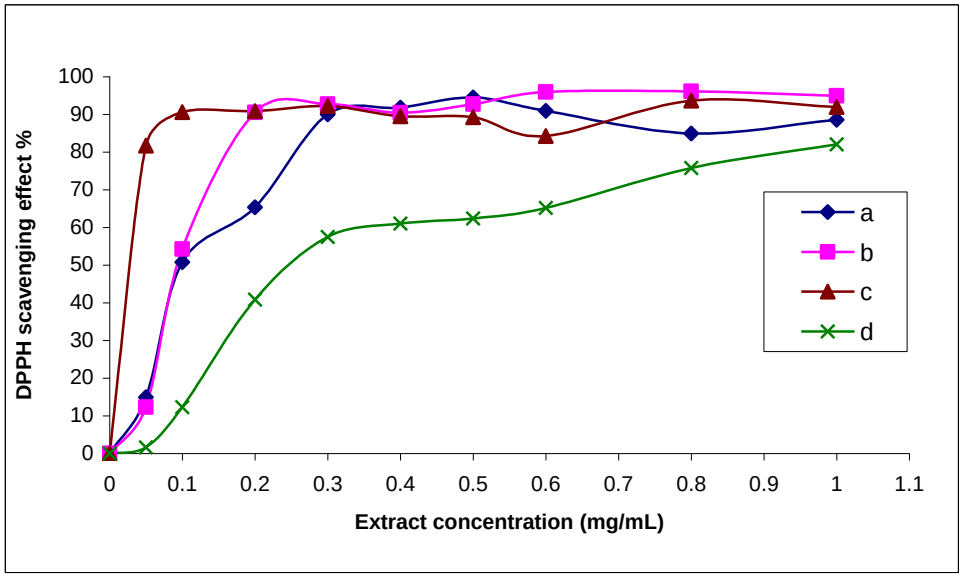

Figure.1: Pourcentage of inhibition as function of the extract concentration of *Limoniastrum monopetalum*. a: ethyl acetate extract, b: Chloroform extract, c: ethanol extract, d: hexane extract.

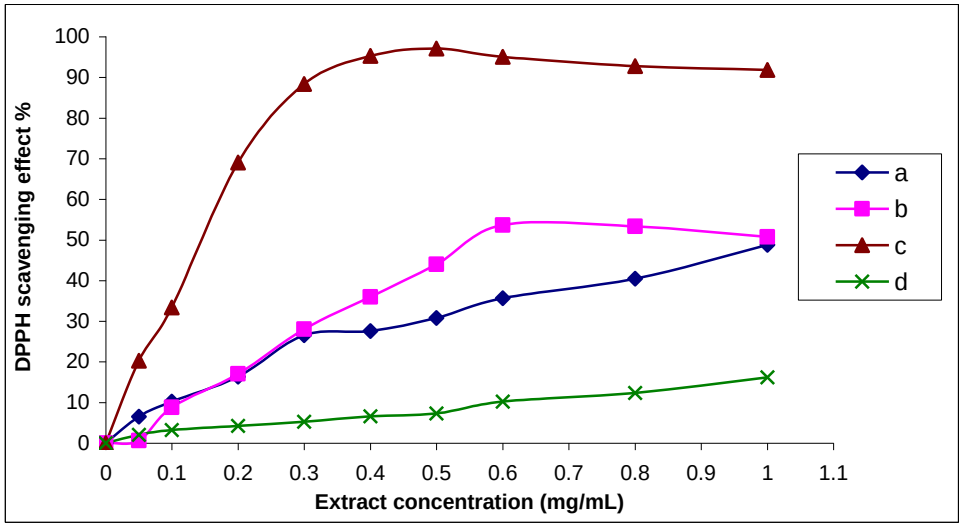

Figure.2: Pourcentage of inhibition as function of the extract concentration of *Echinops spinosus*. a: ethyl acetate extract, b: Chloroform extract, c: ethanol extract, d: hexane extract.

In fact, the ethanol extract showed the highest ability to reduce DPPH radicals. The IC_{50} values are 30 and 147 µg/mL for *Limoniastrum monopetalum* and *Echinops spinosus* extracts respectively. The phenolic content in ethanol extract of aerial part of *Limoniastrum monopetalum* is higher than that of roots of *Echinops spinosus* (Figure 3 and Figure 4) and the radical scavenging activity is likely to be due to the phenolics however, phenols may not be solely responsible in the case of aerial part of *Limoniastrum monopetalum* due to a low correlation of $R^2 = 0.40$ between the phenolic content and % inhibition of DPPH.

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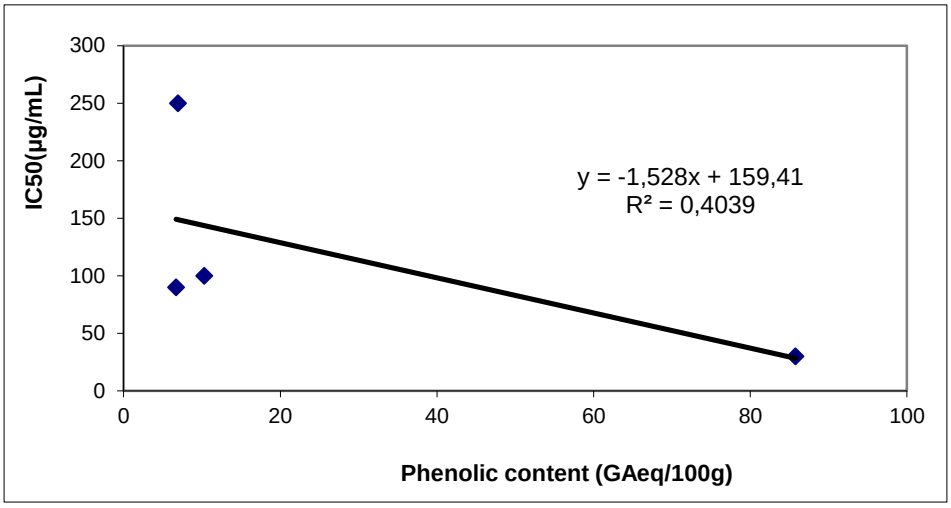

Figure.3: Correlation between total phenolic content and antiradical activity (IC_{50} values) of aerial part of *Limoniastrum monopetalum*.

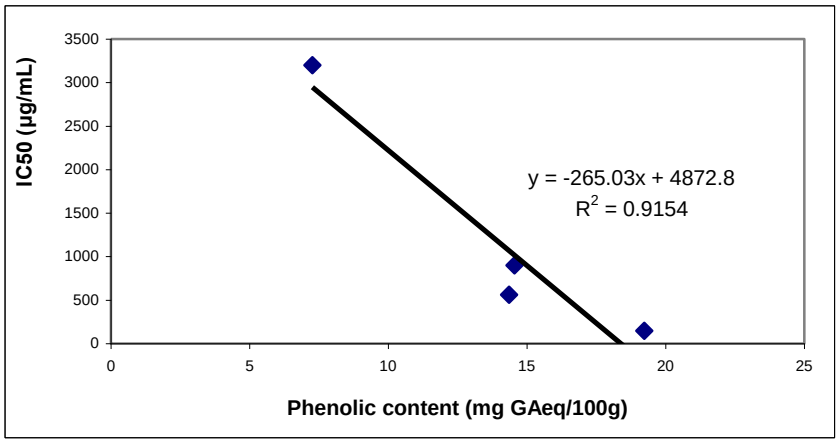

Figure.4: Correlation between total phenolic content and antiradical activity (IC_{50} values) of roots of *Echinops spinosus*.

A low correlation of R^2=0.46 obtained for the aerial part of *Limoniastrum monopetalum* extract and R^2 = 0.43 for roots of *Echinops spinosus* implies that flavonoids are not likely to be responsible for the antioxidant activity of the two plants (Figure 5 and Figure 6). It is also known that only flavonoids of a certain structure and particular hydroxyl position in the molecule determine antioxidant properties. This property depends on the ability to donate hydrogen or electron to a free radical [5]. There was a good correlation between the condensed tannins content and the DPPH assay R^2 = 0.93 for roots of Echinops spinosus, but a low correlation of R^2 = 0.44 was obtained in the case of aerial part of *Limoniastrum monopetalum* (Figure 7 and Figure 8). This indicates that tannins condensed present in the extract are involved in the free-radical scavenging activity of the *Echinops spinosus*, but other phytochemicals may also be responsible in the case of *Limoniastrum monopetalum.*

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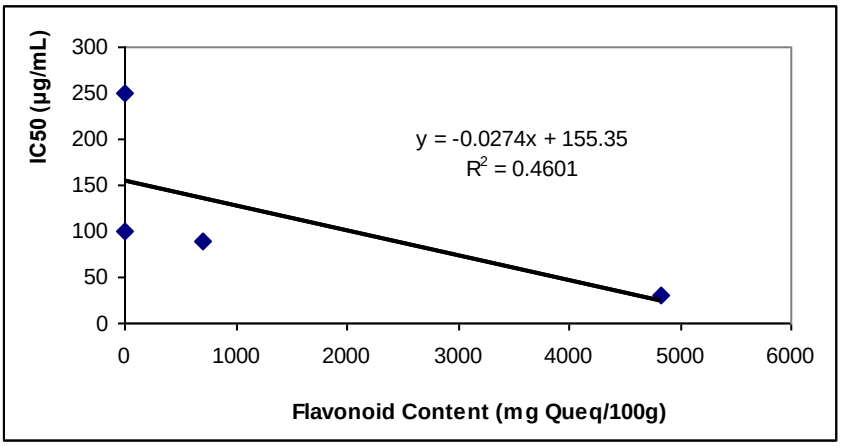

Figure.5: Correlation between flavonoid content and antiradical activity (IC_{50} values) of aerian part of *Limoniastrum monopetalum*.

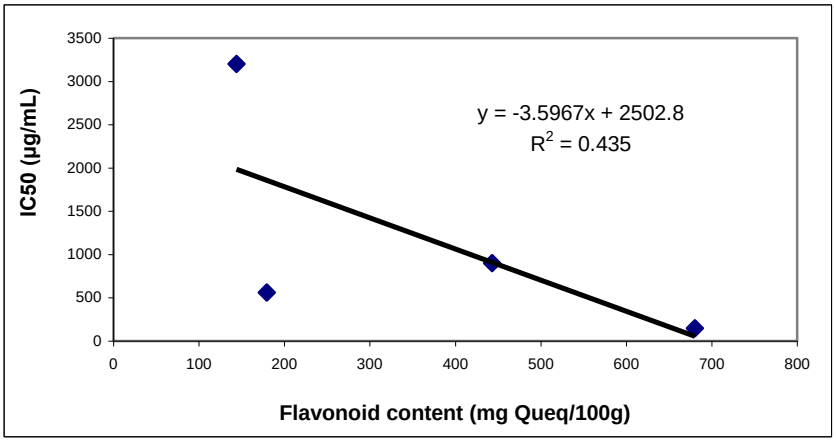

Figure.6: Correlation between flavonoid content and antiradical activity (IC_{50} values) of roots of *Echinops spinosus*.

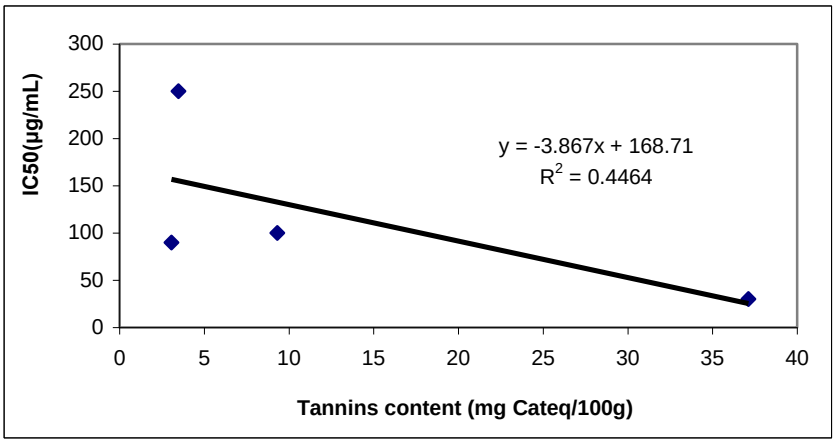

Figure.7: Correlation between condensed tannins content and antiradical activity (IC_{50} values) of aerial part of *Limoniastrum monopetalum*.

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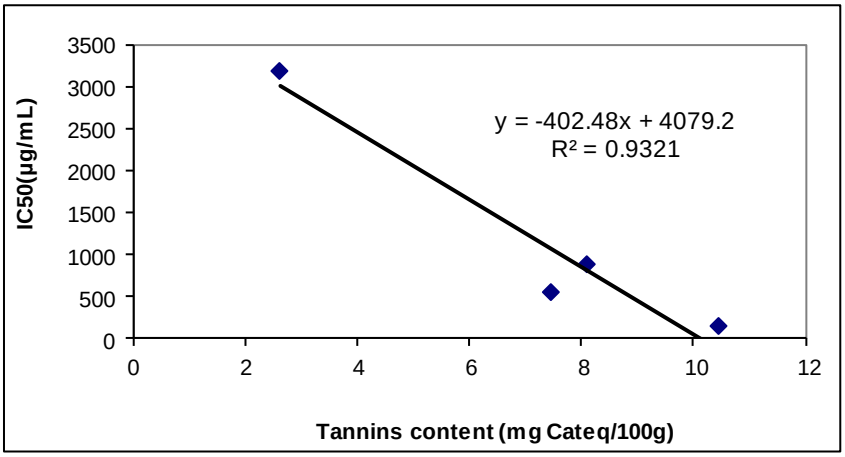

Figure.8: Correlation between condensed tannins content and antiradical activity (IC_{50} values) of roots of *Echinops spinosus*.

CONCLUSION

Analyses of the phenolic content and antioxidant activities in roots of *Echinops spinosus* and aerial part of *Limoniastrum monopetalum*, extracted with solvents of varying polarities, are useful in providing information on the potential of those plants as a source of phenolic antioxidants. At the same time, they also provide data on the characteristics of the antioxidants present in the two plants. In fact, it can be concluded that the extracts obtained using higher polar solvents were more effective than less ones. From this study, it was found that the ethanol extract of the two plants contained the most antioxidant activities and that the antioxidants were mainly polar compounds. Ethanol seems to be recommended solvent for phenolic extraction as well as the appreciation of antioxidant activities for *Limoniastrum monopetalum* and for *Echinops spinosus*.

The tannins and the flavonoids seems to be responsible for many of the above actions as oxidative damage is implicated in most disease processes.

For Echinops spinosus, there is a good correlation, between phenolics, tannins and antioxidant activity. That suggests their use in the prevention and treatment of a number of these diseases. For *Limoniastrum monopetalum*, the correlation is medium between the phenolics, favonoids, tannins and antioxidant activity. That activity can be due to other compounds family.

A number of industrial applications, particularly in the food and medicinal fields might be found in the extract having the best antioxidant.

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