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Study Of The Effect Of Drying Methods On Biochemical Determination Of Some Spontaneous Plants Character Medicinales In The Northen Algerian Sahara

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ABSTRACT

The main objectif of the present study was to investigate he effectect of different draying methods on the biochemical characteristics of tow spontaneous medicinal plants (*Asphodelus tenuifolius* and *Retama retam retam*) in the region of Ouargla and Ghardaïa. In addition to studding their biochemical compounds. The experiments was based on draying the leaves by four different methods, (in the shade, solar dryer, oven (45 °C), by lyophilization), the and fresh plants were used as control. The extraction was performed by using cold maceration with mixter of methanol- water (70/30). Lastly the different extracts were used for the biochemical analysis. The results of polyphenol analysis for plants showed a higher yield for the extracts of *Retama retam* and *Asphodelus tenuifolius* (220,89 µg GAE / g DW and 101,82 µg GAE / g DW) for fresh and Lyophilizerd method respectively. In parallel, their flavonoid content was 37,44 µg QE /g DW at the oven, and 16.10 µg QE / g DW at that Lyophilizerr. Furthermore the contents of tannins that extracted from plants dried by oven was 7.09 µg GAE /g DW *for Retama retam retam*, and 0.78 µg GAE / g DW for *Asphodelus tenuifolius*. Regarding the biological test for alkaloids and reducing sugars, the results showed the presence of these compounds in all extracts except the extracts of fresh samples and solar draying of *Retama retam retam.* Also, the extracts of oven and solar draying from *Asphodelus tenuifolius* were free from the alkaloids. Moreover, regarding the antibacterial activity, the results showed low activity for both plants over the two bacterial strains: *Pseudomonas aeruginosa* and *Escherichia coli*. It was concluded that the method of drying oven is suitable to retain the most of chemical compounds of *Retama retam retam*. However, it was observed that lyophilization was the most conservative method for *Asphodelus tenuifolius.* On the other side, it was concluded that *Retama retam* has low antibacterial activity against *Pseudomonas aeruginosa;* in parallel *Asphodelus tenuifolius* has remarkable antibacterial activity against both bacterial strains studied.

KEYWORDS: medicinal plants, drying methods, extraction, antibacterial, biochemicals.

INTRODUCTION

The Saharan flora, is remarkable for its adaptation to a dry climate and salty soil [1]. It appears to be very poor if we compare the small number of species that inhabit this desert to the enormity of the area it covers only, it to includes 1200 species [2].

Recently, the scientific research was interested in plant compounds that are intended for use in the plant protection field. The molecules derived from so-called natural plants are considered a major source of

drugs; knowing that over 120 compounds from plants are used today in modern medicine and nearly 75% of them are applied according to their traditionals [3].

There are several studies on medicinal plants and spontaneous in the region of Ouargla. As inventories that were made by [4] and [5], studies are accompanied by chemical analyses and other work on phytochemical studies and biological activities of the plant *Limoniastrum guyonianum* [6]. The objectives of this work are designed to study the effect of some drying modes on natural compounds in some wild plants to medicinal character with a simple biochemical assay (determination of total phenols, flavonoids, tannins, biological testing of reducing sugars, alkaloids).

MATERIAL AND METHODS

This work is for contribute to broaden the spectrum of biological active compounds, it may become substitutes for synthetic drugs, and determined to effect the loss of these assets composed, the latter has changed their herbal characteristics, by using different drying modes.

The study focuses on the extraction solutions of 02 medicinal plants (*Retama retam retam, Asphodelus tenuifolius*) and study of their total compounds by assaying a share of total phenols, flavonoids and alkaloids, and other tests proportion of reducing sugars and alkaloids.

The leaves of these plants are subjected to different drying methods are drying in the oven 45°C , shade, , and solar dryer, Lyophilizerr and fresh leaves for each plant order to get 10 different lots.

Sampling of plant species:

The choice of plantes was based on a sample taking into account the structure of the vegetation where ecological floristico- homogeneity criterion is privileged. For good success of the sampling, the season in which the development and floristic diversity is maximum, including annuals is retained. Flowering perennials easy identification[7]. The spontaneous species are harvested in tow areas Ouargla and Ghardaia (Septentrional Sahara) between February and April. The plants was transported to the laboratory in kraft paper which are noted all information about plants.

Preparation of crude extracts (phenolic compounds):

The test plants were macerated in a water-alcohol mixture (methanol / water, 70/30, v / v). Maceration is repeated 3 times with solvent renewal. The three extracts are combined after careful decanting Wattman paper filter, the filtrate is evaporated by rotary evaporator (Buchi) of 45 °C to 60 °C until the total elimination of methanol and dried in the oven with a temperature is not exceeding 40 ºC, after vacuum concentration, the methanol aqueous residue is diluted with methanol, it is put into glass vials, sterile and dark [8].

Biochemical analyzes:

Bioassay of reducing sugars:

Is reacted in the hot test solution on the Fehling previously brought to the boil. The presence of a reducing sugar is manifested by the appearance of a red precipitate [9], [10].

bioassay alkaloids:

2 ml of an extract solution 10% in water containing a drop of concentrated HCl and 3 drops of reagent Bouchardat (iodine 2.5 g, KI and 5 g H_2O 100 ml). A reddish brown precipitation means the presence of alkaloids.

Determination of total phenols:

Secondary metabolites are a wide range of plant molecules, their chemical nature and contents vary widely from one species to another. Several analytical methods can be used for quantification of total phenols. The analysis by the Folin Ciocalteu reagent is used the most.

Polyphenols were determined spectrophotometrically, following the protocol used by **[11]**. 1 ml of the mehanolic extract of the plant is mixed with 5 ml of Folin-Ciocalteu reagent (2M), diluted 10 times and 4 ml of sodium carbonate (Na_2CO_3) at a concentration of 75g/l. The absorbance is measured at 760 nm after incubation for 30 min at Shade. The calibration curve is made by the gallic acid, following the same steps of the assay. All measurements were repeated 3 times.

Determination of flavonoids:

The flavonoids are widely used class of secondary metabolites in the plant kingdom **[12]** Determination of flavonoid Quercetin used the method. 1ml of the extract of the plant is mixed with 1 ml of the solution of aluminum trichloride (20 mg/ml $AlCl_3$). After incubation in the dark for 30 minutes at Shade, the

absorbance of the mixture was measured at 430 nm against a distilled water blank using the same ultraviolet spectrophotometer.

condensed tannins assay (CTLES condensed tannins are determined by the method in the acidic medium vanillin. This method is based on the ability of vanillin reacted with units of condensed tannins in the presence of acid to produce a colored complex measured 500 nm. the reactivity of vanillin with tannin does not imply that the first unit of the polymer. the amounts of tannins are estimated using vanillin method [13]. To quantify the condensed tannins, must be used a standard curve.

Test for antimicrobial activity:

For antimicrobial test was carried out by the disc method [14]. The culture medium consisted of nutrient agar for the isolation and maintenance of parallel bactériennes. En strains, are used the Mueller Hinton agar culture medium for incubating the bacteria with the samples. The microbial support is made of *Escherichia coli*, *Pseudomonas aeruginosa* were isolated from pathological material from Biskra bacteriology laboratory. These strains were cultured in dishes of kneaded containing nutrient agar. After 24 h incubation at 37°C, microbial suspensions were prepared for each microorganism in water physiology.

RESULTS AND DISCUSSION

The principle of drying is simple and involves removing the water in the plant as soon as possible while preserving the species and active ingredients. In general, the taste and qualities of herbs keep well by drying. In general, the taste and qualities of herbs keep well by drying [15].

Test reducing sugars:

The results of biological testing of reducing sugars are shown in Table I. Reducing sugars are detected by a test in the presence of Fehling leading to a brick-red precipitate. Through Table I, we note the presence of reducing sugars in the two species studied in different drying methods.

the total sugar content varies depending on the climate, season and stage of plant development, for example, high temperatures and low precipitation tend to increase the parietal fraction and reduce the soluble content of plants [16]

The study [17] shows the presence of glucomannan polymer type in several species of the Liliaceae family such as Asparagus officinalis, Edymion mutans and Scilla nonscripta. The high percentage of glucose and mannose, shows the type of glucomannan polysaccharide present in the crude extract of water-soluble polysaccharides leaves of *Asphodelus tenuipholius.*

Through the studies [18] The water-soluble polysaccharides from leaves of *Asphodelus tenuifolius* show a predominance of mannose and glucose 39.25% to 31.55%, followed by 10.92% glucuronic acid and 8 , 9% arabinose. Rhamnose and xylose are presented in small percentage with 5.22% and 4.14% respectively.

Table I: Resultats of biologique test of sugars reducteurs

Plant	Drying mode	Sugars reducteurs
Retama retam	Fresh	+
	Shade	++
	Oven	+ + +
	solar dryer	++
	Lyophilizer	+
Asphodelus tenuifolius	Fresh	++
	Shade	+++
	Oven	+++
	solar dryer	+++
	Lyophilizer	+

Test alkaloids:

Table II presents the test results of the presence or absence of alkaloids in the species studied in different drying methods. To perform this test using the reagent Bouchardat. A reddish brown haste signifies the presence of alkaloids. In the species *Retama retam retam*, the presence of alkaloids observed in samples dried in the open air and in the oven. In parallel, for the species *Asphodelus tenuifolius*, the methanol extracts are dried in the open air and fresh plant extract, are marked by the presence of alkaloids. Table II shows that the method of drying in the open air is best for conservative alkaloids in both species studied. According to [19], natural drying barks of Alstonia boonei outdoors could limit losses alkaloids, one of its active ingredients. The action of light and / or heat have an influence on the total alkaloid content in particular and other chemical extracts of this plant groups in general [20].

According to [20], alkaloids rarely exist in the plant, in the free state, usually, they are combined with organic acids (acetic, citric, malic ..) or tannins. Their content is Very variable. Generally, it is between 1% and 2 to 3% (dry weight). Sometimes we find 10% higher contents (bluffs of quinine).

Table II: Resultats of biologique test of alcaloïdes

Plant	Drying mode	Alcaloïdes
Retama retam	Fresh	-
	Shade	+
	Oven	+
	solar dryer	-
	Lyophilizer	-
Asphodelus tenuifolius	Fresh	+
	Shade	+
	Oven	-
	solar dryer	-
	Lyophilizer	+

Determination of total phenols:

Quantitative analysis of extracts polyphenols was performed by [21] the Folin-Ciocalteu reagent. The calibration curve is made by the gallic acid. The total phenol content is reported in mg gallic acid equivalent / g of plant extract, is determined by an equation: y = a x+b. The results of the test plants have shown in (Fig. 1).

From the results obtained in the species *Retama retam retam*, the highest content of polyphenols is recorded in the fresh extract (220.89 µg GAE / g DW). In parallel to the species *Asphodelus tenuifolius* the best values of total phenols are obtained in Lyophilizerd samples (101.82 µg GAE / g DW).

According to [22], the variation of the levels of polyphenols are according to the species *limonaistrum guyonanum* represents contents of 21,291mg Eq gallic acid / g of extract for the hydroalcoholic extract and 15.266 mg Eq gallic acid / g for the aqueous extract.

According to [23], the distribution of secondary metabolites may change during development of the plant. This may be related to the harsh climatic conditions (high temperature, sun exposure, drought, salinity), which stimulate the biosynthesis of secondary metabolites such as polyphenols. In general, the content of polyphenols differ qualitatively and quantitatively from one plant to another, this can be attributed to several factors:

climatic and environmental factors: geographical area, drought, ground attacks and diseases ... etc. [24]

¬ The genetic heritage, the period of harvest and stage of plant are development [11].
¬ The method of extraction and quantification method can be influence the estimation of the content of total phenols [25].

To get the maximum recovery of polyphenols, methanol is suitable solvent [23]. According to [26], water and methanol are both polar solvents especially extract glycosylated flavonoids and tannins. While flavonoid aglycones are extracted with alcohols or water-alcohol mixtures [27].

aqueous methanol 70% is two times more effective than the pure methanol for the extraction of phenolic compounds of rapeseed [28]

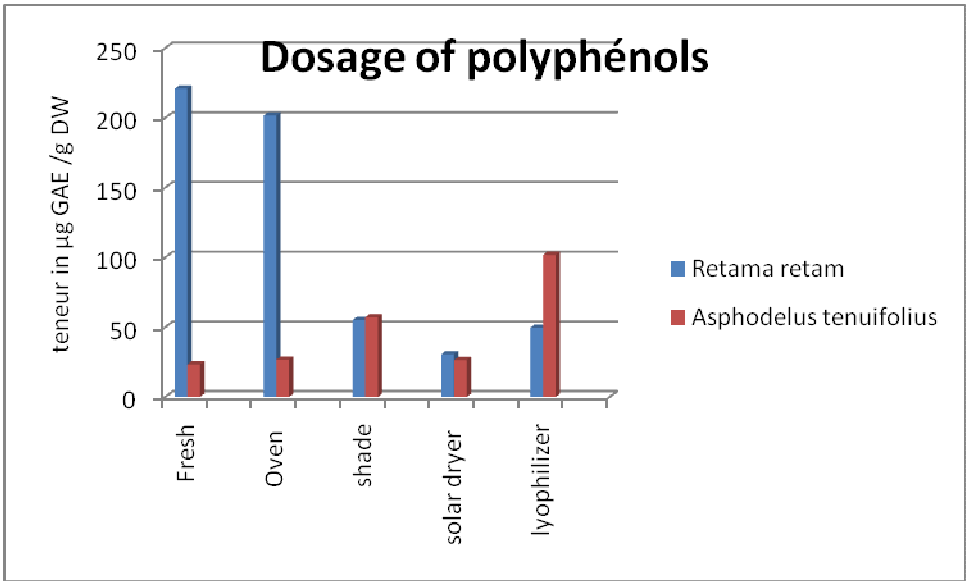

Fig. 1: the content of total phenols in the species studied for different drying methods

Determination of flavonoids:

The assay of flavonoids was performed according to a method of using $AlCl_3$ as the standard method of Quercetin, Fig. 2 shows the concentrations of flavonoids (expressed in µg QE / g DW) in extracts of plants have studied to different drying methods.

A calibration curve was performed with the Quercetin at a wavelength 430 nm. Among the species *Retama retam retam*, the concentration of flavonoids is the highest recorded in the extract of oven 37.44 µg QE / g DW.

Regarding the species *Asphodelus tenuifolius* the best flavonoid contents are marked in extracts of Lyophilizerd dried plants with values of 16.10 µg QE / g DW.

Outside plants that used fresh, it is necessary to very carefully dry those one wishes to keep. It is very important that drying takes place quickly to avoid the damage to plants, their fermentation and loss of their active ingredients (Fig. 2) **[29]**.

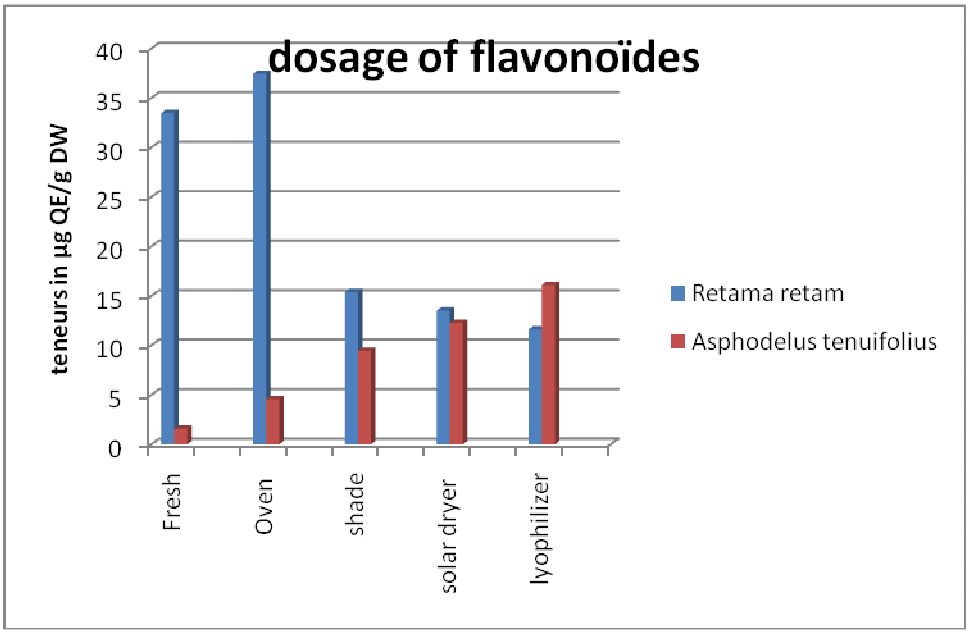

Fig. 2: The tenures of flavonoids in plants studied different drying methods.

condensed tannins assay:

(CTLES condensed tannins are determined by the method in the acidic medium vanillin. This method was based on the ability of vanillin reacted with units of condensed tannins in the presence of acid to produce a colored complex measured 500 nm. the reactivity of vanillin with tannin does not imply on the first unit of the polymer. the amounts of tannins are estimated using vanillin method [13]. To quantify the condensed tannins, must be use a standard curve.

Among the species *Retama retam* and *Asphodelus tenuifolius* oven dried, we see the greatest values of tannins compared to other drying methods that 7,09 µg GAE / g DW and 0.78 µg GAE/g DW respectively (Fig.3).

The study of condensed tannins is currently less advanced; However, they are arguably the most important. It seems definitiVEly demonstrated these tannins are formed by the polymerization of basic molecules with the general structure of flavonoids and which the most important are the flavanols-3 (catechins) and flavanediols-3,4 (leucoanthyocyanidines) [30].

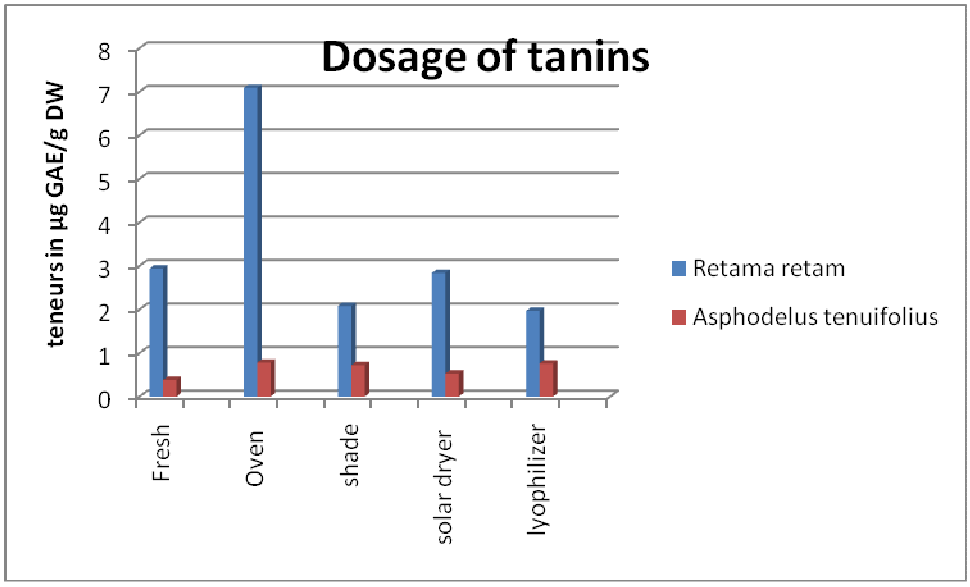

Fig. 3: The tenures of tannins in plants studied to different drying methods.

Antibacterial tests:

The table shows the different results of the antibacterial activity of all species studied in different vis-a-vis methods of drying two bacterial strains.

In the species *Retama retam retam*, there has been a growth inhibition of the bacteria Escherichia coli, they are Very small but there are remarkable circular zones around the disc in the studied extracts which are dried in the open air. But in the same species of plant *Pseudomonas aeruginosa* bacteria test, we see low activity compared to that with Escherichia coli, while no inhibitive observed action against the bacteria fresh plant (Table III).

While, for the species *Asphodelus tenuifolius*, there is weak inhibitory zone for the bacteria *Escherichia coli* or *Pseudomonas aeruginosa* in all drying methods except oven mode and Lyophilizerd to the latter strain which are presented inhibition zones a little remarkable (table VI).

According to [31] and [32] Several studies have also reported that aqueous extracts of different plant family Asteraceae showed no antibacterial activity, while the organic extracts and essential oils of these plants inhibit very significant growth strains tested.

It was reported that the compounds responsible for the antibacterial action seems likely to be the phenolic diterpenoids, which are the main components of the non-polar fraction extracts of plants [33].

As long as the two extracts were the same antioxidant capacity, and that the flavonoid aglycones are insoluble in water whereas glycosylated flavonoids are also readily soluble in water and alcoholic solutions [34], it can be inferred that the two extracts contain glycosylated flavonoids responsible for this activity.

Table III: Resultats of antibactial activity of *Retama retam (Retem)*

	Escherichia coli	*Pseudomonas aeruginosa*
Retama retam F. [1mg/ml]	1 mm	-
Retama retam F. [0,75mg/ml]	2 mm	-
Retama retam F. [0,5 mg/ml]	1 mm	-
Retama retam F. [0,25 mg/ml]	1,5 mm	-
Retama retam oven [1mg/ml]	0,5 mm	0,5-1,5 mm
Retama retam oven [0,75mg/ml]	1 mm	1 mm
Retama retam oven [0,5 mg/ml]	1,5 mm	0,5 mm
Retama retam oven [0,25 mg/ml]	-	1 mm
Retama retam Shade[1mg/ml]	3-4 mm	0,5-1 mm
Retama retam Shade[0,75mg/ml]	3-5 mm	0,5-3 mm
Retem Shade[0,5 mg/ml]	1 mm	2,5-6,5 mm
Retama retam Shade[0,25 mg/ml]	1-4 mm	0,5 mm
Retama retam solar d. [1mg/ml]	-	0,5-2
Retama retam solar d.[0,75mg/ml]	1	0,5-1
Retama retam solar d. [0,5 mg/ml]	0,5	-
Retama retam solar d. [0,25 mg/ml]	1,5-1	2,5
Retama retam Lyophilizer [1mg/ml]	0,5	Traces
Retama retam Lyophilizer [0,75mg/ml]	0,5-2	1-2,5
Retama retam Lyophilizer [0,5 mg/ml]	-	1,5
Retama retam Lyophilizer [0,25 mg/ml]	0,5-1	1-4

Table IV: Resultats of antibactérienne activity of ***Asphodelus tenuifolius***

	Escherichia coli	*Pseudomonas aeruginosa*
Asphodelus tenuifolius F. [1mg/ml]	1-3	3
Asphodelus tenuifolius F. [0,75mg/ml]	Traces	1
Asphodelus tenuifolius F. [0,5 mg/ml]	1,5	0,5
Asphodelus tenuifolius F. [0,25 mg/ml]	1-2	1-2
Asphodelus tenuifolius oven [1mg/ml]	1	1,5
Asphodelus tenuifolius oven [0,75mg/ml]	1,5 -2	3
Asphodelus tenuifolius oven [0,5 mg/ml]	3	1,5
Asphodelus tenuifolius oven [0,25 mg/ml]	-	0,5-2,5
Asphodelus tenuifolius Shade [1mg/ml]	0,5	1,5
Asphodelus tenuifolius Shade [0,75mg/ml]	1,5	1
Asphodelus tenuifolius Shade [0,5 mg/ml]	1	1-5
Asphodelus tenuifolius Shade [0,25 mg/ml]	1	-
Asphodelus tenuifolius solar d. [1mg/ml]	-	-
Asphodelus tenuifolius solar d. [0,75mg/ml]	1,5-4	1,5
Asphodelus tenuifolius solar d. [0,5 mg/ml]	-	Traces
Asphodelus tenuifolius solar d. [0,25 mg/ml]	1,5	1
Asphodelus tenuifolius Lyophilizer [1mg/ml]	0,5-1,5	3
Asphodelus tenuifolius Lyophilizer [0,75mg/ml]	1,5	2
Asphodelus tenuifolius Lyophilizer [0,5 mg/ml]	1,5	0,5
Asphodelus tenuifolius Lyophilizer [0,25 mg/ml]	1	1-3

Conclusion:

This work focuses on studying the method of drying effect on the potentially active in some medicinal plants in the spontaneous Ouargla region. Interest is in medicinal wild plants as natural sources of many active ingredients.

Biochemical analyses of that active substance by the test reducing sugars records their presence in both species *Retama retam* and *Asphodelus tenuifolius* to different modes of drying. It is in parallel, a biological test for tannins, one notices that their absence in *Retama retam* fresh and dried in solar dryer and *Asphodelus tenuifolius* oven dried and solar dryer.

The assay of total phenols by Folin Ciocalteu, leads the dried plants in the oven have the best levels of polyphenols in *Retama retam* Rates (220.89 µg GAE / g DW) and *Asphodelus tenuifolius* Lyophilizerd (101.82 µg GAE / g DW).

Furthermore, the flavonoids assay solution of aluminum trichloride, we find that the oven-drying is the most conservative to the tenures of flavonoids in *Retama retam* costs (37.44 µg QE / g DW) and *Asphodelus tenuifolius* Lyophilizerd with a value of 16.10 µg QE / g DW.

In parallel, the dosage of tannins in the species *Retama retam* and *Asphodelus tenuifolius* oven dried, the largest values of tannins can be seen compared to other drying methods that 7.09 µg GAE / g DW and 0.78 µg GAE / g DW respectively.

On the antibacterial activity, except *Retama retam* dried in the open air and *Asphodelus tenuifolius* Lyophilizerd having a remarkable inhibition against *Escherichia coli* and *Pseudomonas aeruginosa*, respectively, the other extracts of these plants in different drying methods have low activities or for both bacterial strains.

Drying plants for medicinal spontaneous character is important to avoid fermentation plants. In summary, drying improves conservation of products, facilitate their transport, reduce product loss risk after harvest and especially to expand the marketing of these products making them available throughout the year.

It was concluded that the method of drying oven is suitable to read *Retama retam* to retain the most of their assets principes, against it lyophilization the most conservative to *Asphodelus tenuifolius.*

On the other side, it was concluded that *Retama retam* has low antibacterial activity against *Pseudomonas aeruginosa;* in parallel *Asphodelus tenuifolius* has remarkable antibacterial activity against both bacterial strains studied.

Finely, this work might help scientists and practical persons in determining the suitable techniques of draying the medicinal plants. And this is very important to develop field of traditional medicine.

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