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Article

Onopordum nervosum ssp. *platylepis* Flowers as a Promising Source of Antioxidant and Clotting Milk Agents: Behavior of Spontaneous and Cultivated Plants under Different Drying Methodologies

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Abstract: This study aims to evaluate the effect of different drying methodologies (room, microwave, convective, oven and freeze-drying) on the chemical composition, the microbiological quality of extracts and the biological activities namely clotting milk and antioxidant activities for both spontaneous and cultivated *Onopordum nervosum ssp. platylepis*. The results showed that the drying methodology has significantly affected the phenolic composition. Freeze dried flowers showed the best amounts of total phenols, flavonoids and condensed tannins followed by the microwave dried flowers. However, the latest presented the lowest protein content. Finally, the biological activities were significantly dependent on the used drying process. The effective concentration EC_{50} values let to classify the samples according to their decreasing power to inhibit DPPH free radicals: dryer<oven<room <microwave<freeze-drying. The drying process significantly affected the clotting milk activity, freeze dried flowers showed the highest activity. All the obtained results do not reveal a significant difference between cultivated and spontaneous plants.

Keywords: *Onopordum nervosum ssp. platylepis;* spontaneous plant; cultivated plant; drying; chemical composition; clotting milk activity; antioxidant activity

1. Introduction

Asteraceae is the largest family of the flowering plants, the most known and used thistle species are *Cynara cardunculus*, *Cynara humilis*, *Cynara scolymus* and *Onopordum acanthium* as a source of coagulant agent in the manufacture of raw ovine and/or caprine milk.[1,2] Furthermore, the thistle species are used since ancient times as food and medicinal plant. Several species have different biological properties such as antidiabetic, choleretic, diuretic, cardiotonic, anti-hemorrhoidal, antioxidant, anti-inflammatory cytotoxic and antimicrobial activities. [2,3] The different organs of thistle plants contain many bioactive compounds, particularly mono- and dicaf-feoylquinic acids, anthocyanins, and other flavonoids, such as apigenin and luteolin derivatives.

Onopordum L. is a genus of about 60 species of thistles belonging to the Asteraceae family, native to Europe (mainly the Mediterranean region: France, Spain, Italy, Greece), northern Africa (Tunisia, Algeria, Libya), the Canary Islands, the Caucasus and southwest and central Asia.[3] Several *Onopordum* are widely used in traditional medicine. Flowering branches of *Onopordum acanthium* are used as diuretic and antipyretic and roots for diuretic, antipyretic, appetizing and abdominal pain. Onopordum tauricum seeds are used for the treatment of kidney disease in Turkey.[3]

Onopordum nervosum ssp platylepis is an endemic spicy of Tunisia. [4] A few researches have been conducted on this plant. The main important results reported the chemical composition of seed oil including triglycerides, sterols, tocopherols and tocotrienols. [4]

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In recent decades the number of industrial applications of the thistle species has been gradually increasing,[2] which needs two important reflection axes, the first one is about the plant cultivation and the other is how to preserve the plant material after harvesting to avoid its deterioration during storage. Drying is the ancient common processing method used to preserve plant materials.[5,6] However, the selection of drying method has a great influence on the quality, the chemical composition and the biological activities of dried plants.[7] Different plant drying methodologies have been studied such as room, hot air, freeze, vacuum, microwave drying etc. For aromatic and sensitive plants, the natural drying method is used, but recently, with the advancements in technology, many new methods were developed and used for industrial-scale drying processes.[6] Over time, several reports have proven the close relationship between the chemical composition of the species and the drying methodology. To our knowledge there is no study reported on the drying effect on thistle flower's quality.

In this context, the current study aims to evaluate the influence of five drying methodologies on the chemical composition, the antioxidant and the clotting milk activities of wild and cultivated *O. nervosum platylepis* growing in central Tunisia.

2. Material and Methods

2.1. Plant Material

The used samples are spontaneous and cultivated *Onopordum nervosum ssp. platylepis* flowers from the High Agronomic Institute of Chott Mariem (ISA-CM), Sousse, Tunisia plots. With the localization coordinates: Latitude 35°55'01°N; 35°55'02°N and longitude 10°33'41"E; 10°33'48"E for spontaneous and cultivated plants respectively. The plant material was identified by Prof. Rabiaa Haouala a botanist from ISA-CM, University of Sousse.

The cultivated plants are grown on spaced lines (1.2 m) with spacing between the plants of 1.5 m. The plant materials were harvested during the flowering season (end of May 2021). At this stage of maturation, the flowers were completely open with a purple color.

2.2. Drying Methodology

The drying methods include the following: the room drying (RD) is achieved with natural air circulation. Plant materials are spread in thin layers in trays and mixed or turned frequently. The average temperature of the room is 22 ± 2°C. The drying time is about 5 days. For Oven drying (OD), samples are dried by circulating hot air at a temperature of 45°C for 48 hours. The Freeze drying (FD) is performed at a temperature of - 40°C and the vacuum was maintained at a pressure of 10^{-3} Torr for 36 h. The Microwave (MD) treatment is performed at 300 W for 3 min and finally a convective dryer (CD) is used for flowers drying at 30°C during 20 hours.

The samples of each population are divided into three lots and collected until reaching stabe water content. They are then ground into fine powders using a mill and preserved in spittoons, which are provided with the date of collection, name of the population and batch number.

2.3. Aqueous Extract Preparation

The extraction is based on the aqueous maceration method which consists of leaving the powder of the plant material in prolonged contact with a solvent to extract the active ingredients. It is performed at room temperature.

The distilled water is used, one gram of powder from each batch is mixed with 20 ml of distilled water. The resulting mixture was placed on a magnetic stirrer at room temperature for 2 hours. Then, each batch was centrifuged twice at 4°C and 8000 rpm for 10 minutes, and the supernatant was collected and stored at 4°C for future use.

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2.4. Chemical Characterization of O. nervosum ssp. platylepis Aqueous Extracts

2.4.1. Dry Matter

An empty capsule is weighed and its mass is noted. In this capsule, 1 ml of the extract is added, then is placed in the oven at 105°C for 24 hours.

2.4.2. Total Phenols Content

The adopted method is that of Singleton and Rossi,[8] based on the quantification of the total concentration of hydroxyl groups that characterizes the polyphenols in the extract. A volume of 100 µl of each extract was added to 500 µl of Folin-Ciocalteu reagent diluted 10 times and 1000 µl of distilled water. This mixture was stirred at room temperature for 1 min. 1500 µl of sodium carbonate (Na_2CO_3) 20% solution, was added to this mixture. The whole is incubated for 2 hours at room temperature to give the blue coloration, degraded according to the concentration of polyphenols in each solution. The absorbance of the mixture was measured by an UV-Vis spectrophotometer (ZUZI model 4201/50) at a wavelength: λ = 760 nm.

2.4.3. Flavonoids Content

The determination of the flavonoid level is carried out according to the method described by Kim et al. [9] It is based on the formation of a stable complex between aluminum chloride $AlCl_3$ and oxygen atoms located on carbons 4 and 5 of flavonoids to give a yellowish-red coloration. A volume of 1250 µl of distilled water and 75 µl of sodium nitrite $NaNO_2$ at 5% were added to 250 µl of each extract. After 5 min, 150 µl of $AlCl_3$ was added to the mixture. After 6 min, 500 µl of NaOH were added with 775 µl of distilled water to reach a total volume of 3000 µl. Without any incubation, the color of the mixtures immediately changes to yellow. The absorbance of the mixture was measured by spectrophotometer at a wavelength: λ = 510 nm against a blank.

2.4.4. Condensed Tannins

The condensed tannins determination was carried out using the method of Tiitto[10] which is based on the reaction of vanillin with HCl. Vanillin reacts with the terminal flavonoid group of condensed tannins and forms a pinkish-red complex. Tannins, in fact, turn into anthocyanidols when they react with vanillin giving the pinkish-red coloration. A volume of 50 µl of each extract were added to a volume of 3000 µl of a 4% vanillin solution and then stirred. A volume of 1500 µl of concentrated HCl was added to this mixture. The latter is incubated in the dark for 15 min at room temperature. The absorbance of the mixture was measured by spectrophotometer at a wavelength: λ = 500 nm.

2.4.5. Proteins Content

The proteins content in the *O. nervosum platylepis* extracts is determined according to the Bradford [11] method, based on the staining of the proteins of the solution by the blue of Coomassie G250. Once bound to proteins its color turns from brown to blue. The intensity of the staining is proportional to the amount of protein present in the sample. The colouring reagent is prepared by dissolving 100 mg of bright blue of Coomassie G-250 in 50 ml of 95% ethanol. A volume of 130 ml of phosphoric acid (85%) is added and the solution is supplemented to 1 L with distilled water and filtered on filter paper. The pH value of the dye reagent was adjusted by adding concentrated phosphoric acid to a value of 0.4. The reagent was stored at 4°C, protected from light.

For protein determination, a volume of 200 µl was mixed with 2 ml of Bradford reagent. The contents of the tube were thoroughly mixed, and the absorbance was measured after 5 min by UV-Visspectrophotometer at 595 nm using distilled water as blank.

The protein concentration is determined using a calibration curve of Bovine Serum Albumin (BSA) and expressed in mg of protein/g of extract.

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2.5. Microbiological Characterization

To assess the microbiological quality of the extracts, the enumeration of total mesophilic aerobic flora (TMAF), was conducted using plate count agar (PCA) with an incubation period of 48h at 30°C. [12]

2.6. Biological Activities

2.6.1. Clotting Milk Activity

Clotting milk activity is measured according to the method cited by Libouga.[13] The protocol consists of adding 1 ml of crude extract to 10 ml of prepared milk by spearing of 12 g of milk powder in 100 ml of distilled water then, 0.01 M of $CaCl_2$ are added with stirring. The tubes are placed in a water bath at 35°C. The coagulation time corresponds to the time required for the appearance of the first flakes in a thin film of milk flowing on the wall of the tube.

It is expressed either by the coagulant activity unit (C.A.U.) also called rennet unit (U.P) according to the method of Berridge.[14]

The coagulant activity unit (CAU) is defined as the amount of enzyme per milliliter of enzymatic extract that causes the flocculation of 10ml of Berridge substrate in 100 s at 35°C.

It is calculated according to the Formula (1):

$$CAU=100V /10T. V' \quad (1)$$

Where

V: Volume of milk to coagulate (10ml).

V': Volume of the enzymatic solution (1ml).

T: Flocculation time in seconds.

2.6.2. Antioxidant Activity

The antioxidant activity of *O. nervosum ssp. platylepis*is evaluated using radical scavenging tests of DPPH and ABTS radicals and the measure of the iron reducing power method (FRAP).

DPPH Assay

The adoptedmethod in the DPPH test is that of Lee et al., [15] one ml of the methanolic solution of DPPH (0.1 mM) is added to an equal volume of each aqueous solution of the extracts at different concentrations. The mixtures are left in the dark at room temperature for 30 minutes. The absorbance is measured by UV-Vis spectrophotometer against a blank at λ = 517 nm.

The inhibition percentage of DPPH radicals is given by the following Formula (2):

$$IP\ (\%) = (1-A_{sample}/A_{control}) * 100 \quad (2)$$

$A_{control}$: absorbance of the control

$A_{control}$: absorbanceof the sample

The values of the effective concentration (EC_{50}) represinting the antioxidant concentration needed to reduce DPPH by 50%, are graphically determined by logarithmic regression.

ABTS Assay

The ABTS radical scavenging capacity was evaluated using the method of Re et al.[16] The radical cation $ABTS^{\bullet+}$ is generated by mixing a volume of 0.5 ml of a stock solution of ABTS at 7 mM and 0.5 ml of a solution of potassium persulfate $K_2S_2O_8$ at 2.45 mM, protected from light at 4°C for 12 to 16 h before use. The obtained solution is diluted with methanol to reach an absorbance of 0.7 to 734 nm. Then, 0.5 ml of the aqueous extract at different concentrations is mixed with 0.5 ml of the prepared solution. The mixtureare left in the dark at room temperature for 10 minutes. The absorbance is measured by UV-Vis spectrophotometer against a blank prepared at λ = 734 nm.

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The inhibition percentage of ABTS is given by the same method as described for DPPH test and the EC_{50} values are determined graphically by logarithmic regression.

FRAP

The followed experimental protocol is that of Yildrim et al., [17] indeed, 0.5 ml of the aqueous extract at different concentrations is mixed with 0.625 ml of phosphate buffer (0.2 M, pH = 6.6) and 0.625 ml of a solution of potassium ferricyanide $K_3Fe(CN)_6$ at 1%. The whole is incubated in a water bath at 50°C for 30 minutes. Then, 0.625 ml oftrichloroacetic acid (TCA) at 10% is added to stop the reaction. After 10 minutes, an aliquot of 1.250 ml is mixed with 1.250 ml of distilled water and 0.250 ml of an aqueous solution of $FeCl_3$ at 0.1 %. The absorbance is then determined at 700 nm using an UV-Vis spectrophotometer.

The EC_{50} is determined graphically, it corresponds to the concentration that gives an absorbance of 0.5.

2.7. Statistical Analysis

All data were calculated by three replicates and expressed as mean ± standard error of mean. The SPSS version 20 statistical software package was employed for all statistical analysis. The ANOVA analysis of variance was performed and the Duncan test was used to determine significant differences between the different samples at the 5% level. Results were considered significant if the associated P value was less than 0.05. Correlation analysis was conducted to evaluate the relationship between the chemical composition and the biological activities. XLSTAT 2023 software was used to performe principle component analysis (PCA) and classification test (Dendrogram).

3. Results and Discussion

3.1. Effect of Domestication and Drying Methodology on Chemical Composition and Microbiological Properties of O. nervosum platylepis Aqueous Extract

3.1.1. Dry Matter of Aqueous Extract

The results of the determination of total solids for *O. nervosum platylepis* crude extracts are shown in Table 1. The values vary from 1.30 to 2.0%. Based on the statistical analysis, there is a significant difference between solids depending on the flower drying methodology (P=0.026). However, no significant difference (P>0.05) is revealed for crop type (cultivated and spontaneous).

Table 1. Physicochemical composition and microbiological properties of different dried flowers extracts from spontaneous and cultivated *O. nervosum ssp. Platylepis*

Drying methods	**RD**		**OD**		**FD**		**MD**		**CD**	
Parameters	**SF**	**CF**	**SF**	**CF**	**SF**	**CF**	**SF**	**CF**	**SF**	**CF**
DM (%)	1.60±0.04^{b}	1.33±0.03^{b}	1.77±0.06^{a}	1.73±0.04^{a}	1.93±0.06^{a}	2.00±0.02^{a}	1.33±±0.06^{b}	1.67±±0.06^{b}	1.03±0.04^{c}	1.17±0.03^{c}
TP (mg GAE/g	4.15±0.50^{b}	4.14±0.77^{b}	3.62±0.21 b^{c}	3.75±0.61 b^{c}	6.19±0.50^{a}	5.54±0.67^{a}	4.94±0.15^{ab}	4.20±0.16^{ab}	3.01±0.32^{c}	2.96±0.22^{c}
TF (mg QE/g)	0.45±0.03^{c}	0.51±0.02^{c}	0.18±0.02^{d}	0.19±0.02^{d}	2.35±0.07^{a}	2.12±0.09^{a}	0.88±0.05^{b}	0.99±0.05^{b}	0.09±0.01^{d}	0.08±0.02^{d}
CT (mg CE/g)	0.013 ±0.002^{bc}	0.013 0.001^{bc}	0.015 ±0.003^{bc}	0.017 ±0.002^{bc}	0.038 ±0.002^{a}	0.043 ±0.001^{a}	0.021 ±0.003^{b}	0.019 ±0.001^{b}	0.011 ±0.00^{c}	0.009 ±0.001^{c}
PC (mg BSAE/g)	84.53 ±10.41^{b}	99.84 ±9.84^{b}	29.85 ±1.16^{c}	28.99 ±3.60^{c}	263.05 ±18.67^{a}	261.23 ±16.38^{a}	3.58 ±0.62^{d}	4.15 ±0.70^{d}	79.02 ±6.63^{b}	78.87 ±4.12^{b}
TAMF	1.82±0.20^{a}	1.77±0.30^{a}	1.62±0.16 b	1.63±0.10 b	1.38±0.12^{c}	1.35±0.14^{c}	abs^{d}	abs^{d}	1.60±0.22^{b}	1.57±0.25^{b}

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(logUFC)

RD: room drying, OD: oven drying; FD: freeze drying; MD: microwave drying; CD: convective drying; SF: spontaneous flowers; CF: cultivated flowers; DM: dry matter; TP: total phenols; TF: total flavonoids; CT; condensed tannins; PC: protein content, TAMF: total aerobic mesophilic flora. [a,b]: different letters indicate significant difference (P<0.05).

3.1.2. Phenolic Composition

According to Table 1, variability in polyphenols, flavonoids and condensed tannins levels is observed in the studied plant material depending on drying method. The total phenol contents in *O. nervosum platylepisis* flower extracts range from 2.956 mg EAG/g to 5.54 mg EAG/g and from 3.01 mg EAG/g to 6.19 mg EAG/g, flavonoids values are between 0.09 to 2.35 and 0.08 to 2.12 and cultivated and spontaneous samples, respectively. Small quantities of tannins were registred for both plant materials. No significant differences are recorded depending on the origin of the plant, this indicates that the chemical composition is not affected by the cultivation since it was conducted in smilar condition as spontenous growth. According to these results, it is found that *O. nervosum ssp. platylepis* extracts are globally characterized by significant amounts of phenolic compounds in comparison with species of thistles. These results are comparable to the levels registred by Pandino et al.[18] for *Cynara cardunculus var. Scolymus* with a total phenol content of 4.43 mg/g DM. However,–they are less important than that found by Habibatni et al. [19] performed on *O. acanthium*, reveals higher values compared to our results where total phenol content of the extract was 8.93 ± 1.33 mg EAG/g. It is the case also, for the artichoke flower (*Cynara scolymus* L.), a research carried out by Mahmoudi et al. [20] indicated a total phenol content of 23.7 mg EAG/g). For condensed tannins, our results are consistent with the levels found by Mahmoudi et al. [20] who reported a CT value of 0.035 µg EC/g in *C. scolymus* L. flowers. This variability in results, for thistle plant materials, can be explained by the plant species and the extraction conditions which can affect significantly the obatained values.

The high significant variation (P = 0.000) of polyphenol contents depending on the drying method of the flowers indicated that the freeze and microwave drying are the best technologies for polyphenols extractability however elctric convective dryer and oven seem to be increasing the degradation of these substances. Those findings are in agreement with many researches, according to Lim and Murtijaya[21], the freeze and microwave drying of *Phyllanthus amarus* provided the same contents of total phenols (12.10 ± 0.56 mg EAQ/g and 12.70 ± 1.17 mg EAQ/g) respectively. The highest total phenolic content was also recorded for jasmine freeze dried flowers. [6] However, the present results are in disagreement with those of Miao et al.,[22] who found that freeze drying provides the lowest total flavonoids value in dried tea flowers. Broadly, freeze drying usually more effective in preserving active components from degradation in plants, flowers and fruits than hot-air drying.[6,23]

Maghsoudlou et al., [24] indicated that the increase in total polyphenol content in dried plants can be explained by the fact that heat treatment promotes the release of phenolic compounds from the solid matrix. However, the lower amounts can be attributed to oxidation and thermal degradation of phenolic compounds with increased heat intensity and heat treatment duration.[22]

3.1.3. Proteins Content

The analysis of the recorded results (Table 1) reveals avariability in protein content in the plant subjected to different drying methods (P=0.000). The most important protein contents were registred in flower extracts obtained by freeze and convective drying. The values were about 261.22 and 79.02 mg EBSA/g, respectively. While the microwave and oven dried flowers represented the lowest values with 3.58 and 28.99 mg EBSA/g respectevily. The results do not reveal a significant difference (p>0.05) in terms of crop type (cultivated and spontaneous). In comparison with a study conducted on the species Onopordum tauricum belonging to the same family, Mozzon et al.[25] revealed protein contentlevels comparable to our results which are about 132.61mg EBSA/g. Based in our research, the freeze drying is the most protective treatment for proteins, however, the microwave drying is the

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most destructive for these substances. Those findings are in agreement with previous research which suggests that microwave heating has a more destructive effect than conventional heating.[26]

3.1.4. Microbiological Properties of *O. nervosum platylepis* Aqueous Extract

For microbiological analysis, the enumeration TAMF results are presented in Table 1. The evaluation showed that the microbiological quality of the studied extracts are significantly depending on the drying methodology (P = 0.047). It has been observed that *O. nervosum platylepis* extract obtained with microwave heated flowers was completly decantaminated. However, the room dried flowers have registred the highest contamination (log UFC=1.8). This can be explained by the drying short time with MW (3min) in opposition to the room drying which takes more time (5 days) that increases the risk of the plant material contamination. In the other hand, it has been proven that MW radiation is effective in killing bacteria. [27] The results do not reveal a significant difference (p>0.05) between cultivated and spontaneous plants. The use *O. nervosum platylepis* as a replacer of chemical additives should guarantee an acceptable microbiological quality. From the obtained results it can be concluded that all the drying methods have provided an hygienic quality of *O. nervosum platylepis* extracts within the standards.

3.2. Effect of Domestication and Drying Methodology on Biological Activity of O. nervosum Platylepis Aqueous Extract

3.2.1. Clotting Milk Activity

The clotting milk activity is expressed in coagulation time and rennet units (UP), the results of the analysis are presented Table 2.

According to the obtained results, the drying methodology affects significantly the coagulant activity of *O. nervosum platylepis* flower extracts (P=0.000). The most potent activity was obtained for freeze-dried flowers (CAU=0.833), followed by the air dried flowers extract and the convective dryer with the value of 0.556. The extract obtained from the oven dried flowers showed a low coagulation activity however the microwave dried samples were unable to coagulate the milk. The results were comparable for both cultivated and spontaneous plants.

Our results are lower than that obtained by Ben Amira et al.[28] for the flowers of *C. cardunculus* a variation in the values of coagulant activity between 1.11 and 2.40 CAU/ml depending on repining stage. The study conducted by Mozzon et al. [26] indicates that *O. tauricum* flowers exhibited coagulation properties.

Table 2. Clotting milk and antioxidant activities of different dried flowers extracts from spontaneous and cultivated *O. nervosum ssp. Platylepis.*

Drying methods	**RD**		**OD**		**FD**		**MD**		**CD**	
Parameters	**SF**	**CF**	**SF**	**CF**	**SF**	**CF**	**SF**	**CF**	**SF**	**CF**
ClT (s)	180	180	490	480	120	120	ND	ND	180	190
CAU	0.556 ^{b}	0.556 ^{b}	0.204 ^{c}	0.208 ^{c}	0.833 ^{a}	0.833 ^{a}	0 ^{d}	0 ^{d}	0.556 ^{b}	0.526 ^{b}
EC_{50}_DPPH (mg/ml)	0.78±0.08^{b}	0.91±0.04^{b}	1.02±0.02^{a}	1.20±0.02^{a}	0.48±0.02^{c}	0.41±0.03^{c}	0.67±0.09^{b}	0.73±0.04^{b}	1.34±0.08^{a}	1.16±0.01^{a}
EC_{50}_ABTS (mg/ml)	0.20±0.02 ^{b}	0.21±0.01 ^{b}	0.39±0.03 ^{a}	0.37±0.03 ^{a}	0.16±0.04 ^{b}	0.17±0.01 ^{b}	0.14±0.01 ^{b}	0.15±0.02 ^{b}	0.51±0.01 ^{a}	0.49±0.02 ^{a}
EC_{50}_FRAP	2.50±0.03^{d}	2.76±0.12^{d}	3.42±0.06^{b}	3.38±0.09^{b}	1.52±0.19^{c}	1.65±0.06^{c}	1.70±0.07^{c}	1.90±0.05^{c}	3.45±0.03 ^{a}	3.98±0.09^{a}

RD: room drying, OD: oven drying; FD: freeze drying; MD: microwave drying; CD: convective drying; SF: spontaneous flowers; CF: cultivated flowers; ND: not determined; ClT: clotting time; CAU: coagulant activity unit; EC: effective concentration. ^{a,b}: different letters indicate significant difference (P<0.05).

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3.2.2. Antioxidant Activity

From the current point of view, in vitro antioxidant activity should be assessed using multiple methods to cover all aspects of antioxidant effectiveness, in the present study three tests were performed to evaluate the antioxidant properties of *O. nervosum platylepis* flowers extract and the results are presented in Figure 1 and Table 2.

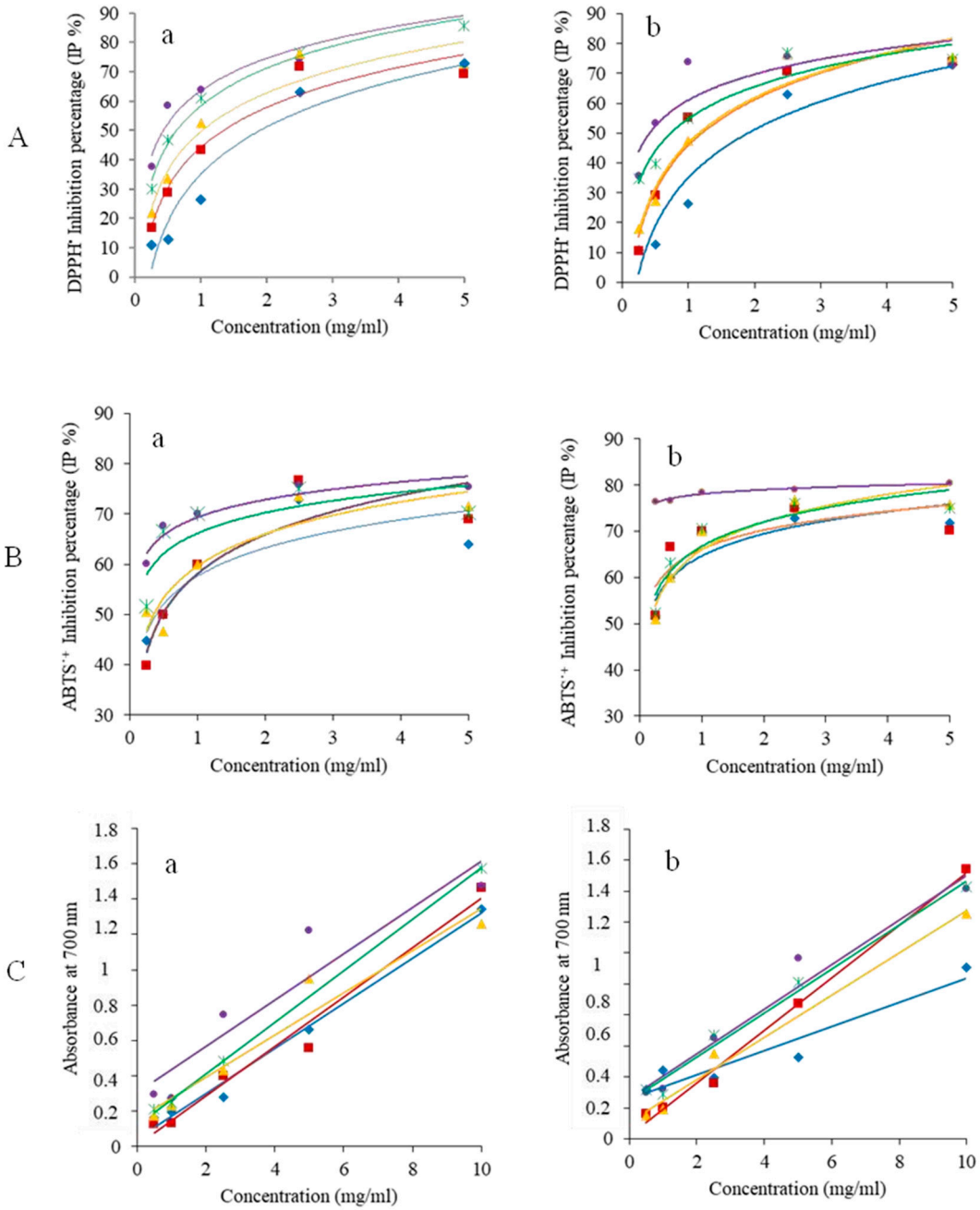

Figure 1. Antioxidant activity of *O. nervosum ssp. platylepis* dried flowers aqueous extract, Freeze drying (FD●), Microwave drying (MD ×), Room drying (RD ▲), Oven drying (OD ■) and Convective drying (CD ◆); DPPH• test (**A**), ABTS•+ test (**B**) and FRAP test (**C**); spontaneous (**a**) and cultivated plants (**b**).

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The percentages of DPPH free radical inhibition as a function of crude extract concentrations of cultivated and spontaneous *O. nervosum platylepis* flowers are shown in Figure 1(a,b).

The results showed that all extracts have radical scavenging activity depending on extract concentration the higher extract concentrations let to the higher inhibition percentages. It has been observed that the used drying methodology significantly affect the radical scanvenging activity of *O. nervosum platylepis* flowers (P = 0.000). The Freeze dried flower extracts showed the best DPPH radicals inhibition percentage for both spontaneous and cultivated plants.

The efficient concentration (EC_{50}) which is inversely related to antioxidant activity, meaning that the smaller the EC_{50} value, the more potent the extract is considered has registred values ranging from 0.41 to 1.34 mg/ml, a high significant difference was recorded for the antioxidant capacity. Regarding the drying method, the extracts with the strongest DPPH• scavenging activity were obtained from freeze-dried and microwave-dried flowers with the EC_{50}values of 0.41 mg/ml and 0.67 mg/ml, respectively. However, the extracts obtained from dried flowers using the oven and the convective dryer showed the lowest DPPH• inhibition, could inflict losses on antioxidant activity. The results do not reveal a significant difference ($p>0.05$) in terms of crop type (cultivated and spontaneous) (Figure 1A(a,b)).

The reported results for the genus Onopordum indicate its radical scavenging ability, the study conducted by Habibatni[19] (2017) reported the antioxidant properties of different *Onopordum acanthium* flower extracts with EC_{50} values of 134.4 µg/ml for the DPPH test.

The present results are in agreement with those of Cheng et al. [29] who indicated the significant effect of drying method. The EC_{50} of native lignin of brauns extracted from quince fruit subjected to different drying methodologies (microwave, sun and freeze drying) about 0.14mg/ml, 0.15mg/ml and 0.18mg/ml, respectively.

Total phenols, flavonoids and tannins are considered potentially antioxidants, their presence indicates that the extracts have antioxidant activity, which varies depending on the drying method.

The obtained results for $ABTS^+$ test (Figure 1B(a,b)) are similar to the DPPH• test, the crude extracts of freeze and microwave dried flowers showed the highest percentages of inhibition at the same concentrations of spontaneous and cultured plants. Based on the obtained results (Table 2), the extracts of microwave and freeze-dried flowers have the highest $ABTS^+$ radical scavenging capacity (EC_{50}=0.14 mg/ml and 0.16 mg/ml, respectively).

The evaluation of antioxidant activity showed that the studied extracts possessed antioxidant capacities, significantly dependent on the drying methodology (P = 0 .000). However, the results do not reveal a significant difference ($p>0.05$) in terms of crop type (cultivated and spontaneous). Our results are in agreement with those obtained by Lim and Murtijaya,[21] who found the best EC_{50} (0.154 ± 0.012 mg/ml and 0.154 ± 0.065 mg/ml) for freeze and microwave dried *Phyllanthus anarus*, respectively.

Finally, for the FRAP test, the results of the variation of absorbance at λ = 700 nm as a function of the concentration of spontaneous and cultivated *O. nervosum platylepis* flower extracts are illustrated in Figure 1C(a,b). We notice, that all the extracts were able to reduce the iron but with different capacities. There is an increase in absorbance from 0.29 to 1.47 and from 0.29 to 1.61 nm for an increase in the concentration of extracts from 0.5 to 10 mg/ml for the spontaneous and cultivated lyophilized extract, respectively. This is explained by an increase in the ability to reduce Fe^{3+} to Fe^{2+} and subsequently better antioxidant activity.

As it can be seen in Table 2, the highest ferric reducing power is registered for the extracts abtained from freeze and microwave dried flowers with EC_{50} values of 1.52 mg/ml and 1.70 mg/ml respectively, followed by the room dried flower extract.

The statistical analysis revealed that there are no significant differences between the EC_{50} values of cultivatedand spontaneous plant extracts ($P>0.05$). These results are similar to the values found by El Hamdaoui et al.,[30] who indicated no significant difference between the EC_{50s} of essential oils obtained from cultivated and spontaneous lavender plants for the same tests.

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3.3. Correlation, PCA and Classification Analysis

Due to the differences in the chemical composition, both antioxidant and clotting milk activities showed significant differences. It is well known that different drying methods and drying temperatures can affect the chemical composition and biological activities of plants (Miao et al., 2022).[22] Correlation analysis (Table 3) was conducted between chemical composition (TP, TF, CT and PC) of *O. nervosum platylepis* aqueous extract and the biological activities (EC_{50} of DPPH•, $ABTS^{•+}$, FRAP, ClT and CAU). The results showed a negative correlation between TP, TF, CT and the EC_{50} of DPPH and ABTS radicals inhibition and FRAP with coefficient values varying between -0.540 and -0.894 with a high significant effect. Higher levels of TP, TF and CT in flower extracts were associated with higher antioxidant activity and lower EC_{50} values, suggesting that these substances may play an important role in free radical scavenging capacity and ferric reducing power.

There was a negative correlation between protein content and antioxidant activity, suggesting that some proteins in *O. nervosum platylepis* aqueous extract can contribute to the antioxidant activity. Those findings are consistent with those obtained by Yu et al.,[23] in which TF contributes significantly to antioxidant activity. The high concentration of flavonoids and phenols in *G. jasminoides* flower stamens and pistils results in high antioxidant capacity.

Negative and positive correlations were observed between protein content and the clotting milk activity represented by the ClT and the CAU parameters with the coefficient values of -0.733 and 0.921, respectively.

Table 3. Pearson correlation between chemical composition and antioxidant and clotting milk activities of spontaneous and cultivated *O. nervosum ssp. platylepis* flowers extracts.

		EC_{50}_DPPH	EC_{50}_ABTS	EC_{50}_FRAP	ClT	CAU
TP	*Coefficient*	-0.894**	-0.769**	-0.864**	-0.375	0.666**
	Sig	0.000	0.000	0.000	0.071	0.000
TF	*Coefficient*	-0.876**	-0.750**	-0.848**	-0.560**	0.803**
	Sig	0.000	0.000	0.000	0.004	0.000
CT	*Coefficient*	-0.780**	-0.540**	-0.689**	-0.387	0.684**
	Sig	0.000	0.002	0.000	0.062	0.000
PC	*Coefficient*	-0.530**	-0.275	-0.432*	-0.733**	0.921**
	Sig	0.003	0.141	0.017	0.000	0.000

TP: total phenols; TF: total flavonoids; CT; condensed tannins; PC: protein content; ClT: clotting time; CAU: coagulant activity unit; EC: effective concentration. **. Correlation is significant at the 0.01 level. *. Correlation is significant at the 0.05 level.

The PCA analysis (Figure 2) of the results for eight studied variables (Pr, TPC, TFC, CT, EC_{50} of DPPH•, $ABTS^{•+}$ and FRAP and CAU) showed that the correlation matrix has very strong coefficients, which indicates a dependent evolution of these variables. The axes 1 and 2 absorb 72.55 and 21.93% of the overall variation, respectively. The graphical representation of the variables in the plane formed by the axes 1 and 2 showed that the axis1 opposes the antioxidant activity to the chemical composition, however, the clotting milk activity is closely related to the protein content. These results are consistent with those issued previously by the correlation analysis. There is a tendency to isolate three groups of individuals regrouping the RD and CD, OD and MD however the FD flowers represent a separated group. We believe that the chemical and biological activities variables have a significant power in the separation of the *O. Nervosum platylepis* extracts.

These results are also proven by the classification test (dendrogram representation) which indicated the presence of three groups.

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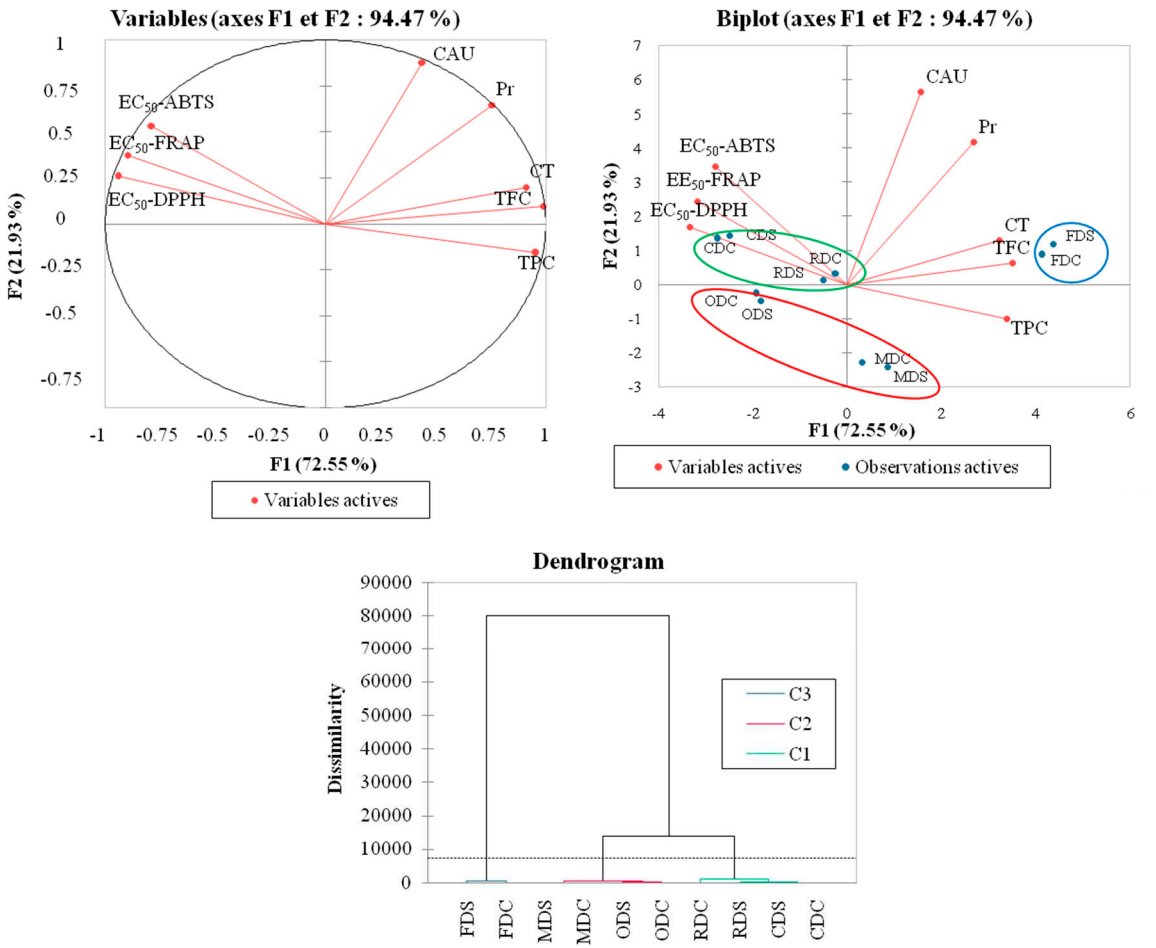

Figure 2. Principle component analysis (PCA) and classification analysis of *O. nervosum ssp. platylepis* dried flowers (FDS: Freeze dried; MD: Microwave drying; RD: Room drying; OD: Oven drying and CD: Convective drying; S spontaneous and C: cultivated, EC: effective concentration; CAU: clotting activity unit; Pr: proteins; CT: condensed tannins; TFC: total flavonoids content and TPC: total phenols content).

4. Conclusions

The present results allowed us to conclude that *O. nervosum platylepis* can be used as a source of antioxidants and clotting milk substances. However, the drying method to stabilize the plant material should be carefully selected. In one hand, significant effects of drying method were observed in both chemical composition and biological activities of *O. nervosum platylepis* flowers. In the other hand, the results did not reveal a significant difference in terms of culture type (cultivated and spontaneous) which could be a potential opportunity for *O. nervosum platylepis* domestication which is an endemic plant of Tunisia and its presence is very threatened by the climate change.

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