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I J N R E

Contribution to the pharmacological valorization of the roots of *Capparis spinosa* L. from Mila region

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Résumé:

Ce travail a été fait pour valoriser les extraits méthanoïques et aqueux des racines de *Capparis spinosa* L. de la région de Mila. D'une part, de faire un screening phytochimique préliminaire (étude qualitative). Et d'autre part de faire une analyse quantitative des polyphénols, flavonoïdes et flavanols. Ensuite, l'activité antioxydante de l'extrait de racines a été évaluée à l'aide de trois tests complémentaires ***in vitro*** (ABTS, FRAP et DPPH). Le criblage phytochimique préliminaire des extraits aqueux et méthanoïques de racines de *C. spinosa* L. a indiqué la présence de polyphénols, d'alcaloïdes et de tannins. Une absence totale de saponines, flavonoïdes, terpènes et mucilages de deux extraits. En termes d'activités antioxydantes, l'extrait méthanoïque a montré la plus grande activité antioxydante en ABTS, FRAP et DPPH (IC50=254.62±5.03µg/mL ; A05=207.30±27.93µg/mL et IC50=364.49±13.57µg/mL) respectivement. En comparaison avec les standards utilisés : BHT, BHA, acide ascorbique et α-Tocophérol. L'activité antioxydante qui a été enregistrée à partir de cet extrait est probablement liée à la teneur en composés polyphénoliques (29.98±mg GAE/g). En conclusion, les résultats obtenus dans cette étude montrent que l'extrait méthanoïque des racines de l'espèce *Capparis spinosa* L. pourrait être utilisé comme produit thérapeutique.

Mots clés : *Capparis spinosa* L., activité antioxydante, racines, région de Mila.

Abstract:

This work was done to valorize the methanolic and aqueous extracts of the roots of *Capparis spinosa* L. from Mila region. In the one hand, to determine a preliminary phytochemical screening. And on the other hand to make a quantitative analysis of polyphenols, flavonoids and flavonols. Then, the antioxidant activity of the roots extract was assessed using three complementary ***in vitro*** tests (ABTS, FRAP and DPPH). Preliminary phytochemical screening for aqueous and methanolic extracts indicated the presence of polyphenols, alkaloids and tannins. A total absence of saponins, flavonoids, terpens and mucilages of two extracts. In terms of antioxydant activities, the ME showed the highest antioxydant activity in ABTS, FRAP and DPPH (IC50=254.62±5.03µg/mL ; A05=207.30±27.93µg/mL and IC50=364.49±13.57µg/mL) respectively. In comparison with the standards used : BHT, BHA, ascorbic acid and α-Tocopherol). The antioxidant activity that was recorded from these extracts is probably related to the content of polyphenolic compounds (29.98±mg GAE/g). In conclusion, the results obtained in this study show that the methanolic extract of *Capparis spinosa* L. roots could be used as therapeutic products.

Keywords: *Capparis spinosa* L., Antioxydant activity, Roots, Mila region.

1. Introduction

Since ancient times, medicinal plants have been recognized as one of the most effective therapeutic agents for the treatment of human diseases [1, 2]. Scientists have recently discovered the importance of secondary metabolites that perform a number of protective functions in the human body. They can strengthen the immune system, protect the body against free radicals, kill pathogenic germs, and much more keep the body fit [3].

The majority of current research is focused on the isolation, purification, identification and characterization of active ingredients from crude extracts. The widespread use of plants by the Algerian and world population requires a precise chemical elucidation. Among these plants, the Caper or *Capparis spinosa* L. of the family Capparaceae is native to the Mediterranean region. In Algeria the *C. spinosa* is common throughout the country, it grows naturally in the wild in sunny places and on skeletal, rocky, dry and stony soil. This species has economic, ecological and medicinal properties [4].

The caper is known in the traditional phyto-medicine that has exploited its properties for several purposes as tonic, aperitive, diuretic and anti-inflammatory. It was one of the most used and appreciated condiment plants of the Greeks and Romans [5].

In Algeria, the different parts of the plant are used to treat used to treat itching, mosquito bites, hives and also to treat asthma and digestive problems. The main objective of the present study is was to evaluate the content of phenolic, flavonoid and flavonol compounds as well as to evaluate the antioxidant activity of methanolic and aqueous extracts of roots of this important species of the capparaceae family.

2. Materials and Methods

2.1. Plant material

The roots of *Capparis spinosa* L. were collected during the flowering season in Zeghaia wilaya of Mila, eastern *Algeria*, located at a longitude 6° 10’ 21” East and a latitude 36° 28’ 04” North. The plant was harvested in August 2018 and the biological tests were conducted a month later. Taxonomic identification of the plant sample was carried out by the Laboratory of Natural Sciences and Materials (LSNM) at the University Center of Mila.

Table 1. Scientific classification of *Capparis spinosa* L. [6]

Kingdom : Plantae
Sub-kingdom : Eukaryotes
Phylum : Spermaphytes
Sub-phylum : Angiosperms
Class : Dicotyledons
Sub-class : Polypetales
Order : Thalamiflora
Sub-order : Parietales
Family : Capparidaceae
Genus : Capparis
Species : *Capparis spinosa* L.

2.2. Preparation of extracts

2.2.1. Aqueous extract

Aqueous extraction of roots of *Capparis spinosa* L. was performed by adding 200mL of distilled water to 10g of powder. After 24 hours of maceration in distilled water under magnetic stirring, the aqueous extract was filtered through Whatman N°3 filter paper and the filtrate was evaporated and dried by a rotary evaporator at 40°C. The residue obtained was stored at -20°C in the dark until their analysis [7].

2.2.2. Metanolic extract

About 20g of roots of *Capparis spinosa* L. was mixed with 100 mL of pure methanol for 48h, then filtered through a Whatman N^{o}3 filter paper and the filtrate was evaporated and dried using a rotary evaporator at 35°C. The residue obtained was stored at -20°C in the dark until their analysis [8].

2.3. Qualitative analysis

In order to detect secondary metabolites, present in aqueous and methanolic extracts of roots of *Capparis spinosa* L. a phytochemical screening was performed using standard techniques based on the observation of color changes in the initial mixture or precipitate formation [9, 10].

2.4. Determination of the content of phenolic compound (TPC)

The content of phenolic compound (TPC) of *C. spinosa* L. extracts was evaluated spectrophotometrically by the Follin-Ciocalteu (FC) method following the protocol described by [11] and [12]. Briefly, into glass hemolysis tubes, 200 µl of the plant extracts were mixed with 1 mL of diluted Folin-Ciocalteu reagent solution. After 5 min in the dark, we added 800µl of sodium carbonate solution (7.5%). The absorbance was read at 765 nm After incubation for 2h in the dark. Total phenols were calculated using a gallic acid standard curve (concentration range: 1-10µg/mL). The coefficient of determination was R^2 =0.992.

2.5. Determination of total flavonoids content (TFC)

In hemolysis tubes, one milliliter of extract of each part of plant was added to one milliliter of methanolic solution of $AlCl_3$ (2%.) Spectrophotometric reading was taken at 430nm after ten minutes of incubation in the dark. Quercetin was used as a standard at a concentration of 2.5-25µg/mL [13]. The flavonoid concentration was calculated using the linear regression equation obtained from the quercetin curve. The coefficient of determination was R^2=0.994.

2.6. Determination of flavones and flavonols (TFoC)

The method used for the estimation of flavonol levels is the one described by Kosalec, (2004) [14]. The determination of flavones and flavonols is based on the same principle as that of total flavonoids [15]. In the hemolysis tubes a volume of 500µl of plant extract was put; then the addition of 1500µl of ethanol, 100µl of 10% methanolic aluminium chloride solution ($AlCl_3$), then 100µl of sodium acetate (5%) and 2800µl of water, after 30min of incubation, the absorbance is read at 415nm. All operations are performed in triplicate. The concentration of flavones and flavonols contained in the plant extracts is calculated in relation to the calibration curve (y= ax+b) obtained by using quercetin as a standard at different concentrations (10-200µg/mL) under the same conditions as the samples. The coefficient of determination was R^2 =0,992.

2.7. Antioxidant activity

Antioxidant activity of roots extracts of *C. spinosa* L. was detected using three assays: DPPH radical scavenging assay, $ABTS^+$ scavenging and reducing power assay (FRAP).

2.7.1. DPPH radical scavenging assay.

The free radical scavenging activity of different samples was evaluated by 1,1-diphenyl-2-picrylhydrazyl ($DPPH^•$). A colorimetric technique developed by Blois [16]. In presence of the evaluated sample, a characteristic wavelength was used to accurately estimate the rate of bleaching of the stable free radical $DPPH^•$. In its reactive form, this latter has an important capacity of absorption at 517nm.

On the other hand, during the reduction by an antioxidant or a radical species, the absorption declines noticeably. Briefly, in a dark place, a one-tenth mM solution of $DPPH^•$ was prepared in ethanol. 160µL of the prepared $DPPH^•$ solution was placed in each well of the microplate, then, a volume of 40µL of each extract at different concentrations (200-3,125µg / mL) was added. After 30 min of incubation at room temperature, the absorbance was measured at 570nm. High free radical scavenging activity implies a decrease in the absorbency of the reaction mixture. A calibration curve was used to evaluate the concentration of $DPPH^•$ in the reaction medium. The following equation quantifies the scavenging power of the $DPPH^•$ radical.

$$\textbf{DPPH}^• \textbf{ scavenging effect (\%)} = (A_{Control} - A_{Sample}/A_{Control}) \times 100 \qquad \textbf{Equation (1)}$$

Where $A_{Control}$ is the absorbance of the control reaction and A_{Sample} is the absorbance in presence of different extracts of *Capparis spinosa* L. roots or of standards [21]. The inhibitory concentration of 50%

of the DPPH activity (IC_{50}) of different samples was thereafter calculated from the equation that determines the percentage inhibition versus concentration of inhibitor. It was expressed as µg / mL and compared with that of BHA, BHT, and α tocopherol.

2.7.2. ABTS radical cation decolorization assay

The ABTS+ radical of different samples was determined according to Re et al., (1999) [17] with slight modifications. Briefly, 160µl of ABTS solution was added to 40µl of different concentrations of plant samples (12.5-800 µg/mL) in 96-well microplates. The whole was incubated for 10 min at room temperature after that the absorbance was read at 734 nm using the microplate reader. The percentage of inhibition was calculated using the following equation:

$$\%I = (A_1 - A_2/A_1) \times 100 \qquad \text{Equation (2)}$$

The results were compared to BHA and BHT standards. Where: A_1 and A_2 are the absorbances of the negative control and the different extracts of*Capparis spinosa* L. respectively. The results were expressed as inhibition concentration (IC_{50}).

2.7.3. Ferric-reducing antioxidant power (FRAP) assay

The reducing power activity (FRAP) of the different samples was detected by applying the protocol described by Oyaizu et al., (1986) [18]. For this purpose and in 96-well microplates, 10 µL of samples (3.125-200µg/mL) were added to the reaction mixture consisting of 40µL of phosphate buffer (0.2 M, pH 6.6) and 50 µL of potassium ferricyanide (1%). After incubation time for 20 min at 50°C in the oven, 50 µL of trichloroacetic acid (TCA) (10%), 40 µl of distilled water, and 10µl of ferric chloride $FeCl_3$ (0.1%) were added to the previous mixture. The absorbance was read at 700 nm against a blank. The absorbance was measured in a microplate reader at 700nm against a blank (methanol or distilled water depending on the type of extract). The standard used in this assay is Ascorbic acid and the results were expressed as $A_{0.50}$, which corresponds to the concentration producing 0.500 absorbance.

2.8. Statistical Analysis

The results obtained are reported as the mean ± SD value of three measurements; statistical analysis of parametric data for IC_{50} and A_{05} were carried out using GraphPad Prism 6 software by one-way ANOVA followed by Turkey's post hoc test for multiple comparisons. The differences with $P< 0.05$ were considered statistically significant.

3. Results and Discussions

3.1. Phytochemical screening

As shown in Table 1, qualitative analysis of roots extracts of *Capparis spinosa* L. revealed the great presence of tannins, alkaloids and polyphenols of two aqueous and methanoic extracts. However, flavonoids, terpens, saponosids and mucilages were not detected (negative) in the two extracts aqueous and methanolic.

These results are similar to those of the previous study, carried out on *C. spinosa* L., and they revealed the presence of polyphenols and alkaloids and the absence of sterols and triterpenes [2, 19, 20]. A number of components have been identified in *Capparis spinosa* L. by other authors, including flavonoids [21], alkaloids [22, 23] and terpenoids [24].

Table 2. Results of the qualitative study (phytochemical screening) of roots extracts of *Capparis spinosa* L.

Phytochemicals	ME	AqE
Polyphenols	++	+
Flavonoids	-	-
Tannins	+++	+
Terpenes	-	-
Alkaloides	++	+
Saponosides	-	-
Mucilage	-	-

Symbols (-,+,++,+++) represent the following : (-) = negative, (+)= few concentration,(++)= moderate concentration, (+++)= high concentration.

3.2. Quantitative analysis

The total phenol content of the *C. spinosa* L. revealed that the methanolic extract had the highest concentrations of polyphenols and flavonoids compared to the aqueous extract. The detection of TPC in the extracts of *C. spinosa* L. showed that the ME (29.98mg ± GAC/g) had the highest content, followed by the aqueous extract (24.55±mg GAC/g).

Meddour et al, (2013) [25] also found that the methanolic extract of the flower buds of *Capparis spinosa* is rich in polyphenols (29.01±0.84mg eqAG/g). Also, Yue-lan et al, (2010) [26] found that the flavonoid content of the ethanolic extract of the fruits of *Capparis spinosa* L. is 5.439 ± 0.73 milligrams of rutin equivalents per gram of extract. This variation can be due to several elements such as geographical position of the plants, ecological conditions and climate.

Also, Bonina et al. (2002) [27] found that the freeze-dried methanolic extract of *C. spinosa* flower buds was rich in polyphenols (65.13 ± 5.53 mg RE/g extract), high value compared to our results, this difference is explained by the difference in the plant parts studied as well as the extraction method which plays an important role in phytochemical valuation. Arrar et al. (2013) [28] and Rajhi et al. (2019) [29] found that polyphenols and flavonoids are more abundant in leaves, flowers and fruits and finally roots. But both studies confirm the richness of *C. spinosa* in these active molecules.

Evaluation of TFC in the extracts showed that the methanolic extracts had higher flavonoid content (12.06±mg EQ/g), followed by the aqueous extract (02.17±mg EQ/g). Khojasteh Rad et al, (2020) [30]; found that the hydro-methanolic extract of the leaves of C. spinosa from Iran was rich in flavonoids (128.88 mg ER/g extract), high value compared to our results. The highest TFoC was recorded in the aqueous extract (9.77±mg EQ/g), followed by the methanolic extract (4.51±mg EQ/g).

Table 3. Content of phenolic compound (TPC), flavonoids (TFC) and flavonols (TFoC) of root extracts of *Capparis spinosa* L.

Extract		TPC (mgGAE/g extract)[1]	TFC (mgQE/g extract)[2]	TFoC (mgQE/g extract)[2]
Roots	**AqE**	24.55±1,45	02.14±0,17	9.77±0,2
	ME	29.98±8,39	12.06±0,82	4.51±0,30

Results are expressed as means ± SD of three measurements

1 milligrams of gallic acid equivalents per grams of sample powder

2 milligrams of quercetin equivalents per grams of sample powder

3.3. *In vitro* antioxidant capacity

To evaluate the potential reducing power and antioxidant activity of *Arctium minus* roots, several approaches with different reaction principles were adopted, in other words: the DPPH radical scavenging assay, ABTS cationic radical decolorization and reducing power assay. The percentage inhibition or absorbance of the various extracts and standards are shown in Table 4, while the IC_{50} and $A_{0.5}$ values are reported in Table 5.

The results of the *in vitro* antioxidant activities revealed that *Capparis spinosa* L. species has significant antioxidant activities at different levels, especially the methanolic extract.

The IC_{50} and $A_{0.5}$ values varied according to the polarity of the extracts. The aqueous extract showed low antioxidant activity.

ABTS cation radical decolorization

C. spinosa L. showed low ABTS radical scavenging activity. The two extracts of the plant studied for ABTS radical scavenging, showed antiradical activity against ABTS radical, but this antiradical activity is still lower than that expressed by the standard. The methanolic extract showed the highest antioxidant activity (IC_{50}=254.62±503µg/mL) followed by the aqueous extract (IC_{50}= 346.34±8.75µg/mL). The extract with the lowest IC50 value has the most powerful antiradical activity. Our study is the first performed by using the radical trapping method (ABTS) for the species *Capparis spinosa* L.

In this activity, the extracts showed a better free radical scavenging power than in the antiradical activity to DPPH this can be justified by the ability of the radical cation ABTS to be more versatile than DPPH because it is soluble in water and organic solvents, which allows an evaluation of antiradical activity for both hydrophilic and lipophilic compounds [31].

Reducing power assay (FRAP)

This activity was represented as $A_{0.5}$ values. The best result was given by the methanolic extract (IC_{50} = 207.30±27.93µg/mL). On the other hand, the aqueous extract showed a lower activity, with an IC_{50} value significantly different from that of the standards (414.62±59.73µg/mL).

DPPH radical scavenging assay.

The results obtained revealed that only the methanolic extract showed antiradical activity with IC50=364.49±13.57 µg/mL (Table 2). On the other hand, the aqueous extract showed no antiradical activity (Table 5). Many researches have been done to study the antioxidant power of *Capparis spinosa* extracts in Italy Bonina et al, (2002) [27] showed that freeze-dried methanolic extract of fresh berries of *C. spinosa* has a very high antioxidant activity with an IC50 of 34 µg/mL. Bouriche et al, (2011) [32] also made the same finding as the latter on Algerian Caper with an IC50 of 53.53µg/mL.

4. CONCLUSION

This study was designed to investigate the phytochemical characterization and to evaluate the *in vitro* antioxidant activity of *Capparis spinosa* L. by applying different methods. The qualitative study of bioactive substances showed the existence of several bioactive compounds such as alkaloids and polyphenols. The present research suggests that the tested samples possess relatively different, but dose-dependent antioxidant activities. In addition, the methanolic extract showed the highest antioxidant activity compared to the aqueous extract. This could be due to the richness of total phenolic compound and alkaloids in this extract. These data lead us to further studies to determine the chemical composition of these extracts and to understand the structure-activity relationship of the molecules responsible for the antioxidant activity.

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