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Research Article

Phytochemical Study, Antibacterial and Antioxidant Activities of Extracts of *Capparis spinosa L.*

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ABSTRACT
The *Capparis spinosa* L. is a species which has a great importance in the field of traditional medicine in both arid and semi-arid regions whose nutritional and medicinal value remains ambiguous in Morocco. It is in this context that our work is aiming at the recovery of this species through a phytochemical study and an evaluation of antibacterial and antioxidant activities of flower buds of Capparis spinosa L. spontaneous collected and sold by Nour cooperative within the region of Zerhoun, Morocco. We achieved the solid-liquid extraction by two different methods in order to optimize the extraction yields of polyphenols; maceration and soxhlet by using two mixtures of solvents: methanol / water and acetone/water at 70%. The best yields are obtained with methanol / water mixture; 46% for soxhlet against 31% for maceration. The quantitative analysis of phenolic is being performed by colorimetry, extracts showed that the soxhlet method is more effective than the maceration. The qualitative analysis performed by HPLC / UV-ESI-MS also reported the existence of the same flavonoid glycosides in both hydromethanolic extracts in which the most important are the derivatives of kaempferol, quercetin and isorhamnetin. The antioxidant activity is determined by the DPPH test, showed that the aqueous-methanolic extracts obtained by soxhlet and maceration expressed some approximate antioxidant powers with IC50 of respectively 0.73± 0.01 and 0.84± 0.01 mg / ml. However, the antibacterial activity evaluated against pathogenic strains such as *Staphylococcus aureus, Escherichia coli, Klebsilla pneumoniae,* revealed the ineffectiveness of various extracts against the most tested strains.

Keywords: *Capparis spinosa* L., Zrhoune, polyphenols, flavonoids, HPLC, antibacterial, antioxidant activity.

INTRODUCTION

The family of *Capparidaceae* contains the *Capparis* genus, including the species of *Capparis spinosa* L. which is called in Arabic "Kabbar". It was once used by the Sumerians, Greeks and Romans throughout history because of its therapeutic qualities. In Iranian traditional medicine[1], the fruit and root are used as antirheumatic, diuretic, tonic, astringent but the fresh aerial parts of the caper are widely used in the field of culinary. Once preserved in vinegar or in an acidic brine[2], these parts are used as a condiment in Mediterranean cuisine where the flower buds are one of the most popular spices thanks tao their bitter and spicy flavors[3].

Scientifically, the phytochemical studies of capers extracts showed the presence of many chemical families with very interesting biological activities. These molecules such as alkaloids, fatty acids, phenolic acids, flavonoids, aldehydes, esters, vitamins and glucosinolates[4,5,6]. Other studies by Ali-Shtayeh & Abu-Ghdeib (1999)[7] proved that the aqueous extract of Capparis spinosa has antifungal properties with a percentage of inhibition greater than 90% against T. violaceum which is responsible for ringworm, and which is widespread in Africa and Asia. Gadgoli and Mishra (1999)[8] also revealed the antihepatotoxic activity in vitro and in vivo models of p-methoxy-benzoic acid, which isolated from the methanolic fraction of the aqueous extract of Capparis spinosa L. Also various studies of the different parts of C.spinosa L. showed that the latter have several biological activities including anti-inflammatory effects[9], antidiabetic effects[10], antihistamine effects and antiallergic effects[11]. For the first time, Brevard et al. (1992)[12] were able to identify and determine, by the GC-MS, the sulfur content in the volatile natural capers. This study showed that they contain sulfides, isothiocyanates and cyclooctasoufres (S8).

Worldwide various studies were conducted on caper. In Italy, it has been shown that its flower buds contain the following flavonoids: quercetin, rutin, quercetin 3-O-rutinoside, kaempferol-3-O-rutinoside, kaempferol-3-rhamnosyl rutinoside[3]. In Algeria, Bouriche et al.[13]

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confirmed that the methanol extract of the flower buds of caper can be considered a good source of antioxidants and antibiotics for therapeutic and nutraceutical industries.

In view of so many interesting properties, the economic importance of this species has increased considerably especially in the Mediterranean region. For Morocco, it is a species of xérophyte with an ecological, socioeconomic and medicinal importance which is integrated in sustainable development program. Moreover, Morocco is considered among the top producing countries of this species in the world. However, the number of research studies on the chemical profile of Morocco's capers is very limited. To our knowledge, extracts of capers from Morocco have not been a subject to any previous study focusing on their chemical composition or their biological properties. It is in this perspective that we undertook this research which is the contribution to the enhancement of bioextraits from this medicinal plant of the Zerhoun area by the characterization of chemical constituents and the evaluation of their antibacterial and antioxidant powers.

MATERIALS AND METHODS

Material

Plant Material

The caper was harvested in Zerhoun, Morocco (Nour cooperative) in full bloom during the month of June 2014. Capparis spinosa L. was identified in Rabat Scientific Institute by Prof. M. Bentatou. The buds are dried at room temperature in the dark for 13 days and then finely ground by using an electric grinder.

Microbiological material

The used bacterial strains are responsible for various infections such as skin and urinary tract which are a major problem for public health. These are Gram-Positive Bacteria (Staphylococcus aureus) and Gram-Negative Bacteria: Escherichia coli, Pseudomonas aeruginosa. They were provided by the bacteriology laboratory of the hospital Mohammed V of Meknes. These bacteria have been isolated from patients and were maintained by subculture on agar media (Chapman Mackonkey).

Methods

Phytochemical screening

The chemical compounds of groups detection tests were performed according to the protocol of Harborne (1973)[14], Amadou (2004)[15], Judith (2004)[16] Bruneton (2009)[17] and N ' N'Guessan et al. (2009)[18].

The presence of alkaloids is prepared by the precipitation of salts and revelation with the reagent Mayer and Dargendorff. The characterization of gallic tannins is carried out by adding Stiasny reagent, sodium acetate and ferric chloride. The catechol tannins are detected by isoamyl alcohol and hydrochloric acid. Cyanidin reaction allows detection of free flavonoids but the leucoanthocyanes are also revealed by the reaction of cyanidin and without adding magnesium chips. Anthocyanins are detected by the addition of sulfuric acid at 10% and 25% NH_4OH. Anthracene derivatives are highlighted by using dilute ammonia to 25%. Cardiotonic glycosides are identified by using potassium hydroxide and the appropriate reagents, while for the sterols and triterpenes, they are revealed by using concentrated sulfuric acid. The saponins are characterized by their foaming power in aqueous solution by measuring the index of foam. The characterization of mucilage is reassured by the addition of absolute ethanol to the aqueous decoction. And finally, monosaccharides and holosides are highlighted by means of concentrated sulfuric acid and a saturated solution of thymol in ethanol.

Extraction and Splitting of Polyphenols from Capparis spinosa L.

Extraction by maceration

The herbal drug (30g) and 300 ml of acetone or methanol with 70% are introduced into a flask. The extraction is carried out by maceration for 24 hours at room temperature and sheltered from light. The obtained filtrate after the maceration is retained and the pomace is extracted again under the same conditions until a total of three macerations. The final volume (accumulated filtrate) is concentrated in rotary evaporator.

Extraction by Soxhlet

The Soxhlet extraction undergoes the same steps listed above (extraction by maceration). 30 g of plant material Capparis spinosa L. turned powder then placed in a paper cartridge filter inside the Soxhlet extraction chamber. The vegetal material is extracted with 350 ml of solvent consisting of acetone or methanol with 70%. A total of five cycles is necessary for the exhaustion of the vegetable material. After filtration, the solvent is removed by evaporation in vacuo. The resulting residue is the crude polyphenol extract.

Splitting

Splitting Capparis spinosa L. polyphenols (the crude extracts obtained above) is performed according to the Bruneton protocol[17] in order to make further slight modifications. It is based on the sharing of polyphenols according to their degree of solubility between increasing polarity solvents. The splitting of the extracts is carried out by using ethyl acetate and n-butanol. In addition to the hydro-methanolic or aqueous acetone crude extract (F0), the other three fractions are obtained: the ethyl acetate fraction (F1), the butanol fraction (F2) and the remaining aqueous fraction (F3). The extracts are stored until their use.

Determination of Phenolic total

The dosage of Capparis spinosa L. total polyphenols is performed according Dehpour Abbas Ali et al.[19], through slight modifications by using the Folin-Ciocalteu reagent. From a stock solution prepared aqueous gallic acid mass concentration 50 mg / l, the test solutions are prepared at concentrations ranging from 0.08 mg / ml to 1.28 mg / ml 20 ml of each solution or extract polyphenols, are introduced into 100 ml volumetric flasks containing 1.5 ml of Folin-Ciocalteu 10%. After 6 min, a volume of 1.5 ml of sodium carbonate Na_2CO_3 75 mg / ml is added. The solutions are kept in the dark for two hours at room temperature and the concentrations are determined by using a UV / Visible kind LNICAM-DISCPD2000-1. The absorbance is measured at a wavelength of 765 nm. The total polyphenol concentration of the extracts is calculated from the regression equation of the calibration range of

between 20-140 µg / ml established with gallic acid (y = 0,095x + 0.003). The results are expressed in ug equivalent gallic acid per milligram of dried plant material (µg GAE / g of plant). The content of total phenols is calculated by using the following formula:

$$T = \frac{(C \times V)}{m(\text{dried matter})} \times D$$

C: Concentration measured according to the calibration curve (Figure 2); V: volume of the aggregate sample and D: dilution factor.

Determination of flavonoids

Quantification of flavonoids is carried out by a colorimetric method adapted by Djeridane et al.[20].

From the methanolic solution (0.1 g / l) of quercitrin, a concentration ranges from 5, 10, 15, 20, 25, 30µg / ml was prepared in volumetric flasks of 50 ml by adding to each solution, an amount of 20 ml of distilled water. After 5min, 100 ml of aluminum trichloride ($AlCl_3$) to 10% (w / v) are added. The solutions are adjusted to 50ml with pure methanol, shaken immediately and are kept in the dark for 30 minutes at room temperature. The absorbance of each concentration is determined at 333nm with a spectrophotometer mentioned above for the determination of total phenols. The quantitative analysis of flavonoid extracts of our samples is carried out by adapting the same procedure used for the preparation of the calibration curve, replacing quercetin by a volume of extract until the appropriate concentration. The concentrations of flavonoids of each extract is calculated from the regression equation of the calibration range established with quercetin (y = 0,073x - 0,081).

$$T = \frac{(C \times V)}{m(\text{dried matter})} \times D$$

C: Concentration measured by using the calibration curve (Figure 4); V: Volume of the overall sample and D: Dilution factor

HPLC / UV-ESI-MS analysis

The chromatographic analysis of polyphenolic extracts of Capparis spinosa L. is made by a HPLC chromatograph equipped with a UV / Vis detector. The used column is the C18 reverse phase. The mobile phase for the elution of the molecules is a solvent mixture of acetonitrile and a solution of phosphoric acid concentration of 0.05 M. The solvent gradient in this experiment is as follows: 2% of acetonitrile (isocratic) 0-3 min, 2-30 % of acetonitrile in phosphoric acid (linear gradient) 3-19 min, 30-80% of acetonitrile in phosphoric acid (linear gradient) 19-23 min, 80% of acetonitrile in phosphoric acid (isocratic) 23-28 min, 80-2% of acetonitrile in phosphoric acid (linear gradient) 28-32 min 2% of acetonitrile (isocratic) 32-40 min. The flow rate is 1.5 mL / min. The injection volume is 20.mu.l. The UV detection is performed through scanning in the wavelength range between 200-400 nm then three acquisition wavelengths of 254 nm, 280 nm and 349 nm.

We then use the electrospray method to ionize the molecules in the mass spectrometry. The standards used are: gallic acid, ellagic acid, tannic acid, quercetin and coumarin.

The analysis of the eluted compounds is done first by comparing the retention times of the individual peaks extracts with retention times of the peaks corresponding to the standards and then by analyzing the mass spectra of the eluted molecules.

The antibacterial activity

In order to verify the susceptibility of the bacteria which is subject to the different extracts of Capparis spinosa L., we adopted the method of dissemination. This involves the use of Whatman paper disks of 6 mm in diameter and Mueller-Hinton agar as a medium for culture. We filed three discs in which the first one is impregnated with the extract (1 mg / ml), the second others are reserved as witnesses: positive (ciprofloxacin) and negative (distilled water) in a surface of a medium by swabbed microbial suspension. The plates were incubated in an incubator at 35 ± 2 ° C for 18 to 20 hours. The manifestation of the antibacterial activity of the extracts is asserted by a halotranslucide around the disk which is identical with that of the sterile agar and whose diameter is measured and expressed in centimeters.

DPPH radical-scavenging activity

The radical scavenging activity of different extracts of the flower buds of C. spinosa L. is performed by the method based on the DPPH (1,1-diphenyl-2-picrylhydrazyl) as a relatively stable radical.

The solution of DPPH is prepared by solubilizing 2.4 mg of DPPH in 100 ml of ethanol. The extracts were prepared by dissolution in ethanol at a rate of 2,8mg / ml. This stock solution called mother-solution will then undergo a series of dilutions in order to obtain the following concentrations: (0.08, 0.16, 0.32, 0.48, 0.64, 0.8, 0.96, 1.12, 1.28, 1.44, 1.6, 1.7, 1.8, 1.9, 2.08, 2.16, 2.32, 2.64, 2.8 mg / ml). The test is conducted by mixing 200 .mu.l of the test compound and 2,8ml of DPPH solution. These concentrations were prepared with ascorbic acid (vitamin C) and butylhydroxyanisole (BHA) to serve as positive controls. A white has also been realized through the absolute ethanol alone. The samples are then left in the dark for 30 minutes, and discoloration compared to the negative control containing only the solution of DPPH was measured at 517 nm. The results were expressed as a reduction percentage of DPPH (AA%):

$$AA\% = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \times 100$$

AA%: Percentage of antioxidant activity; A (control): absorbance of the solution containing only DPPH radical solution and A(sample): absorbance of sample solution to be tested in the presence of DPPH.

The graph of change in absorbance as a function of the concentration of extract was used to determine IC_{50}. The values of concentrations for inhibiting or reducing 50% of the initial concentration of DPPH (IC50) were graphically determined by linear regression. Since there is no absolute measurement of the antioxidant capacity of a compound, the results are often worn with respect to a reference

antioxidant such as ascorbic acid or butylated hydroxyanisole (BHA).

RESULTS AND DISCUSSION

Phytochemical screening

the phytochemical tests were done on various extracts which are prepared from the flower buds of Capparis spinosa L. by using specific revealing reagents. The results of screnning phytochique extracts from the Capparis spinosa L. buds are summarized in Table 1.

Flower caper buds are rich in alkaloids, flavonoids, gallic tannins, sterols and triterpenes, glycosides cardiac, leucoanthocyanins, and holosides. On the other hands, they lack narcotics, catechin tannins, anthocyanins and free and combined anthraquinones. These results are similar to those of other researchers who have demonstrated the presence of flavonoids[21,22], alkaloids[23], terpenoids[24]. As for Meddour et al,[25], they reported the absence of catechin tannins. According Sawadogo (1986)[26], the species Capparis corymbosa Lam revealed positive tests for alkaloids, flavonoids, gallic tannins, saponins, sterols and triterpenes.

Polyphenols extraction yields of C. spinosa L.

It is clear through mere observation of the extraction yields in Figure 1 that the hydro-methanol mixture (MeOH / water) gives the best extraction yield in which an average of 38.23% for the Capparis spinosa L., while the water-acetone mixture (AcO / water) gives the lowest yield (average 18.50%). It should be noted that whatever kind of the used extraction solvent is, the yields which are obtained by the Soxhlet extraction method are higher than those which are obtained by maceration. This difference in performance between the previews can be related to the nature of the solvent and the extraction temperature. In fact, the more we increase the polarity of the solvent or the extraction temperature, the more yield increases.

The extractions by Soxhlet and by maceration are standard procedures which are commonly used to remove the phenolic compounds from plant sample. Many previous studies[27,28,29] confirmed that these methods - which are simple, effective and requires a relatively less expensive equipment - allowed obtaining very high yields of phenolic compounds. Similarly, Castro-Vargas et al.[28] reported that the method of soxhlet showed a so high yield of total phenolic extract of guava seed with grades ranging from 0.380 to 6.730%. In another study done by Weidner et al.[30], it was reported that the extraction of phenolic compounds from seeds of three wild vines is successfully using the soxhlet technique.

In spite of many positive aspects in the operation of these extraction techniques, they still have significant drawbacks, such as the need to use large amounts of hazardous organic solvents, with very adverse impacts on health and the environment, on the time of extraction as well as on the interference and degradation of the components due to internal and external factors such as light, air, high temperatures and enzymatic reactions[31].

Determination of total phenols

The quantitative analysis of the total phenols is determined through the equation of the linear regression of the calibration curve expressed as µg which is equivalent to gallic acid by g per dry matter (Figure 2).

The contents of the total polyphenols obtained by the two extraction methods are represented in Figure 3. It appears from the analysis of all the obtained results that the polyphenol extracts C.spinosa L. varies according to the polarity of the used solvent and the extraction temperature. In addition, the extracts which are obtained by the Soxhlet extraction method are the richest in total phenols compared to those which are obtained by the maceration method. Indeed, the content of polyphenols in the crude extract, which are obtained by the Soxhlet, records the highest value being 876.37µg GAE / g of DM at mean against 746.27 µg GAE / g of DM at mean which is obtained by maceration.

We find that the Soxhlet extraction in the presence of hydror-methanolic mixture and extraction by maceration in the presence of hydro-acetonic mixture can be considered as the best extraction conditions of polyphenols.

The lowest polyphenol content is observed in the case of the fraction of ethyl acetate obtained through the soxhlet method with a value of 179.27 µg GAE / g of plant.

We can infer that these results are lower than those obtained by Bonina et al. (2002)[32] since the methanolic freeze-dried extract of L. spinosa C. with a polyphenol content of 65.13 mg / g: rutin milligram equivalent per extract gram. This vast difference may be related to the influence of several factors such as the method of extraction, the standard used for determining the polyphenols, the extrinsic factors (geographical and climatic), the genetic factors and the plant growth stage.

Determination of flavonoids

The quantitative analyzes of the flavonoids are determined through the equation of the linear regression of the calibration curve expressed in mg equivalent of quercetin per gram of the dry matter (Figure 4).

The results of flavonoids contents of the Capparis spinosa L. extracts obtained by the different solvents (Figure 5) show that the fractions (F0) hydro-methanolic and hydro-acetonic from Soxhlet recorded the highest levels namely 232.28; 133.33 ug equivalent to quercetin / g MVS respectively compared to those obtained by maceration (163.49; 85.58 µg EQ /g of DM at mean). Therefore, we agree that the best method to extract the flavonoids is the soxhlet method in combination with the hydro-methanolic mixture.

Concerning the various fractions (F1, F2, F3) obtained by liquid-liquid extraction, there is a variation in the total flavonoids content; the mentioned butanol fraction (F2) has a maximum of flavonoids (129,71 µg EQ /g of DM at mean), while the residual fraction (F3) and ethyl acetate fraction (F1) have contained lower concentrations (56.50; 1.20 µg EQ /g of DM at mean). This suggests that some solvents are better suited for the extraction of flavonoids than others.

In addition, all of these results is consistent with the chromatographic analysis which indicated the presence of flavonoids in the fractions (Fo).

Table 1: Results of phytochemical screening of Capparis spinosa L.

Chemical groups			Reagents / Reaction	*Capparis spinosa L.*
Alkaloids			Valse-Mayer Reagent	+++
			Dragendorff Reagent	+++
Poly-phenols	Total	gallic	Reagent Stiasny	++
	tannins	catechin	HCl	---
	anthocyanins		acid-base Reaction	---
	free flavonoids		Cyanidin with Mg	+ + +
	Leucoanthocyanes		Cyanidin without Mg	+ + +
Free anthracene derivatives			Bornträger Reaction	---
combined anthracene derivatives		O-glycosides	Color reaction	---
		C-glycosides		
Sterols and triterpenes			Libermann-Burchard Reaction	+++
Mucilage			Precipitation Reaction	---
Saponosids			Foam Index (FI)	---
cardiac glycosides			Kedde Reaction	+ + +
Narcotics			Color reaction	---
Oses and holosids			Color reaction	+ + +

(+++) Strong presence; (++) Moderately present; (+): Slightly present; (-): Negative test

Table 2: List of the compounds identified by mass spectrometry in the extract of L. C.spinosa polyphenols obtained by Soxhlet in the presence of 70% of methanol

No.	Name	Molecular formula	Mass	Ion M/Z	Fragmentations
1	Isorhamnetin	$C_{16}H_{12}O_7$	316	317	317
2	isorhamnetin-rutinoside	$C_{28}H_{32}O_{16}$	624	625	479, 317
3	Isorhamnetin-3-glucoside rutinoside	$C_{34}H_{42}O_{21}$	786	787	641, 317
4	Quercetin	$C_{15}H_{10}O_7$	302	303	303
5	Quercetin glucoside	$C_{21}H_{20}O_{12}$	464	465	465, 303
6	Quercetin-rutinoside	$C_{14}H_{12}O_5$	610	611	303
7	Quercetin-7-glucopyranoside rhamnopyranosyl-rhamnopyranoside	$C_{22}H_{24}O_{12}$	756	757	611,465, 303
8	Kaempferol	$C_{15}H_{10}O_6$	286	287	287
9	Kaempférol-glucoside	$C_{21}H_{20}O_{11}$	448	449	449, 287
10	kaempferol rutinoside	$C_{27}H_{30}O_{15}$	594	595	449,287
11	kaempferol rhamnopyranosyl-glucopyranoside- rhamnopyranoside	$C_{33}H_{40}O_{19}$	740	741	595, 449,287
12	Methoxy-kaempferol glycoside	$C_{22}H_{21}O_{12}$	478	479	317, 287
13	13-methoxy-kaempferol rutinoside	$C_{28}H_{32}O_{16}$	624	625	479,317, 287

Indeed, Meddour et al[25] found that the flavonoid content of the water-methanol extract of Capparis spinosa L. is 5.97 ug equivalent to rutin / mg of the extract. In addition, Cao et al.[33] indicated a flavonoid content of the ethanol extract of the fruit Capparis spinosa L. about 5.44 ug equivalent of the rutin. We see that our results show that flavonoid levels are much higher than those obtained by Cao and Meddour. This increase may be due to several factors: On the one hand, there is temperature and the association methanol with water to weaken the hydrogen bonds and the release of molecules of interest; on the other hand, there are extrinsic factors (such as geographic and climatic factors), genetic factors, and also the degree of maturation of the plant and the duration of storage[34,35].

Qualitative analysis of extracts of C.spinosa L. through HPLC / UV-ESI-MS

Identification of phenolic compounds extracted by Soxhlet

The chromatographic profiles (HPLC), shown above, illustrate the peaks of the standard phenolic compounds and compounds from the extract hydro-métanolic C.spinosa L. and their retention times and their relative abundances.

Following the analytical and the spectral data (chromatograms and mass spectra), we detected and identified 13 compounds whose names and empirical formulas are presented in Table 2.

The chromatogram of the methanol extract obtained through the method of Soxhlet reveals the presence of the majority peaks which range between 22.22 and 24.95 min. The analysis of mass spectra and their comparison with the results reported in the literature were used to determine the existence of flavonoids like molecules O-glycosides with ions: a pseudo-molecular ion $[M + H]^+$ and fragments $[M-X + H]^+$ corresponding respectively to a loss of a hexose (X: 162) or rhamnose (X: 146) or both rutinoside (X: 308).

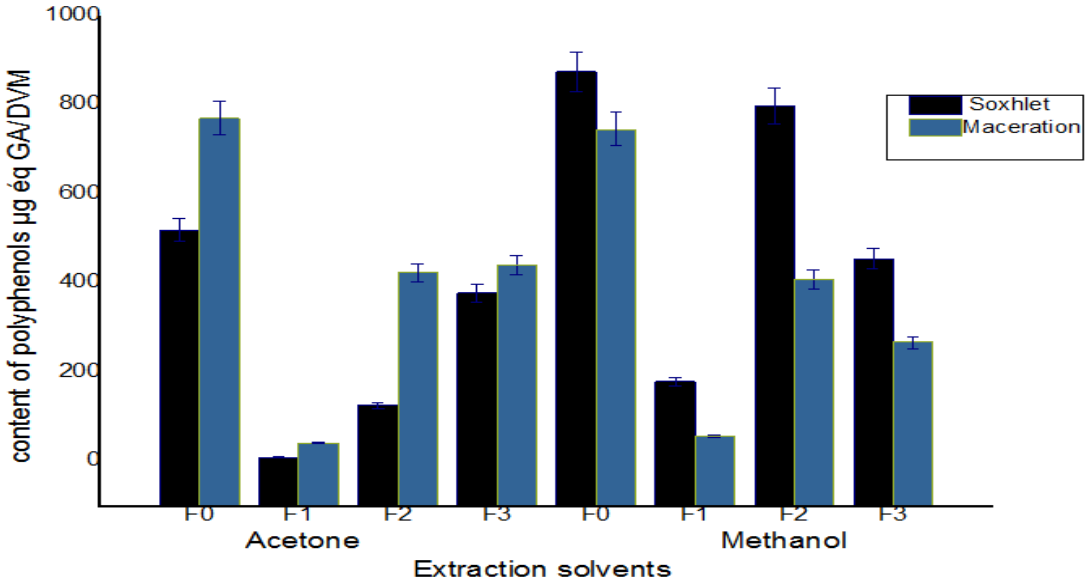

Figure 3: Levels of the total polyphenols (TP) in the hydro-methanolic extracts and hydro-acetonic of *C. spinosa* L.

The most important compounds are the kampferol-rutinoside, the kampferol-glucoside, methoxy-khampferol-rutinoside, quercetin-rutinoside, quercetin-glycoside and quercetin-rhamnopyranosyl-glucopyranoside-rhamnopyranoside.

Besides these compounds which are previously mentioned[32,4,5,21,22], other compounds belonging to the same groups were detected for the first time in the methanolic extract of this species. These are isorhamnetin-rutinoside and isorhamnetin-rhamnosyl diglucoside. Other authors[36,37] have indicated the presence of isorhamnetin-rutinoside in other parts of the plant, which may therefore enhance our supposition. Regarding the gallic acid and coumarin, we have not characterized the ions when analyzing the level of the extract. As for the ellagic acid, despite the retention time indication 24, 34 min, it was undetectable in an obvious way in the mass spectra, which can be explained by its low abundance or its total absence in the extract.

Identification of the phenolic compounds extracted by maceration

The chromatographic profile (HPLC) reveals the richness of this extract in various molecules. Comparing its chromatographic (HPLC) and spectral (MS) characteristics with those which are obtained previously (Figure 6), we notice that there is an overall similarity of their chemical profiles in a qualitative manner. Indeed, both extracts revealed the common compounds which are representing the same majority peaks. It seems that most of the identified compounds are flavonoids that are found in the form of glycoside in which the most important are the derivatives of kaempferol, quercetin, rutin and isorhamnetin. Thus, we notice the absence of gallic tannins, coumarin and ellagic acid. Table 3 summarizes the nature of the compounds identified in maceration extract, pseudo-molecular ions, plus the ions obtained by the different fragmentations.

The quantitative analysis of two extracts allowed us to identify 13 compounds with percentages of 65.21% for the extract which is obtained by the maceration method and 75.42% for that of the soxhlet method of the overall chemical composition. The retention time and their relative percentages are given in Table 4.

According to the results, we notice that there is only a quantitative difference in the hydro-méthanolic extracts of flavonoids derived by maceration and Soxhlet method. Both extracts show that kaempferol is the main compound with higher rate in the extract which is obtained by Soxhlet.

However, the hydromethanolic extract obtained by maceration contains the strongest rate of quercetin with 9.48% compared to that of Soxhlet (7.78%). As for isorhamnetin, it seems more important in the extract which is obtained by Soxhlet (2.11%) than in the extract of maceration (0.88%).

These results reflect a correspondance with those of flavonoids assays in which the methanolic extract obtained by Soxhlet reported the highest content followed by that of maceration.

The kaempferol and quercetin are flavonols which are known to contain various pharmacological effects. They are used as food supplements on account of their high antioxidant activity. Many preclinical studies have shown to have a wide range of biological activities, including anti-inflammatory, antimicrobial, anticancer, cardioprotective, anti-allergic, neuroprotective[38,39] and analgesics[39,40].

By analyzing the spectral results obtained in this study, we notice that glycolysed flavonoids appear. The presence of kaempferol-rutinoside is marked by its peak at m / z 595 $[M + H]^+$, and string ions at m/z = 449 ($[M + H\text{-}146]^+$, loss of rhamnose) and the peak at m / z = 287 representing the kaempferol in ($[m + H\text{-}162]^+$, loss of a hexose). Kaempferol glucoside is indicated by the molecular ion nickname at m /z = 448 ($[M + H]^+$) and his string ion at m / z = 287 (loss of a hexose [M + H 162] +). The ion at m/z

Table 3: Identification by HPLC -MS of the phenolic compounds in the extract of C.spinosa L obtained through maceration and methanol with 70%

No.	Name	Molecular formula	Mass	Ion M/Z	Fragmentations
1	Isorhamnetin	$C_{16}H_{12}O_7$	316	317	317
2	isorhamnetin-rutinoside	$C_{28}H_{32}O_{16}$	624	625	479, 317
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8	Kaempferol	$C_{15}H_{10}O_6$	286	287	287
9	Kaempférol-glucoside	$C_{21}H_{20}O_{11}$	448	449	449, 287
10	kaempferol rutinoside	$C_{27}H_{30}O_{15}$	594	595	449,287
11	kaempferol rhamnopyranosyl-glucopyranoside- rhamnopyranoside	$C_{33}H_{40}O_{19}$	740	741	595, 449,287
12	Methoxy-kaempferol glycoside	$C_{22}H_{21}O_{12}$	478	479	317, 287
13	13-methoxy-kaempferol rutinoside	$C_{28}H_{32}O_{16}$	624	625	479,317, 287

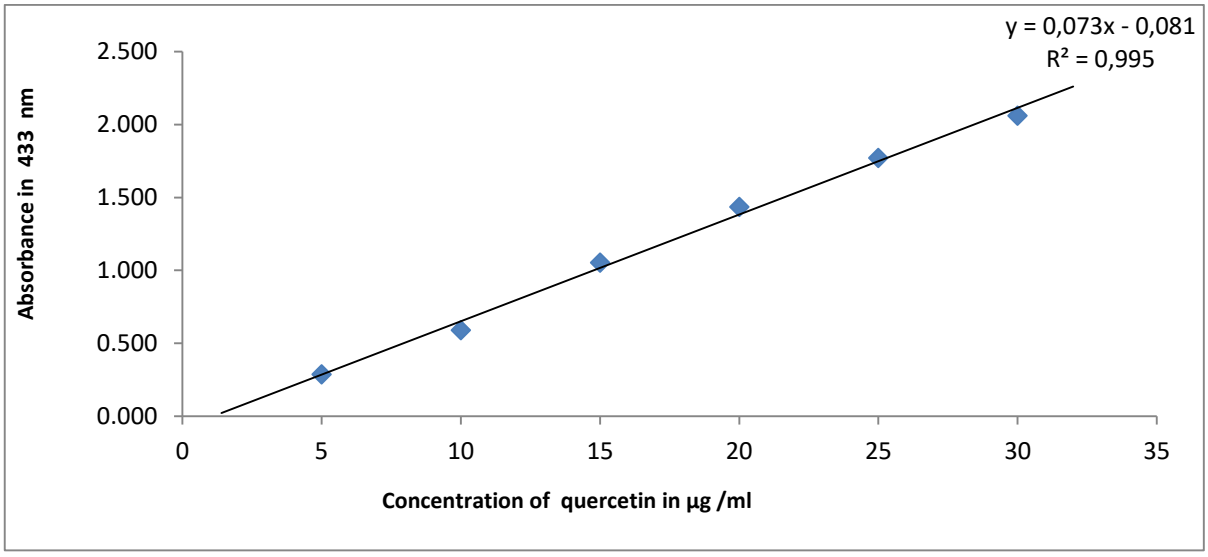

Figure 4: Calibration curve of quercetin.

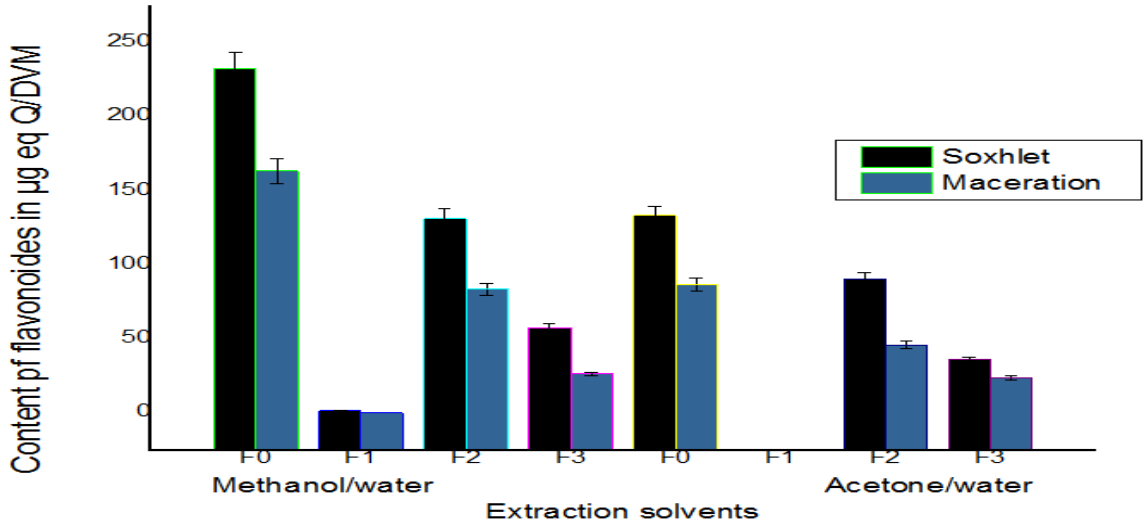

Figure 5: Levels of flavonoids (LF) in the various hydro-methanolic extracts and hydro-acetonic C. spinosa L.

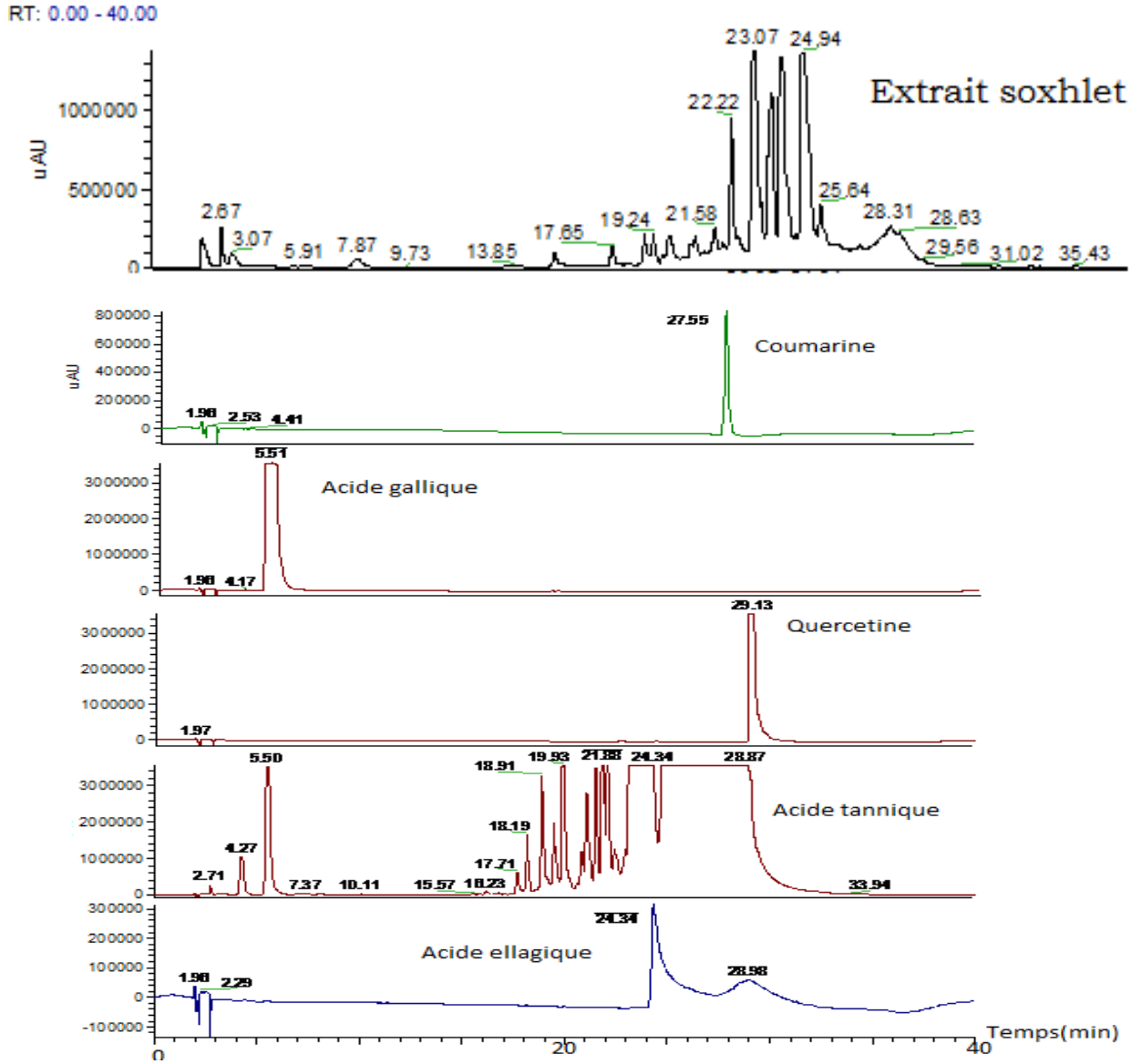

Figure 6: HPLC chromatograms of polyphenols obtained through *C.spinosa* L. soxhlet in methanol-water (70%) and the standards (Coumarin, gallic acid, quercetin, tannic acid, ellagic acid)

= 625 ([M + H] $^{+}$) confirms the presence of the methoxy-kaempferol-rutinoside with string ions at m/z = 479 (loss of rhamnose at m / z = 146) followed by m / z = 317 (loss of a hexose at m / z = 162) and m/z = 287 (loss of a methoxy at m / z = 30). The kaempferol-rhamnosyl-rutinoside is characterized by the ion at m / z 741 and fragment at m / z = 595 ([M-146 + H] +, loss of rhamnoside), m / z = 287 (loss of rutinoside; [M-308 + H] +).

Quercetin-rhamnosyl-glucopyranoside-rhamnoside is characterized by a peak at m / z 756 which in turn produces ion rutin at m / z = 611 ([M + H-146], loss of rhamnose) and a string ion at m / z = 465 quercetin-rhamnose, and thereafter an ion m / z = 303 which is quercetin. The pseudo-molecular ion m / z 467 [M + H] $^{+}$ is the quercetin glucoside and a string ion m / z = 303 ([M-H-162] $^{+}$, loss of a hexose] +.

As far as isorhamnetin-rutinoside is concerned, this is the first time it is identified in the aerial part of this species, showing a peak pseudo-molecular at m / z = 625 [M + H] $^{+}$, followed by m / z = 479 [m + H] $^{+}$ (loss of rhamnose: [m-146 + H] $^{+}$) and the peak m / z = 317 [M + H] $^{+}$ refers to isorhamnetin (due to a loss of a hexose: 162). The ion m/z = 787 ([M + H]$^{+}$), on the other hands, showed fragmentations at m / z = 641 ([M-146 + H] $^{+}$, loss of rhamnoside) and at m / z = 317 that of isorhamnetin (loss of two hexose units at m/z = 324), this confirms the probable presence of isorhamnetin-rutinoside-glucoside.

In positive mode, some compounds donesn't fragment such as quercetin, kaempferol and isorhamnetin. Our results are consistent with those published by other authors[4,5,21,22,32,41]. These analytical results can somehow justify the many biological properties of the species proved by several studies and thus confirm its use in traditional medicine.

Antibacterial activity

The results of the antibacterial activity of the tested extracts in the obtained C. spinosa L. are negative. The extracts have no effect against the tested bacteria (E. coli, Kleibseilla pneumoniae and Satphylococcus aureus

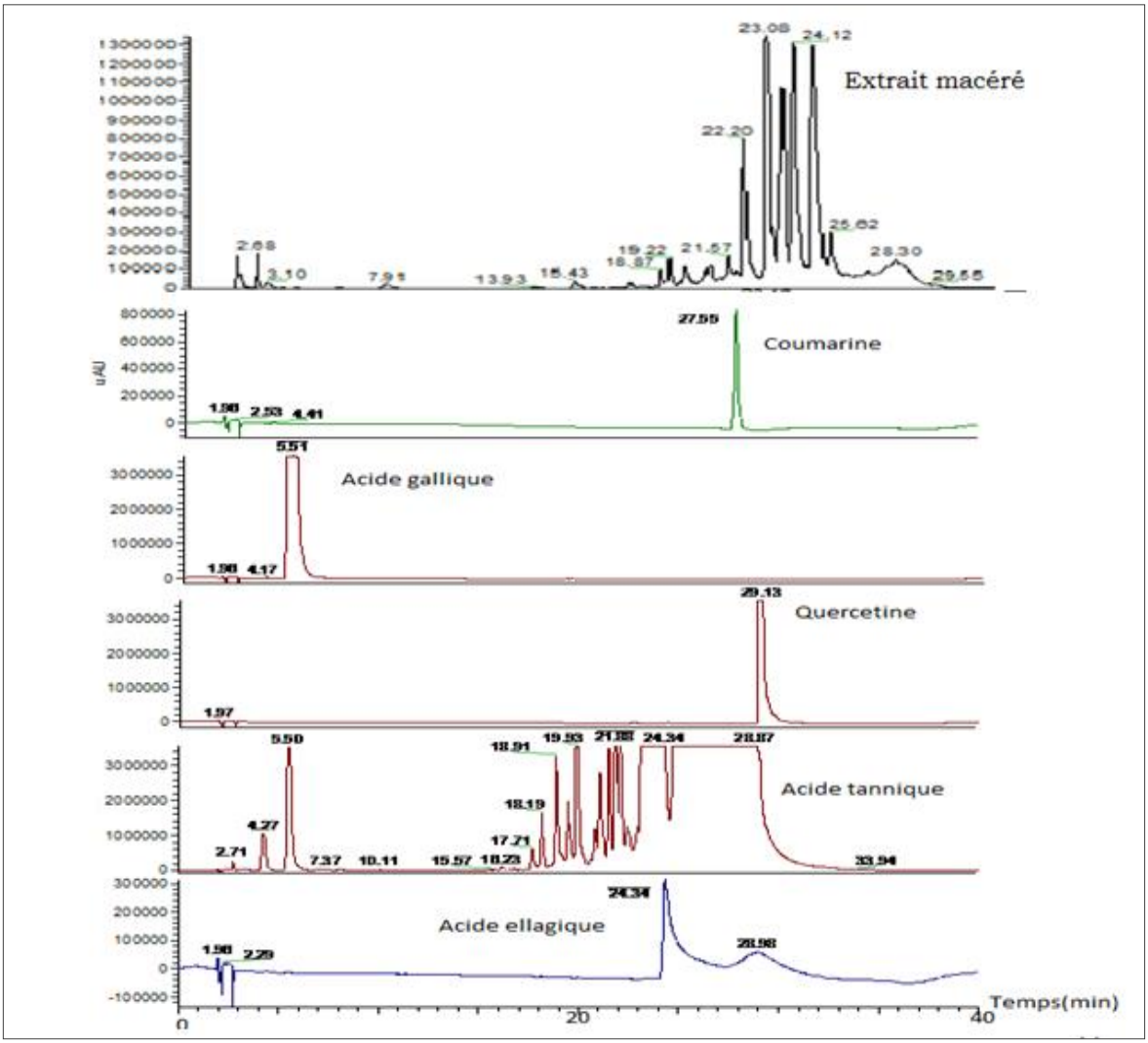

Figure 7: shows the chromatographic profile (HPLC) of the methanol extract of C.spinosa L obtained by maceration.

sensitive Métis) regardless of the dose, which shows the strength of the tested strains.

However, there are certain classes of polyphenols such as flavonoids, tannins which are known for their role in the plant defense against microorganisms, insects. Indeed, several studies have demonstrated the antibacterial tannins, flavonoids such as catechin and myricetin[42]. Referring to all the bibliographic data, we can conclude that the antibacterial activity of extracts C.spinosa L. varies from one study to another. The set of the obtained results in this study does not, moreover, strengthen and deepen our research on the anti-microbial power of caper that could reveal this species.

According to Meddour[25], the hydro-methanolic extract of C. spinosa L. proved to be inactive on stems: E. coli and P. aeruginosa and S. aureus whatever the dose (125 mg / ml - 1000mg / ml). The aqueous extract is inactive on E.coli strains and P. aeruginosa. However, at a dose of 1 g / ml, this extract was found to be active on S. aureus with a zone of inhibition of 9.50 ± 0.50 mm. Similarly, Mahasneh[43] proved that the aqueous and ethanol extracts of L. C.spinosa are inactive on E. coli and P. aeruginosa, but on S. aureus, they respectively presented the inhibition zones of 10 and 13 mm.

Proestos and his colleagues (2006)[44] reported that the extract from the leaves of C. spinosa L are inactive on E. coli but exhibit a low activity on S. aureus.

Studies on other species of the genus *Capparis* show that the root bark extracts of C. decidua have a great activity against *P. aeruginosa, S. aureus* and *E. coli*[45].

We can conclude that these results are consistent with those which we obtained in our study. There are several factors influencing the determination of antibacterial activity as: the body of the used plant by Natarajan et al, (2005)[46], the extraction methods by Turkmen et al, (2007)[47], the type and concentration of the extract, the nature and chemical structure of bioactive molecules present in the extract, the type of the tested strains, the method of evaluation of the antibacterial activity[42,48].

Antioxidant activity

The chemical compound 2,2-diphenyl-1-picrylhydrazyl (DPPH) is one of the most used free radicals for the direct evaluation of the antioxidant activity (AA) thanks to its radical shape of stability and its simplicity of analysis[49].

Table 4: Percentage of three major flavonoids of flower buds of C.spinosa (E.M.S: hydromethanol extract obtained by Soxhlet, E.M.M: hydromethanol extract obtained by macerating)

flavonoid Compounds	relative abundance (%) obtained by E.M.M	Relative abundance (%) obtained by E.M.S
Quercetin-7-glucopyranoside rhamnopyranosyl-rhamnopyranoside	6,13	7,35
Quercetin glucoside	0,48	1,07
Rutin	13,18	8,15
kaempferol rhamnopyranosyl-glucopyranoside- rhamnopyranoside	20,56	14,42
kaempferol rutinoside	22,01	35,22
Kaempferol-glucoside	0,63	0,73
methoxy-kaempferol rutinoside	0,12	3,33
Methoxy-kaempferol glycoside	0,26	0.56
isorhamnetin-rutinoside	1,28	1,98
Isorhamnetin-3-glucoside rutinoside	0,56	2,61
Pourcentage des composés identifiés	65,21%	75,42%
Percentage of unidentified compounds	34,79%	24,58%
Percentage of kaempferol	19,18%	24,97%
Percentage of quercetin	9,48%	7,78%
Percentage of isorhamnetin	0,88%	2,11%
Percentage of flavonoides	29,54%	34, 86%

generally in a positive mode $[M + H]^+$ by losing the sugar units.

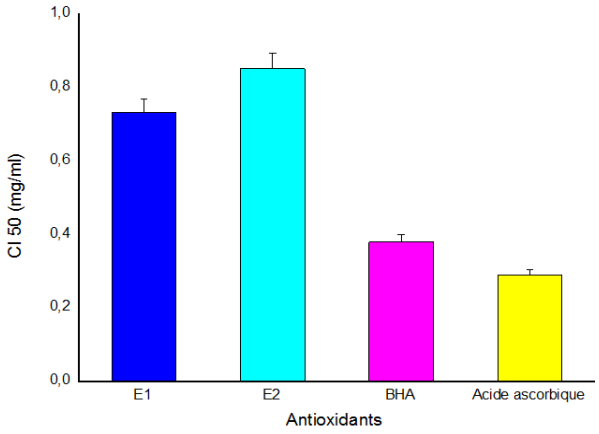

Figure 8: the IC_{50} values found for the extracts and standards in mg / ml with E1: extract of Soxhlet and E2: extract of maceration.

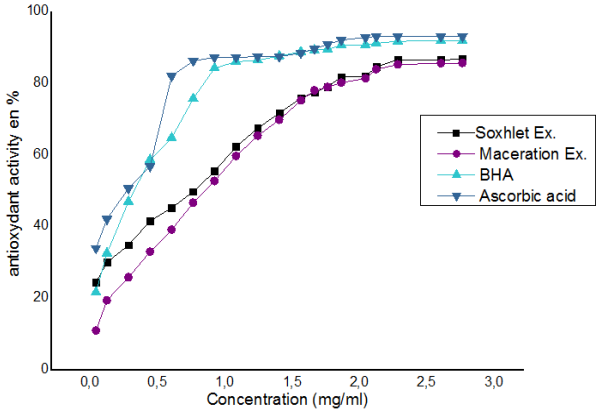

Figure 9: Antioxidant activity of the different extracts of buds of *C. spinosa L.*

The radical DPPH is initially purple, then becomes yellow in the presence of phenolic compounds which yield an H atom on DPPH and turns it into a yellow stable molecule. This change in color shows the ability of phenolic compounds to scavenge free radicals, therefore, this reaction provides information on the direct anti-radical power of extracts. This test is followed by the UV visible spectrophotometry, by measuring the decrease in absorbance at 517 nm[50]. The results of the antioxidant activity of the hydro-methanol extracts obtained by the methods of maceration and soxhlet as well as those of the two used reference antioxidants (butylated: BHA and ascorbic acid: vitamin C) are illustrated in Figure 8. In figure 9, we find that the tested extracts of L. C.spinosa have a dose-dependent anti-radical activity. The more we increase the concentration, the more antioxidant activity increases until it reaches the plate. So, the hydro-methanol extract which isobtained by the Soxhlet method revealed the highest antioxidant activity (AA = 86.88%), followed by the one from the maceration method with a percentage inhibition of 85,79%. To better characterize this anti-radical power, we determined the IC50 (concentration of the substrate that causes an inhibition of 50% of the DPPH activity) of each of the different extracts. According Kadiri et al.[51], the more value of the IC 50 is low, the more antioxidant activity is high. According to the recorded results, the hydro-methanol extracts obtained by the two methods of maceration and soxhlet, have significant antioxidant powers. Their respective IC50 is in the range of 0.84± 0.01 and 0.73± 0.01 mg/ml but low enough to those of the ascorbic acid and the BHA whose values are of the order of 0.29± 0.01 and 0.38± 0.01 mg / ml respectively. Also, the difference between the extraction methods used

in obtaining the extracts led to a slight significant difference in the antioxidant activity. Indeed, the results obtained during dosing phenolics reported that the extract obtained by Soxhlet contains a greater amount of phenolic compounds in relation to the extract obtained by maceration. Our results are superior to those of Meddour et al. (2013)[24] who found that the methanolic extract of flower buds, of flowers and of immature fruit of C. spinosa L. exhibited a
scavenging activity of about 78.34%.

Several authors have reported that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins act as donors of hydrogen atoms or electrons[52]. By invoking the qualitative analyzes carried out by HPLC-MS, we can establish a relationship between the antioxidant activity and the extracts tested in the presence of the group flavonols such as kaempferol, quercetin isorhamnetin. These are known as natural antioxidants widely distributed in the plant kingdom as aglycones or glycosides. A study by Germano et al.[4] suggested that the antioxidant power of the methanol extract of Capparis spinosa L. buds is attributed to the presence of rutin whose presence was proved by HPLC analysis. Similarly, Inocencio et al.[4] have detected the existence of derivatives of quercetin, kaempferol in the methanolic extract of L. C.spinosa, and they considered this plant as a source of antioxidant molecules.

CONCLUSION

This study reports a rational justification on flower buds of L. C.spinosa of Moulay Idriss Zerhoun which are widely exported and used for their flavors and their virtues. The phytochemical screening showed the richness of flower buds of L. C.spinosa secondary metabolites. It is apparent that the extraction by the Soxhlet in the presence of 70% of methanol and the maceration with acetone at 70% gives the best yields of the phenolic compounds. The spectrophotometric determination of the phenolic compounds clearly shows a significant difference between the two extracts since the hydro-methanol extracts obtained by Soxhlet and those of hydro-acetone produced by maceration have recorded the highest levels of the phenolic compounds. Similarly, for the total flavonoids of soxhlet in the presence of hydro-methanolic mixture seems to be the best method of extraction. The qualitative study by HPLC-MS showed that hydro-methanol extracts derived by both methods contain the same flavonoid molecules whose majorities are derivatives of kaempferol, quercetin, rutin and isorhamnetin. Indeed, the use of both extraction methods did not lead to a qualitative difference, but we noticed a greater presence of kaempferol and isorhamnetin hydromethanol in the extract obtained by Soxhlet and the quercetin in extract from hydromethanol by maceration. Concerning the antibacterial activity, no activity was seen against the tested strains. On the other hand, the test DPPH says that the extracts obtained by the two methods have anti-radical activity almost similar which are due to the existence of flavonols in the plant. These biomolecules are of paramount importance in the life of the plant. Indeed, the plant synthesizes because they are involved in its position vis-à-vis protection of various environmental aggressions and in the adaptations to the arid and semi-arid climate. The results obtained in this work are only a first step in the recovery of this species, additional tests will be needed to identify, isolate and purify the constituents responsible for several therapeutic and nutritional virtues.

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Erratum: Few lines of Table 2 remained hidden behind figure 8 in earlier version of manuscript, which are resolved in the present copy. No, other data changed.: Erratum Performed on 5th February, 2017