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Antioxidant, Antifungal and Phytochemical Investigations of *Capparis spinosa* L.

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Abstract: The antioxidant and antifungal activities of crude hydro-ethanolic extract from *Capparis spinosa* L. (Capparidaceae) leaves and their fractions, obtained by liquid-liquid extraction (LLE) using solvents with increasing polarity (hexane, diethyl ether, ethyl acetate, butanol, and water), were investigated. The crude extract and the obtained fractions were characterized by colorimetric analysis, pyrolysis-gas chromatography (GC)-mass spectroscopy (MS), Fourier Transform Infrared Spectroscopy, and their antioxidant and antifungal capacity were determined. It was observed that the ethyl acetate fraction was enriched in polyphenols, the butanol fraction resulted in purified from proteins and the residual aqueous fraction contains more hydrophobic compounds. The evaluation of the antioxidant activity revealed that the ethyl acetate fraction possesses an interesting capacity 1,1-diphenyl-2-picrylhydrazyl(DPPH) radical scavenging with a percentage of inhibition of 84.02% at a concentration of 2 mg/mL and better ferric reducing antioxidant power (FRAP) 4.275 ± 0.011 mmol/g of dry sample than the other fractions tested. Regarding the antifungal activity, the diethyl ether fraction showed the highest activity against *Aspergillus niger* with 58.78% of inhibition. The results obtained in this work showed the relevance of the valorization of the leaves of *Capparis spinosa* L., given its richness in bioactive molecules can be regarded as a natural source of antioxidant and antifungal and may be considered in the future to replace synthetic preservatives in food, pharmaceutic products and cosmetic.

Keywords: *Capparis spinosa* L.; fractionation; natural products; hydroxyl-aromatic; protein; antifungal; antioxidant

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1. Introduction

There is much proof that the consumption of vegetables, fruits, and other derived plant products, is beneficial for human health because of the presence of a wide variety of bioactive molecules. These substances are secondary metabolites, biosynthesized by plants to prevent UV stress, pathogen attack or to attract pollinator insects [1]. The phenolic compounds including phenolic acid, flavonoids, and tannins are a major group of phytochemicals that present strong antioxidant and antifungal activities [2]. Moreover, because of the toxicity of food and cosmetic preservatives (i.e., parabens, butylated hydroxytoluene, and butylated hydroxyanisole), there is an increasing demand for the search of new bioactive substances from natural sources [3,4].

The therapeutic virtues of plants are showing renewed interest thanks to the improvement of extractive techniques and the progress of structural analysis methods for

the discovery of new active substances. The exhaustive extraction is usually carried out with different solvents of increasing polarity to best extract the most active components with the highest biological activity [5]. The liquid-liquid extraction with organic solvents allowed a first selection and separation of secondary metabolites and also a depletion of the plant organic matter. Each organic solvent will allow, depending on its selective extraction and solubilization power, one or more types of substances, thus different solvents will give different compositions of extracts [6]. *Capparis spinosa* L. is recognized for its phytochemical richness, is a nutritional and therapeutic potential, which is particularly interesting to exploit, and has proven to be a model plant suitable for the extraction of bioactive compounds.

Capparis spinosa L. (the caper) is a perennial shrubby plant that is widespread in Mediterranean countries. It belongs to the family Capparidaceae and the genus *Capparis* [7]. Indeed, *Capparis spinosa* L. is well known for its high metabolic potential primary and secondary active [8]. Whatever the forms in which they are metabolized, these substances are extremely complex in structure and chemical composition and, having a recognized therapeutic activity that is listed in many pharmacological activities, have also been shown potential applications for human health [9,10].

The literature provides plentiful information about the health benefits and chemical composition of *Capparis spinosa* L. leaves. For instance, they are well known for their antimicrobial and antioxidative properties, attributed to lipophilic and hydrophilic compounds including carotenoids, polysaccharides, amino acids, flavonoids, and proanthocyanins [11,12]. In traditional medicine, the leaves were used to treat gout and arthritis.

Despite the well-documented *Capparis spinosa* L. fruits and roots, data on the other *Capparis spinosa* L. organs remain unequal disparate. In this sense, we focused our work on leaves, which were investigated for their phytochemical contents and therapeutic potential.

The objective was (i) to investigate the composition of hydro-ethanolic extract of leaves of *Capparis Spinosa* L. and fractions by pyrolysis-gas chromatography/mass spectrometry (Py-GC-MS) (ii) to assess the antioxidant activity of hydro-ethanolic extract and fractions via the commonly 1,1-diphenyl-2-picrylhydrazyl(DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP) methods, and (iii) to evaluate the antifungal capacities of hydro-extract and fractions against *Aspergillus niger*.

2. Materials and Methods

2.1. Crude Material

Aerial parts of *Capparis spinosa* L. were collected in July 2017 in Siliana Mountain, northwestern Tunisia. The freshly harvested leaves were separated from the branches and dried in the shade for 20 days at room temperature. The dried material was mechanically pulverized using a knife mill and stored in a closed container.

2.2. Extraction

The leaves (100 g) of *Capparis spinosa* L. were macerated in 500 mL of a hydro-alcoholic solution (80% ethanol, v/v) for 24 h with stirring at room temperature. After filtration, the solution was evaporated to dryness under reduced pressure in the RSLAB 100-PRO rotary evaporator and the resulting hydro-ethanolic extract was named CSECE.

2.3. Liquid-Liquid Partition

The crude hydro-ethanolic extract (15 g) was dissolved in 200 mL of hot water (40 °C) and subjected to a series of liquid extractions in separating funnels with solvents immiscible with the aqueous extract. This operation allows the separation of one or more constituents using their unequal distribution in two immiscible liquids.

It consists of adding 3 × 300 mL of hexane to the aqueous extract to eliminate lipids and chlorophyll. Then, the aqueous phase is mixed with diethyl ether (v/v) to obtain an organic phase containing flavonoids aglycones and methoxylated aglycones. The remaining aqueous phase, in turn, undergoes three extractions with ethyl acetate in order to recover

in the organic phase certain flavonoids aglycones but especially monoglycosides and eliminates the monosaccharides. The remaining aqueous phase is mixed with n-butanol to recover flavonoids and triglycosides. The final aqueous phase contains especially the more polar glycosylated flavonoids.

The extracted fractions with hexane (CSHex), diethyl ether (CSDE), ethyl acetate (CSAC), n-butanol (CSBu), and the aqueous fraction (CSAQ) were dried at low pressure at 45 °C and stored at 4 °C.

The extraction yield is defined as the ratio between the mass of the dry extract obtained after evaporation and the initial mass of the plant material. The yield is calculated by the following Equation (1):

$$Yield\ (\%) = m_0/m_1 \times 100 \quad (1)$$

where m_0 is the mass in grams of the evaporated dry residue and m_1 corresponds to the mass in grams of the initial dry plant matter.

2.4. Phytochemical Screening

2.4.1. Determination of the Total Phenolics Compounds

The quantitative determination of total polyphenols was carried out by the Folin Ciocalteau method [13]. An aliquot of each fraction (100 µL) was mixed with 500 µL of Folin Ciocalteau reagent (10%). After stirring the sample, followed by a rest for 6 min, 400 µL of an aqueous solution of Na_2CO_3 (7.5%) was added and finally incubated for 30 min in the dark. The reading was performed against a blank using a spectrophotometer (Lambda 800 Spectrophotometer, Perkin Elmer, Waltham, MA, USA) at 765 nm. The amount of total polyphenols was estimated from a calibration curve carried out in parallel under the same operating conditions using gallic acid as a positive control. The phenolics content is expressed in milligrams of gallic acid per gram of dry weight (mg GAE/g DW). All measurements were performed in triplicate.

2.4.2. Total Flavonoid

The principle of this method is based on the formation of a complex between flavonoids and aluminum chloride [14]. Firstly, 250 µL of the appropriately diluted extract was added to 75 µL of an aqueous solution of $NaNO_2$ (15%). The mixture was stirred well and left for 6 min before adding 75 µL of freshly prepared aluminum chloride ($AlCl_3 \cdot 6H_2O$, 10%). After standing for 5 min at room temperature, 1000 µL of NaOH (1 M) was added to the mixture. Finally, the mixture was adjusted to 2500 µL with distilled water. The absorbance of the mixture was read at 510 nm. The standard range was prepared with quercetin. The total flavonoid content of the fractions is expressed in milligrams of quercetin per gram (g) of dry weight (mg EQ)/g DW). All determinations were performed in triplicate and averaged.

2.4.3. Total Condensed Tannin

The amounts of condensed tannins were estimated using the vanillin method in an acidic medium, the condensed tannins depolymerize [15]. In brief, 350 µL of the diluted extract was treated with 750 µL of vanillin (1% in 7 M H_2SO_4) in an ice-water bath. After homogenization, the mixture was incubated at room temperature for 15 min and the absorbance measured at 500 nm. The contents of condensed tannins were expressed in mg of catechin equivalent per range of dry matter (mg ECA/g DW). All measurements were performed in triplicate.

2.4.4. Py-GC-MS

The samples were subjected to pyrolysis coupled with gas chromatography and mass spectrometry, which is a powerful molecular characterization tool. It consists of a decomposition of complexes by heat, in order to obtain smaller fragments that are volatile and therefore more easily analyzed [16]. This technique allows a qualitative interpretation, but also a relative quantitative estimation of the contribution of polyphenols that may be

present in the *Capparis spinosa* L. leaves. The analyses were carried out with a CDS analytical Pyroprobe5150 (CDS analytical, 465 Limestone RD, Oxford, PA, USA). The temperature of the samples is raised to 600 °C for 15 s with a heating rate of 20 °C/ms. The effluents produced are driven out of the interface by a carrier gas (helium) to GC (7890A) Agilent (5301 Stevens Creek Blvd. Santa Clara, CA, USA),via an injector (split/split less 50:1). The chromatography was carried out on a capillary column HP-5MS (length: 30 m, internal diameter: 0.25 mm, thickness: 0.25 µm) stationary phase (5% phenyl methylsiloxane, 95% methylsiloxane). The oven program started at 50 °C and was held for 2 min at this temperature. Then, it was raised to 120 °C at 8 °C min^{-1}, held for 5 min, raised to 280 °C at 8 °C min^{-1}, and finally increased to 300 °C at 10 °C min^{-1} and held for 10 min. The chromatograph is coupled to a mass spectrometer (5975C inert MSD Triple-Axis Detector, Agilent Technologies, Santa Clara, CA, USA), which allows each compound to be identified by its retention time GC and its mass spectrum. Identification was made possible by comparing the mass spectra obtained with those of NIST computer libraries as well as those reported in the literature [17].

2.4.5. Infrared Analysis

Fourier transform IR spectroscopy analysis was carried to determine the nature and structure of different functional groups of the bioactive compounds present in the five fractions as well as the crude extract. The infrared spectra were obtained using a Perkin Elmer spectrometer equipped with ATR (Attenuated Total Reflectance) system, with an internal reflection diamond crystal lens. The acquisitions were made by performing 32 scans between 4000 and 600 cm^{-1}, with a resolution of 4 cm^{-1}. After scanning, the organic functional groups were identified through the location of the different bands on the FTIR spectrum and compared with those given in the literature [18,19]. FT-IR analysis was used to obtain the qualitative information regarding functional groups present in extracts, and to validate the results of Py-GC-MS.

2.5. Antioxidant Activities

In our study, the in vitro antioxidant activity of *Capparis spinosa* L. was demonstrated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging and reducing power (FRAP, ferric reducing antioxidant power).

2.5.1. DPPH Assay

The radical scavenging capacity, using a free radical DPPH radical (1,1-diphenyl-2-picrylhydrazyl) test, was carried out according to a modified version of the radical method described by [20] on a Perkin Elmer, Lambda 800 Spectrophotometer. 0.1 mL of different concentrations (0.25, 0.5, 1, and 2 mg/mL) of each sample (CSECE, CSHex, CSDE, CSAC, CSBu, and CSAQ) was added to 0.9 mL of methanolic solution of DPPH (0.1 mM). After incubation, 30 min in the dark at room temperature (25 ± 2 °C), the absorbance was measured at 517 nm against a methanol blank and the DPPH solution. The results were expressed as a percentage of DPPH inhibition (*IP*%) according to the following Equation (2):

$$IP\,\% = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100 \quad (2)$$

The quercetin was used as a positive control, the absorbance of which was measured under the same conditions as the samples and for each concentration. The test was repeated three times.

2.5.2. FRAP Assay (Ferric Reducing-Antioxidant Power)

The reducing power of *Capparis spinosa* L. extracts has been determined using the Iron reduction method (FRAP) described by [21] corresponds to the reduction of a ferric tripyridyltriazine complex [(Fe (III)-TPTZ) 2] into a ferrous tripyridyltriazine complex [(Fe (II)-TPTZ) 2] by an antioxidant (AH), at a pH of 3.6 to maintain the solubility of iron.

FRAP reagent is prepared by mixing a buffer solution Sodium acetate (300 mM, PH 3.6), a solution of 2,4,6-tris (2-pyridyl)-1,3,5-s-triazine TPTZ (10 mM) and a solution of $FeCl_3$ in the proportions 10:1:1. A volume of 50 µL of the extract is mixed with 1500 µL of FRAP reagent, after incubation at 37 °C for 30 min in the dark, the absorbance was measured at 595 nm against the blank. A standard range is produced with an aqueous solution of iron sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$). The results were expressed in $FeSO_4 \cdot 7H_2O$ mM equivalent per gram of dry extract (mM/g DE). All measurements were in triplicate.

2.6. Antifungal Activity

The fungus *Aspergillus niger* Tiegh (MB284309) (CBS-KNAW, Utrecht, The Netherlands) was cultured on potato dextrose agar (PDA, Merck, Keniworth, NJ, USA) for 7 days at 25 ± 1.5 °C and used in this test. Extracts samples were diluted in dimethylsulfoxide (DMSO) at final concentrations of 25 mg/mL and then appraised by two methods.

In the first method, an aliquot (10 µL and 20 µL) of each extract was pipetted to the center of a Petri dish filled with PDA, and around that was aseptically inoculated an aliquot of spores. The Petri dishes were sealed and incubated for seven days at 25 ± 1.5 °C. Three repetitions of each sample and control (without extracts) were prepared. After incubation, the growth intensity (GI) was determined by visual assessment using a numerical scale according to ISO 846 (Table 1). In parallel, Whatman paper (2 cm × 2 cm) was soaked with 20 µL of each extract and placed on Petri dishes with PDA (10 mL) and 0.4% streptomycin. Each PDA dish was inoculated with suspension 10^6 spores/mL and incubated for 7 days at 25 ± 1.5 °C. Then, absorbent papers were extracted from Petri dishes and washed with sterile Ringer's solution. The obtained spore solution was vortexed and stained (Lactophenol blue) to count the spores concentration and life viability with a Cellometer® Mini (Nexcelom Bioscience LLC, Lawrence, MA, USA) automated cell counter by putting 20 µL of each spore solution inside counting chambers and using Cellometer® Mini software for the analysis. The fungal growth inhibition (*FGI*) was calculated as the concentration of spores (conidia) per milliliter, according to Equation (3):

$$FGI = \frac{\mathrm{Cg} - \mathrm{Tg}}{\mathrm{Tg}} \tag{3}$$

Table 1. Evaluation of growth intensity according to ISO 846 [22].

Growth Intensity (GI)	Evaluation
0	No apparent growth under microscope
1	No growth to the naked eye, but visible to the microscope
2	Visible growth, covering up to 25% to the test area
3	Visible growth, covering up to 50%
4	Visible growth, covering up to 75%
5	Significant growth, covering more than 75%

Cg is the average concentration in the control samples and Tg is the average concentration in the treated sample [22,23].

2.7. Data Analysis

The results are presented as the mean (n = 3) ± the standard deviation. In order, the statistical analysis was performed by the IBM SPSS Statistics 26 software (IBM, Armonk, NY, USA) on the values obtained. The differences are tested for significance by the analysis of variance (ANOVA) followed by the Tukey test. A probability of *p*-value less than 0.05 has been adopted as a criterion of significant differences. The software used to draw the graphs was Origin Pro 2016 (Origin Lab, Northampton, MA, USA).

3. Results

3.1. Phytochemical Screening

The extraction used in the first place is of the solid–liquid type, in this method the solvent must cross the barrier of the solid–liquid interface and dissolve the active ingredient (organic compounds) that the solvent contains inside. Secondly, a liquid–liquid extraction was carried out which makes it possible to diffuse the phenolic compounds into a liquid by applying solvents of increasing polarity allowing a selection and separation of the secondary metabolites contained in the crude extracts.

According to Figure 1, the crude hydroethanolic extract represents a high yield of 17.9 ± 1.177%. In the work of Kalantari et al. [9], hydro-alcoholic extract of *Capparis spinosa* L. leaves had a higher percentage (15.75%) yield of extraction compared to the ethyl acetate and chloroform extracts, which is the same in relation to our results.

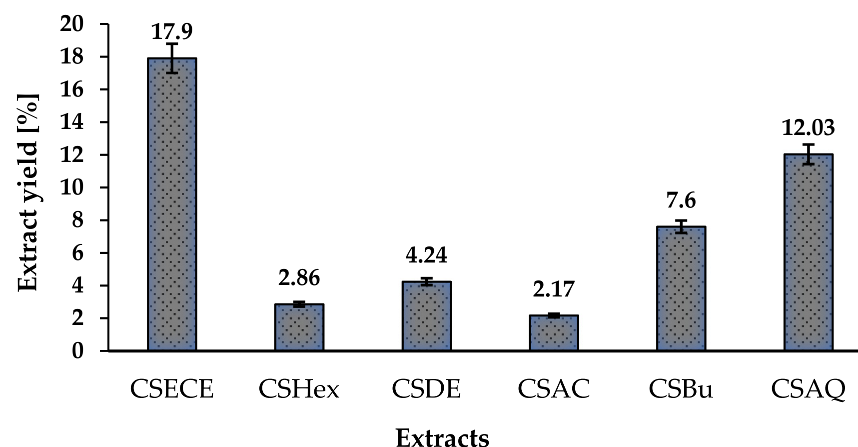


Figure 1. The yield of different extracts of *Capparis spinosa* L. leaves. CSECE: crude extract. The fractions: CSHex: hexane, CSDE: diethyl ether, CSAC: ethyl Acetate, CSBu: n-Butanol, CSAQ: aqueous.

The extraction yields by fractionation of the raw extract of leaves of *Capparis spinosa* L. fluctuate significantly between 2.17 ± 0.149% and 12.03 ± 1.621% in the following increasing order: ethyl acetate (CSAC), hexane (CSHex), diethyl ether (CSDE) n-butanol (CSBu) and aqueous (CSAQ). These results indicate a relatively interesting for the extraction carried out by butanol and the fraction of the residual aqueous solution. This could presume the richness of the leaves of *Capparis spinosa* L. in compounds of high polarity including phenolic compounds, given their richness in hydroxyl groups [24]. Therefore, the difference between the extraction yields obtained with the five fractions depends on the chemical properties of the molecules to be extracted, the physicochemical characteristics of the solvents used, and in particular their polarity which influences the solubility of the chemical constituents of a sample [5].

The crude extract and the five fractions were quantitatively characterized by spectrophotometer, in order to determine their contents in total polyphenols (TP), flavonoids (TF), and condensed tannins (TC), then underwent phytochemical characterization by FT-IR and Py-GC-MS.

The results of the colorimetric analysis of the phenolic compounds are summarized in Table 2. The results revealed that the crude hydro-ethanolic extract is rich in a significant number of bioactive compounds. When compared to other extracts, the hydro-ethanolic crude fraction presented significantly higher values of condensed tannins (17.93 ± 0.15 mg CE/g) ($p < 0.5$). This can be explained by the differential solubility of the bimolecular extracted from the plant material and the selectivity of the solvents used. The liquid-liquid extraction method can dilute or on the contrary increase the contents of phenolic compounds in the crude extract [25,26].

Table 2. Results of the determination of phenolic compounds.

Samples	TPC [1]	TFC [2]	Tannins [3]
CSECE	82.031 ± 2.113 [a]	48.446 ± 2.209 [a]	17.929 ± 0.152 [a]
CSHex	61.919 ± 0.731 [b]	31.458 ± 1.547 [b]	10.024 ± 0.051 [b]
CSDE	78.889 ± 1.707 [a]	35.729 ± 2.210 [b]	11.357 ± 0.253 [c]
CSAC	259.31 ± 6.177 [c]	107.291 ± 8.650 [c]	16.786 ± 0.101 [a]
CSBu	62.72 ± 1.138 [b]	22.1881 ± 0.085 [d]	8.036 ± 0.253 [d]
CSAQ	54.253 ± 0.406 [b]	13,546 ± 1.547 [d]	10.645 ± 0.556 [b]

[1] mg of gallic acid equivalent per gram of dried extract (mg GAE/g), [2] mg of quercetin equivalent per gram dried extract (mg QE/g), [3] mg of catechin equivalent per gram of dried extract (mg CE/g). CSECE: crude extract. The fractions: CSHex: hexane, CSDE: diethyl ether, CSAC: ethyl Acetate, CSBu: n-Butanol, CSAQ: aqueous fraction. Values are the means ± SD of three measurements. One-way analysis of variance (ANOVA): values with different letters are significantly different ($p < 0.05$).

Khojasteh Rad et al. [27] reported that the content of condensed tannins in the hydroalcoholic extract of *Capparis spinosa* L. leaves harvested in July 2016, Iran is 2.54 mg CE/g DM. This quantity of condensed tannins is less important compared to our results.

However, we found that the values determined in the fractions depended significantly on the solvents used. The ethyl acetate fraction (CSAC) showed the significantly highest levels of polyphenols and total flavonoids 259.31 ± 6.177 mg GAE/g and 107.291 ± 8.65 mg QE/g, respectively ($p < 0.05$). This suggests that the hydroxyl-aromatic concentrates in the fraction (CSAC). While the lowest amount of these compounds was found in the aqueous fraction (CSAQ) with a content of 54.253 ± 0.406 mg GAE/g for polyphenols and 13.546 ± 1.547 mg QE/g for flavonoids which means that this fraction is the least hydroxyl-aromatic. Our results are in agreement with research by Hyun et al. [28], who found that the ethyl acetate fraction, n-butanol fraction, and crude extract were more efficient in recovering phenolic compounds. Furthermore, previous studies showed that the flavonoids of *Capparis* leaves mainly concentrate in the ethyl acetate extract (1.6 ± 0.016 mg RE/g MS), followed by the hexane extract (0.61 ± 0.009 mg ER/g MS), and a low amount was detected in the aqueous extract (0.3 ± 0.018 mg RE/g MS) [29]. These results are similar to the conclusions drawn by our study. The difference in the amount of total phenolic compounds may be due to differences in environmental conditions, plant growth, and experimental conditions [30].

The results of the quantitative analysis of the condensed tannins indicate that these molecules are important constituents of the phenolic compounds of the different fractions, their quantity varies between 8.036 ± 0.253 mg EC/g and 16.786 ± 0.101 mg EC/g.

In some cases, the analysis by gas chromatography is unsuitable due to the non-volatility of the heaviest compounds. In this case, the GC-MS can be coupled upstream to the pyrolysis (Py-GC-MS). In this technique, the sample is pyrolyzed, which causes the degradation of the macromolecular structures creating volatile fragments. Thus, this method makes it possible to quantify and identify the thermal degradation products of the samples [31–33]. The four organics fractions were analyzed by Py-GC-MS. To facilitate the comparison of the organic fractions, we grouped all the pyrolysis fragments identified and classified according to several categories: phenols, proteins, polysaccharides, oxygenated compounds (fatty acids, fatty acid ester, alcohols, and furan), terpenes, halogenated compounds, aliphatic hydrocarbons (alkanes and alkynes) steroids, as well as the compounds that could not be identified. All these results are represented in Tables 3 and 4. The pyrolysis components are partially similar, the most relevant difference, between the distributions of compounds, comes from the fact that the ethyl acetate fraction (CSAC) is richest in phenols with a relative content of 36.50% and contain more polysaccharides (2.66%) and furanics (coumaran, 5-methylfurfural, furfural) (10.19%). Hexane fraction (CSHex) has lower amounts of phenolic compounds (8.43%) and more ester fatty acids (11.65%) and steroids (2.43%) as well as slightly fewer furanics (1.37%) and significant protein (38.95%) and halogenated compounds (5.74%). This suggests that hexane is a good solvent for separating the organic fraction of hydroxyl-lean residuals (for example, terpenes, waxes, fatty acids,

oils) from the raw mixture. Fractions CSBu and CSDE were the most protein condensed compared to the samples analyzed with proportions 62.68%, 60.09%, respectively, and contain other organic constituents, in smaller proportions. Our results are similar to previous works which have demonstrated the richness of *Capparis spinosa* L. in biologically active substances such as phenols, fatty acids, terpenes, sterols, polysaccharides, flavonoids [34], alkaloids [35], and proteins [12], [36].

Table 3. Normalized relative percentage grouped by class of functional group of the Py-GC/MS peak areas at 600 °C.

Functional Groups	CSHex	CSDE	CSAC	CSBu
Phenolic (%)	8.43	11.76	36.50	5.82
Proteins (%)	38.95	60.09	4.86	62.68
Polysaccharides (%)	1.33	0.55	2.66	-
Fatty acid (%)	6.70	0.70	4.52	9.59
Fatty acid ester (%)	11.65	5.58	4.29	0.94
Furanes (%)	1.37	4.47	10.19	2.91
Terpene (%)	1.86	-	1.75	0.23
Halogenated compounds (%)	5.74	-	-	-
Hydrocarbons (%)	4.52	4.89	1.86	4.92
Alcohol (%)	-	-	14.25	-
Steroid (%)	2.43	-	0.90	1.69
Others (%)	17.02	11.96	18.22	11.22

Organic fractions: CSHex: hexane, CSDE: diethyl ether, CSAC: ethyl acetate, CSBu: n-butanol.

Table 4. Identification of the compounds resulting from Py-GC/MS at 600 °C of the organic fractions.

Peak Number	Compound	CSHex	CSDE	CSAC	CSBu
		RT (min)			
1	Pyrrolidine, 1-methyl	3.214	3.237	-	3.214
2	3-Penten-1-yne, 3-methyl-, (Z)	3.820	3.820	-	-
3	Isopropyl alcohol	-	-	4.253	-
4	Furfural	-	-	5.044	
4	Pyrrole, 4-ethyl-2-methyl	-	6.673	-	6.673
5	5-Methylfurfural	-	7.205	7.245	7.205
6	Phenol	7.528	7.580	7.534	7.765
7	Benzyl alcohol	-	-	8.469	-
8	Proline, N-methyl-, butyl ester	8.527	8.539	8.585	8.568
9	Guaiacol	9.330	9.324	9.365	9.330
10	Methyl benzoate	9.428	9.428	-	9.434
11	Phenylethyl alcohol	-	9.757	9.815	9.763
12	Benzoic acid	-	-	11.629	-
13	1H-Pyrrole, 2,3-dimethyl	11.779	11.773	-	-
14	Benzoic acid, 2hydroxy-, hydrazide	-	-	11.865	-
15	Coumaran	11.946	11.946	12.044	11.992
16	1-Methoxycyclohexane	-	13.003	-	-
17	2-Methylpiperidine	-	-	-	13.009
18	Isonaline 70	-	-	14.014	-
19	Indole	14.430	14.424	14.493	-
20	Proline, N-methyl-, butyl ester	-	-	-	14.597
21	2-Methoxy-4vinylphenol	15.024	15.013	15.094	15.030
22	Phenol, 2,6-dimethoxy	16.041	16.041	16.122	16.047
23	5-Methoxy-1H-indole-3-carbaldehyde	17.837	17.831	-	17.837
24	Phenol, 2-methoxy-4-(1-propenyl)	18.247	18.241	18.270	-
25	1,6-Anhydro-beta-d-glucopyranose	18.894	-	-	-

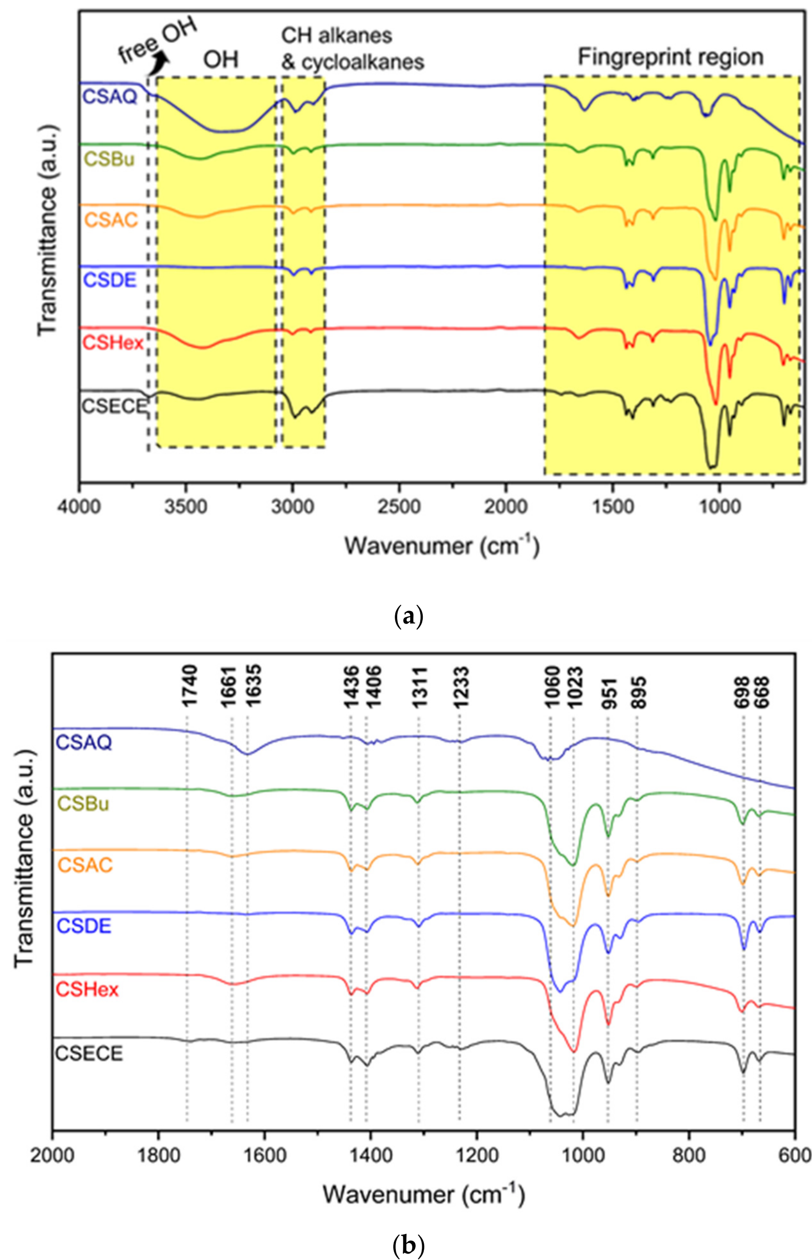
Table 4. *Cont.*

Peak Number	Compound	CSHex	CSDE	CSAC	CSBu
		RT (min)			
26	ButylatedHydroxytoluene	-	-	19.402	-
27	Diethyl phthalate	20.684	20.684	20.696	20.690
28	cis-Pinane	23.676	23.670	23.682	-
29	3,7,11,15-Tetramethyl-2-hexadecen1-ol	-	-	24.149	-
30	Hexadecanoic acid, methyl ester	24.600	24.594	24.606	24.594
31	n-Hexadecanoic acid	25.016	24.987	25.016	25.062
32	alfa-Methyl linolenate	26.367	26.361	26.356	26.356
33	Phytol	26.465	-	26.459	26.465
34	Nonacosane	-	33.511	-	403.318
35	Hexadecane, 1-iodo	33.517	-	-	-
36	Octadecane, 1-iodo	36.670	-	-	-
37	Hentriacontane	-	36.647	36.659	-
38	Docosane	-	-	-	36.670
39	Eicosane	40.326	40.314	40.326	38.686
40	Tetratriacontane	-	-	-	40.332
41	Beta-Sitosterol	40.961	-	40,456	40.967

RT: Retention time, (-): Not detect, CSHex, CSDE, CSAC and CSBu correspond respectively to hexane, diethyl ether, ethyl acetate and n-Butanol fractions.

The analysis of the composition showed the presence of numerous secondary metabolites with or without pharmacological and/or therapeutic activity, belonging to different chemical classes could represent a fingerprint for the evaluation of the quality of the different organic fractions of the hydro-alcoholic extract of the leaves.

The chemical structures of the different samples were analyzed through FTIR and their corresponding spectrums are shown in Figure 2a. For a better understanding, the fingerprint region of the spectrums is represented in Figure 2b. The analysis of different samples shows several similarities. However, the IR spectrum of the crude extract shows a band at 3679 cm^{-1} corresponding to the –OH group of free alcohols, the peak at 1740 cm^{-1} indicates the presence of the carbonyl group, which are not visible in fractions [37]. Comparing the five spectra we noticed that they have the same organic functions but with different transmissions. Indeed, all the spectra have shown a wide absorption band at 3430 cm^{-1} which is associated with the presence of –OH stretching vibrations which is attributed to the content of bioactive compounds (alcoholic and phenolic compounds [38]), and intense bands around 2991 cm^{-1} and 2903 cm^{-1} could be assigned to C–H of alkanes and cycloalkanes. The main differences between the fractions were found first in the CSDE fraction, exhibiting less intensity on the –OH bands at 3430 cm^{-1}, on vibrator elongation band C=C at 1636 cm^{-1}, and more intensity on the band 1023 cm^{-1} characteristic of the C–O vibrator which confirms the richness of the CSDE fractions with the hydrocarbons. In the second, the CSAQ fraction presented the widest band located at 3320 cm^{-1}, most intense band at around 1635 cm^{-1} corresponds to the vibrator C=C and show disappearance of the bands included in the region of 1000–650 cm^{-1}. The spectra of CSHex, CSDE, CSAC, and CSBu showed a signal at 1311 cm^{-1}, 1023 cm^{-1}, 951 cm^{-1}, and 895 cm^{-1} was respectively characteristic of the –OH, C–O, C–H, and deformation of the C–H groups indicating the predominance of phenolic such as tannins and flavonoids as well as oxygenated substances [39,40]. These results are in agreement with the Py-GC-MS results.



(**a**)

(**b**)

Figure 2. (**a**) FTIR spectra of crude extract and corresponding fractions obtained from *Capparis spinosa* L. CSECE: crude extract. The fractions: CSHex: hexane, CSDE: diethyl ether, CSAC: ethyl acetate, CSBu: n-butanol, CSAQ: aqueous. (**b**) Fingerprint region of FT-IR spectra of crude extract and fractions; CSHex: hexane, CSDE: diethyl ether, CSAC: ethyl acetate, CSBu: n-butanol, CSAQ: aqueous of *Capparis spinosa* L.

3.2. Antioxidant Activity

The antioxidant capacity of the CSECE extract and the five fractions of this extract could inhibit the free radical DPPH, in comparison with positive antioxidant control, quercetin. The results are illustrated in Figure 3. The antioxidant activity exerted on the free radical DPPH by the extracts and fractions is dose dependent.

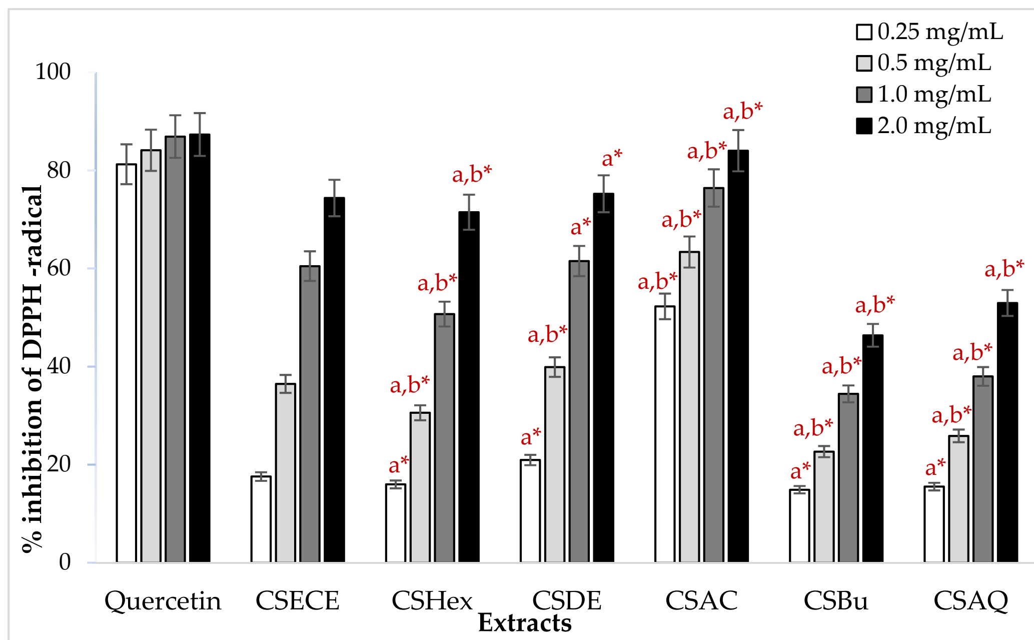


Figure 3. DPPH test: Histograms, expressed as a percentage of inhibition (%), illustrating the antioxidant activity of the standard, the crude extract (CSECE) and its fractions at different concentrations, a and b mean statistically different from standard and CSECE respectively for the same concentration, * indicates significance ($p < 0.05$). The fractions: CSHex: hexane, CSDE: diethyl ether, CSAC: ethyl acetate, CSBu: n-butanol, CSAQ: aqueous.

All the extracts can reduce the stable radical DPPH to 2,2-diphenyl-1-picrylhydrazine. The results indicate that the CSECE extract has a significant and active antioxidant capacity or even less than the positive control. Among the fractions, it appears that they have a significant antioxidant capacity (anti-DPPH) and that the activity is not found in the same way in all the fractions at the same concentration tested. At the concentration of 2 mg/mL, the maximum scavenging activity of the free radical DPPH was observed for all the samples. Our result clearly indicated that the ethyl acetate fraction presented an inhibition percentage (84.02%) quite higher than the other fractions and close to the reference antioxidant ascorbic acid (87.32%) at the same concentration. These findings are in agreement with the results reported in the literature [29] indicating better DPPH free radical scavenging activity in the ethyl acetate solvent. The high antioxidant capacity could be attributed to the presence of high amounts of phenolic compounds, which exceed 36% in this fraction. While the activity of CSHex, CSDE, and CSAQ was 71.47%, 75.23%, and 52.97% respectively. With the exception, the CSBu fraction was the less effective at neutralizing the DPPH radical with 46.37% inhibition at 2 mg/mL, this might be attributed to the low amount of phenolic compounds (5.82%). The antioxidant activity is expressed significantly in a dose-dependent manner, each time the concentration of extract is increased, the percentage of inhibition is significantly increased. This phenomenon is interpreted by the transfer of single electrons which are localized in the external orbital of the DPPH, and after having reached a given concentration, the antioxidant will react completely with the radical, and when we increase the concentration, the activity antioxidant will remain constant since this is accompanied by the saturation of the electronic layers of the radical [41]. The inhibition rates of the DPPH radical recorded in the presence of the hydroalcoholic extract and the various fractions are lower than those of ascorbic acid. This could be explained by the presence of methoxy groups which increase the accessibility of the center of the DPPH radical to ascorbic acid [42–44]. This result could also be interpreted as the total content of phenolic compounds in the raw extract that does not incorporate all the antioxidants [45]. Moreover, the fact that the synergistic effect of polyphenols makes the antioxidant activity of extracts weaker than that of isolated natural phytochemicals [46]. The results of our study illustrate

that the antioxidant capacity of the extracts of the leaves of *Capparis spinosa* L. was strongly affected by the extraction solvent.

The antioxidant activity of *Capparis Spinosa* L. extracts was assessed using the FRAP method. It is based on the capacity of the extracts to reduce ferric iron Fe^{3+} to ferrous iron Fe^{2+}. The results of the FRAP tests (Table 5) were in agreement with those of the DPPH tests, the ethyl acetate fraction having stronger antioxidant activity, with a reducing power of 4.275 ± 0.011 mmol Fe^{2+}/g of sample, followed by the diethyl ether fraction 1.38 ± 0.024 mmol Fe^{2+}/g of sample and the hydroethanolic extract 1.354 ± 0.043 mmol Fe^{2+}/g. while, the CSHex, CSBu, and CSAQ fractions showed a comparatively lower activity with reducing values of 1.11 ± 0.060, 0.898 ± 0.047, and 0.744 ± 0.045 mmol of Fe^{2+}/g of sample, respectively. According to the literature, these results are in agreement with the data found by Mohebali et al. [47], which revealed that the extract of leaves of *Capparis spinosa* L. has a significant reducing activity using the FRAP test (3606.75 ± 0.01 µmol/g). The reducing power of *Capparis spinose* L. is probably due to the presence of a hydroxyl group in phenolic compounds which can serve as an electron donor. Additionally, these results suggest that the FRAP values reported in our study may be related to the content of thiols and sulfur compounds [48]. Thus, antioxidants can be considered as reductants, and inactivate oxidants by reductants can be demonstrated as redox reactions [49]. Some previous studies have also shown that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity [50].

Table 5. Results of the FRAP test of different samples.

Samples	FRAP (mmol Fe^{2+}/g Dry Sample)
CSECE	1.354 ± 0.043 [a]
CSHex	1.110 ± 0.060 [a]
CSDE	1.380 ± 0.024 [a]
CSAC	4.275 ± 0.011 [b]
CSBu	0.898 ± 0.047 [c]
CSAQ	0.744 ± 0.045 [c]

CSECE: crude extract, CSHex: hexane fraction, CSDE: diethyl ether fraction, CSAC: ethyl acetate fraction, CSBu: n-butanol fraction, CSAQ: aqueous fraction. The results are expressed as mean ± standard deviation (n = 3). Means with different letters are significantly different($p < 0.05$).

3.3. Antifungal Activity

The methods used to study the antifungal effect of CSECE and its fractions against food contaminants were designed to assess the effectiveness of the compounds in inhibiting fungal growth and secondly, to study the sensitivity of microorganisms with extracts and to measure their antifungal power. The results of exposure of *Aspergillus niger* to various doses of leaf samples of *Capparis spinosa* L. are presented in Figure 4. The results of Figure 4 indicate that the samples studied did not inhibit fungal growth in a dose-dependent manner. The crude extract and the fractions (CSHex, CSBu) tested at a dose of 20 µL were effective in controlling the fungal growth (growth intensity = 1). At a dose of 10 µL, the CSAQ fractions were more effective than CSAC and CSDE (growth intensity = 2, 3, respectively).

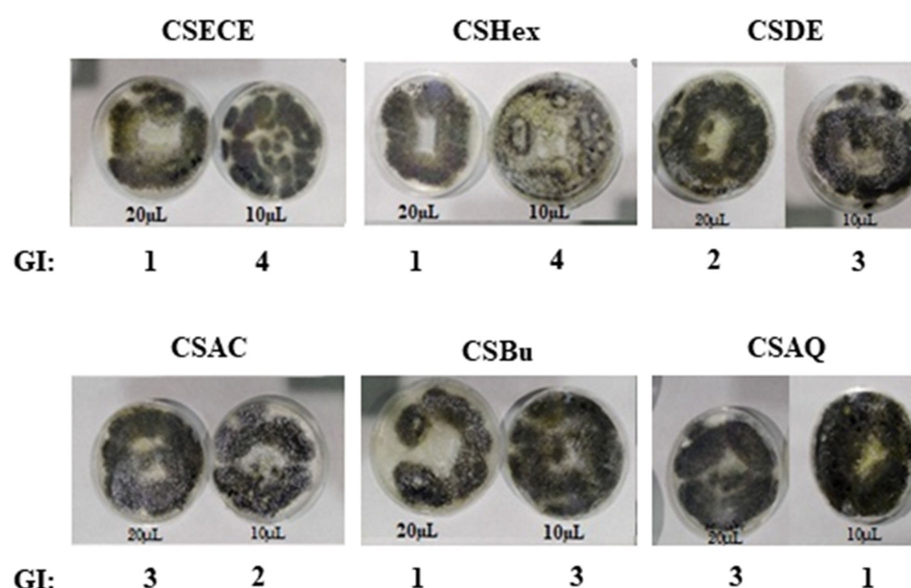


Figure 4. Antifungal activity of the crude extract and the fractions (CSHex, CSDE, CSAC, CSBu, and CSAQ correspond respectively to hexane, diethyl ether, ethyl acetate, n-butanol and aqueous fractions.) against *A. niger* by the PDA agar method. GI: Growth intensity.

Table 6 shows the percentage inhibition of fungal growth, and the viability measured after 7 days of incubation at 25 °C using the Cellometer® software. It was clear from the obtained results that the inhibition of fungal growth depended on the type of solvent used. The crude hydro-ethanolic extract inhibited 46.56% of fungal growth. CSHex, CSBu, CSAQ revealed considerable inhibition with 57.9%, 52.18%, and 58.01%, respectively. Regarding the CSAC fraction, did not show inhibition (FGI, 12.71%) not considered fungistatic, in contrast of CSDE fraction showed a higher inhibition effect (FGI, 58.78%) against the growth of *Aspergillus niger*. The positive behavior of CSHex, CSBu, and CSDE against *A. niger* was probably due to its high percentage of protein which was 38.95%, 60.09%, and 62.68% respectively. These results were consistent with previous research that concluded that the proteins extracted from *Capparis spinosa* L. act as antifungal agents that cause growth retardation in the fungal strain [36,51]. The antifungal efficacy of extracts is particularly due to the composition of these in various bioactive compounds belonging to different chemical classes, which can be used to reduce the process of biodegradation [52,53].

Table 6. Results of antifungal activity against *Aspergillus niger* of CSECE, CSHex, CSDE, CSAC, CSBu, and CSAQ.

Sample	FGI (1) [%]	Viability (2) [%]
Control	-	78.31
CSMCE	46.56	62.55
CSHex	57.09	73.00
CSDE	58.78	55.45
CSAC	12.71	56.06
CSBu	52.18	60.21
CSAQ	58.01	60.21

(1) Fungal growth inhibition, (2) Lives spores/total spores. CSECE: crude extract. The fractions: CSHex: hexane, CSDE: diethyl ether, CSAC: ethyl acetate, CSBu: n-butanol, CSAQ: aqueous.

4. Conclusions

The present study demonstrates the richness in phenolic compounds as well as the important antioxidant and antifungal activities of the crude ethanolic extract and fractions of *Capparis spinosa* L. leaves.

In order to elucidate bioactive molecules crude extract of *Capparis spinosa* L. was partitioned by LLE. When fractions were analyzed, the bioactivity was mainly found in the ethyl acetate fraction CSAC. The phytochemicals were present diversely in different solvents. The Py-GC-MS analysis corroborated that the CSAC fraction mainly constituted phenolic compounds while CSBu and CSDE fractions were most condensed in protein. The FT-IR spectra analysis showed that there is a similarity between the fractions, which confirms the results obtained by colorimetric analysis and Py-GC-MS. The study of antioxidant antifungal activities demonstrates that the ethyl acetate and hexane fractions of *Capparis spinosa* L. leaves should serve as potential sources of new antioxidant and antifungal products. Nevertheless, further works are needed to insulated and identify active compounds, especially in ethyl acetate and hexane fraction, which will allow them to be used in human health and pharmaceutical fields. In vivo studies are also needed to understand the involvement of phenolic compounds in regards to antioxidant and antifungal mechanisms.

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