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Phytochemical profile and antimicrobial activity against phytopathogenic strains of Hydro-methanolic extracts of Caper (*Capparis spinosa* L). From the region of Souk Ahras (Algeria)

Sabrina. AMIRAT[a*], Nina. SADOU[a], Samar. MAKNI[b], Ratiba. SERIDI[a], Latifa Assia. BENSEGHIR[a], Djawhara. CHOHRA[c], Abdelmoumen. BOULEBNANE[d], Yahia. KHELEF[e], Lakhdar. DJEDILAT[f], Sahar. KESKES[b], Hanane. DHOUIB[b], Maissa. CHAKROUNb Houari, BENAMAR[g] and Slim. TOUNSI[b].

[a] Laboratory of Plant Biology and Environment (LBVE) «Medicinal Plants» Axis, Faculty of Sciences, Department of Biology, Badji Mokhtar University, Annaba, Algeria.

[b] Laboratory of Biopesticides (LB Pest), Center of Biotechnology of Sfax, University of Sfax, P.O. Box 1177, 3018 Sfax, Tunisia.

[c] Laboratory of Synthesis and Organic Biocatalysis, Department of Chemistry, Badji Mokhtar University, Annaba, Algeria.

[d] Department of English, University of Algiers 2, Abou El Kacem Saâdallah, Algeria.

[e] Laboratory Biology, Environment and Health (LBEH), Department of Cellular and Molecular Biology, Faculty of Natural Science and Life, University of El Oued, Algeria

[f] Valorization of Biological Resourced Laboratory (VRBN). Campus ELBez, Algiers Road, Setif 19137, Algeria.

[g] Laboratory of Research in Arid Areas, Faculty of Biological Sciences, University of Science and Technology Houari Boumediene, PO Box 32, El Alia, Bab Ezzouar, 16111 Algiers, Algeria

***Corresponding author: sabrinaamirat@univ-annaba.org**

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ABSTRACT

The use of different types of synthetic pesticides has significantly contributed to the management of agricultural production losses caused by plant diseases. However, their use can induce numerous environmental and health problems which necessitate exploring safer solutions. Relying on plant biomass as a source of bioactive molecules can be an excellent alternative.

In order to enhance the Hydro-methanolic extracts of the aerial parts (Leaves, Fruits) of *Capparis spinosa* L; As a potential source of bioactive molecules, having undergone two methods of extractions: Ultrasonic assisted extraction and maceration extraction.

A phytochemical profile by FT-IR; HPLC-DAD; GC/MS; was established to better understand the different phytochemical components of the plant.

The FT-IR analysis of the powders of the studied parts and their extracts revealed the presence of several chemical functions including the grouping of phenols.

On this basis, this research focused on a quantification of phenolic compounds by HPLC-DAD, the majority being the catechol followed by coumaric respectively. The rate in the leaves extract from the maceration is (40.687 µg/g extract) while the rate in the fruits extract from the ultrasonic extraction is (41.326 µg/g extract).

GC/MS analysis of the fractions obtained after silylation revealed the presence of 73 compounds for the Ethyl Acetate and Hexane fractions of the dry extracts of the studied leaves while noting the presence of 32 compounds in the Ethyl Acetate and Hexane fractions for the dried extracts of the fruits studied.

The antimicrobial activity tested on reference strains showed a significant power of the dry extracts of the plant on the one hand against*: Botrytis cinerea* LPAP630. *Fusarium oxysporum* CTM 10402. *Aspergillus niger* CTM 10099 and on the other hand against: *Agrobacterium tumefaciens* B6. Agrobacterium *tumefaciens* C58.

The results show the promising capabilities and efficiency of *Capparis spinosa* L in the field of biopesticide. Moreover, they highlight the open wide horizons for possible application through innovative bioprocesses for sustainable development.

Keywords: *Capparis spinosa* L; FT-IR; HPLC-DAD; GC/MS; Antimicrobial activity; Biopesticide; Sustainable development.

Introduction

Capparis spinosa L(Capparacea) is widely distributed in Europe, Africa, Asia and Oceania [1] And it is considered among the most important commercial crops in the Caper family.
It is spontaneous, xerophyte and a plant endowed with «multivalence», with a rare ecological capacity of adaptation, notably marked adaptation to marginal soils [2] During its initial growth phase, this ancient plant is vulnerable to salt stress, and using a bio stimulant may enhance plant biomass, proline, and the activity of soil enzymes boosted caper bioflavonoid and raised the Capers have a high phenol concentration

It is a species with interesting and proven potential, not only in terms of its ecology, but also in terms of its nutritional aspect, particularly its phytopharmacological traits [3].

Due to the increased need for phenolics as natural antioxidants and food stabilizers, various studies have recently focused on the extraction of these biomolecules. There have been several biological actions demonstrated, including antioxidant, anti-diabetic, anti-microbial, and anti-inflammatory properties [4] provided in the literature. Furthermore, the methanolic extract showed the highest phenolic and flavonoid contents [5].
According to research, *Capparis spinosa* L has a very broad spectrum of medicinal and economic applications in different medical systems for instance, leaf extract has been shown to have hepatoprotective [6]and nephroprotective [7] properties. It also has anthelmintic and antiallergic effects [8, 9]

In fact, C. spinosa continues to inspire researchers all around the world, and its subject is still crucial [5, 10, 11]

The high cost of pesticides, the demands of international markets in terms of the quality of agricultural products, consumer health and environmental concerns push us to develop inexpensive and safe methods.

It should be mentioned that only a few numbers of research studies have been conducted on *Capparis spinosa* L extracts biopesticide activities, especially on microbial pathogen [3] which are insufficient in light of the plant's abundance.

Materials and Procedures

Study Area Presentation

The selected study area during our field trips is located in the Ouled Moumen wilaya of Souk-Ahras Forest.

The Souk-Ahras region is located in the north-eastern part of Algeria. Geographically, it sits in a basin, surrounded by mountains with complex mountainous relief (500 to 1400 m). It is part of the Tellian Atlas in the North and the High Plains in the South. It is characterized by a continental climate with Mediterranean and desert influences, with rainfall ranging from 300 to 1000 mm/year [36].

The originality of the work focused on the materialization of station, which has not been the subject of an inventory study of this species, and which is distributed as follows in Table 1. Figure 1

Table 1: Geographic coordinates of the station studied

Site	Date of Harvest	Locality	Geographical coordinates	Site accessibility	Ecotype
FRINA	**20/07/2020**	**Ouled Moumen**	**N 36° 22/ 48,72//** **E 8° 22/ 48,72//**	**Accessible**	**Spiny**

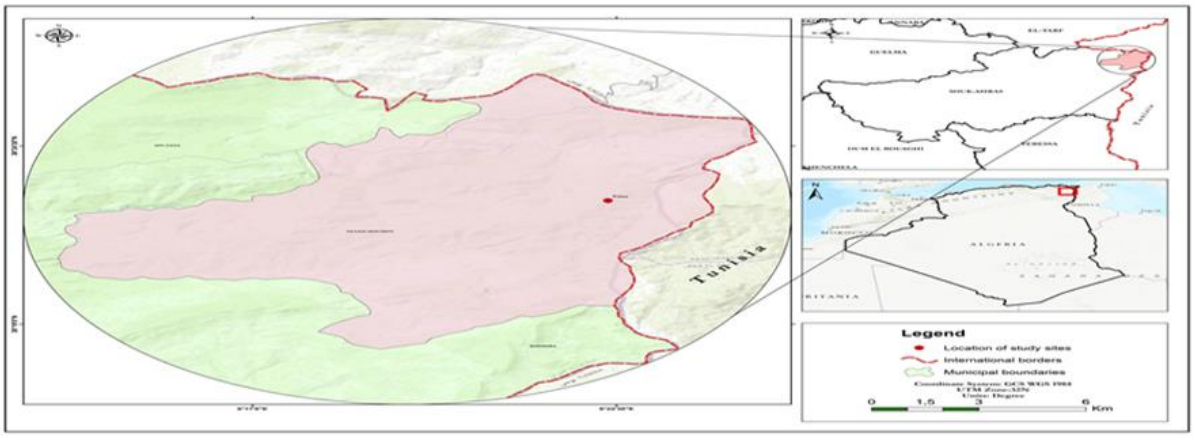


Figure 1: Study site location map

Plant Material:

Plant material of C. spinosa L(Figure2) was collected from their natural habitat during the period mentioned (Table 1). The plant was identified by Pr. HAMEL T, botanist at Badji Mokhtar University, Annaba, Algeria. The samples were sorted, dried and powdered.

Figure 2: *Capparis spinosa* L in its natural habitat.

Biological Material:

The biological material used was supplied by the Laboratory of Biopesticides of the Center of Biotechnologies of Sfax (CBS) Tunisia; Our study focused on 5 reference strains, namely three fungal strains: *Botrytis cinerea* LPAP630. *Fusarium oxysporum* CTM 10402. *Aspergillus niger* CTM 10099 and two bacterial strains: *Agrobacterium tumefaciens* B6. *Agrobacterium tumefaciens* C58.

Preparation of Extracts

The preparation of the extracts was carried out only on the powder samples of the leaf parts and fruit.

Ultra Sound Extraction

Extraction was carried out according to **[19].** 200ml of MeOH80% solvent was added to 20g of each studied part of the plants. Then they were put in the ultrasonic bath Type (ISOLAB) at 30° for 30 min. After filtration, the filtrate was evaporated by steam rotavapor (BUCHI Rotavapor R 200) at a temperature of 55°.

A yield evaluation was carried out, and dry extracts were kept at 4°C until being used.

ELUS for the dry extract of the leaves and EFUS for the dry extract of the fruits were mentioned (Table 2).

Extraction by Maceration

Maceration consists of letting the powder of the plant material in prolonged contact with a soil-front to extract the active ingredients. It is an extraction that is done at ambient temperature and has the advantage of preserving the thermosensitive substances.

200ml of solvent MeOH80% is added to 20g of each part of the plant and let macerate for 24H under continuous agitation. Subsequently, they are filtered and evaporated as for sonication, with an evaluation of yields. Dry extracts were kept at 4°C until being evaluated.

ELMA refers to the dry extract of the leaves, and EFMA refers to the dry extract of the fruits (Table 2).

Evaluation of yields

The yields of dry extracts obtained by the two extraction methods were calculated using the following formula: $$R\% = \frac{P1-P2}{PE} \times 100$$

R%: Yield.

P1: Bag weight + sample after evaporation in (g).

P2: Empty bag weight (g).

PE: Test portion (g).

Analysis by FT-IR

Fourier Transformed Infrared Spectroscopy (FT-IR) is a fast and inexpensive characterization technique. The extracts of the different parts of the plant as well as the powders of each part were tested by a device type: Shimadzu, IR Affinity, Japan in an interval of 400-4000 cm-1.

HPLC-DAD Analysis of Phenolic Compounds

The analysis of phenolic compounds was carried out using an HPLC model Agilent series 1260, coupled to a diode array detector (HPLC-DAD), and equipped with an injector and a 25°C thermostatically-controlled column oven. The separation was performed on a C18 Kinetex PFP column (100 4.6 mm, 2.6 µm, Phenomenex). The column and sampler were set to 25°C, with a flow rate of 1.5 ml/min.

The injection volume is 20 µl. The mobile phase consists of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). The gradient used was: 5% B for the first six minutes, followed by 20% B for the following 7 min, then 60% B for the next 4 min, 100% B for the subsequent 2 min, then a column wash for 1 min to 100% B, followed by a return to the original conditions (5% B) in 1 min, and 5% regeneration of B for 4 min. The flow rate was 1.5 ml/min and the injection volume was 20 µl. The identification of each compound was based on its retention time and the comparison of spectral data with reference products at 250, 280, 315 or 370 nm.

Analysis by GC/MS

A number of methods have been proposed for the separation and quantification of phenolic compounds. Some authors have proposed gas chromatography coupled with mass spectrometry (GC-MS) as a method that can provide accurate polyphenol composition results.

However, the analysis of non-volatile and thermolabile phenolic compounds by GC-MS assumes conversion to volatile and thermotolerant substances by chemical derivation (The dry hydromethanolic extracts underwent successive fractionations by two solvents: Hexane and Ethyl acetate. The fractions are then evaporated and kept a 4°C until use.

Twenty mg of each fraction was trimethylsilylated using 50 µl N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) and 50 µl pyridine at 60°C for 1 hour, then analyzed by GC-MS. The GC oven temperature was maintained at 100°C for 1 minute and was then programmed from 100°C to 260°C at 4°C/min and maintained for 10 minutes. The split/splitless (splitless mode) injector temperature was set to 280°C. Components were identified by careful examination of fragmentation patterns and spectral data obtained from the Wiley and NIST libraries.

Antimicrobial Activity.

The antimicrobial activity of the Hydro-methanolic extracts was identified by the determination of MIC (Minimum Inhibitory Concentration), MFC (Minimum Fungicidal Concentration) for fungi, and MBC (Minimum Bactericidal Concentration) for bacteria. (Microdilution method).

Antibacterial Activity

The Minimum Inhibitory Concentration (MIC) corresponds to the lowest concentration of the extract capable of inhibiting any microbial growth visible to the naked eye. It, therefore, measures a bacteriostatic effect but does not provide information on the state of the bacterial population. It is not revealed whether the bacteria have been killed in part or in whole, or whether they have only stopped multiplying.

The MIC for the dry extracts studied was determined on a 96-well ELISA plate using the liquid micro-dilution method. Microbial inoculum was added at a rate of 10 µl (10^7 CFU/ml) in each well. The plates were subsequently incubated at the optimum growth temperature of the microorganism. For ease of reading, MTT (3-(4,5-Dimethyl-2-thiazolyl) -2,5-diphenyl-2 H-tetrazoliumbromide) was used as an indicator of microorganism viability by adding 25 µl of MTT (0.5 mg/ml sterile distilled water) in each well, and the mixture was incubated at 37°C for 30 min. The wells, where microbial growth was inhibited, remained colorless after incubation with MTT while those with bacterial growth turned blue [38] In contrast, the Minimum Bactericidal Concentration (MBC) corresponds to the lowest concentration of extract capable after 18 to 24 hours of contact with a bacterial population, leaving a percentage of a surviving bacteria of less than or equal to 0.01%. In order to determine MBC, a sample (3 µl) was taken from all wells without visible microbial growth, seeded in a solid medium and incubated at 30°C for 18 hours.

Antifungal Activity

For the determination of MIC and MFC, we proceeded with the same process for antibacterial activity without the use of MTT.

Results and Discussion

Evaluation of Yields

Table 2: Yields of hydromethanolic extracts from both extraction methods.

Extraction Methods	Parts Studied	Symbole	Yields
Ultra Sound	**Leaves**	**ELUS**	**12.26%± 0,64**
	Fruits	**EFUS**	**14.83%± 1,37**
Maceration	**Leaves**	**ELMA**	**18.86%± 0,50**
	Fruits	**EFMA**	**21.31%± 1.08**

The results are expressed as an average of triplicate standard deviation.

The yield results are presented in Table 2, which shows that the yields of extracts obtained from maceration were higher than those obtained from sonication, with the highest recorded value being (21.31% ± 1.08%) for fruit methanol extract.

These results are in agreement with the results mentioned by [14] with a value of 21.58% for the methanol extract of the flowering buds, flowers and immature fruits of C. spinosa of the Batna region obtained by 24H maceration.

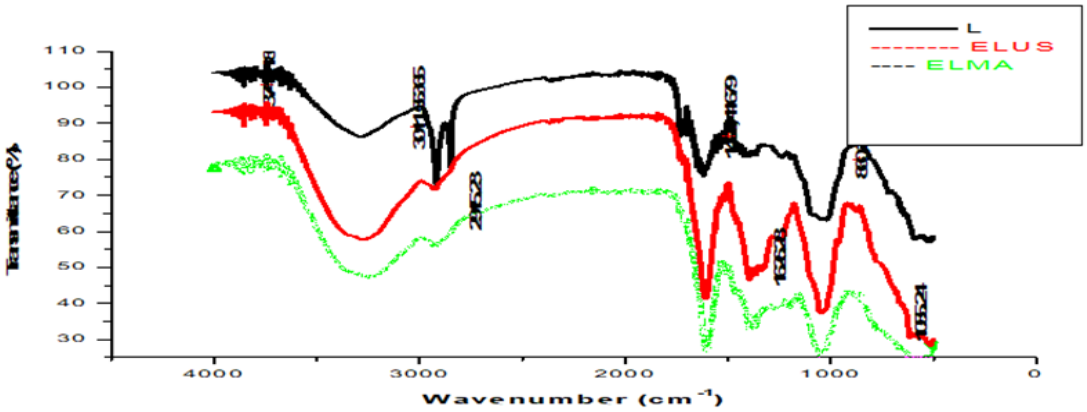
Also, in the work of [12], in a study of crude hydro-methanolic extracts of leaves and fruits, obtained by maceration for 48 hours of the species of the region of M'zyzle (Morocco), a significantly lower value of 6.93% for leaf extract and a value closer to ours with 17.33% for fruit were found.

Our results are in perfect agreement with those reported by [16] during the study of extracts of C. spinosa leaves from the Siliana region (Tunisia), obtained by 24-hour maceration, which reported a yield of the hydro-ethanol extract of (17.9% ± 1.177%).

Similarly, [17]in their work found a worth of 15.75% for the hydro-alcoholic extract of the leaves. It is also noted that [18] reported a value of 13% for the methanol extract of the aerial portion, and 15% for the roots of the Cholistan (Rohi) Desert plant in Pakistan after 72H maceration.

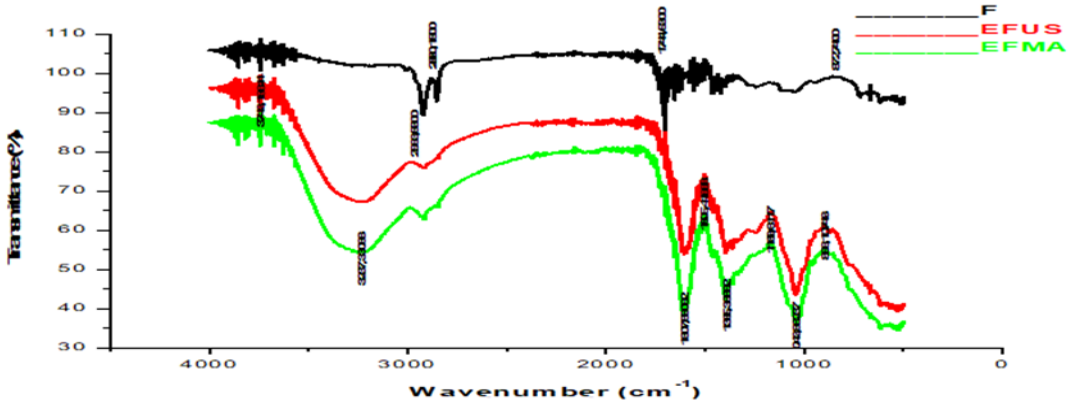
Several studies may have mentioned an improvement in the performance of the extracts subjected to ultrasound [19, 20]. This divergence in rates of return also depends on several factors, namely the nature and solvent polarity, extraction time and temperature, chemical composition, and environmental conditions, as well as the organ studied [21]. Therefore, the variation in yields between the two extraction methods and the different parts of the plant in our results should be highlighted [22].

Chemical Composition by FT-IR



L: Powder of Leaves; ELUS: Extract of Leaves by Sonication; ELMA: Extract of Leaves by Maceration

Figure 3: FT-IR profile of C. spinosa leaves powder and ELUS; ELMA



F: Powder of Fruits; EFUS: Extract of Fruits by Sonication; EFMA: Extract of Fruits by Maceration

Figure 4: FT-IR profile of C. spinosa Fruits Powder and EFUS; EFMA

The FTIR analysis carried out on the powders of the aerial parts of the plant, compared to the various extracts tested reveals, after study, spectra of similarities in both figures: Figure 3 and Figure 4. However, for the two studied parts, we noted the presence of the peaks of 732.42 cm^{-1} and 886.11 cm^{-1} in an interval of (675-995) corresponding to the Alkene group (C-H). 1025.44 cm^{-1} (1000-1050) corresponding to the Aliphatic fluor compounds group (C-F). 1158.66 cm^{-1} (1050-1300) of the function Ester, Ethers, Alcohols, Carboxyl acids (C-O). 1382cm^{-1} (1300-1410), Phenols or tertiary alcohol (O-H). 1512.97 cm^{-1} (1485-1555) Aromatic nitro compounds (C-H). 2920.39 cm^{-1} (2850-3000), Alkane pods (C-H). 3038.50 cm^{-1} (2500-3300), Carboxylic acid (O-H). 3038.50 cm^{-1} (3200-3600) Alcohols, Phenols (O-H). Our results are consistent with those found [23] We draw the conclusion that the comparison of the spectra of raw powder and extracts revealed the same organic activities but with various transmissions.

Chemical Composition by HPLC-DAD

Results in Figure 5 show the presence of the following compounds: Hydroxycortisol, Caffeic acid, Catechol, Ellagic acid, Coumaric acid, Ferrulic acid, Vanniline; and an absence of compounds: Gallic acid, Rutin and Quercitin.

The main compound is Catechol with (40.687 µg/g extract) for the extract of the leaves from the maceration, and a rate of (41.326 µg/g extract) for the extract of the fruits from the ultrasonic extraction.

Many studies have reported the presence of Rutin as a majority compound [24-26] Nevertheless, the results of this study corroborate those found by [18] regarding the total absence of Rutin in the extracts studied.

This gives more information about the diversity and richness of the plant in phenolic compounds and their variations at the same species.

The changes in the chemical composition of the plant were linked to meteorological factors, the location of the collection, and other factors, as was previously shown [27, 28]

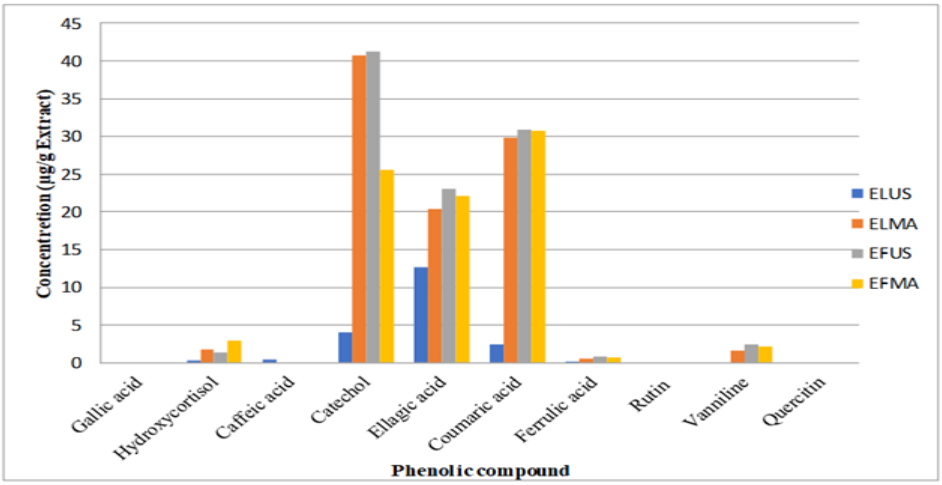


Figure 5: Identification and concentration of phenolic compounds by HPLC-DAD

Chemical composition by GC/MS.

Table 3: Identification of chemical compounds for Fractions leaves studied.

	No	Compounds	A	RT	B	RT	C	RT	D	RT
Fatty acid										
Ester	**1**	**3,6-heptanooxepin-4,5-dicarbonsaure-dimethylester**	**-**		**-**		**+**	**12,102**	**-**	
	2	**Trimethylsilyl ester of glycine**	**+**	**13.5683**	**-**		**-**		**-**	
	3	**Ethyl Oleate**	**-**		**+**	**21,713**	**+**	**21,778**	**-**	
	4	**(3.beta.) 9,19-Cyclolanost-24-en-3-ol, acetate**	**-**		**+**	**44,376**	**-**		**-**	
Acid ester	**5**	**Malic acid, tris(trimethylsilyl) ester**	**-**		**-**		**+**	**5,254**	**-**	
	6	**Benzeneacetic acid, .alpha.,3,4-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester**	**+**	**7.951**	**-**		**-**		**-**	
	7	**Azelaic acid, bis(trimethylsilyl) ester**	**-**		**-**		**+**	**8,711**	**-**	
	8	**Dodecanoic acid, trimethylsilyl ester**	**-**		**-**		**+**	**6,657**	**-**	
	9	**Tetradecanoic acid, trimethylsilyl ester**	**-**		**+**	**9,599**	**-**	**9,614**	**-**	
	10	**Pentanedioic acid, 3-methyl-, bis(tert-butyldimethylsilyl) ester**	**+**	**10.4920**	**-**		**-**		**-**	
	11	**7,10-Hexadecadienoic acid, methyl ester**	**-**		**-**		**-**	**10,895**	**-**	
	12	**Hexadecanoic acid, ethyl ester**	**-**		**-**		**+**	**13,475**	**-**	
	13	**Palmitelaidic acid, trimethylsilyl ester**	**-**		**+**	**14.693**	**+**	**14,742**	**-**	
	14	**D-Gluconic acid, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, trimethylsilyl ester**	**-**		**-**		**+**	**15,261**	**-**	
	15	**Hexadecanoic acid, trimethylsilyl ester**	**+**	**18,5856**	**+**	**15.533**	**+**	**15,596**	**-**	
	16	**Trichloroacetic acid, dodec-9-ynyl ester**	**-**		**-**		**+**	**17,669**	**-**	
	17	**(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester**	**-**		**-**		**+**	**17,994**	**-**	

	18	**Heptadecanoic acid, trimethylsilyl ester**	**-**		**-**		**+**	**20,549**	**-**	
	19	**Linoleic acid, ethyl ester**	**-**		**+**	**21.119**	**+**	**21,187**	**-**	
	20	**(Z)-9-Octadecenoic acid, methyl ester**	**-**		**+**	**21.422**	**+**	**21,488**	**-**	
	21	**Phosphoric acid, dioctadecyl ester**	**-**		**-**		**+**	**22,179**	**-**	
	22	**Octadecanoic acid, ethyl ester**	**-**		**-**		**+**	**22,726**	**-**	
	23	**(Z,Z)-9,12-Octadecadienoic acid, trimethylsilyl ester**	**-**		**-**		**+**	**23,579**	**-**	
	24	**Oleic acid, trimethylsilyl ester**	**+**	**21,0737**	**+**	**23,766**	**-**		**-**	
	25	**Linolenic acid, trimethylsilyl ester**	**-**		**-**		**+**	**23,843**	**+**	**23,555**
		Octadecanoic acid, trimethylsilyl ester	**-**		**+**	**24,819**	**+**	**24,868**	**-**	
	26	**trans-9-Octadecenoic acid, trimethylsilyl ester**	**+**	**26,9792**	**-**		**-**		**-**	
	27	**Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester**	**-**		**-**		**+**	**34,836**	**-**	
	28	**(Z)-9-Octadecenoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester**	**+**	**40,9724**	**-**		**+**	**38,421**	**-**	
Carboxylic acid derivatives	**29**	**2,3,4-Trihydroxybutyric acid tetrakis(trimethylsilyl) deriv.**	**-**		**-**		**+**	**5,879**	**-**	
	30	**Gulonic acid, 2,3,5,6-tetrakis-O-(trimethylsilyl)-, lactone**	**-**		**-**		**+**	**11,068**	**-**	
	31	**Gulonic acid, 2,3,5,6-tetrakis-O-(trimethylsilyl)-, lactone**	**-**		**-**		**+**	**14,537**	**-**	
	32	**1H-Indole-1-acetic acid**	**-**		**+**	**24.032**	**+**	**24,098**	**-**	
Olefin	**33**	**(Z)-7-Hexadecene**	**+**	**7,4040**	**-**		**-**		**-**	
	34	**1-Octadecene**	**+**	**10.3744**	**-**		**-**		**-**	
	35	**1,2-dimethyl-3,5-bis(1-methylethenyl) cyclohexane**	**-**		**+**	**27,08**	**-**		**-**	
	36	**(E, Z)-1,3,12-Nonadecatriene**	**-**		**+**	**36,934**	**-**		**-**	

	37	**Cholesta-3,5-diene**	**-**		**+**	**39,812**	**-**		**-**	
Alkanes	**38**	**Nonadecane**	**-**		**+**	**32,861**	**-**		**-**	
Heterocyclic	**39**	**2-cyclohexyl-Piperidine,**	**-**		**-**		**-**		**+**	**7,493**
	40	**Spiro[cyclopropane-3-(3,4-dihydro-1H-2-thianaphthalene)]**	**-**		**-**		**+**	**11,375**	**-**	
Alcohol	**41**	**Pluchidiol**	**-**		**-**		**-**		**+**	**8,821**
	42	**-(-) Isoeugenol; Cyclohexanol, 5-methyl-2-(1-methylethenyl)**	**-**		**-**		**-**		**+**	**17, 661**
	43	**(Z)-3,7-dimethylocta-2,6-dien-1-ol**	**-**		**-**		**-**		**+**	**21, 18**
	44	**3-tert-Butyl-4-methoxycyclohexanol**	**-**		**-**		**-**		**+**	**26, 113**
	45	**Alpha.Santalol; 5-(2,3-dimethyltricyclo[2.2.1.0(2,6)]hept-3-yl)-2-methyl-2-Penten-1-ol, stereoisomer**	**-**		**-**		**-**		**-**	
	46	**alpha.-D-galactopyranoside, hexa-(trimethylsilyl)-2-O-Glycerol**	**-**		**-**		**+**	**29**	**-**	
	47	**14-methyl-, (3.beta.,5.alpha.)-9,19-Cyclocholestan-3-ol**	**-**		**+**	**35,82**	**-**		**-**	
	48	**(3.beta) Cholest-5-en-3-ol**	**-**		**+**	**39,997**	**-**		**-**	
	49	**14-methyl-(3.beta.)Cholest-7-en-3-ol**	**-**		**+**	**40,493**	**-**		**-**	
	50	**Vitamin E**	**-**		**+**	**41.112**	**-**		**-**	
	51	**Ergost-5-en-3.beta.-ol**	**-**		**+**	**43,004**	**-**		**-**	
	52	**4-methyl-(3.beta.,4.alpha.,5.alpha.) Cholest-7-en-3-ol**	**-**		**-**	**43,26**	**+**		**-**	
	53	**4,4-dimethyl-(3.beta.,5.alpha.)Cholest-8(14)-en-3-ol**	**-**		**+**	**43,611**	**-**		**-**	
	54	**(3.beta.)Lup-20(29)-en-3-ol**	**-**		**+**	**44,194**	**-**		**-**	
	55	**(3. beta.) Lanost-8-en-3-ol**	**-**		**+**	**44,867**	**-**		**-**	
	56	**(3.beta.,24S)Stigmast-5-en-3-ol**	**-**		**+**	**45,768**	**-**		**-**	
	57	**24-propylidene-(3.beta.) Cholest-5-en-3-ol**	**-**		**+**	**46,292**	**-**		**-**	
	58	**Cycloartanol**	**-**		**+**	**46,552**	**-**		**-**	
Alcohol	**59**	**pentakis-O-**	**-**		**-**		**+**	**7,776**	**-**	

derivatives		**(trimethylsilyl)-Arabinitol**								
	60	**1,2,3,4,5-pentakis-O-(trimethylsilyl)-L-Sorbopyranose**	**-**		**-**		**+**	**9,603**	**-**	
	61	**Glucose oxime hexakis(trimethylsilyl)**	**-**		**-**			**12,574**	**-**	
	62	**1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-muco-Inositol**	**-**				**+**	**13,376**	**-**	
	63	**1,2,3,5-tetrakis-O-(trimethylsilyl)-Arabinofuranose**	**-**		**-**		**+**	**13,735**	**-**	
	64	**1,2,3,4,6-pentakis-O-(trimethylsilyl)-beta-D-Galactopyranose**	**-**		**-**		**+**	**14,414**	**-**	
	65	**1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-myo-Inositol**	**-**		**-**		**+**	**19,36**	**-**	
Ketone	**66**	**2-(1-methylpropyl)-Cyclopentanone**	**-**		**-**		**-**		**+**	**18, 256**
Ketone dérivatives	**67**	**1,3,4,5,6-pentakis-O-(trimethylsilyl)-D-Fructose**	**-**		**-**			**9,268**	**-**	
	68	**1,3,4,5,6-pentakis-O-(trimethylsilyl)-D-Fructose**	**-**		**-**		**+**	**9,427**	**-**	
	69	**Per(trimethylsilyl)-L-sorbose**	**-**		**-**		**+**	**16,496**	**-**	
	70	**2',4-Dimethoxy-6'-nitro-3,5-di-t-butylbiphenyl 1,1'-Biphenyl, 3',5'-bis(1,1-dimethylethyl)-2,4'-dimethoxy-6-nitro- (CAS)**	**+**	**37.525**	**-**		**-**		**-**	
	71	**Tocopherol-. gamma. -tms-derivative (high mass adjustment=100%);**	**-**		**+**	**42,436**	**-**		**-**	

A: ELAEUS; B: ELAEMA; C: ELHUS; D: ELHMA

GC/MS analysis of the four fractions of C. spinosa L leaves extracts are represented in the table 3. It reveals the presence of 73 compounds. Including 24 Acid Ester, 18 Alcohol, 7Alcohol derivatives, 5 Olefins, 5Keto derivatives, 4 Carboxylic acid derivatives, 3 Alkane Heterocyclic, 3 Ester, 1 Ketone and 1 Fatty acid. Therefore [23] reported the presence of only 25 phytoconstituents compounds in the methanolic extract of leaves analyzed by GC/MS.

The presence of Tocopherols, which belong to the vitamin E group of compounds were mentioned in leaves and flower buds by [29]. Numerous studies have connected plants containing these chemicals with a reduced risk of several chronic and degenerative diseases, including cancer, cardiovascular diseases, and atherosclerosis.[30]

These results indicate that the leaves of C. spinosa are rich in organic compounds and clearly show the influences of the used organic solvents and the extraction method in the profiles of the extracts [31].

Table 4: Identification of chemical compounds for Fractions fruits studied.

	No	Compounds	E	RT	F	RT	G	RT	H	RT
Fatty acid	**1**	**(Z, Z) -9,12-Octadecadienoic acid**	**+**	**28,27**	**-**		**-**		**-**	
Ester	**2**	**Ethyl 9-hexadecenoate**	**-**		**+**	**12,769**	**+**	**12,769**	**-**	
	3	**Ethyl Oleate**	**-**		**+**	**21,73**	**-**		**-**	
	4	**1-Monooleoylglycerol trimethylsilyl ether**	**-**		**-**		**+**	**38,398**	**-**	
Acid ester	**5**	**Malic acid, tris(trimethylsilyl) ester**	**-**		**-**		**-**		**-**	
	6	**Dodecanoic acid, trimethylsilyl ester**	**-**		**-**		**-**		**+**	**6,669**
	7	**Tetradecanoic acid, methyl ester**	**-**		**-**		**-**		**+**	**7,533**
	8	**Tetradecanoic acid, trimethylsilyl ester**	**-**		**+**	**9.593**	**+**	**9,593**	**+**	**9,628**
	9	**(Z)-9-Hexadecenoic acid, methyl ester**	**-**		**-**		**-**		**+**	**10,898**
	10	**7,10-Hexadecadienoic acid, methyl ester**	**-**		**-**		**-**		**+**	**10.989**
	11	**14-methyl-Pentadecanoic acid, methyl ester**	**+**	**11,396**	**-**		**-**		**+**	**11,407**
	12	**Hexadecanoic acid, ethyl ester**	**+**	**13,467**	**+**	**13,433**	**+**	**13,444**	**-**	
	13	**Palmitelaidic acid, trimethylsilyl ester;**	**-**		**+**	**14,709**	**+**	**14,709**	**-**	
	14	**Hexadecanoic acid, trimethylsilyl ester**	**+**	**15.573**	**+**	**15.578**	**+**	**15,578**	**+**	**15.59**
	15	**(Z,Z)-9,12-Octadecadienoic acid, methyl ester**	**+**	**17,661**	**-**		**-**		**+**	**17,707**
	16	**(Z,Z,Z)-9,12,15-Octadecatrienoic acid,**	**+**	**17,993**	**-**		**-**		**-**	

		methyl ester								
	17	**Octadecanoic acid, methyl ester**	**-**		**-**		**-**		**+**	**19,384**
	18	**Linoleic acid, ethyl ester**	**-**		**+**	**21,134**	**+**	**21,14**	**-**	
	19	**(Z)-9-Octadecenoic acid, methyl ester**	**-**		**+**	**21,438**	**+**	**21,444**	**+**	**18,302**
	20	**9-Octadecenoic acid, ethyl ester**	**-**		**-**		**+**	**21,735**	**-**	
	21	**Phosphoric acid, dioctadecyl ester**	**+**	**22,176**	**-**		**-**		**-**	
	22	**(Z,Z)-9,12-Octadecadienoic acid, trimethylsilyl ester**	**-**		**+**	**23,589**	**+**	**23,584**	**+**	**28,442**
	23	**Oleic acid, trimethylsilyl ester**	**-**		**+**	**23,83**	**+**	**24,11**	**-**	
	24	**Linolenic acid, trimethylsilyl ester**	**+**	**23,87**	**+**	**14,846**	**+**	**14,846**	**-**	
	25	**Octadecanoic acid, trimethylsilyl ester**	**-**		**+**	**24.82**	**+**	**24,831**	**-**	
	26	**Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy] propyl ester**	**-**		**+**	**34,799**	**+**	**34,81**	**-**	
Alcohol	**27**	**d-ISOMENTHOL**	**+**	**18,651**	**-**		**-**		**-**	
	28	**SOLANESOL**	**-**		**+**	**29,431**	**-**		**-**	
	29	**Dodecamethylpentasiloxane**	**+**	**3,236**	**-**		**-**		**-**	
	30	**alpha.-Santalol; 2-Penten-1-ol, 5-(2,3-dimethyltricyclo [2.2.1.0(2,6)] hept-3-yl)-2-methyl-, stereoisomer**	**-**		**+**	**28,533**	**-**		**-**	
Alcohol derivatives	**31**	**Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-, L-**	**-**		**-**		**-**		**-**	
Olefin	**32**	**Stigmastan-3,5-diene**	**-**		**+**	**38,055**	**-**		**-**	

E: EFAEUS; F: EFAEMA; G: EFHUS; H: EFHMA

The results of the GC/MS analysis of the four fruit extracts of C. spinosa L are presented in Table 4, which revealed variations in the profiles of the four extracts. A total of 32 compounds were detected; 22 Acid Ester, 4Alcohol,3Ester,1Fatty acis,1Alcohol,1Olfin.

Comparing to the leaves fractions extracts we note the absence of Carboxylic acid derivatives and Alkane Heterocyclic, Ketone and Ketone derivatives.

[32] In a study of chloroform and hexane extracts by GC/MS, 29 compounds were reported of distribution of secondary metabolites in the organs of the plant were also mentioned by [33].

the results of GC/MS in both of parts studied confirm those already found by the FT-IR.

Antifungal activity

Table 5: MIC and MFC values of leaf extracts and fruit against *Botrytis cinerea* LPAP,630.

Extracts	MIC	MFC	MIC/MFC
ELUS	**1.25**	**1.25**	**1**
ELMA	**0.625**	**0.625**	**1**
EFUS	**2.5**	**2.5**	**1**
EFMA	**2.5**	**2.5**	**1**

Table 6: MIC and MFC values of leaf and fruit extracts against *Aspergillus niger* CTM 10099.

Extracts	MIC	MFC	MFC/MIC
ELUS	**10**	**10**	**1**
ELMA	**5**	**5**	**1**
EFUS	**5**	**5**	**1**
EFMA	**5**	**5**	**1**

Table 7: MIC CFM values for leaf and fruit extracts against *Fusarium oxysporum* (CTM 10402).

Extracts	MIC	CFM	CFM/MIC
ELUS	**1**	**10**	**1**
ELMA	**5**	**10**	**2**
EFUS	**2**	**>10**	**>4**
EFMA	**5**	**5**	**1**

The results recorded in Tables 5;6; and 7 show absolute antifungal activity where MFC/MIC=1 ratios are recorded.

The ELMA extract is considered to be an exception where there is MFC/MIC <4 fungal activity. Added to that, there is no activity for the EFUS extract against all fungal strains tested [34]

Antibacterial activity:

Table 8: MIC and MBC values of leaf and fruit extracts against *Agrobacterium tumefaciens* B6

Extracts	MIC	MBC	MBC/MIC
ELUS	**2.5**	**5**	**2**
ELMA	**2.5**	**>10**	**>4**
EFUS	**5**	**5**	**1**
EFMA	**5**	**5**	**1**

Table 9: CMI & and MBC values of leaf and fruit extracts against *Agrobacterium tumefaciens* C58

Extracts	MIC	MBC	MIC/ MBC
ELUS	**2.5**	**2.5**	**1**
ELMA	**1.25**	**5**	**4**
EFUS	**5**	**5**	**1**
EFMA	**5**	**5**	**1**

Analysis of the Table 8 show that the ELUS extracts; EEFUS; EFMA are bactericidal agents against the pathogenic tested bacteria lead to the MBC/MIC ratios<4 [34].

Hence, the extracts of EFUS and EFMA are absolute bactericidal agents because we record a ratio MBC/MIC=1.

Similarly for table 9; The results obtained show that the extracts ELUS; EFUS, EFMA have an absolute bactericidal activity with a ratio MBC/MIC =1[35].

Conclusion

This study shows that the geographical and environmental variables, harvest time, storage practices, genotype, and extraction and treatment methods have a significant impact on the chemical fingerprints and biological activities of *Capparis spinosa* L from the region of Souk-Ahras, north-east of Algeria.

This is the first work that, to the best of our knowledge, provides the identifications and qualifications of polyphenols contents in dried extracts and their biopesticides proprieties in Algeria.

Additional studies should be taken into consideration in light of these findings to highlight the potential for creating a new biological product for the protection of ecosystems to ensure a sustainable development.

Conflict of Interest: The authors declare that they have no conflict of interest.

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