



RESEARCH ARTICLE

Exploring the potential of bioactive compounds of *Capparis spinosa* L. from Morocco: Unveiling its antioxidant and antifungal powers against *Alternaria alternata*

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ARTICLE HISTORY

Received: 09 July 2024

Accepted: 17 September 2024

Available online

Version 1.0 : 04 November 2024



Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

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Chiboub B, Maatougui A, Aboukhalid K, Khallou A, Khallouf H, Nazih A, Loubna Esseghir, Mourad Baghour. Exploring the potential of bioactive compounds of *Capparis spinosa* L. from Morocco: Unveiling its antioxidant and antifungal powers against *Alternaria alternata*. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.4074>

Abstract

Capparis spinosa L. is a Mediterranean shrub and one of the most important species of the genus *Capparis* due to its ecological, medicinal and economic importance. This species has strong adaptation characteristics to regions with fluctuating climates. This work aims to evaluate the effect of the locality's climate and plant's parts on the content of polyphenols, flavonoids and antioxidant and antifungal activities of caper leaves and seeds. The present study was carried out in 7 localities in 2 Moroccan regions, Oriental and Fez-Meknes, where we collected leaves and seeds samples. Phenolic compounds were determined by the Folin Ciocalteu method, antioxidant activity was studied by DPPH and ABTS tests and an *in vitro* test of antifungal activity was achieved using *Alternaria alternata*. The analysis of variance showed no significant effect of locality, plant's parts and their interaction on the phenolic compounds, antioxidant and antifungal activities of *C. spinosa*. Methanolic leaves extracts demonstrated the most favorable outcomes, yielding a maximum polyphenol content of 124.48 ± 0.05 mg EAG/g DW. Additionally, the maximum flavonoid content was recorded at 24.51 ± 0.01 mg EQ/g DW. The evaluation of the antioxidant activity showed that the minimum inhibitory concentration at 50 % (IC₅₀) is 2.06 ± 0.05 mg/mL and 2.05 ± 0.02 mg/mL using DPPH and ABTS tests respectively. These extracts exhibited the highest % of inhibition, 30.59 % at 1000 ppm against *A. alternata*. The richness of the caper plant in bioactive compounds reveals an interest in its therapeutic and pharmaceutical virtues. In addition, it could be an alternative to chemicals for the control of phytopathogenic fungi on fruits or vegetables.

Keywords

Capparis spinosa L.; polyphenols; flavonoids; DPPH; ABTS; *Alternaria alternata*

Introduction

For a long time, aromatic and medicinal plants (AMP) have been used as therapeutic agents to cure diseases, as they possess bioactive components beneficial for health (1). According to the World Health Organization, 2.4 % of the world's population primarily depends on traditional medicine. The AMP sector is one of the richest in the world due to its diversity as well as its development and valorization potential. However, the majority of species used come from spontaneous populations. Excessive exploitation of species

and climate change associated with the fragility of natural environments (drought, overgrazing, etc.) are gradually leading to a profound change in the structure and dynamics of populations, which are increasingly threatened.

According to the conservation and valorization of medicinal plants of the Moroccan flora, our study focused on a plant of high-added value: the caper plant (*Capparis spinosa* L.). It has a crucial ecological role and a high capacity for germination (2). It is also used for landscaping and erosion control. It offers an opportunity to explore its medicinal properties and contribute to a better understanding of its active compounds.

The caper plant has been used for centuries in traditional herbal medicine as an antifungal, anti-inflammatory, antihepatotoxic, hypoglycemic, diuretic, antihypertensive, etc. (3). It also has significant antioxidant activity. In addition to its multiple therapeutic virtues and its aromatic qualities, which are highly appreciated in Mediterranean kitchens, the caper plant is increasingly being requested for the quality of its essential oils by alternative medicine and the pharmaceutical, cosmetic and food industries (4).

In Morocco, it is present in several localities and is cultivated by farmers in the regions of Fez, Taounate, Meknes, Safi and Marrakech, also in the areas of Settat, Nador, Missouri, Alhoucima, Taza, Ouarzazate and Taroudant (5). However, several difficulties hinder its development, including a lack of precise knowledge about its agroecological characteristics and other multitude of uses.

Thus, the main objective of this study is to contribute to the valorization of bio-resources. The specific objectives are to (i) evaluate the total polyphenols and flavonoid content of extracts from the seeds and leaves of *C. spinosa*, (ii) evaluate the antioxidant activity of these extracts and (iii) determine their antifungal activity.

Materials and Methods

Plant material

The leaves and seeds used in this work were collected on July 15, 2023, in 7 localities belonging to 2 Moroccan regions: Oriental and Fez-Meknes. A total of 14 samples were collected: 7 samples of leaves and 7 samples of seeds, 6 from Oriental and 8 from Fez-Meknes (Table 1 and Fig. 1). The climate of the Oriental region is both Mediterranean in the North, with rare precipitation, often falling in the form of stormy rains at high intensity and Continental in the South, sensitive to Saharan factors, characterized by cold winter,

hot and dry summer with frequent winds. Over the year, the average temperature is 16.7 °C and the average precipitation is 287.2 mm. The dominant soil types in this region are lithosols, entisols and other calcareous soils. In a slightly contrasting environment, the Fez-Meknes region is subject to 3 types of climate: Continental, Mediterranean semi-arid and sub-humid climates, with average annual rainfall ranging from 500 mm to 600 mm, exhibiting a mild north-south gradient. The average annual temperature is around 12 °C. Predominant soil types include vertisols, calcareous chernozems and alluvial regosols.

The geographical coordinates for every station were recorded (Table 1 and Fig. 1). Each sample contained at least 100 g of intact leaves and fruits (which contain seeds) collected from 3 shrubs in a limited area. All the samples were carefully placed inside labeled plastic boxes, ensuring proper identification. They were then transported to the laboratory of the National Institute of Agronomic Research (INRA). The collected leaves and seeds were carefully cleaned and dried at a temperature of 30 °C for a period of 4 days. Once completely dried, they were ground to obtain powder using an electric grinder. This powder was used for the preparation of the extracts necessary for the various analyses.

Preparation of extracts

The extracts were prepared by dissolving 5 g of powder from each plant organ (seeds and leaves) with 50 mL of solvent-water mixture (80/20) and the solvent used was methanol. The extraction is carried out by maceration at room temperature and darkness for 24 h. Then, filtration is achieved to separate the solid extract from the solvent. The filtrates were subjected to vacuum evaporation using a rotary evaporator at 50 °C. The extracts are stored at freezing temperature (4 °C). Obtained extracts served for the quantification of polyphenol components, flavonoid content and antioxidant and antifungal activities.

Total polyphenols determination

Total polyphenols were measured by a colorimetric method using the Folin-Ciocalteu reagent according to the method (6). 50 µL of extract was added to 200 µL of Folin-Ciocalteu reagent and 1.35 mL of distilled water. After 4 min incubation at 25 °C, 400 µL of 20 % (w/v) sodium bicarbonate was added. The absorbance is read at 750 nm after 20 min of incubation. Gallic acid is used as a standard and results are expressed as mg of gallic acid equivalent per g of dry weight.

Flavonoids determination

The determination of flavonoids was carried out based on the chelating properties of $AlCl_3$ (7). 200 µL of each

Table 1. Geographic coordinates of the 7 prospected locations.

Region	Locality	Locality code	X	Y
Oriental	Zayo	ZY	34.55589	-2.34505
	Chwihya	CH	34.80775	-2.65761
	Gafait	GF	34.23909	-2.41032
Fez-Meknes	Taza	TA	34.20808	-4.01084
	Gueldamane	GLD	34.15883	-3.94776
	Missour	MIS	33.0126	-4.03975
	Njil	NJ	33.11847	-4.32603

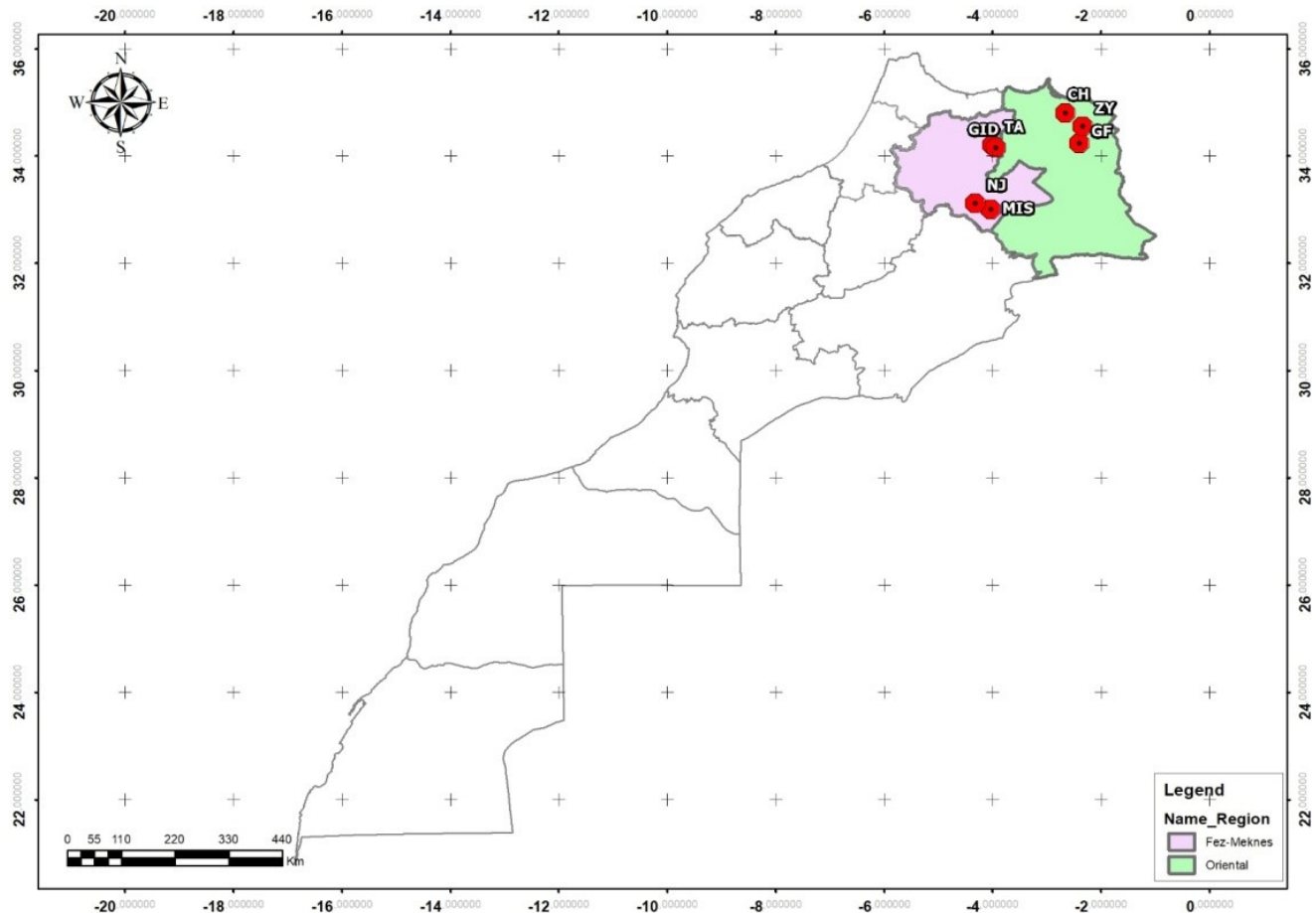


Fig. 1. Location of sampling sites and geographic origin of the *Capparis spinosa* L. surveyed populations.

ethanolic and methanolic extractor standard was added to 750 μL of AlCl_3 solution (2 %). The mixture was vigorously shaken and incubated for 15 min at room temperature and the absorbance was read at 430 nm. The results were expressed as the mg equivalent of quercetin per g of dry weight.

Antioxidant activity determination with DPPH test

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) test measures the antioxidant activity of compounds capable of transferring hydrogen atoms. The compound (DPPH) is a colorful and stable purple radical cation. 20 μL of various concentrations of the extracts were added to 180 μL of a 0.004 % methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm (8). Inhibition percent I (%) of free radical by DPPH was calculated according to the following equation:

$$I (\%) = \left[\frac{\text{ABS control} - \text{ABS test}}{\text{ABS control}} \right] \times 100 \quad (\text{Eqn. 1})$$

Where, ABS control: is the absorbance of the control at the wavelength 517 nm; ABS test: is the absorbance of the sample at the same wavelength 517 nm.

The IC_{50} value (50 % inhibitory concentration) is defined as the sample concentration necessary to achieve a 50 % reduction in DPPH radicals. To calculate the IC_{50} value, a linear regression is usually used, where the concentration of the tested compounds is represented on the x-axis (X) and the inhibition percent is represented on the y-axis (Y).

Antioxidant activity determination with ABTS test

The ABTS radical method is based on the neutralization of a radical cation resulting from mono-electron oxidation of synthetic chromophore 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). 100 μL of various concentrations of the extracts (or ascorbic acid used as a positive control) were added to 900 μL of the diluted ABTS* + solution (9). The absorbance is measured after 10 min of incubation at room temperature ($20 \pm 2^\circ\text{C}$) at 734 nm. The ABTS radical trapping activity A (%) is calculated using the following equation:

$$A (\%) = \left[1 - \left(\frac{A_{\text{extract}}}{A_{\text{control}}} \right) \right] \times 100 \quad (\text{Eqn. 2})$$

Where, A control: corresponds to the absorbance of the ABTS solution (negative test); A extract: corresponds to the absorbance of the sample solution with ABTS.

The IC_{50} value (50 % inhibitory concentration) is defined as the sample concentration necessary to achieve a 50 % reduction in ABTS radicals. To calculate the IC_{50} value, a linear regression is usually used, where the concentration of the tested compounds is represented on the x-axis (X) and the ABTS radical trapping activity is represented on the y-axis (Y).

Antifungal activity

The fungal strain *A. alternata* was isolated from infected potato leaves exhibiting black spot symptoms using a baiting method (10). The strain was cultured on potato dextrose agar (PDA), prepared by mixing 200 mL of potato extract, 20 g of dextrose, 20 agar and 800 mL of deionized

water, followed by autoclaving. After cooling the medium to 40 °C in a water bath, the methanolic extract of leaves and seeds was added to achieve a final concentration of 100 mg/mL. Control plates contained only PDA.

For each treatment, 90 mm petri dishes were prepared by pouring the sterile molten PDA (100 µL per plate) mixed with the extracts. After solidification, 6 mm plugs from 7-day-old *A. alternata* cultures were inoculated onto the plates. The plates were incubated at 28 °C for 10 days, with 3 replicates per treatment. Colony diameters were measured every 2 days to assess fungal growth. The percentage inhibition (PI) of mycelial growth was calculated when the control plates reached the edge of the petri dishes. The formula used for the calculation was:

$$PI (\%) = dc - dt/dc \times 100 \quad (\text{Eqn. 3})$$

Where, dc: Average increase in mycelial growth in control; dt: Average increase in mycelial growth in treatment.

This procedure was used to evaluate the antifungal activity of the extracts by measuring the growth inhibition of *A. alternata* under various concentrations.

Statistical analysis

All data were submitted to an analysis of variance (ANOVA) with 2 factors of variation using SPSS version 26 software for Windows. The Tukey test compared the means to determine homogeneous groups at $\alpha = 0.05$.

Results

Statistical analysis

From the results of the statistical analysis, we observed that the 2 factors (locality and parts of the plant) studied have no significant effect on the analyzed parameters (phenolic compounds, antioxidant and antifungal activities).

Total polyphenols content

The analysis of variance showed no significant effect of locality, parts of plant and their interaction on the total polyphenols content of *Capparis spinosa*. Fig. 2 shows the polyphenol contents obtained from the different parts of the plant (leaves and seeds) using methanol as an extraction solvent. The highest polyphenol content was

obtained in leaves from GF (124.48 ± 1.03 mg EAG/g DW), followed by leaves from ZY (86.83 ± 0.26 mg EAG/g DW). Concerning seeds, the highest polyphenol content was obtained in those from GLD (43.5 ± 0.12 mg EAG/g DW), followed by seeds from GF (41.84 ± 0.09 mg EAG/g DW). The lowest polyphenol content (14.31 ± 0.05 mg EAG/g DW) was observed in leaves from NJ.

Flavonoids content

The analysis of variance showed no significant effect of locality, parts of the plant and their interaction on the flavonoid content of *C. spinosa*. Fig. 2 shows the flavonoid content obtained from the different parts of the plant (leaves and seeds) using methanol as an extraction solvent. The highest flavonoid content was obtained in leaves from ZY (24.51 ± 0.69 mg EQ/g DW), followed by leaves from CH (22.9 ± 0.61 mg EQ/g DW). Concerning seeds, the highest flavonoid content was obtained in those from GF (6.65 ± 0.11 mg EQ/g DW), followed by seeds from NJ (6.35 ± 0.1 mg EQ/g DW). The lowest flavonoid contents was 0.22 ± 0.01 mg EQ/g DW, which was observed in seeds from CH.

Antioxidant activity

The analysis of variance showed no significant effect of locality, parts of the plant and their interaction on the antioxidant activity of *C. spinosa* using DPPH and ABTS tests. The antioxidant activity of the methanolic extracts from the leaves and seeds was demonstrated using 2 chemical methods: DPPH free radical trapping and ABTS radical trapping. The percentage of inhibition I (%) is calculated for each concentration. Fig. 3 represent the variation in the percentage of inhibitory power depending on the concentration of each extract. According to the results obtained (Fig. 3), the % of free radical inhibition increases with higher concentrations for both chemical methods.

Using the DPPH test, the highest percentage of inhibition was obtained in 10 mg/mL concentration of leaves (L) extract from GF 87.23 ± 1 %, followed by leaves from MIS 85.89 ± 1 %. Concerning seeds (S), the highest % of inhibition was obtained in 10 mg/mL concentration of those from NJ at 78.78 ± 1 %, followed by seeds from ZY at 76.42 ± 1 %. The lowest percentage of inhibition was obtained in 3 mg/mL concentration of seed extract from

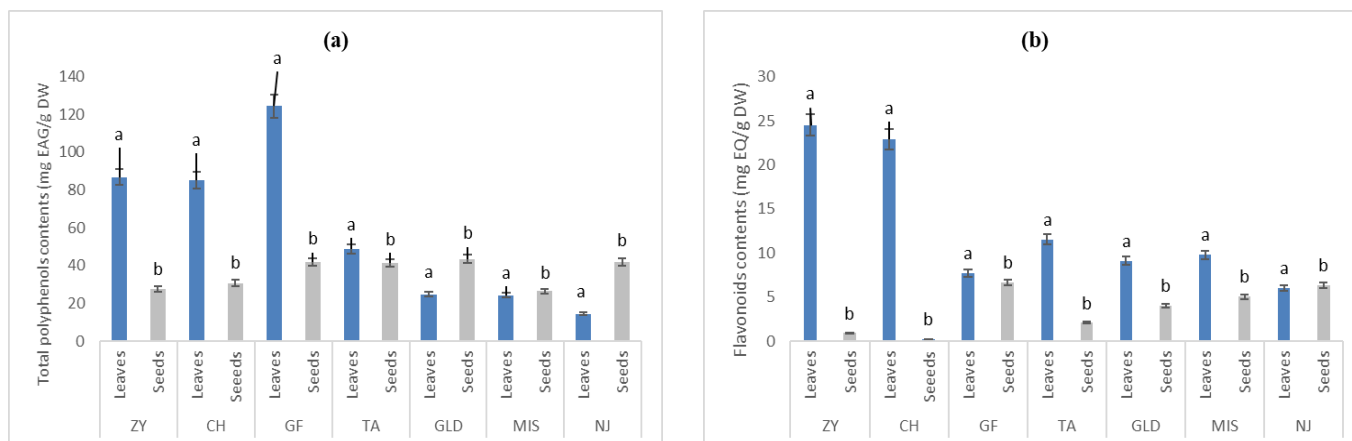


Fig. 2. Total polyphenols (a) and flavonoids (b) contents of different plant's parts of *Capparis spinosa* L., depending on the locality. Bars with the same letters indicate no statistically significant differences according to Tukey's test.

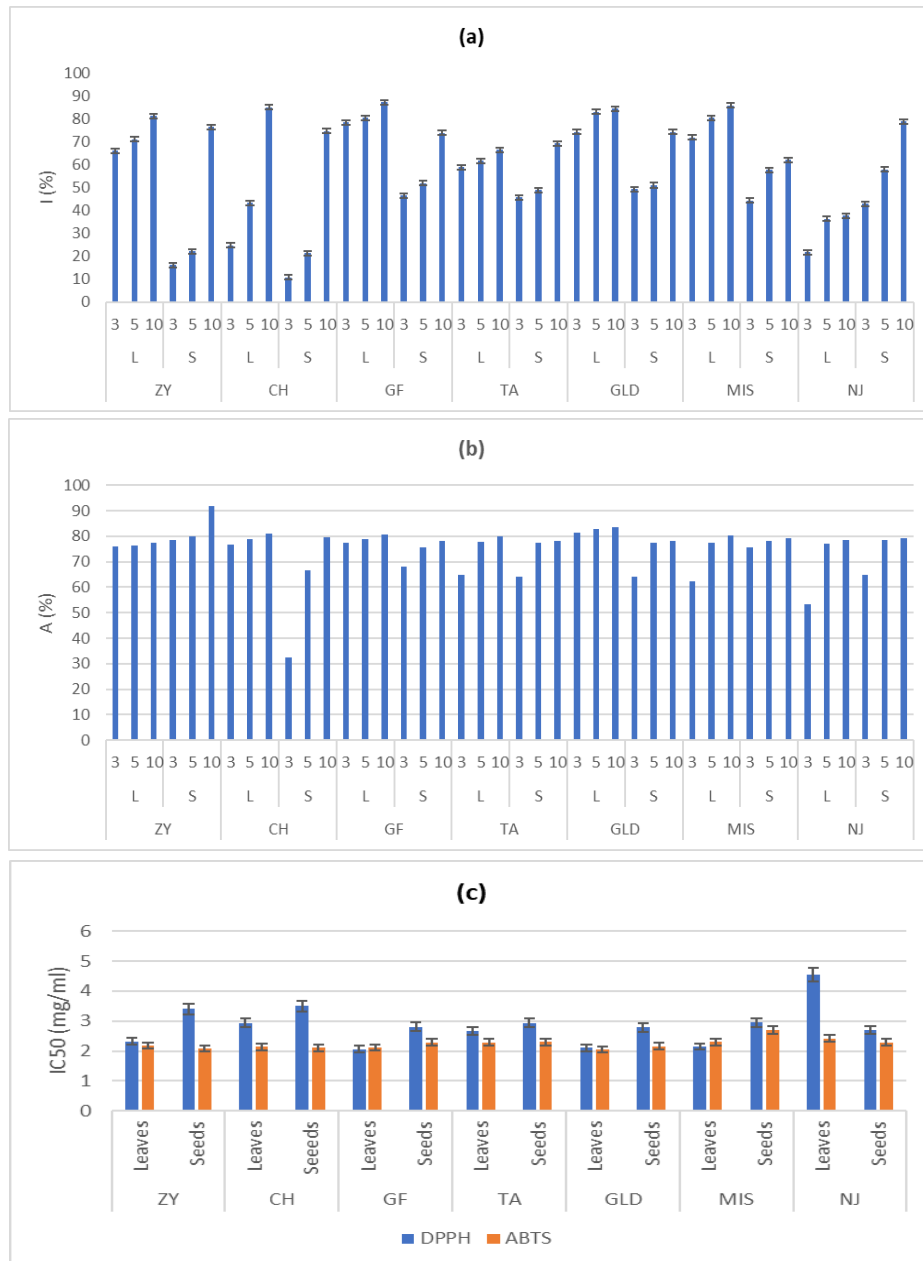


Fig. 3. Inhibition (a & b) and IC₅₀ (c) of extracts of different plant's parts of *Capparis spinosa* L. determined by the DPPH and ABTS tests. CH 10.81 ± 1 %.

Using the ABTS test, the highest % of inhibition was obtained in 10 mg/mL concentration of seed extract from ZY 91.95 ± 1 %, followed by leaves from GLD 83.59 ± 1 %. The lowest percentage of inhibition was obtained in 3 mg/mL concentration of seed extract from CH 32.47 ± 1 %. Fig. 3 also presents the results of IC₅₀ (half inhibition of concentration) of extracts of leaves and seeds of *C. spinosa*, using methanol as a solvent; the extract that has the lowest IC₅₀ value exerts the most powerful anti-radical activity.

Leaves extracts from GF and GLD showed the highest antioxidant activity, with an IC₅₀ of 2.06 ± 0.05 mg/mL against 0.05 ± 0.01 mg/mL for ascorbic acid using as a reference and 2.05 ± 0.02 mg/mL against 0.06 ± 0.01 mg/mL for ascorbic acid using DPPH and ABTS tests, respectively. Leaves extract from NJ and seeds extracts from MIS recorded the highest IC₅₀ values were 4.54 ± 0.06 mg/mL and 2.7 ± 0.06 mg/mL using DPPH and ABTS tests, respectively. There is a correlation between the polyphenol content and the antioxidant activity of methanolic extracts from

different parts of the caper plant (leaves and seeds) collected from different localities. The high number of hydroxyl groups in these extracts is associated with their antioxidant activity.

Antifungal activity

The analysis of variance showed no significant effect of locality, parts of the plant and their interaction on the antifungal activity of *C. spinosa*. Results (Fig. 4) revealed that all the extracts tested exhibited a wide range of radial mycelial growth of *Alternaria alternata* (Table 2 and Fig. 5). The average radial mycelial growth of the test pathogen using leaf extracts ranged from 5.9 mm (CH) to 8.3 mm (GF). Concerning seed extracts, the average radial mycelial growth of the test pathogen ranged from 7.03 mm (TA) to 8.37 mm (CH). Average mycelial growth inhibition of the test pathogen using leaf extracts ranged from 2.35 % (GF) to 30.59 % (CH). Concerning seed extracts, the average mycelial growth inhibition of the test pathogen ranged from 1.57 % (CH) to 17.25 % (TA). All extracts inhibit the mycelial growth and pigmentation of *A. alternata*.

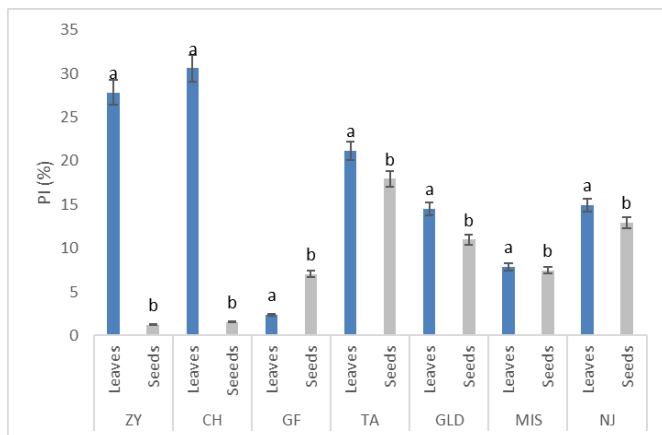


Fig. 4. Percentage inhibition of mycelial growth of *Alternaria alternata* responding to application of *Capparispinosa* L. extracts. Bars with the same letters indicate no statistically significant differences according to Tukey's test.

Table 2. *In vitro* evaluation of *Capparispinosa* L. extracts against mycelial growth of *Alternaria alternata*.

Locality code	Part of plant	Colony diameter of test pathogen (mm)
ZY	Leaves	6.13
	Seeds	8.33
CH	Leaves	5.9
	Seeds	8.37
GF	Leaves	8.3
	Seeds	7.9
TA	Leaves	6.7
	Seeds	7.03
GLD	Leaves	7.27
	Seeds	7.57
MIS	Leaves	7.83
	Seeds	7.87
NJ	Leaves	7.23
	Seeds	7.4

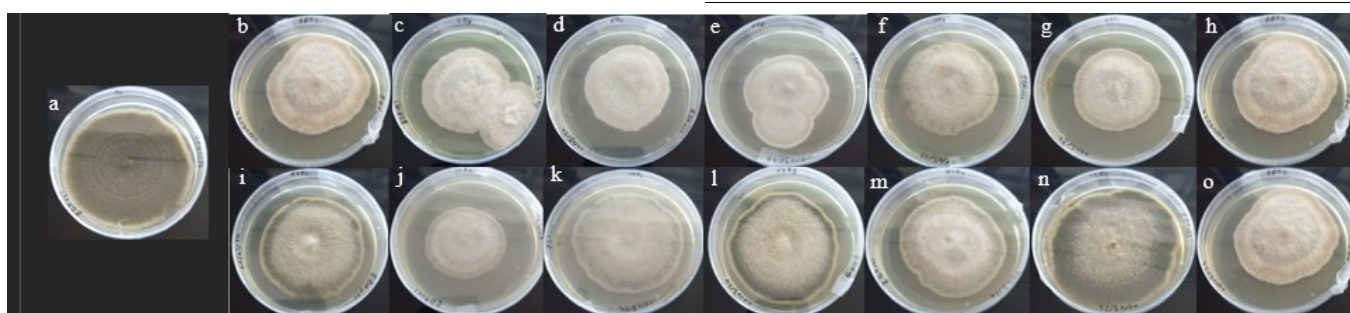


Fig. 5. *In vitro* effect of leaves and seeds extracts on growth and inhibition of *Alternaria alternata*.

a: control, b: leaves extract from ZY, c: leaves extract from CH, d: leaves extract from GF, e: leaves extract from TA, f: leaves extract from GLD, g: leaves extract from MIS, h: leaves extract from NJ, i: seeds extract from ZY, j: seeds extract from CH, k: seeds extract from GF, l: seeds extract from TA, m: seeds extract from GLD, n: seeds extract from MIS, o: seeds extract from NJ.

Discussion

In this study, we obtained that leaves and seeds extracts of *Capparispinosa* are very rich in phenol compounds and have important antioxidant activity, regardless of the locality's climate, because the caper plant has an ideal adaptation and resilience to climate change (11). Also, the extracts inhibit mycelial growth and pigmentation of *Alternaria alternata*, which could be explained by disturbing its metabolism with *C. spinosa* extracts.

Methanol extraction allows to obtain a high concentration of polyphenols and flavonoids in several organs like leaves, roots, seeds, buds, flowers and fruits; this is confirmed (12), who found that the lyophilized methanolic extract of *C. spinosa* buds is rich in total polyphenols and who showed that the methanolic extracts inhibited the peroxidation of the linoleic acid more than aqueous extracts for all parts of the plant (13). This author also noted that the polyphenol content of seeds in the methanolic extract is higher than roots (29.016 ± 10.5 mg EAG/g of extract and 9.2 ± 2.2 mg EAG/g of extract respectively), also for flavonoids (3.4 ± 1.2 mg EQ/g of extract and undetectable respectively).

In general, the total polyphenols and flavonoids compounds were found in higher concentrations in the aerial part than in the root extract. It was proved that the total polyphenols content of aqueous extracts of *C. spinosa* was higher in the flowering stage than in the vegetative stage, ranging from 67.29 mg EAG/g dry powder in flower buds to 33.55 mg EAG/g dry powder in leaves (14). A similar

trend was observed for flavonoid contents; higher values were found in flowers (27.54 mg EQ/g dry powder) compared to leaves (13.97 mg EQ/g dry powder). It was also proved that a total phenolic content of 116.3 ± 0.06 mg GAE/g DM and 18.9 ± 0.08 mg RE/g DM for flavonoids were obtained for aqueous extract of *C. spinosa* leaves (15). Also, studies revealed that polyphenols and flavonoids are more abundant in leaves, flowers, fruits and finally roots (13, 16). The results suggested that the polyphenols content of the methanolic extract of buds flowers was 29.01 ± 0.84 μ g EAG/mg of extract and that of flavonoids was 5.97 ± 0.42 μ g EQ/mg of extract (17). All of these studies confirm the richness of *C. spinosa* in these active molecules. This difference compared with our results is explained by the difference in the parts of the plant studied, which plays an important role in phytochemical valorization (18).

Leaves and seeds tested in our study have a high antioxidant activity. Indeed, a previous study showed that the flowers and fruits of *C. spinosa* present low antioxidant activity with IC_{50} of 70.10 ± 2.32 mg/mL and 137.14 ± 2.08 mg/mL, respectively, compared with ascorbic acid, which was used as a positive control (16). Several factors can influence the polyphenol content and antioxidant activity in plants, such as the age of the plant, the physicochemical characteristics of the soil, the phenological stage, etc.

It was proved that the highest antioxidant activity was found in the fruit and root of the caper plant using the DPPH method (19). The ABTS method's measurement of antioxidant activity resulted in the same results and

indicated that fruits had the highest activity. We also note that there is a correlation between the content of polyphenols and the antioxidant activity in the parts of the plant (leaves and seeds). This result shows that the antioxidant activity depends on the concentration of polyphenols but also on the nature and structure of the antioxidant molecules present in the extract (20), the presence of other constituents in small quantities and the synergy between them (21). Generally, polyphenols with a high number of hydroxyl groups exhibit very significant antioxidant activity. In this sense, several studies in the literature have shown a correlation between anti-radical activity and polyphenol content (22).

The percentage of inhibition recorded for leaves aqueous extract of *C. spinosa* against *Triticum aestivum* was 18.36 % (23). The antifungal activity of ethanolic extract of *C. spinosa* was investigated *in vitro* against *A. alternata*, *Fusarium oxysporum*, *Phoma destructiva*, *Rhizoctonia solani* and *Sclerotium rolfsii* and produced fungal growth inhibition (24). The antifungal activities of *C. spinosa* organs extracts are attributed to chemical compounds belonging to secondary metabolites groups such as phenols, flavonoids, steroids, alkaloids and other compounds. The richness of the *C. spinosa* with the total phenolic compounds, tocopherols, carotenoids and vitamin C could be the main factor in its antifungal effects (25). Previous chemical studies have reported that polyphenols, flavonoids and glucosinolates were isolated from caper extract (26). The analysis showed an interest in *C. spinosa* leaves bioactives that are similar to those of fruits, which shows the possibility of valorization as a condiment in different dishes or as a food complement (27).

Conclusion

As part of the valorization and conservation of Moroccan biodiversity, the phytochemical properties of *Capparis spinosa* L. were studied. It is a xerophytic plant that presents morphological and physiological characteristics, allowing it to tolerate the climatic conditions of arid and semi-arid zones. This work aims to enhance the understanding of this particular plant species. In addition, this study demonstrated that the ability of *C. spinosa* organ extracts could be used as a biopesticide as an alternative management method against plant diseases and control the growth of plant pathogenic fungi and may be applied as an alternative method to reduce fungicides. In conclusion, all the results obtained are complementary to each other and their optimal combination remains the safest way to conserve and promote this species accurately.

Acknowledgements

We would like to express our sincere gratitude to our thesis director and co-supervisors for their contributions and guidance. The financial support was provided by the National Institute of Agronomic Research, Oujda and Laboratory of the Lagoon of Marchica, which is attached to the Multidisciplinary Faculty of Nador, Mohammed First University, Nador.

Authors' contributions

BC carried out the experiments, participated in drafting the manuscript, AM participated in coordination and critically revised the manuscript, KA participated in performing the statistical analysis and participated in drafting the manuscript, AK carried out the experiments of antifungal activity, HK carried out the experiments, AN participated in the interpretation of the results, LE carried out the experiments of antioxidant activity, MB conceived of the study and revised critically the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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