Chemical investigation, the antibacterial and antifungal activity of different parts of *Capparis spinosa* extracts

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**ABSTRACT**

*Capparis spinosa* (*Capparidaceae*), is one of the most used medicinal plants worldwide. It is used for the treatment of various diseases because of its biological and pharmacological effect, such as antioxidant, anticancer, antihypertensive, antidiabetic and antibacterial. Phytochemical analysis of the plant showed that it is a rich source of bioactive constituents, including alkaloids, glucosinolates, tocochromanols, carotenoids and polyphenols, which have led to *C. spinosa* being considered as a promising medicinal plant. Our study aims to detail the chemical profiles of the present bioactive responsible for the pharmacological effects of *C. spinosa*; it also aims to experimentally demonstrate the presence of polyphenols in different parts of the plant as well as their antimicrobial effects. For this, we used methanolic and aqueous extracts of the different parts of the plant picked in Beni Aziz in the Sétif region (North-east of Algeria). The extracts subjected to TLC and HPLC showed that they were rich in flavonoids and phenolic acids. This led to find that rutin was the most dominant compound in most of our extracts. On the other hand, the antimicrobial effect was tested by the disk diffusion method on three bacterial strains: *E. coli*, *P. aeruginosa* and *S. aureus* and two fungi: *C. albicans* and *A. flavus*. *Candida albicans*’ antifungal effect of our extracts was absent. While the same extracts showed a slight inhibitory activity against *Aspergillus flavus*. As for the antibacterial effect, it exists only for the methanolic extract of the twigs against *Staphylococcus aureus*. While the other extracts only showed a slight inhibitory activity against the same strain. The other strains of bacteria were resistant to all extracts at any used concentration.

**Keywords:** *Capparis spinosa*, Pharmacological effect, Chemical investigation, antibacterial activity, phenolic compounds, antifungal activity.

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**INTRODUCTION**

Caper (*Capparis spinosa* L.) belongs to the Capparaceae family native to the Mediterranean region. *C. spinosa* is one of the most widespread aromatic plants that grows everywhere, on slopes, rocky and stony and generally well adapted to the basin of the dry zones. The wild species of *Capparis* are found in the surrounding Mediterranean countries extending as far as the great desert in North Africa and in the dry regions of western and central Asia. In the Mediterranean coast, the caper tree can grow wildly only in the Algerian coast. Where it prefers light, well-draining soils with a neutral to alkaline pH. It can also be found in light, sandy or loamy soils (pH = 7.5-8). ¹ The harvest’s perfect time of caper in Algeria is June, and it is mainly used for traditional remedies. ² Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, biopesticides ³ and food additives. As a result of accumulated experience from the previous generations, today, all the world’s cultures have an extensive knowledge of herbal medicine. *Capparis spinosa* which was commonly used as medicinal plant, contains many biologically active chemical groups including, alkaloids, glycosides, tannins, phenolics, flavonoids, triterpenoids steroids, carbohydrates, saponins and a wide range of minerals and elemental electrolytes. It exerted many pharmacological effects including antimicrobial ⁴, cytotoxic, antidiabetic ⁵, ⁶, anti-inflammatory...
Common names

*Capparis spinosa* L (Capparidaceae) commonly known as the El-Kabbar caper plant in Algeria.

Arabic: Kabbar, Assef; Berber: Tayloulut, Tailoulout, Amserlih, Ouailoulou; English: Caper Bush, Caperbush, Caper, Caperberry; French: Câprier, Caprier commun, Câpres, Fabagelle, Tapana, German: Kapper, Kapernstrauoch; Italian: Cappero, Capperone (fruit); Spanish: Alcaparra, Caparra, Tapana; Alcaparron, Caperberries; Swedish: Kapris; Telugu: Kokilakshmu; Urdu: Kabar Family: Capparidaceae.

Botanical status: The botanical status of *Capparis spinosa* L is given in Table 1.

MATERIALS AND METHODS

Plant material

*Capparis spinosa* has been collected from the region of Beni Aziz (Sétif region) North-east of Algeria and identified by Pr H. Laouer, from Sétif 1 University, Algeria. A voucher specimen has been preserved in the laboratory. Leaves, seeds and capers (fruits) of *C. spinosa* have been dried in the shadow and powdered before the extraction. (Figure 1)

Preparation of *Capparis spinosa* extracts

To obtain the aqueous extracts, 10 g of the powder of each of the different plant parts (roots, leaves, flowers, seeds and fruits) we have mixed with 100 mL of distilled water, heated for 15 min, and stirred 24 hours in the darkness at 4°C. We have filtered the aqueous extract through a glass with cotton to remove particles. The filtrate has been lyophilized and stored at –20°C until to use.

We have put the dried plant material in a blender and subsequently mixed with a 10–20 volumes of 85% aqueous methanol. The slurry has been placed at room temperature for one week and we filtered the extract through a Buchner funnel. The methanol has been removed by rotary evaporation.

Table 1: Botanical situation of the *Capparis* species

| Reign     | Vegetal               |
|-----------|-----------------------|
| kingdom   | Plantae               |
| sub-kingdom | Tracheobionta         |
| phylum    | Magnoliophyta         |
| Class     | Magnoliopsida         |
| Sub-class | Dilleniidae           |
| Order     | Capparales            |
| Family    | Capparaceae           |
| Species   | Capparis spinosa      |

TLC thin layer chromatography

The support used in this study is a silica gel plate: (20 × 20cm, 60F254). The migration system used is: dichloromethane / methanol (86/14) (V / V) for the aqueous and methanolic extracts is (H₂O, MeOH). Four phenolic compounds were also applied to the plate as standards: three flavonoids: Quercetin, (Quer), Rutin, (Rut), and Catechin (Cat), and a phenolic acid: Gallic Acids (AGal) (2mg of each standard in 1ml of methanol).

Before analyzing aqueous and organic samples, we have applied them in a small spot in the form of 5 μl points by a micropipette 1 cm from the end of the silica gel plate. (the organic sample consisting of 10 mg of the caper extract, seeds, leaves, roots, flowers, twigs and root bark, all are dissolved in 1 ml of methanol, the aqueous sample consists of 10 mg of the different aqueous extracts of capers, seeds, leaves, roots and flowers dissolved in 1 ml of distilled water).

Once it has been impregnated with the different extracts, the plate constituting of the stationary phase is then dried and deposited vertically inside the chromatographic tank where it is traversed by a mobile phase (Dichloromethane /
Methanol (80/20) (v/v). The flow between the two phases causes migration and separation of the different compounds. After the development of the chromatogram (approximately 20 minutes); the plates have been further dried and examined under a UV lamp (λ = 254 nm). Our chromatographic tank containing the mobile phase has been saturated beforehand for a period of one hour to allow good elution. Each migrating substance is characterized by its frontal ratio (RF), and its fluorescence after revelation by iodine.

**Analysis method by high performance liquid chromatography (HPLC)**

High performance liquid chromatography (HPLC) analysis is arguably the most useful analytical technique for the characterization of phenolic compounds because it exhibits high resolution, high reproducibility, and long life. Relatively short analysis. For the analysis of polyphenols, HPLC: Agilent 1100 colomne C18 monomeric columns with polar grouping are preferable because they allow better selectivity. The wavelength range of flavonols, flavones and isoflavones ranges from 254-200 nm. The maximum absorption is detected at 270 nm. The organic samples consist 10 mg of the extract of (Capers, Seeds, Leaves, Roots, Flowers, Twigs and Root Bark) each dissolved in 1 ml of methanol, the aqueous sample consists 10 mg of different aqueous extracts of (Capers, Seeds, Leaves, Roots and Flowers) dissolved in 1 ml of distilled water. The six standards: Quercetin, (Quer), Rutin, (Rut), Catechin (Cat), Gallic acid (A Gal), Ferulic acid (Afer) and Tannic acid (AT) are dissolved at a rate of 2 mg in 1 ml of methanol. 5 μl of each extract were injected in a C18 column of dimension (250 × 4.6 mm) and a particle diameter equal to 5 μm. The elution solvent consists two phases: • Phase A: (water / Formic acid) (v/v) (99.9 / 0.1) • Phase B: Pure acetonitrile (HPLC gradient). The elution method applied is of the gradient type spread over 60 minutes with a flow rate of 1 ml / min as follows: - from 0 to 10 min: 5 - 25% B, - from 10 to 25 min: 25 - 40% B - from 25 to 35 min: 40 - 60% B - from 35 to 45 min: 60 - 80% B - from 45 to 60 min: 80% B. The detection is done by a UV-Visible detector at a wavelength of 270 nm.

**Antimicrobial susceptibility test Bacteria and Fungi strains**

The antibacterial tests were carried out using reference strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and clinical origins strains: *Pseudomonas aeroginosa*, *Aspergillus flavus* and *Candida albicans* obtained from the Laboratory of Bacteriology at Sétif hospital.

**Antibacterial activity**

The evaluation of antimicrobial activity has been performed by the agar disc diffusion method cited by. The aqueous extracts are dissolved in water with the dosages: 1g/ml, 500 mg/ml, 250 mg/ml and 125 mg/ml. The methanolic extracts are dissolved in water with the dosages: 1g/ml, 500 mg/ml, 250 mg/ml and 125 mg/ml. The bacterial strains are subcultured by the streak method, then incubation at 37° C for 18 to 24 h in order to obtain a young culture and isolated colonies. A colony well isolated has been mixed with 5 ml of sterile distilled water in a test tube in order to have an initial cell density or a turbidity close to 0.5 Mc Farland (Abs: 0.08-0.1 at 625 nm). Inoculation is carried out by using a swab dipped in the inoculum, and spread over the entire surface of the Mueller-Hinton agar. The operation is repeated two more times by turning the box 60° each time to ensure a homogeneous distribution of the inoculum.

Finally, the swab is passed around the edge of the agar surface. Then 6 mm diameters of Whatman filter paper N3 discs were prepared and impregnated with 30 μl of the different concentrations of extracts of *Capparis spinosa*. The impregnated discs are then gently deposited by sterile forceps on the surface of the inoculated agar. A disk impregnated with DMSO / distilled water is also deposited as a negative control, as well as a ready-to-use disk of Gentamicin (10 mg) serving as a positive control. The petri dishes are incubated in an oven at 37 °C between 18 and 24 hours. The diameter of inhibition zone has been measured by transparent ruler to nearest mm. Inhibition zone with diameter was less than 12 mm. were considered as having no antibacterial activity, diameter between 12 and 16 mm. were considered moderately active, and these with > 16 mm. were considered highly active.

**Antifungal activity**

Antifungal susceptibility test in vitro has been tested against pathogenic human fungi *Candida albicans* and *Aspergillus flavus*. We used Sabouraud dextrose medium containing chloramphenicol for the fungal suspension from three to five days old, it was made in sterile distilled water and its turbidity was adjusted to 0.5 Mc Farland (10⁶ CFU/ml). An aliquot of 0.1 ml of this fungal suspension was spread over the surface of agar plate. The disc technique was used for the antifungal activity; sterile paper discs of 6 mm diameter impregnated with 30 μl of plant extract. The same procedure was used as described previously. The discs impregnated with DMSO and Fluconale 10 mg / ml are respectively used as negative and positive controls.

**Statistical analysis**

All determinations were conducted in triplicate and all the results were calculated as mean ± standard deviation (SD). Statistical analysis was performed using Student’s t test for significance. Differences were considered significant at p ≤ 0.05.

**RESULTS AND DISCUSSION**

**Thin Layer Chromatography (TLC)**

The thin layer chromatograms of *Capparis spinosa* made it possible to separate some substances which were visualized as visible spots (UV 254 nm). Each component is characterized by its frontal ratio.

The extracts with many spots are the richest in polyphenols and flavonoids. Caper extracts: aqueous (4 compounds) and methanolic (5 compounds). Leaf extracts: aqueous (3 compounds) and methanolic (4 compounds). The other extracts have fewer compounds than these last two with: 2 apparent compounds for each of the extracts: aqueous and methanolic from flowers, methanolic from seeds and methanolic from the root bark. 1 single apparent compound for: the aqueous and methanolic extract of the root, the aqueous extract of the seed and the methanolic extract of the twigs (Figure 2)

Calculation of Front-End Reports (Rf) it is the ratio of the distance traveled by the solute (X) / the distance traveled by the solvent (Y). \[ Rf = \frac{X}{Y} \] with \( Y = 6.6 \) cm

**Table 2:** Calculation of the frontal ratios of the standards.

| Standards     | X     | Rf  |
|---------------|-------|-----|
| Quercetin     | 5.2   | 0.78 |
| Rutin         | 2     | 0.3  |
| Catechin      | 4.2   | 0.63 |
| Gallic acid   | 4.3   | 0.65 |
Comparison between the Rf of the different standards (Table 2) and the Rf of the compounds of the plant extracts, we have observed that: Quercetin: present in the aqueous and methanolic extract of caper. Rutin: present in most extracts (the aqueous and methanolic extract of caper, the aqueous and methanolic extract of the leaves; the aqueous and methanolic extract of the flowers; the aqueous extract of the root and the methanolic extract seed, bark and twigs). The rutin was the dominant phenolic in it. This study was designed to assess the phenolic composition, including phenolic acids and in vitro biological activities as antioxidant and mineral analysis of C. spinosa L., as well as to evaluate their nutritional and medicinal potentials. Catechin: as with quercetin, it is present in the aqueous and methanolic extract of caper. Gallic Acid: Didn't have any matching spots.

**Identification of Phenolic Profile**

In the technique of identifying molecules by HPLC, each compound is characterized by its retention time (TR), which is the time that elapses between the injection of the sample and the appearance of the chromatographic peak. Our qualitative analysis, use of different standards that marked peaks at different retention times is used to detect the presence that mentioned before in all of our extracts.

| Standard | Gallic acid | Ferulic acid | Catechin | Rutin | Quercetin | Tannic acid |
|----------|-------------|--------------|----------|-------|-----------|------------|
| Retention time (min) | 6.1 | 10.35 | 10.49 | 13.22 | 22.37 | Non detectable |

Note that the tannic acid chromatogram cannot be interpreted, due to its belonging to a very heterogeneous subfamily of polyphenols, we talk here about a tannin bump. The comparison between the retention times of the standards (Figure 3) with those recorded in the chromatograms of the various methanolic and aqueous extracts allowed the probable identification of certain flavonoids and phenolic acids in the different parts of the C. spinosa plant, summarized in the table 4 below. follows:
This result joins to the TLC to confirm that rutin is the most abundant flavonoid in the plant, with a presence in most of the extracts. They also confirm the richness of the extracts of capers and leaves, given that they are the parts that have contained the most standard polyphenols. (Table 4) Gallic acid is emerging in HPLC detection as opposed to TLC because it is more sensitive technique with a broad spectrum of detection.

In these results, we find that the methanolic extract is richer in phenolic products compared to other extracts. (Figure 4 and 5)
Antibacterial activity

We have studied the power of extracts from different parts of the plant of *C. spinosa* by the method of diffusion in agar medium by impregnation of the discs by different concentrations, (Mueller-Hinton for the antibacterial test. The antimicrobial activity was estimated by the zone of inhibition around the discs (diameter of inhibition) of the extracts tested on three bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). All bacterial strains showed sensitivity for the standard antibiotic used (Gentamicin 10 mg). The inhibitory effects of the growth of germs are manifested by a single extract which is the methanolic extract of the Twigs vis-à-vis *S. aureus*. (Table 5)

Other methanolic extracts of (Flowers, Capers, Leaves and Roots) exhibited dose-dependent areas of inhibition against strains of *Staphylococcus aureus*. While the aqueous extracts showed no zone of inhibition on all strains regardless of the dose used. The range of the zone of the inhibition was from 11 to 20 mm. Maximum inhibition zone of 25 mm was obtained with methanolic extract of *Capparis spinosa* twigs against *Staphylococcus aureus*. (Table 5)

The aqueous and methanolic extracts did not show any antibacterial effect at all, the doses used either against *E. Coli* or *P. aeruginosa*. (Table 5)

Our results obtained are in agreement with those of [25] on the same strains, where he found that the ethanolic and aqueous extracts are inactive for *E. Coli* and *P. aeruginosa*, while the butanolic extract exhibited an inhibitory effect of bacterial growth for the three strains used.

A research undertaken worked on the leaves of *Capparis spinosa*, found that the extract is inactive on *E. coli*, but has suspicious activity on *Staphylococcus aureus*. [27]

The results obtained for the biological activity (diameter of inhibition [mm]) are collated in tables and the values 1, 1/2, 1/4 and 1/8 correspond to the various dilutions of the initial concentration of dry residue of the extract in DMSO which is 1 g/ml.
### Table 5: Antibacterial activity methanolic extract of different parts of Capparis spinosa.

| Test Bacteria     | Inhibition Zone in mm | Twigs       | Flowers     | Capers      | Leaves      | Roots        |
|-------------------|------------------------|-------------|-------------|-------------|-------------|--------------|
|                   |                        | 1/2         | 1/4         | 1/8         | 1/2         | 1/4          | 1/8          |
| Pseudomonas aeruginosa | 25.12±0.14             | 21.15±0.22  | 15.17±0.23  | 10.23±1.13  | -           | -            | -            |
| Staphylococcus aureus | 20.22±0.45             | 15.55±0.18  | 10.22±0.10  | 8.75±0.11   | -           | -            | -            |
| Escherichia coli    | 17.12±0.22             | 12.50±0.40  | 10.13±0.03  | 7.75±0.10   | -           | -            | -            |

Values (means ±SD) are average of three samples of each bacterium, analyzed individually in triplicate. The values 1, 1/2, 1/4 and 1/8 correspond at different dilutions of the initial concentration of the dry residue of the extract in DMSO, which is 1 g / ml.

### Antifungal test

The extracts used (Flower, Capers, Leaf, and Root) at different concentrations 1g/ml, 500 mg/ ml, 250 mg/ml 125 mg/ml prove inactive on two strains used (C. albicans and A. flavus) whatever the dose used. Methanolic extracts from twigs showed low activity against Aspergillus flavus. (Table 6)

### Table 6: Antifungal activity of methanolic twigs extracts against Aspergillus flavus.

| Test Bacteria     | Inhibition Zone in mm | Dilution 1 | 1/2         | 1/4          | 1/8 |
|-------------------|------------------------|------------|-------------|--------------|-----|
| Aspergillus flavus| 8.43±0.38              | 7.12±1.44  | 7.73±0.29   | -            |

Values (means ±SD) are average of three samples of each fungal, analyzed individually in triplicate.

### CONCLUSION

Our attention was focused on Capparis spinosa, which is a Mediterranean plant that is widely used in Algeria, particularly in the region of Sétif, where it was collected for this study.

Many in vivo and in vitro studies are being carried out around the world in order to be able to evaluate the clinical and pharmacological applications of C. spinosa with the aim of developing a new natural drug with less toxic and undesirable effects.

Besides that, other studies are done to characterize and quantify the different bioactive molecules present in the plant by using different techniques, such as HPLC and TLC which we used in our study and which have showed that the methanolic and aqueous extracts exhibit rich source of antioxidant such as flavonoids and phenolic acids with the presence of Rutin and Quercetin in considerably high amounts.

The extracts showed significant effects on bacteria: Staphylococcus aureus. Capparis spinosa L could be used as a potential source of natural antimicrobial agents with beneficial therapeutic effects.

A moderate anti-fungal activity was observed against Aspergillus flavus. Our current research offers the possibility of developing strategies for controlling human pathologies with natural extracts or bioactive metabolites of medicinal plants. Further, phytochemical studies are ongoing to define the chemical structure and characteristics of bioactive compounds especially present in flowers and twigs of this botanical genus.

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