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Evaluation of the Antimicrobial Activity and Phytochemical Study of the Ethanolic Extracts of the Leaves and Flower Buds of the Caper «*Capparis spinosa* L. »

Nesrine BENKESSIRAT [1*], Yamina MOUAS[1], Fatma Zohra OUKARA [2], Lydia CASASNI[3], Houssem DEGAICHIA [4], Cherifa CHAOUIA [1]

[1] Department of Agro-ecological Biotechnology, Faculty of Natural and Life Sciences, Saad Dahleb University, B.P. 270, road of Soumaa, Blida 09000, Algeria.
[2] National Forest Research Institute (INRF), BP 37,Chéraga, Algiers, Algeria.
[3] Department of Biological Sciences, Faculty of Natural and Life Sciences and Earth sciences, Djilali Bounaam University, N14 Khemis Miliana, Algeria.
[4]Research Center in Agro-Pastoralism (CRAPast), Djelfa, Algeria.
Email :nesrinebenkessirat@hotmail.com
Phone: 00 213 774123946

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Abstract
The caper plant «*Capparis spinosa*» is a spontaneous medicinal species commonly foundthroughout the Mediterranean region. The objective of this study is to assess in vitro antimicrobial activity of ethanolic extracts of the caper organs(including leaves and flower buds) against three bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) and one fungal strain (*Candida albicans*). Furthermore, the study aims to identify the chemical compounds present in theethanolic extracts and quantify the total polyphenols and flavonoids contained therein. The prepararion of the ethanolic extracts was carried out using a Soxhlet extraction, whereas the antimicrobial activitywas assessed using the agar disk diffusion method. The chemical composition was identified through HPLC, while the quantification of totalpolyphenols and flavonoids was conducted using the Folin-Ciocalteu method and aluminumchloride colorimetric assay, respectively.
The ethanolic extract of the flower buds demonstrated the most significant antibacterial activity against *Pseudomonas aeruginosa*. HPLC analyses revealed the presence of rutin in all extracts tested, with the ethanol extract from the leaves exhibiting the highest total content of polyphenols and flavonoids.
The results of this study indicate that ethanolic extracts of the caper organs contain phenolic compounds that exhibit antimicrobial activity against all tested strains.

Keywords: *Capparis spinosa*, ethanolic extract, antimicrobial activity, total polyphenols, total flavonoids.

Introduction

Medicinal plants encompass a diverse range of plant species utilized for treating various diseases **(Sereme et al., 2011).** One such plant is the caper (*Capparis spinosa*), a spontaneous

xerophyte that is widely spread in the Mediterranean region, possessing significant medicinal properties employed in traditional medicine **Benseghir-Boukhari et Seridi (2007).** This plant species contains many biologically active chemical groups, including alkaloids, glycosides, tannins, phenols, flavonoids, triterpenoids, steroids, and saponins, as well as a wide range of minerals **(Benzidane et al., 2020)**. It also has a multitude of pharmacological properties, including cytotoxic, antidiabetic, anti-inflammatory, antioxidant, and antimicrobial **Rahnavard et Razavi (2017).**

In light of the adverse effects of antimicrobial chemicals, researchers have shifted their focus to raw herbal extracts as a promising source of natural bioactive molecules **(Yakhlef et al., 2011).**

The aim of this study is to evaluate in vitro the antimicrobial activity of ethanolic extracts derived from the leaves and flower buds of the caper plant against three bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*) and one fungal strain (*Candida albicans*) that are associated with various diseases. Subsequently, a qualitative analysis will be conducted using High-Performance Liquid Chromatography (HPLC), along with a quantitative evaluation that involves measuring the total polyphenols and flavonoids present in the ethanolic extracts obtained from the various organs of the caper plant, specifically the leaves and flower buds.

MATERIALS AND METHODS

The caper plant material, comprising of leaves and flower buds, was collected during the vegetative and reproductive phase in July 2020 from the region of Hammam Melouane in eastern Blida, Algeria. Following the harvest, the samples were dried in the shade at room

temperature of 35 ± 1°C for a period of 15 days. Subsequently, the dried plant material was ground using an electric mill and the resulting powders were stored in kraft paper bags.

Extract preparation

The caper's leaves and flower buds were subjected to Soxhlet extraction for approximately 1h30 min to obtain ethanol extracts. The modified protocol involved using 20g of plant powder from each organ, along with 300ml of 90% ethanol **Hebi et Eddouks (2015).**

The ethanolic extracts were then evaporated using a rotary evaporator, and the resulting dry residue was stored until it was required for use.

Calculation of yield

The yield (%) of the extract was determined using the following formula **(Arab et al., 2018)**

$\%yield = (M - M_0) / Mt \times 100$

Where: %yield represents the yield of the extracted material,

M: denotes the combined mass of the beaker and the extract,

M_0: indicates the mass of the empty beaker,

Mt: refers to the mass of the plant powder.

Evaluation of antimicrobial activity

The microorganisms employed in this study are ATCC (American type culture collection) strains that were supplied by the bacteriology department at Frantz Fanon hospital Blida in Algeria. The characteristics of the tested strains are illustrated in Table 1.

Table 1: The characteristics of the strains tested

Tested strains	**Bacteriological characteristics**	**References**
Bacterial strains		
Escherichia coli	Gram –	ATCC25922
Pseudomonas aeruginosa	Gram –	ATCC27853
Staphylococcus aureus	Gram +	ATCC25923
Fungal strain		
Candida albicans		ATCC10231

The agar diffusion method was used to evaluate the antimicrobial activity of the extracts from the caper's organs at a concentration of 125 mg/ml **(Meddour et al., 2013)**

Microbial culture

The strains were cultured using the streak plate method and then incubated in a 37 1°C lab oven for 24 hours to establish a young culture and isolate colonies for the preparation of the inoculum **(Moroh et al., 2008).** Following the incubation period, colonies were handpicked using a platinum loop, and then transferred to a tube containing physiological water.

The density of the inoculum was determined by measuring the optical density with a densitometer, aiming for a density close to 0.5 McFarland for bacterial strains **(Meddour et al., 2013)** and 1.8 McFarland for the fungal strain.

Preparation of the medium and application

The nutrient agar and Sabouraud agar serve as culture mediums for bacterial and fungal strains, respectively. The medium is dispensed into Petri dishes at a volume of 18 ml per dish. A 6 mm diameter disc of sterile Whatman paper, saturated with a dry extract dissolved in dimethylsulfoxide (DMSO) at a concentration of 125 mg/ml, is placed on the surface of the Petri dish that has been inoculated with the test strain, using a forceps. The Petri dishes are then incubated for 24 hours at a temperature of 37 ± 1°C for bacterial strains, and for 48 hours at 25 ± 1°C for fungal strains. Each test was conducted in triplicate.

The reading is conducted by measuring the diameter, in millimeters, of the inhibition zone surrounding each disc that contains the extract, with the help of a graduated ruler. Antibiotics such as ampicillin, vancomycin, and econazole were employed as positive controls in this study.

High-performance liquid chromatography (HPLC)

All extracts derived from the caper plant were subjected to qualitative analysis of polyphenols and flavonoids using High-Performance Liquid Chromatography (HPLC).

Principal

The methodology conducted in this study involved adhering to the protocol established by (**Benzidane et al., 2020)** which entails weighing 10 mg of each sample.

The six standard substances, namely gallic acid, tannic acid, salicylic acid, quercetin, rutin, and vanillin, were dissolved in ethanol at a ratio of 2 mg per 1 ml.

5 µl of each extract was injected into a C18 column with dimension of (250 × 4,6 mm) and a particle diameter of 5 µm. The detection was carried out using a UV-Visible detector set to a wavelength of 270 nm.

Determination of phenolic compounds

Total polyphenols content

The quantification of total polyphenols was conducted through the use of the Folin-Ciocalteu method as adapted by (**Aichi-Yousfi et al., 2016)** the ethanolic extracts derived from the leaves and flower buds (50 µl, 1 mg/ml) were combined with 1.58 ml of distilled water and 400 µl of Folin-Ciocalteu reagent. Subsequently, 300 µl of Na2CO3 were introduced to the solution after a 5-minute rest at room temperature, followed by an incubation period at 20°C in the absence of light for 30 minutes. The absorbance was then determined at 765 nm.

The quantification of polyphenols was performed based on a calibration curve y = 0.0091 x established using a standard "gallic acid" at varying concentrations ranging from (0 - 100 µg/ml). The results were reported in micrograms of gallic acid equivalent per milligram of extract (µg GAE/mg of extract).

Total flavonoid content

The total flavonoid content was determined using the aluminium trichloride AlCl3 method adapted by (**Stefanucci et al., 2018)** In this procedure, ethanolic extracts from the leaves and flower buds of the caper, at a concentration of 1 mg/ml (1 ml), were combined with an equal volume of 2% aluminium trichloride. A blank was then prepared, consisting of 1 ml of AlCl3 mixed with 1 ml of ethanol. Subsequently, absorbance readings were conducted at a wavelength of 430 nm following a 10-minute incubation period at room temperature.

The quantification of flavonoids was performed on a calibration curve y = 0.0089 x established using a standard «quercetin» at various concentrations (0-120µg/ml). The total flavonoid content was reported in micrograms of quercetin equivalents per milligram of extract (µg QE/mg of extract).

Statistical analyses

The statistical analysis was conducted using SPSS© version 20.0.0 for Windows™. An analysis of variance (ANOVA) was performed, accompanied by a post-hoc Tukey test at the 5% threshold.

RESULTS AND DISCUSSION

Extraction yields

The application of Soxhlet extraction with 90% ethanol on the powdered material derived from caper organs produced the yields presented in Table 2.

Table 2: Extract Yield.

Aerial part	**Yield (%)**
Leaves	15.1
Flowerbuds	21.05

The obtained yields differ depending on the organ, with recorded percentages of 15.1% for leaves and 21.05% for flower buds.

The results demonstrate a strong correlation between the extraction yield achieved and the plant organs that were used. This conclusion is substantiated by the study conducted by (**Sabiou et al., 2019)** in which they propose that the fluctuations in yield could be attributed to the affinity between the solvent and the molecules present in the various samples. Furthermore, the authors propose that the disparity in yield among different plant organsis associated with the specific roles fulfilled by each part of the plant (**Sabiou et al., 2019).**

Antimicrobial activity

The application of the agar diffusion method provided insights into the influence of ethanolic extracts, specifically at a concentration of 125 mg/ml, on the tested strains.
The inhibition zone diameters of the strains growth range from 7 to 12.6 mm, as indicated in Table 3.

Table 3: Antimicrobial activity of ethanolic extracts derived from caper organs

	Escherichia coli	***Pseudomonas***	***Staphylococcus***	***Candida albicans***

		aeruginosa	***aureus***	
Leaves	7.33 ±0.57	8.33 ±0.57	7 ±1	8,33±0,57
Flower buds	7.77 ±1.01	12.66 ±0.57	10 ±0	8 ±1,73
Antibiotic	16	R	20	R

R: Resistant

The ethanolic extracts derived from the leaves and flower buds of the caper plant exhibit notable antibacterial effects against *Pseudomonas aeruginosa* and *Candida albicans* when compared to the positive control (antibiotic and antifungal disc). Specifically, the extracts demonstrate antibacterial properties against *Pseudomonas aeruginosa*, yielding inhibition zones of 8.33 mm and 12.66 mm for the leaves and flower buds, respectively.

The analysis of variance indicates a very highly significant difference ($P<0.001$)

Furthermore, the ethanolic extracts also display antifungal activity against *candida albicans*, with inhibition diameters of 8.33 mm and 8 mm for the leaves and flower buds, respectively.

The analysis of variance indicates a very highly significant difference ($P<0.001$)

The ethanolic extracts tested exhibited minimal effectiveness against *Escherichia coli*, displaying inhibition diameters of 7.33 mm for leaves and 7.77 mm for flower buds, in comparison to the antibiotic (positive control). Similarly, *Staphylococcus aureus* demonstrated lower susceptibility to ethanolic extracts, resulting in inhibition zones of 7 mm and 10 mm for leaves and flower buds, respectively, when compared to the positive control.

The variance analysis corroborates these findings by indicating a very highly significant difference ($P < 0.001$).

The evaluation of antimicrobial efficacy revealed that the activity is contingent upon the organ under scrutiny and the strain tested.

The variation in sensitivity of the strains tested in response to ethanolic extracts can be attributed to the ability of active ingredients to penetrate the cell wall and membrane structures **(Cox et al., 2000).**

The strain *Pseudomonas aeruginosa* exhibits resistance to multiple antibiotics **(Yakhlef et al., 2011)**. It, however, displayed sensitivity to all extracts tested, including those derived from leaves and flower buds. The ethanol extract from flower buds demonstrated the highest inhibition diameter of 12.66 mm, possibly attributed to the permeability of the strain's cell wall to the extracts, facilitating the penetration of the active ingredients through the cell membrane. These results indicate the potential use of the tested extracts of *Capparis spinosa* as a natural alternative in the field of therapeutics.

Several studies have been conducted to determine the antimicrobial efficacy of specific extracts derived from *Capparis spinosa*, as demonstrated in the research of **Mahasneh (2002)** the study revealed that the ethanolic extract obtained from the aerial part of the caper plant exhibited antimicrobial properties against *Staphylococcus aureus* and *Candida albicans*, with inhibition zones measuring 13 mm and 14 mm respectively. However, this extract was found to be ineffective against *Escherichia coli* and *Pseudomonas aeruginosa***.**

Likewise, **(Mahasneh et al., 1996)** conducted a study demonstrating that crude extracts derived from the entire aerial parts of the caper, including methanol, hexane and butanol extracts, exhibited antimicrobial activity against *Escherichia coli, Staphylococcus aureus*, and *Candida albicans,* with inhibition zones ranging from 7.13 to 9.13 mm.

Moreover, another study revealed that the ethanol extract derived from the bark of the root of *Capparis decidua* exhibited antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* ***(*Rathee et al., 2010).**

The findings obtained corroborate with the research endeavors undertaken, demonstrating that the ethanol extracts from the caper's organs (leaves and flower buds) exhibit activity against all tested strains.

In contrast, the results obtained in this study are more significant than those reported by (**Meddour et al., 2013)** the various extracts tested with different solvents specifically petroleum ether, dichloromethane, methanol, and aqueous solutions were found to be inactive

at a concentration of 125 mg/ml against all bacterial strains examined, including *Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*. However, the petroleum ether extract demonstrated activity against *Staphylococcus aureus*, with an inhibition diameter of 10.17 mm.

According to a recent study conducted by (**Benzidane et al., 2020)** the methanolic extracts acquired from various parts of the caper plant (twigs, leaves, flower buds, flowers, and roots) did not exhibit any inhibition zones against the *Escherichia coli* and *Pseudomonas aeruginosa* strains tested at a concentration of 125 mg/ml. However, the *Staphylococcus aureus* strain exhibited sensitivity to all extracts, with inhibition zones ranging from 7.11 to 10.23 mm. On the other hand, the fungal strain *Candida albicans* demonstrated resistance to the extracts from leaves, flower buds, flowers, and roots.

The results observed may be explained by the variation in the chemical composition of the tested extracts **(Yakhlef et al., 2011).**

Consequently, various research efforts have indicated that several parameters may influence the determination of antimicrobial activity, such as the organ studied **(Natarajan et al., 2005),** the type of extract **(Bouzid et al., 2010),** the nature of the chemical composition of the extracted bioactive molecules as well as the type of microorganisms studied **Cushnie et Lamb (2011)**

High-performance liquid chromatography HPLC

The qualitative analysis employing various standards, including gallic acid, salicylic acid, tannic acid, rutin, vanillin, and quercetin, has successfully identified specific flavonoids and phenolic acids in ethanolic extracts derived from leaves and flower buds. This identification was achieved by comparing the retention times of the extracts with those of the established standards. The standard retention times are presented in Table 4.

Table 4: Standard retention times

Standards	**Gallic acid**	**salicylic acid**	**tannic acid**	**Rutin**	**Vanillin**	**Quercetin**

Retention time (min)	2.77	9.71	1.92	3.41	5.55	10.13

The chromatograms of the various ethanolic extracts of caper are illustrated as follows:

Chemical composition of the ethanolic extract from leaves

The HPLC analyses reveal that gallic acid is the predominant constituent of the ethanolic extract derived from the leaves, comprising 45.76%, while rutin and tannic acid are present in lesser amounts at 34.45% and 19.79%, respectively (Figure 1).

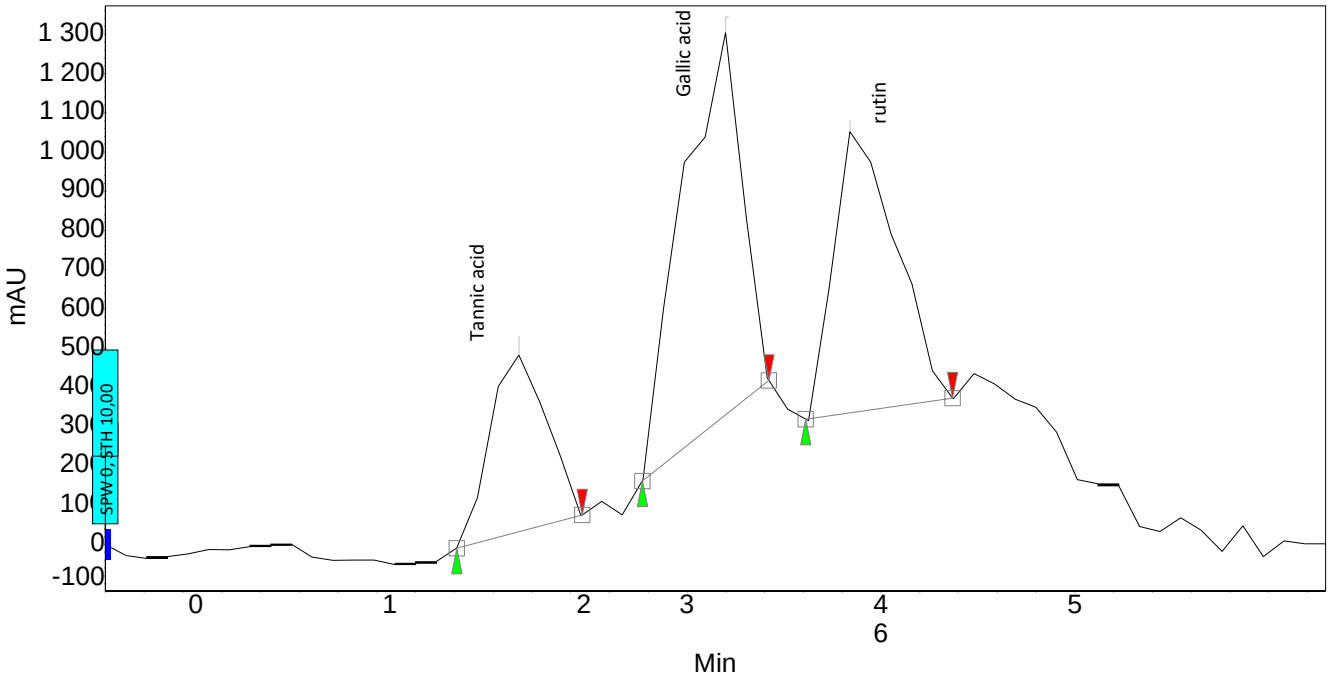

Figure 1: Ethanolic extract of caper leaves (HPLC chromatogram)

Chemical composition of the ethanolic extract from flower buds

The primary constituent found in the ethanolic extract of flower buds was gallic acid, making up 40.37% of the total composition, while tannic acid followed with a composition of 33.24%, and rutin constituted 26.40% (Figure 2).

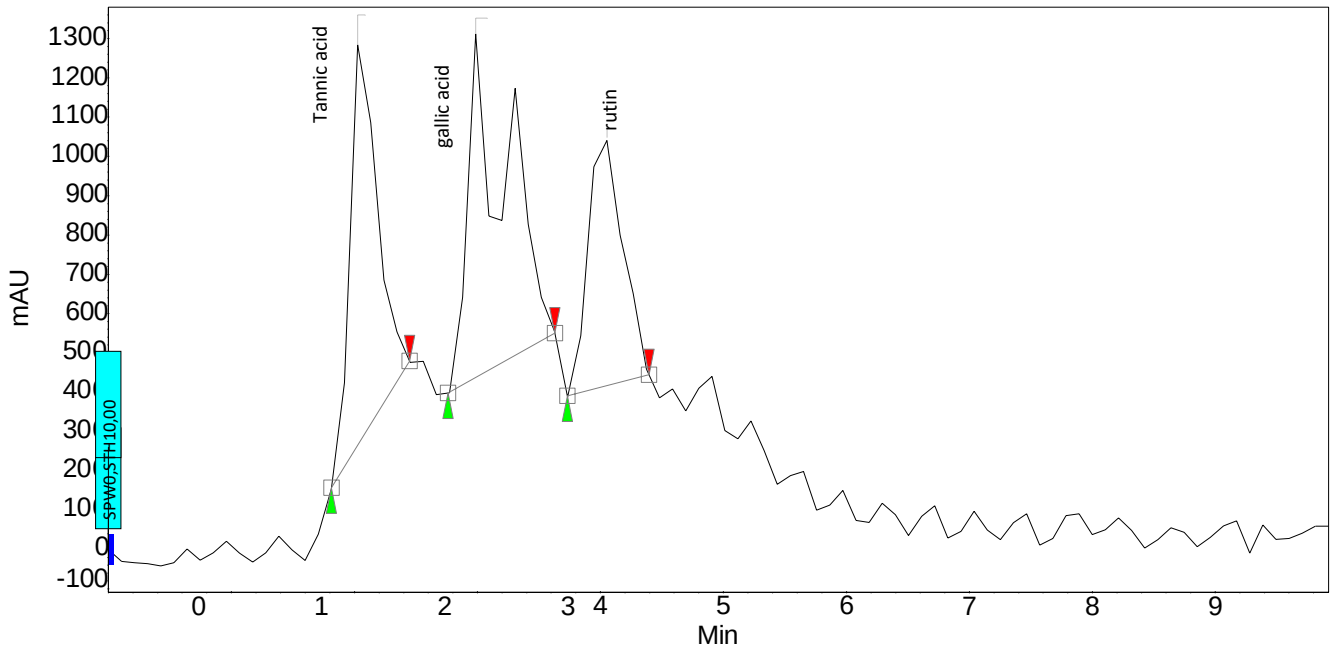

Figure 2: Ethanolic extract from caper flower buds (HPLC chromatogram)

The results indicate that rutin is present in all tested ethanol extracts of caper, albeit at different concentration levels. This finding aligns with the study conducted by **(Aichi-Yousfi et al., 2016)** which demonstrated that rutin is the predominant constituent in the ethanol-based extract from the leaves of *Capparis spinosa* harvested in Tunisia.

In a related study, it was found that rutin constitutes the predominant flavonoid in various parts of the caper plant harvested from the Beni Aziz Sétif area, being detected in the majority of the extracts analyzed, both methanolic and aqueous **(Benzidane et al., 2020)**

A different research indicates the absence of rutin, while other compounds, including Hydroxycortisol, Caffeic acid, Catechol, Ellagic acid, Coumaric acid, Ferrulic acid, and Vanillin are indentified to be present **(Sabrina et al., 2024**). The same researchers elaborate that variations in the plant's chemical composition are associated with meteorological conditions, the harvesting site, and additional factors, as previously demonstrated.

Determination of phenolic compounds

Total polyphenols content

Polyphenols in ethanolic solutions were quantified through the Folin-Ciocalteu method. The concentration of polyphenols in caper leaves and flower buds is established by using a calibration curve derived from the linear equation y = 0.0091x (Figure 3).

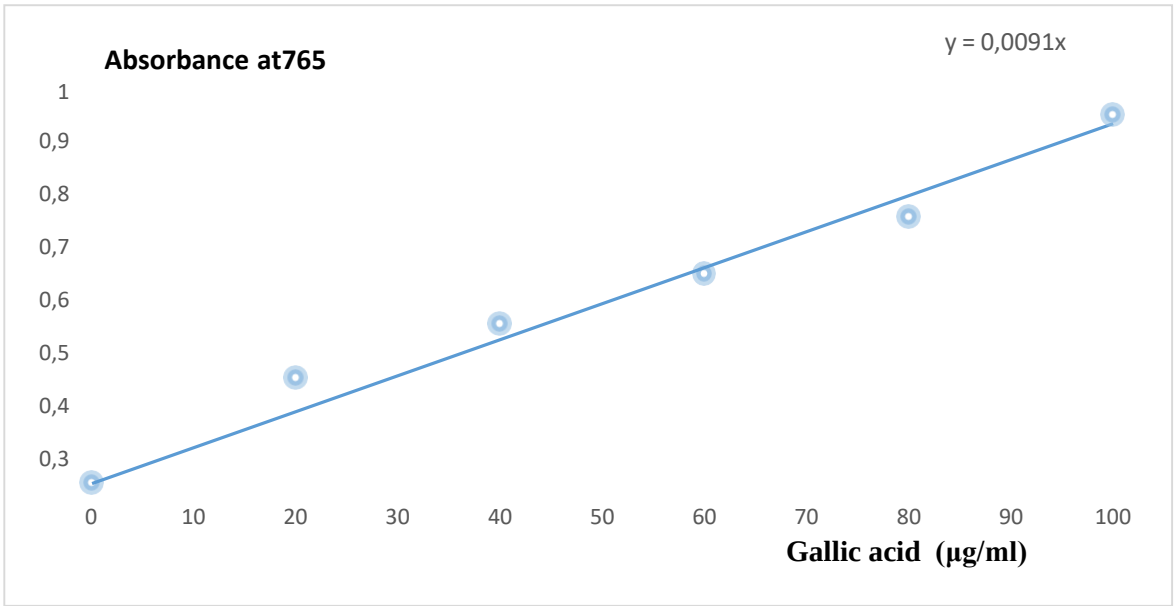

Figure 3: Calibration curve of gallic acid

Polyphenols content is denoted in microgram equivalent of gallic acid per milligram of extract (µg EQ/mg of extract) (Table 5)

Table 5: Total polyphenol contents of ethanolic extract

Test organ	**Polyphenol content (µg GAE/mg extract)**
Leaves	35.6 ± 0.06
Flowerbuds	22.59 ± 0.38

Variations in results were observed across different organs, with the ethanol extract derived from the leaves exhibiting a higher polyphenol content of 35,6 µg GAE/mg of extract in comparison to the flower buds, which contained 22,59 µg GAE/mg extract.

The analysis of variance (ANOVA) indicates a very highly significant difference ($P < 0.001$). Several studies have been conducted to assess the polyphenol content of specific *Capparis spinosa* extracts. Notably, a study by **(Aichi-Yousfi et al., 2016)** demonstrated that the ethanolic extract of *Capparis spinosa* leaf exhibited the highest polyphenol content at 857,22 mgGAE/gDM, in contrast to the aqueous extract with a content of 763.90 mgGAE/gDM.

Furthermore, caper's methane extract is found to contain the highest polyphenol content, measuring at 29.01 µg GAE/mg of extract, notably surpassing other aqueous extracts such as dichloromethane and petroleum ether, which have polyphenol contents ranging from 05.30 to 25.68 µg GAE/mg of extract **(Meddour et al., 2013)**

Numerous studies have indicated that there is an intricate correlation between the content of phenolic compounds in a plant and various factors, namely the type of solvent utilized, the organ under scrutiny, the extent of reaction to biotic and abiotic stressors in its environment (**Stefanucci, A et al., 2018),** and the geographical areas where the plant is harvested (**Aichi-Yousfi et al., 2016).**

Total flavonoid content

The AlCl3 method was used to estimate the flavonoid levels in ethanolic extracts derived from the leaves and flower buds of the caper.

The concentration of flavonoids in the extracts is established by using the calibration curve derived from the linear equation y = 0.0089x (Figure 4).

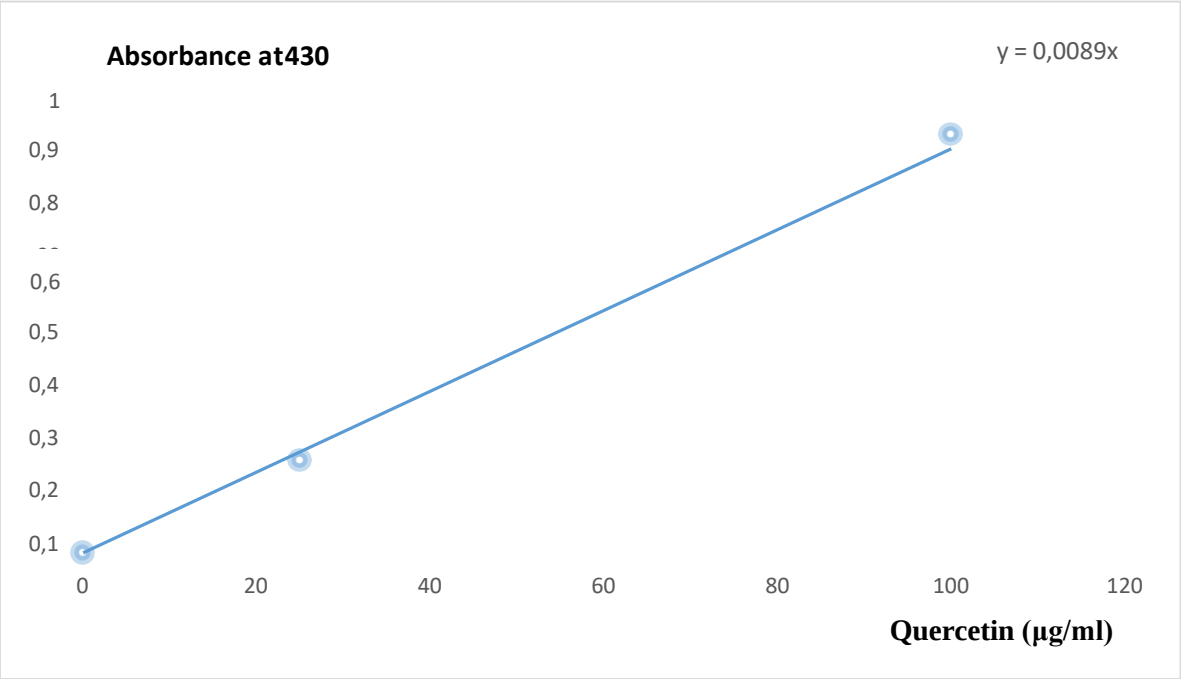

Figure 4: Calibration curve of Quercetin

The content of flavonoids is denoted in microgram equivalent of quercetin per milligram of extract (µg EQ/mg of extract) (Table 6).

Table 6: Total flavonoid content of ethanolic extracts

Test organ	**Flavonoid content (µg EQ/mg extract)**
Leaves	166.73 ± 1.45
Flowerbuds	49.43 ± 0.9

The acquired results demonstrate inconsistency and variation among different organs. The highest flavonoid content is observed in the ethanolic extract obtained from leaves, with a measurement of 166.73 µg EQ/mg of extract, whereas the ethanolic extract of flower buds shows a concentration of 49.43 µg EQ/mg extract.

The analysis of variance (ANOVA) indicates a very highly significant difference ($P < 0.001$). The findings achieved hold greater significance than those presented by **(Meddour et al., 2013)** where in they highlighted that the aqueous extract demonstrated the highest concentration of flavonoids, measuring at 11.82 µg RE/mg of extract, surpassing the levels found in alternative extracts such as methanolic, dichloromethane, and petroleum ether, which ranged between 0.08 and 5.97 µg RE/mg of extract.

The ethanolic extract of *Capparis spinosa* leaves demonstrated a higher flavonoid content of 82.84 mgEQ/gDM compared to the aqueous extract, which yielded a lower content of 78.02 mgEQ/gDM (**Aichi-Yousfi et al., 2016)**

The resulting variations may be attributed to several factors, namely the region of harvest, the complex characteristics of these compounds in relation to extraction methods, and the concentration level of the solvent used (**Arab et al., 2018).**

CONCLUSION

The evaluation of the antimicrobial activity of *Capparis spinosa* organs has demonstrated the antimicrobial effectiveness of the ethanolic extracts obtained from caper organs (leaves and flower buds) against bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) and a fungal strain (*Candida albicans*) with varying levels of susceptibilities. Notably, the flower buds have enabled the recording of the highest yield of 21.05% and displayed prominent antibacterial activity against *Pseudomonas aeruginosa*, resulting in an inhibition diameter of 12.66 mm, in contrast to the leaves. The HPLC results revealed the existence rutin in all samples analyzed, with varying contents of total polyphenols and flavonoids. It thus was observed that the ethanolic extract obtained from the leaves exhibited the highest concentration compared to the flower buds.

Further studies are required to locate the site of action of the bioactive molecules responsible for antimicrobial activity at the cell level, thereby elucidating the therapeutic effects of *Capparis spinosa*.

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Conflict of Interest:

The authors affirm that there are no conflicts of interest to disclose.

Declaration Statement:

The authors declare that this manuscript has not been previously submitted for publication.

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