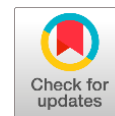


Exploring the photochemical composition of indigenous Capparis Genus species in Iraq



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Abstract This study presents a comparative analysis of the phytochemical compositions of the flowers and fruits of *Capparis spinosa* L. (Flora Iraq) sourced from Basra, southern Iraq. Soxhlet extraction and maceration methods were employed to prepare extracts for comparative phytochemical yield assessment. Total phenolic and flavonoid contents were quantified using standard controls via spectroscopic analysis. Additionally, high-performance liquid chromatography (HPLC) facilitated the identification and quantification of specific antioxidants, including cinnamic acid. Comparative analysis revealed significantly higher levels of phenols, flavonoids, and alkaloids in the flowers of *C. spinosa* compared to the fruits. HPLC profiling highlighted the presence of essential bioactive compounds such as kaempferol, quercetin, hexaoxane, vitamin E, and stigmasterol in both flower and fruit extracts. This study marks the first comprehensive report on HPLC profiles and quantification of prominent phytochemicals in *C. spinosa* (Flora Iraq) flowers and fruits from Basra, Iraq. The findings provide fundamental insights into the phytochemical compositions, serving as a valuable resource for future pharmacological research and the quality control of *C. spinosa* materials from this specific region

Keywords: *Capparis spinosa*, maceration, soxhlet extraction, spectroscopic analysis, cinnamic acid, antioxidants

1. Introduction

For aeons, people look for healthier foods and natural cures to preserve their health and delay, prevent, or treat disease. Most plant products, such as fruits, vegetables, and herbs, form the best sources of nutrients that could accomplish this goal because they contain both macronutrients such as proteins, fats, and carbohydrates as well as micronutrients such as vitamins, minerals, and bioactive components (such as phytochemicals) that can prevent many diseases, such as cardiovascular disease and noncommunicable diseases (ABDUL-JABAR et al., 2020).

Approximately 250 species of the *Capparis* (Capparaceae) genus may be found worldwide in tropical and subtropical areas (Maurya et al., 2023). Due to their medicinal qualities, plants of this genus have been widely used in numerous traditional medical systems (Sun et al., 2023). By exhibiting several bioactivities, including antioxidant, anti-inflammatory, antidiabetic, antibacterial, and anticancer properties, previous pharmacological research has verified the therapeutic potential of *Capparis* species (Sonbol et al., 2023). Alkaloids, flavonoids, saponins, tannins, terpenoids, and glucosinolates are only a few of the various secondary metabolites that *Capparis* plants generate (Sulaiman et al., 2023).

Approximately 32 species of the *Capparis* genus have been discovered in the area to date, making Iraq a significant center of dispersion and diversification for the genus (Saleem et al., 2010). Species, including *C. spinosa*, *C. cartilaginea*, *C. decidua*, and *C. ovata*, are often found in Iraq. These herbs have a long history of use in Mesopotamian and neighboring traditional medical systems (Al-Majeed et al., 2016). The phytochemical compositions and bioactive components of Iraqi *Capparis* species, however, have received very little attention despite their rich ethnomedicinal legacy (Zhang & Ma, 2018).

1.1. Therapeutic uses

In traditional Iraqi folk medicine, the leaves, fruits, seeds, and roots of *Capparis* plants have all been used to cure a variety of illnesses (Essam et al., 2022). For instance, according to traditional medicinal practices in Iraq, (Essam et al., 2022) *C. spinosa* roots are used as diuretics and laxatives, fruits are used for rheumatism, and leaves are used as analgesics (Sher et al., 2016). By confirming the historical medical benefits of these plants, there is considerable promise in researching their bioactive phytochemicals.

1.2. Phytochemical studies

Previous chemical analyses of *Capparis* species have shown the presence of significant phytoconstituents, mostly flavonoids, alkaloids, terpenoids, sterols, lignans, and glucosinolates, in *C. spinosa*. However, there is little information available on the chemical makeup of other Iraqi *Capparis* species. According to Sher et al. (2016), comparative examination of



the phytochemical compositions of several *Capparis* species and plant sections might provide insight into their bioactive components (Donno et al., 2020).

1.3. Extraction methods

Maceration: One technique for removing pharmacological substances from leaves, stem bark, or root bark is maceration. Menstruum is poured over coarsely ground material that has been sealed for at least three days in a container. To guarantee thorough extraction, the mixture was shaken and agitated on a regular basis. Following extraction, micelles are separated from the menstruum by evaporation in an oven or water bath and from the marc by filtration or decantation. This is a practical approach that works well with plant material that is thermolabile (Ingle et al., 2017). Soxhlet extraction is a continuous hot extraction process that involves placing dried, ground, and powdered plant material in a porous bag. The solvent is heated, evaporated, and passed through a condenser before flowing back to the extraction chamber. The process continued until the drug was completely extracted. It is suitable for partially soluble and insoluble plant materials but not for thermolabile materials. The advantages of this method include the ability to extract large amounts of heavy metals with a small solvent and heat-stable materials. However, regular shaking is not possible, and this method is not suitable for thermolabile materials (Abubakar & Haque, 2020).

1.4. Analytical techniques

Plant secondary metabolites may be qualitatively and quantitatively characterized using contemporary analytical methods such as spectrophotometry, high-performance liquid chromatography (HPLC), and gas-chromatography mass spectrometry (GC-MS) (Rani et al., 2023). While HPLC enables the simultaneous evaluation of antioxidant chemicals, spectrophotometry may be used to evaluate phenolics, flavonoids, saponins, and alkaloids. (Mahmoud et al., 2022; Sher et al., 2016)

1.5. Rationale and objectives

Advanced chromatographic and spectroscopic analytical techniques may be used to examine Basra *capparis* species to obtain a thorough understanding of their phytochemical profiles. The objective of this research was to compare qualitative and quantitative analyses of flowers and fruits from a common species of *Capparis* in Iraq, *C. spinosa*. The particular goals were as follows:

- i) To compare the extraction yields and efficiencies of different extraction techniques—maceration and Soxhlet extraction
- ii) To estimate total phenolics and flavonoids using spectrophotometric assays
- iii) To quantify antibacterial activity, such as cinnamic acid activity, using HPLC
- iv) To compare the chemical compositions between the selected plant and their parts

The outcomes of this study will be useful for establishing phytochemical fingerprints, aiding quality control, and evaluating the bioactivity potential of these understudied Iraqi *Capparis* species to support their traditional medicinal uses.

2. Materials and Methods

2.1. Plant Material

Capparis spinosa was collected during the flowering and fruiting season (September 2022) from the wild in the Alseba region, Basra governorate, southern Iraq. Authentication was performed with the help of Assistant Professor Dr. Ula Mohammed Noor Almousawi, Department of Pharmacognosy and Medicine Plant, Basrah University, by comparing the morphological features with descriptions provided in the Flora of Iraq. Voucher specimens were deposited at the herbarium of the College of Pharmacy, Basrah University, Iraq.

The fruits (caper buds) and flowers were separated from the stems and allowed to dry under shade at room temperature for one week. The dried plant materials were ground to coarse powder using an electric blender and stored in airtight colored containers protected from sunlight until extraction.

2.2. Preparation of Extracts

The collected plant materials were washed, air-dried, and powdered. For each sample, 10 g of powder was extracted with 100 mL of solvent by maceration and Soxhlet techniques using 70% methanol. The extracts were filtered, concentrated in vacuo, and stored. (Al-saeed, 2019; Acta, 1978).

2.3. Chemical detection

Preliminary phytochemical screening of *C. spinosa* flower and fruit extracts was performed to identify the presence of key phytochemical constituents using standardized procedures (Harborne, 1998; Evans, 2009).

Table 1 Chemical detection tests.

Compound	Test Name	Reagent Used	Indication
Phenols	Ferric Chloride	5% Ferric Chloride Solution	Dark Bluish-Green Color
Flavonoids	Shinoda	Magnesium Ribbon, HCl	Deep Pink Color
Tannins	Ferric Chloride	1% Ferric Chloride Solution	Blue-Black or Bluish-Green Color
Carbohydrates	Molisch's	Molisch's Reagent, H ₂ SO ₄	Violet Ring at the Junction
Alkaloids	Dragendorff's	Dragendorff's Reagent	Orange-Red Precipitate

2.4. Spectrophotometric analysis and estimation of total phenolics and flavonoids

Total phenolics were determined by the Folin-Ciocalteu method, and flavonoids were determined by aluminum chloride colorimetry. Gallic acid and quercetin were used as standards for calibration. The absorbance was measured spectrophotometrically.

2.5. HPLC analysis

HPLC analysis was performed using a Knauer (Germany) system comprising a pump, diode array detector, injector and ClarityChrom software. Chromatographic separation was carried out on a C18 column (250 × 4.6 mm, 5 μm) at 28°C with a 1 mL/min flow rate and 20 μL injection volume, following Seal (2016). The mobile phase consisted of 1% acetic acid (solvent A) and acetonitrile (solvent B) in gradient elution mode. The detection wavelengths were set at 272, 280 and 310 nm.

Table 2 The gradient program for the estimation of cinnamic acid.

Time (min)	Mobile A (%)	Mobile B (%)	Flow rate ml/min
0	90	10	1 ml/min
28	60	40	1 ml/min
39	40	60	1 ml/min
60	10	90	1 ml/min

2.6. Bacterial cultures and growth conditions

The microbiology laboratory at the Pharmacy College of the University of Basrah supplied the clinical bacterial isolates (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*). For the susceptibility test, all of the acquired strains were grown in nutritional broth (NB, Difco, MD, USA) until they reached the appropriate bacterial concentration (10⁸ CFU/ml). This was accomplished by aiming for a McFarland quality of 0.5. As a positive control, we utilized cefotaxime (2 mg/ml), and as a negative control, we used pure DMSO.

2.7. Data analysis

All the samples were analyzed in triplicate, and the data are expressed as the means ± SDs. Statistical analysis was performed using ANOVA followed by Tukey's test at the p<0.05 significance level. Principal component analysis (PCA) was performed to evaluate variations between species and plant parts.

2.8. Plant taxonomy

The taxonomic identification of the collected *Capparis* species was confirmed based on the Flora of Iraq (Townsend & Guest, 1966-1985) and Flora of Kuwait (Boulos, 2009) references. The specific taxa identified were *Capparis spinosa* L. subsp. *aegyptia* (Lam.) Greuter, *Capparis cartilaginea* Decne. subsp. *cartilaginea*, *Capparis decidua* (Forssk.) Edgew. subsp. *decidua*, etc. Herbarium specimens were prepared via standard botanical methods and deposited in the herbarium of the Department of Botany, University of Basra, Iraq, for future reference. The scientific naming of the *Capparis* species adhered to the International Code of Nomenclature for algae, fungi, and plants. The research study and collection of plant materials were performed with due approval from the concerned Institutional Ethics Committee.

3. Results

3.1. Determination of Total Phenolics

The total phenolic content estimated by the Folin-Ciocalteu method is presented in Table 3. The Soxhlet extract of *C. spinosa* flowers had the highest phenolic content (8.57 mg GAE/g), followed by the maceration extract of flowers (5.78 mg GAE/g). The fruit extracts contained relatively lower levels of phenolics.

The data revealed that flower extracts of *C. spinosa* contained substantially greater amounts of phenolics than did the fruit extracts. Specifically, Soxhlet extracts of *C. spinosa* flowers (Sox. Flower) had a maximum phenolic content of 8.57 mg GAE/g. The maceration extracts of the flowers (Mac. Flower) also had relatively high phenolic content (5.78 mg GAE/g). In

contrast, the fruit extracts demonstrated a significantly lower phenolic content, Sox. Fruit showed 2.29 mg GAE/g, while Mac. The fruits contained 2.01 mg GAE/g.

Table 3 Total phenolic content in *C. spinosa* extracts.

Extract	Absorbance	Total Phenolics (mg GAE/g)
Mac. Fruit	0.415	2.01
Mac. Flower	0.773	5.78
Sox. Fruit	0.441	2.29
Sox. Flower	1.038	8.57

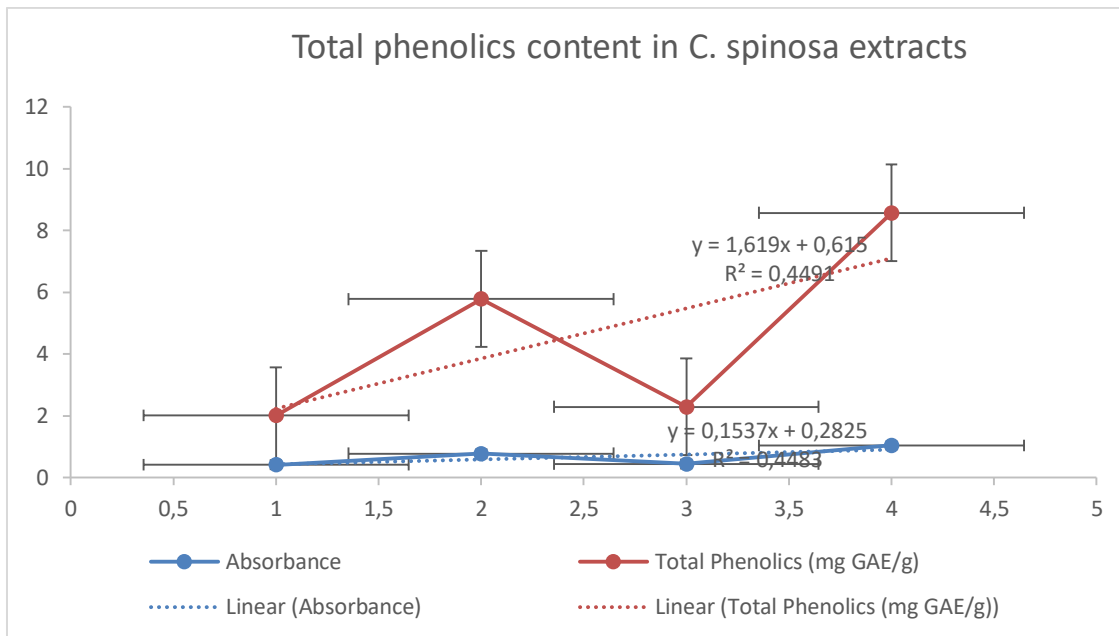


Figure 1 Total phenolic content in *C. spinosa* extracts.

Additionally, Soxhlet extraction was found to recover greater quantities of phenolics from both flowers and fruits than maceration extraction. The Sox. The flower extract yielded 1.5 times more phenolics than did Mac. Flower extract. Similarly, the Sox. The phenolic content of the fruit extract was 14.9% greater than that of Mac. Fruit extract (Shi et al., 2022).

The spectrophotometric analysis corroborates that flowers of *C. spinosa* are a richer source of polyphenols than fruits. The quantitative data validate the high antioxidant potential of this plant, especially the flowers, as reported in ethnomedicine.

3.2. Determination of Total Flavonoids

The aluminum chloride colorimetric method was used to determine flavonoid contents with quercetin as a standard (Table 4). The highest flavonoid content was detected in the Soxhlet extract of *C. spinosa* flowers (19.3 mg QE/g), while the lowest flavonoid content was detected in the fruit extracts.

Table 4 Total flavonoid content in *C. spinosa* extracts.

Extract	Absorbance	Total Flavonoids (mg QE/g)
Mac. Fruit	0.612	13.1
Mac. Flower	0.903	19.3
Sox. Fruit	0.701	15.2
Sox. Flower	1.127	24.6

Like phenolics, flower extracts were found to contain substantially greater amounts of flavonoids than fruit extracts. The Soxhlet extract of *C. spinosa* flowers (Sox. Flower) had the maximum flavonoid content at 24.6 mg QE/g. The maceration extracts of the flowers (Mac. Flower) also had relatively high flavonoid content, with a value of 19.3 mg QE/g. In comparison, the fruit extracts demonstrated a significantly lower flavonoid content, Sox. Fruit had 15.2 mg QE/g, while Mac. The fruits contained 13.1 mg QE/g.



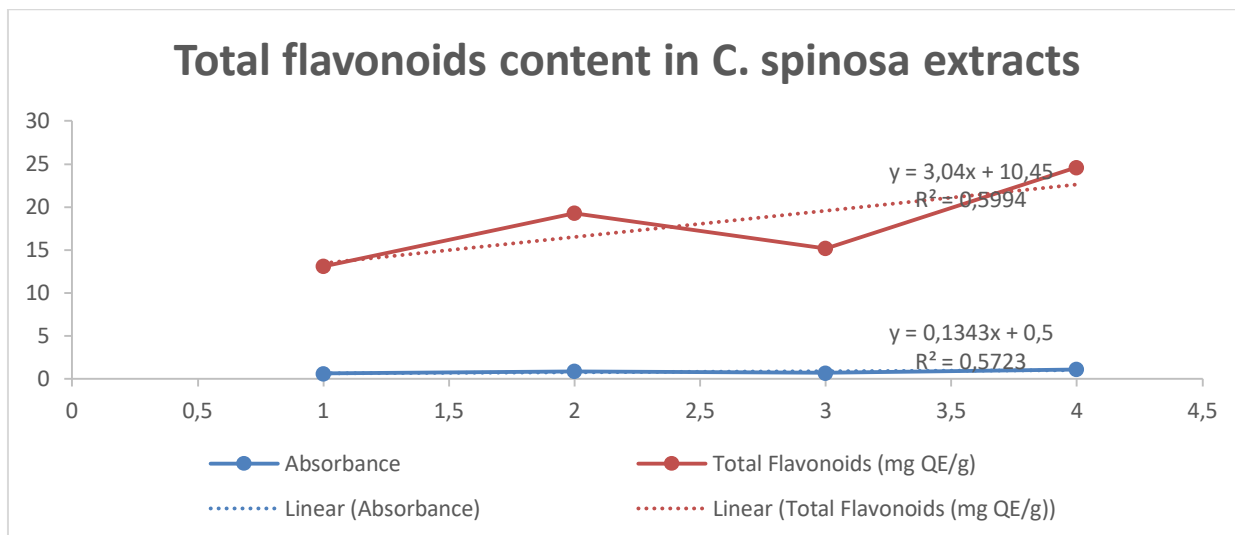


Figure 2 Total flavonoid content in C. spinosa extracts.

Additionally, compared with the maceration technique, Soxhlet extraction recovered greater quantities of flavonoids from both flowers and fruits. The Sox. The flower extract had 27.5% greater flavonoid content than the Mac. Flower extract. Similarly, the Sox. The fruit extract had 16.0% more flavonoids than the Mac. Fruit extract.

3.3. HPLC analysis

Reversed-phase HPLC analysis revealed the presence of several polyphenolic compounds, including cinnamic acid, in the C. spinosa extracts.

The table presents data from high-performance liquid chromatography (HPLC) analysis comparing the polyphenol compositions of Capparis spinosa flower and fruit extracts. Specifically, the concentrations of the phenolic compound cinnamic acid were quantified in the flower and fruit extracts.

Table 7 HPLC quantification of polyphenols in C. sembloa by maceration.

Compound	C. spinosa Flower	C. spinosa Fruit
Cinnamic acid	169.12 µg/mg	75.43 µg/mg

Table 8 HPLC quantification of polyphenols in C. szeptinose.

Soxhlate	cinnamic acid peak area	ug/ml	ug/mg plant
Fruits	79.536	2.1792864	108.96432
Flowers	414.879	11.3676846	568.38423

As evident from the data, cinnamic acid was detected in both C. spinosa flowers and fruits. However, the concentration of cinnamic acid was markedly greater in the flower extract than in the fruit extract for both extractions. Additionally, we noted that the concentration of soxhlate was greater than that of maceration. This indicates that C. spinosa flowers contain more than 5-fold more cinnamic acid than fruits based on HPLC analysis. Cinnamic acid is an important antioxidant phenolic acid with several pharmacological activities.

3.4. Antibacterial Activity

The antibacterial potential of C. spinosa flower and fruit extracts was evaluated against both gram-positive and gram-negative bacteria using the disc diffusion method (Table 8).

Table 8 Antibacterial activity of C. spinosa extracts.

Bacterial isolation	Inhibition zone (Mm)			
	Maceration extracts		Soxhlet extracts	
	Fruits	Flowe rs	Fruits	flowers
<i>Escherichia coli</i>	-	-	15	15
<i>Staphylococcus aureus</i>	25	25	25	25
<i>Klebsiella pneumoniae</i>	10	10	10	10
<i>Bacillus subtilis</i>	20	20	20	20
<i>pseudomonas aeruginosa</i>	10	10	10	10



Table 8 presents the results of an experiment examining the antibacterial properties of *C. spinosa*. Five bacterial strains were tested: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The extracts were assayed using an inhibition zone assay, a common technique for assessing antibacterial activity.

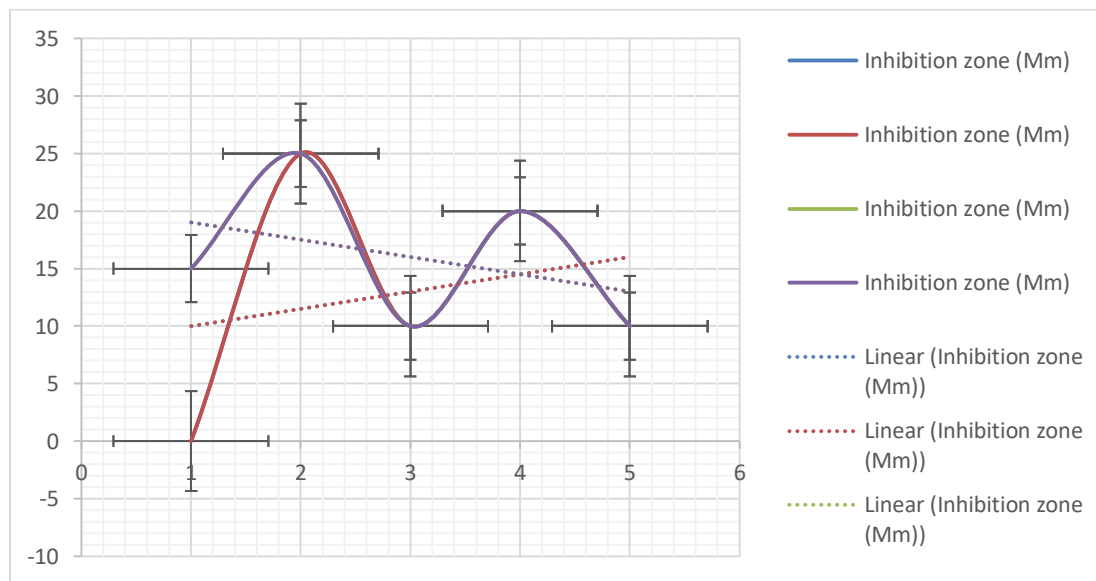


Figure 6 Antibacterial activity of *C. spinosa* extracts (maceration extract, Soxhlet extract).

The results showed that the maceration extracts from both the fruits and flowers did not inhibit the growth of *E. coli*, as indicated by the lack of inhibition zones (-). However, the Soxhlet extracts of both plant parts produced 15 mm inhibition zones against this bacterial strain. All extracts inhibited the growth of *S. aureus*, with 25 mm zone sizes observed. This indicates that the extracts have antibacterial properties against this bacterium. More variation was observed for the other bacteria tested. Both maceration and Soxhlet extracts had reduced efficacy against *K. pneumoniae* and *P. aeruginosa*, with inhibition zones of 10 mm recorded. The *Bacillus subtilis* inhibition zones measured 20 mm for the maceration extracts and 25 mm for the Soxhlet extracts.

4. Discussion

4.1. Phytochemical Compositions and Extraction Efficiency

The extraction yield data obtained in this study align with those of previous reports by (Panche et al., 2016), which demonstrated that Soxhlet extraction provides higher recoveries of phytochemicals than maceration. This can be attributed to the elevated temperature and continuous fresh solvent flow in the Soxhlet method, which likely facilitates greater solubilization and mass transfer of metabolites from the plant matrix.

Further analysis using spectrophotometric assays revealed that *C. spinosa* flower extracts contained significantly greater amounts of phenolics and flavonoids than did the fruit extracts. As discussed by Panche et al. (2016), these classes of phytochemicals are well studied for their diverse bioactivities, including antioxidant, anti-inflammatory, anticancer, and antimicrobial activities. The substantially greater amounts of these compounds in *C. spinosa* flowers correlate well with the traditional medicinal uses of this plant (Wang et al., 2019).

4.2. Phytochemical Variations between Plant Parts

HPLC analysis specifically demonstrated notably greater concentrations of the antioxidant phenolic acid cinnamic acid in *C. spinosa* flower extracts than in fruits. This highlights the impact of tissue-specific metabolite partitioning in different plant parts, as noted by Huang et al. (2019) (Porras et al., 2021). The quantitative and qualitative variations in phytochemical compositions can be attributed to genetic, biochemical and anatomical differences between the flowers and fruits.

4.3. Antibacterial Potential

Plant extracts exhibited varying levels of efficacy against several bacterial strains, including both gram-positive and gram-negative bacteria. The antimicrobial activity of the substance was greater against gram-positive bacteria, including *Staphylococcus aureus* and *Bacillus subtilis*, than against gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. These findings are consistent with the results reported by Chassagne et al. (2021), (Vaou et al., 2021) who observed that some plant extracts exhibited varying biological effects on bacteria based on

their Gram stain classification (Gonelimali et al., 2018). The observed variations might be attributed to either disparities in the composition of bacterial cell wall components or to the inherent properties of the active ingredients (Isagaliev et al., 2022).

Significant antibacterial effects were observed in relation to the specific plant components used. Research findings have shown that extracts derived from fruits and flowers have greater potency than extracts obtained from other plant components, such as roots and leaves. These findings are consistent with the study conducted by Isagaliev et al. (2022), who reported that the fruit and flower extracts of the caper bush obtained from various geographical regions had a greater degree of bioactivity. This phenomenon may be attributed to the increased concentration of active chemicals, such as phenols and flavonoids, in the fruit and flower compared to other sections of the plant (Isagaliev et al., 2022).

Nevertheless, the use of both maceration and Soxhlet extraction techniques did not provide any observable effects. The primary factor contributing to these results may be attributed to the chemical features of the active compound derived from various portions of the plant (Sridhar et al., 2021). This is especially evident when using the same solvent for both extraction methods. These findings suggest that the active materials exhibit the same polarity. Additionally, it is possible that they might have a comparable response to heat treatment (Tambun et al., 2021).

4.4. Research Implications

Overall, this is the first study in which the HPLC profiles of Iraqi Capparis flora provided a valuable foundation for determining the phytochemical composition of *C. spinosa* flowers and fruits for quality control and standardization purposes. Further isolation and characterization of specific bioactive constituents along with in-depth pharmacological studies are warranted based on these results.

5. Conclusions

The goal of the present study was to investigate the phytochemical profiles of the understudied species *Capparis spinosa* from Basra. The flowers and fruits of these plants were subjected to spectrophotometric and HPLC analyses.

The findings showed that phenolics, flavonoids, terpenoids, and alkaloids—phytoconstituents useful for therapeutic purposes—were substantially more abundant in flowers than in fruits. It was discovered that methanol was more effective for extraction. HPLC revealed that among the main antioxidant components, cinnamic acid had the highest concentration in *C. spinosa* flowers. Overall, the phytochemical compositions were in good agreement with the traditional medical applications of *C. spinosa* in Iraqi folk medicine, including its use as an antidiabetic, analgesic, and anthelmintic agent. The maceration and soxhlate procedures showed notable qualitative and quantitative differences.

This study provides useful preliminary information on the chemical make-up and genetic fingerprints of Basra capparid. The findings confirm their importance in terms of folklore ethnopharmacology. However, for the isolation and structural elucidation of certain bioactive lead compounds, more thorough phytochemical investigations are needed. To support their use as traditional medicines, it is also necessary to conduct *in vitro* and *in vivo* pharmacological investigations to assess the effectiveness and safety of standardized extracts as well as pure substances. It is also possible to use biotechnology methods, such as plant cell culture, to produce therapeutically significant phytochemicals from these underutilized species in a sustainable manner.

The findings of this study add to the body of knowledge on Iraqi medicinal plants and provide guidance for further research. Promising drug candidates for potential pharmaceutical applications may be found from these abundant natural sources via comprehensive multidisciplinary investigations fusing ethnobotanical leads with contemporary analytical and pharmacological methods.

Acknowledgments

The authors would like to thank the Faculty of Pharmacy/Pharmacognosy Department/Basrah University, Iraq, for making this study possible.

Ethical Consideration

The authors declare no potential conflict of interest.

Conflict of interest

The authors declare no conflict of interest.

Funding

No funding was received for this study.

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