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Original article

Spectrophotometric Analysis of Carbohydrates, Proteins, Amino Acids, and Metals in Leaf and Stem Extracts of *Cistaceae*

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A B S T R A C T

Although the plants of the *Cistaceae* family have historically been prized for their medicinal qualities, thorough comparative studies of various plant components are frequently lacking. The goal of this investigation was to estimate the total carbohydrate and total protein by spectrophotometer, and to estimate the concentrations of Iron, Copper, and Nickel by atomic absorption, also to compare the phytochemical and biochemical makeup of leaf and stem extracts from a Cistus species to identify the most useful component of the plant for potential uses. Qualitative phytochemical screening for key secondary metabolites was performed on alcoholic and aqueous extracts of leaves and stems. In addition, quantitative analyses were carried out to ascertain the overall content of carbohydrates, proteins, amino acids, and specific minerals (Fe, Ni, and Cu). The phytochemical screening findings demonstrated that leaves have a substantially higher concentration of bioactive ingredients, with aqueous extracts containing high amounts of flavonoids and both extracts containing moderate amounts of tannins and saponins, as compared to stems. Compared to the stems (0.541 and 0.272), respectively, the quantitative analysis also confirmed the superiority of the leaves, which had much higher concentrations of total proteins (1.983 ppm) and amino acids (0.541 ppm). In contrast, the stems, which function in nutrient storage, had a greater total carbohydrate content (0.359 ppm) than the leaves (0.137 ppm). The iron content of the leaves was similar to that of the mineral analysis, but the copper and nickel levels were slightly higher. These data provide conclusive evidence of a distinct biochemical division between the two organs and establish the leaves as a better source of both vital nitrogenous molecules and secondary metabolites of medicinal value. Prioritizing the use of Cistus leaves in the creation of phyto-pharmaceuticals and nutraceuticals is supported by this solid scientific argument.

Introduction

For millennia, natural products derived from medicinal plants have been a cornerstone of traditional medicine and a prolific source for modern drug discovery [1]. The vast chemical diversity inherent in the plant kingdom offers a unique and largely untapped reservoir of bioactive compounds with significant therapeutic potential [2]. These compounds, which are also referred to as phytochemicals or secondary metabolites, consist of important classes like flavonoids, phenolics, alkaloids, and terpenoids that have been shown to have a variety of pharmacological properties, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer effects [3]. Consequently, the systematic investigation of plant-derived natural products continues to be a critical endeavor in the search for novel therapeutic agents [4]. The *Cistaceae* family, commonly known as rockroses, comprises several genera of perennial shrubs predominantly found in the Mediterranean region [5]. Plants belonging to this family, particularly from the Cistus, Halimium, and Helianthemum genera, have a long history of use in folk medicine for treating various ailments, including skin diseases, digestive problems, and inflammatory conditions [6].

Scientific literature has increasingly substantiated these traditional uses, attributing the observed biological activities to a rich profile of phytochemicals [7]. Species within the *Cistaceae* family are known to be abundant sources of polyphenolic compounds, such as flavonoids and tannins, as well as a diverse array of terpenes, including labdane-type diterpenes [8]. These compounds are recognized for their potent antimicrobial and antioxidant properties, making *Cistaceae* species a subject of considerable research interest. The distribution and concentration of phytochemicals can differ significantly not only between

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species but also within the same plant, such as leaves and stems [9]. Genetic factors, developmental stage, and environmental conditions often influence this variation. Leaves are typically the primary sites of photosynthesis and are often rich in phenolic compounds that protect against UV radiation and oxidative stress, while stems provide structural support and are involved in the transport and storage of metabolites [10]. Therefore, a comparative analysis of the phytochemical composition of different plant organs is essential for identifying the most potent source of specific bioactive compounds. Such studies are crucial for optimizing extraction procedures and maximizing the yield of desired molecules for pharmaceutical or nutraceutical applications. Despite the growing body of research on the *Cistaceae* family, many species remain underexplored, and comprehensive comparative studies evaluating the biochemical potential of different plant parts are limited [8,9].

Challenges in phytochemical research, including the need for standardized extraction methods and robust analytical techniques, persist [11]. The study of different plant constituents and their medical applications was conducted in Libya in different studies [12-41]. This study aims to address this gap by conducting a comparative phytochemical screening and biochemical evaluation of leaf and stem extracts from selected *Cistaceae* species. By systematically analyzing and contrasting the phytochemical profiles, specifically the total phenolic and flavonoid contents, and evaluating the associated antioxidant and antimicrobial activities, this research seeks to determine whether leaves or stems represent a richer source of bioactive compounds. The findings are expected to provide valuable scientific data to support the targeted use of specific plant parts from the *Cistaceae* family in the development of new phytomedicines and functional ingredients.

Methods

Plant Collection

In the spring of 2023, fresh aerial pieces of *Cistaceae* were collected from the Al-Gabel Al-Akhdar region. The plant specimens were officially identified and verified at the Seliphium Herbarium in the Botany Department of the Faculty of Science at Omar Al-Mukhtar University.

Samples preparation

To ensure the purity and preservation of the plant material, a rigorous sample preparation technique was employed. To remove any dirt or other pollutants that may be present, the selected plant's leaves and stems were first carefully collected and washed in distilled water. At this point, it's critical to remove any foreign components that could obstruct potential testing. After that, the cleaned plant material was thoroughly dried in a chilly, dark area to prevent deterioration brought on by light and humidity. This drying method eliminates excess water while preserving the integrity of the plant's constituent parts and preventing the growth of microorganisms. After the plant material had dried enough. A mortar and pestle were used to grind it into a fine powder. This grinding process makes it easier to efficiently extract bioactive chemicals in the subsequent analysis by increasing the surface area of the plant material. After that, the crushed plant powder was carefully stored in hermetic polyethylene containers to prevent deterioration and contamination. These bottles were kept in a chilly, dark place to maintain the stability of the plant chemicals until further research could be conducted.

Phytochemical Screening

A qualitative phytochemical screening of the plant extracts was carried out in order to determine whether significant groups of secondary metabolites were present. The analyses were carried out using several well-known, common colorimetric Techniques according to previous studies [20-25].

Sterol and Triterpenoid Testing (Liebermann-Burchard Test)

The chloroform extract was mixed with 0.3 mL of acetic anhydride in a ratio of one milliliter (1 mL). Following this, a few drops of concentrated sulfuric acid were gently administered along the side of the test tube. A reddish-violet ring formed at the interface between the two layers, followed by the emergence of a green tint in the chloroform layer, was considered a strong indication of the presence of sterols and/or triterpenoids.

Flavonoids Test (Alkaline Reagent Test)

Adding a few drops of diluted sodium hydroxide solution rendered a portion of the plant extract alkaline. The presence of flavonoids was indicated by the appearance of a strong yellow hue, which disappeared upon the addition of a few drops of diluted acid.

Alkaloid Testing (Dragendorff's Test)

The plant extract was filtered after being acidified with a dilute solution of hydrochloric acid. With diluted ammonium hydroxide, the acidic filtrate was then meticulously neutralized before being extracted with chloroform. On a piece of filter paper, a few drops of the chloroform extract were placed. The location was

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treated with Dragendorff's reagent after drying. The presence of alkaloids was confirmed by the appearance of a notable orange or reddish-brown precipitate/spot.

Tannin Test (Ferric Chloride Test)
The plant extract was filtered after being diluted with 50% ethanol. A few drops of a 1% ferric chloride ($FeCl_3$) solution were introduced to the transparent hydroalcoholic filtrate. A favorable outcome for the existence of tannins was the formation of a blue-black or greenish-black precipitate.

The Modified Borntrager's Test for Anthraquinones
The plant extract was filtered after being hydrolyzed by boiling with a few drops of dilute sulfuric acid in one milliliter (1 mL) of solution. Chloroform was used to extract the filtrate. An equal volume of diluted ammonia solution was introduced after the chloroform layer was removed. The presence of anthraquinones was indicated by the formation of a rose-pink to cherry-red hue in the lower ammoniacal layer of the mixture as it was shaken.

Test for Saponins
The plant extract, about 1 mL, was diluted in a test tube with 5 mL of distilled water, then vigorously shaken for 5 minutes. The presence of saponins was indicated by the formation of a stable foam layer that was at least 1 cm high and lasted for at least 15 minutes.

Determining the total amount of soluble protein
To determine the total protein (Total N × 6. 25), the soluble protein was calculated by multiplying the total nitrogen by 6. 25, and the protein content was expressed as mg protein/g/g FW.

Estimation of metals
Using the technique outlined by previous studies, the atomic absorption (Perkin Elmer 800) was used to identify the metals of (Cu, Ni, and Fe) at Omar El-Mukhtar University's central lab.

Carbohydrate Identification
0. 2 of the dry sample by weight was initially crushed to ascertain the total carbohydrates, followed by the addition of 5 ml of sulfuric acid. After the samples had completely dissolved, a little barium carbonate (Ba_2CO_3) was added, and the samples were heated once more before being allowed to cool at room temperature. The phenol-sulfuric acid method was used to calculate the total carbohydrate content. The sample solutions were filtered after cooling. To induce color development, 1 mL of a 5% aqueous phenol solution was added to 1 mL of the filtrate, followed by the quick introduction of 5 mL of concentrated sulfuric acid. The absorbance of the colored complex produced.

Results

Phytochemical screening
To determine the existence of significant secondary metabolite classes, a qualitative phytochemical analysis was conducted on both aqueous and alcoholic extracts obtained from the leaves (B1) and stems (B2) of the tested Cistaceae species. The findings, which are based on the strength of colorimetric reactions, are presented in (Tables 1) (aqueous extracts) and 2 (alcoholic extracts). The relative amounts of each phytochemical group were qualitatively assessed and classified as high (++++), moderate (+++), low (++), trace (+), or absent (-).

Aqueous Extracts
The phytochemical study of the aqueous extracts, as seen in Table 1, revealed a rich and diverse composition in both the leaves and stems. The leaf extract (B1) revealed an extremely high concentration of flavonoids (++++), a moderate concentration of tannins and saponins (+++), a low concentration of alkaloids (++), and a trace amount of sterols and/or triterpenes (+). The stem extract (B2), in contrast, showed a moderate presence (+++) of tannins and saponins. Nevertheless, the flavonoid (+++) and alkaloid (++) concentrations were somewhat lower than those seen in the leaves. The concentration of anthraquinones was low (++) in both leaves and stems.

Alcoholic Extracts
A different distribution pattern of phytochemicals was seen during the screening of the alcoholic extracts (Table 2). The leaf extract (B1) had a particularly high concentration of tannins and saponins, both of which were found in moderate concentrations (+++). Additionally, it included trace levels (+) of alkaloids, flavonoids, and sterols and/or triterpenes. In contrast, the phytochemical content of the alcoholic stem extract (B2)

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was typically lower. Only traces of tannins, alkaloids, and saponins were found (+), whereas flavonoids and anthraquinones were found at low concentrations (++). Surprisingly, the alcoholic extract of the stems contained none of the sterols or triterpenes (-). When comparing the screening results, the leaves are generally found to be a better source of the tested phytochemicals, specifically flavonoids in the aqueous extract and tannins and saponins in both the aqueous and alcoholic extracts. The extraction efficiency of these secondary metabolites was also greatly impacted by the solvent used.

Table 1. The phytochemical screening of aqueous extracts of the studied plants

Phytochemical screening test	B1	B2
Tannins	+++	+++
Alkaloid	+++	++
Flavonoids	++++	+++
Anthraquinenes	++	++
Sterols and or Triterpenes	++	+
Saponins	+++	+++

B1: Cistaceae leaves B2: Cistaceae stems

Table 2. The phytochemical screening of Alcoholic extracts of the studied plants

Phytochemical screening test	B1	B2
Tannins	+++	+
Alkaloid	+	+
Flavonoids	+	++
Anthraquinenes	++	++
Sterols and or Triterpenes	+	_
Saponins	+++	+

B1: Cistaceae leaves B2: Cistaceae stems

(+): presence , (++): Moderate contents as color test , (+++) : High contents as color test , (-): Absent

Total carbohydrate, protein, Amino Acid, and Metals

Primary Metabolites and Mineral Content

On the leaf and stem sections of the examined Cistaceae species, primary metabolites—total carbohydrates, total proteins, and total amino acids were quantitatively analyzed in addition to the qualitative screening of secondary metabolites. Additionally, the concentration of key trace elements (Fe, Ni, Cu) was measured.

The entire amount of protein, amino acids, and carbohydrates

(Tables 3, 4, and 5) display quantitative data for the main metabolites. There was a notable difference in the distribution of these substances between the stems and leaves. As seen in (Table 3), the total carbohydrate content of the stems (B2) was much higher (average 0. 359 ppm) than that of the leaves (B1) (average 0. 137 ppm). In contrast, the leaves were shown to have a lot more nitrogenous chemical. The leaves have three times as much protein as the stems, with an average of 1. 983 compared to 0. 541 ppm, respectively (Table 4). In the same way, the leaves had about twice as many total amino acids (an average of 0. 541) as the stems (an average of 0.272 ppm (Table 5). The analysis of selected trace elements revealed comparable levels of iron (Fe) in both the leaves (39.15 µg/g) and stems (39. 85 ppm). However, the leaves (B1) had somewhat higher concentrations of nickel (Ni) (1.571 ppm) and copper (Cu) (2.51 ppm) than the stems (B2), which had 1. 22 ppm of Ni and 2. 16 ppm of Cu (Table 6).

Table 3. The total carbohydrate contents (ppm) of the studied plant extracts:

Content / Sample	C1	C2	C3	Average
Cistaceae leaves	0.136	0.138	0.138	0.137
Cistaceae Stems	0.360	0.359	0.358	0.359

Table 4. The contents of total protein (ppm) of the studied plant extracts.

Contents / Sample	C_1	C_2	C_3	Average

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Cistaceae leaves	1.968	1.994	1.983	1.981
Cistaceae Stems	0.543	0.542	0.541	0.542

Table 5. The contents of amino acids* (ppm) *of the studied plant extracts.

Contents / Sample	C1	C2	C3	Average
Cistaceae leaves	0.542	0.541	0.541	0.541
Cistaceae Stems	0.275	0.272	0.272	0.273

Table 6. Metal contents of the studied plants(ppm).

Elements / Samples	Fe	Ni	Cu
B1	39.15	1.571	2.51
B2	39.85	1.22	2.16

Discussion

The current study examined the chemical composition of alcoholic and water extracts from the leaves and stems of a Cistaceae species. According to the existing scientific literature, there are significant differences in the distribution of secondary metabolites depending on the plant component and the extraction solvent used. Our findings indicated that, in general, the leaves have a higher concentration of essential phytochemicals than the stems. Particularly, the aqueous leaf extract had a very high flavonoid concentration (++++). This is consistent with previous research indicating that leaves, as the primary sites of photosynthesis, accumulate high levels of phenolic compounds like flavonoids to protect against biotic and abiotic stresses, including UV radiation and oxidative damage. A recent comprehensive review on various Cistus species also highlighted those leaves are the main repository for polyphenolic compounds, directly corroborating our observations [42]. The moderate presence of tannins and saponins in both aqueous and alcoholic leaf extracts further underscores the metabolic activity of this plant part. The choice of solvent profoundly influenced the extraction efficiency, a well-documented phenomenon in phytochemical research [43].

In our study, water (aqueous extract) proved to be more effective for extracting flavonoids, whereas the alcoholic extract of the leaves showed higher relative concentrations of tannins and saponins. This differential solubility is expected, as the polarity of the solvent plays a crucial role in determining which compounds are extracted. The high polarity of water makes it an excellent solvent for many polar compounds like flavonoid glycosides, while less polar solvents like ethanol can be more effective for other classes of compounds, which explains the different profiles observed. Interestingly, alkaloids were detected in low to trace amounts in most extracts. While many plant families are known for high alkaloid content, the Cistaceae family is not typically characterized as a primary source. However, their presence, even in small quantities, warrants further investigation, as alkaloids are known to be a scaffold for many therapeutic drugs and exhibit potent pharmacological activities even at low concentrations [44]. Similarly, the detection of anthraquinones, although in low amounts, is significant. These substances are recognised for their possible biological effects, including laxative and antimicrobial effects, which could contribute to the plant's traditional medicinal uses. A key observation from our study is the marked difference between the leaf and stem extracts. The stem extracts consistently showed lower concentrations of most phytochemicals. This is biologically plausible, as different plant organs are specialized for different functions; stems primarily provide structural support and transport, while leaves are major sites for the synthesis and storage of defensive secondary metabolites [45].

The complete absence of sterols and triterpenes in the alcoholic stem extract, while present in the leaf extract, is a clear example of this organ-specific chemical distribution. This finding is critical for traditional medicine and commercial applications, as it suggests that harvesting leaves would be far more efficient for obtaining a high yield of bioactive compounds. This aligns with a review by previous studies [46] which emphasized that different parts of Cistus plants possess varied chemical profiles and, consequently, different biological activities. In conclusion, this comparative study provides valuable insight into the phytochemical landscape of the studied *Cistaceae* species. It confirms that leaves are a superior source of medicinally important compounds like flavonoids, tannins, and saponins compared to stems. Furthermore, it highlights the critical importance of solvent selection in phytochemical analysis. These findings strongly support the traditional use of *Cistaceae* leaves in herbal medicine and provide a scientific basis for prioritizing this plant part for future pharmacological investigations and the potential development of phytopharmaceutical products.

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The physiological roles and nutritional potential of various components of the Cistaceae plant are further revealed by quantitative analysis of main metabolites and minerals. Our research reveals that carbohydrates, proteins, and amino acids are clearly separated between the leaves and stems, which correspond to their unique biological roles. The significantly higher concentration of total carbohydrates in the stems (0.359 ppm) compared to the leaves (0.137 ppm) is a key finding. This is biologically consistent, as stems in perennial shrubs serve as a primary organ for transport and, importantly, the storage of carbohydrates like starch and sucrose. These stored reserves provide energy for survival during dormant periods and for new growth. In contrast, while leaves are the site of carbohydrate synthesis (photosynthesis), they primarily export these sugars to other parts of the plant rather than storing them in large quantities [47]. Conversely, our results show that the leaves are substantially richer in total proteins (1.983 ppm) and amino acids (0.541) than the stems. This is expected, as leaves are the epicenters of metabolic activity. They are densely packed with enzymes required for photosynthesis, carbon fixation (e.g., RuBisCO, one of the most abundant proteins on Earth), and the synthesis of secondary metabolites [47]. The higher protein and amino acid content directly reflects this intense enzymatic and biosynthetic machinery. This finding also highlights the potential nutritional value of Cistaceae leaves as a source of protein. The analysis of mineral content revealed that both leaves and stems are good sources of iron (Fe), with nearly equal concentrations. Iron is a crucial micronutrient for plants, acting as a cofactor in many enzymatic reactions, including chlorophyll synthesis and electron transport chains [48]. The slightly higher levels of copper (Cu) and nickel (Ni) in the leaves are also noteworthy. Copper is another essential cofactor for enzymes involved in photosynthesis and respiration, while nickel is a component of the urease enzyme. The accumulation of these metals in the leaves is likely linked to their role in the active metabolic processes occurring there [45]. The concentrations of these metals are within the typical range reported for many plant species and underscore the plant's ability to uptake essential minerals from the soil. The determination of metals in different samples plants, soils, water and others were studied by used different methods as atomic absorption, X -ray Florescence and spectrophotometer [49-92] most of these studies showed high accuracy and precision, the distribution of primary metabolites follows a clear functional pattern: stems act as carbohydrate storage organs, while leaves serve as the primary centers for protein synthesis and metabolism. This complements our phytochemical findings, painting a comprehensive picture where leaves are the richest source of both bioactive secondary metabolites and essential nitrogenous compounds, making them the most valuable part of the plant for potential nutritional and medicinal applications.

Conclusion

The phytochemical and biochemical makeup of leaf and stem extracts from the studied *Cistaceae* species was successfully compared in this study. The findings clearly show that the leaves contain far higher levels of essential primary metabolites, such as proteins and amino acids, as well as beneficial secondary metabolites, such as flavonoids, tannins, and saponins, when compared to the stems. The stems, on the other hand, were discovered to be the main site of carbohydrate storage. The solvent selection was also proven to be a crucial element that affects how various chemical classes are extracted. These results offer solid scientific evidence in support of the traditional use of *Cistaceae* plant leaves in herbal treatments. From a practical perspective, this study supports targeting leaves rather than the stems for harvesting to maximize the production of bioactive chemicals for use in the pharmaceutical, nutraceutical, and cosmetic industries. Future research should concentrate on evaluating the pharmacological effects of substances isolated and identified from leaf extracts.

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Conflict

No conflict with any other studies for the results recorded in this study.

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