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*Original Article*

# Comparative Phytochemical Screening, Biochemical Evaluation, and Metals of Leaf and Stem Extracts of *Viburnum tinus* plant

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**Abstract**
By using two different solvents (aqueous and alcoholic), this study sought to quantitatively assess the primary biochemical components in the leaf and stem extracts of Viburnum tinus and conduct a qualitative phytochemical screening. Qualitative analysis of the plant extracts revealed the presence of several types of secondary metabolites. with a notable variation in their distribution based on the plant part and the solvent used. Both sections had a large quantity of flavonoids, tannins, and saponins, according to the findings. Among the tested samples, the alcoholic stem extract exhibited a strong presence of flavonoids and saponins. while the water leaf extract had a high concentration of tannins, alkaloids, and saponins. The importance of choosing the right solvent to isolate certain classes of chemicals is highlighted by these results. In terms of quantitative biochemical analysis, the leaves had much greater concentrations of total carbs (average 0. 492 mg/g), total protein (average 0. 862 mg/g), and total free amino acids (average 0. 656 mg/g) than the stems. The essential mineral content was also examined; the concentrations of Iron (Fe), Nickel (Ni), and Copper (Cu) ranged from 39. 15 to 60. 38 ppm, 1. 17 to 1. 57 ppm, and 1. 89 to 5. 73 ppm, respectively, with a relative increase in Fe levels in both leaves and stems. These findings, in conjunction with the plant's potential for pharmaceutical and nutritional applications, demonstrate that Viburnum tinus, with the leaves in particular, represents a valuable source of bioactive compounds and important nutrients.
**Keywords.** Viburnum Tinus, Phytochemical Screening, Biochemical Constituents, Essential Minerals.

## Introduction

The plant kingdom remains the most significant reservoir of novel chemical entities, contributing greatly to the fields of traditional healing practices and contemporary pharmaceutical research [1]. The exploration of medicinal plants is crucial for discovering new bioactive compounds that can address emerging health challenges, particularly in the context of increasing antimicrobial resistance and chronic diseases [2]. Secondary metabolites produced by plants, for instance, alkaloids, tannins, and saponins, flavonoids, are primarily responsible for their therapeutic properties and are often the focus of phytochemical investigations [3]. Viburnum is a genus within the family Adoxaceae and consists of nearly 150–200 species of shrubs and small trees that grow mainly in temperate and subtropical areas [4].

Historically, several members of this genus have played an important role in traditional healing systems for their therapeutic value, particularly as anti-inflammatory, antispasmodic, and calming agents [5]. Among these, Viburnum tinus L., commonly known as Laurustinus, is a Mediterranean evergreen shrub widely cultivated for its ornamental value but also recognized for its traditional medicinal uses. Ethnobotanical research in recent years has reaffirmed the plant's value in traditional healing practices, reflecting its abundant bioactive profile [6]. Phytochemical studies on Viburnum species have revealed a diverse array of secondary metabolites, including iridoids, triterpenoids, phenolic compounds, and essential oils. Specifically, research on V. tinus has indicated the presence of various bioactive components, with some studies focusing on the anti-inflammatory and anti-cancer potential of its leaf extracts. However, the chemical composition can vary significantly depending on the plant part (e.g., leaves, stems, fruits), the geographical origin, the developmental stage, and, critically, the extraction solvent used [7].

The choice of extraction solvent is paramount in phytochemical research, as it dictates the polarity of the compounds isolated and, consequently, the biological activity of the resulting extract [8]. A comparative analysis using solvents of different polarities, such as water (aqueous) and alcohol (alcoholic), is essential to obtain a comprehensive profile of the plant's chemical repertoire [9]. Furthermore, while qualitative phytochemical screening provides a general overview of the classes of compounds present, a quantitative biochemical evaluation is necessary to determine the nutritional and pharmacological value of the plant material [10]. The biochemical composition, including total carbohydrates, proteins, and amino acids, is a key indicator of the plant's overall nutritional quality and metabolic status [11]. Variations in these primary metabolites between different plant organs, such as leaves and stems, can reflect differences in their physiological roles and storage capacities. Moreover, the mineral content of medicinal plants is of


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significant interest, as essential trace elements like Iron (Fe), Copper (Cu), and Nickel (Ni) are vital cofactors for numerous human physiological processes. The concentration of these elements is known to be a complex trait, influenced by the interaction between the plant's genetic makeup and environmental factors like soil type and climate [12].

Despite the recognized traditional uses and preliminary phytochemical findings, a detailed comparative study focusing on the differences in both the qualitative phytochemical profile and the quantitative biochemical composition (macronutrients and essential minerals) between the leaves and stems of *Viburnum tinus* are still limited. Such a comparative evaluation is crucial for identifying the most potent and nutrient-rich part of the plant for potential pharmaceutical or nutraceutical development [13]. The phytochemical screening studies and their applications took place in many investigations in Libya many years ago [14 -54]. Also, estimating the metals, minerals, and some compounds, we have in different samples, such as solis, plants, and others [55 -119]. Therefore, this study was designed to perform a comparative analysis of the aqueous and alcoholic extracts of *Viburnum tinus* leaves and stems. One of the aims of this research was to: (1) investigate the presence of key classes of secondary metabolites through qualitative phytochemical screening and (2) perform a quantitative biochemical evaluation of total carbohydrates, proteins, amino acids, and essential mineral content in both plant parts. This study provides scientific evidence that may assist in the effective use of *Viburnum tinus* for developing therapeutic agents and functional food products.

## Methods

### *Identification and Collection of Plant Material*

From the Al-Gabel Al-Akhdar area, fresh aerial sections of *Viburnum tinus* were gathered in the spring of 2023. At the Seliphium Herbarium in the Botany Department of the Faculty of Science at Omar Al-Mukhtar University, the plant specimens were identified and verified.

### *Preparation of samples*

A careful sample preparation procedure was used to guarantee the purity and preservation of the plant material. The chosen plant's leaves and stems were first carefully gathered and rinsed in distilled water to get rid of any dirt or other pollutants that may be attached. At this step, clearing the sample of any unwanted elements that may disrupt upcoming tests is necessary. Following that, the washed plant matter was carefully dried in a cold, dark environment to reduce deterioration caused by light and moisture. The integrity of the plant components is maintained, and microbial development is inhibited by this drying process, which eliminates extra water. The plant stuff was crushed into a fine powder using a mortar and pestle after it had dried sufficiently. By increasing the surface area of the plant material, this grinding process facilitates the efficient extraction of bioactive substances in the subsequent analysis. Following that, the pulverized plant powder was meticulously kept in airtight polyethylene containers to avoid deterioration and contamination. To preserve the stability of the plant chemicals until more research may be done, these bottles were kept in a cold, dark location.

### *Phytochemical Screening*

To assess the presence of key secondary metabolites, the plant extracts underwent qualitative phytochemical evaluation. determine whether significant groups of secondary metabolites were present. The analyses were carried out using a number of well-known, common colorimetric techniques [34-38].

### *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). Subsequently, concentrated sulfuric acid was slowly added along the inner wall 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 regarded as a strong sign for the existence of sterols and/or triterpenoids [34].

### *Flavanoids Test (Alkaline Reagent Test)*

By adding a few drops of diluted sodium hydroxide, part of the plant extract became alkaline. The emergence of a strong yellow coloration pointed to the presence of flavonoids [34-40], and this color vanished when diluted acid was subsequently added.

### *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 treated with Dragendorff's reagent after drying. The alkaloids existence was confirmed by the appearance of a notable orange or reddish-brown precipitate/spot [34-40].


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***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 [34-40] 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 [34-36].

***Test for Saponins (Froth Test)***
Approximately 1 mL of the plant extract was placed in a test tube with 5 mL of distilled water and shaken thoroughly for roughly five minutes. After shaking, the tube was observed for foam formation. The appearance of a stable foam layer about 1 cm in height that remained for at least 15 minutes was taken as evidence of the presence of saponins.

***Calculating the overall soluble protein content:***
Soluble protein was estimated by applying the conversion factor of 6.25 to the measured total nitrogen, with the protein content expressed as mg per gram of fresh weight (FW).

***Identification of minerals and metals:***
The metals of (Cu, Ni, and Fe) were determined with atomic absorption (Perkin Elmer 800) at the central lab of Omar El-Mukhtar University, following the method described by [30].

***Carbohydrate Determination:***
To determine the total carbohydrates, 0. 2 of the dried sample by weight was first ground, then 5 ml of sulfuric acid was added after the samples had completely dissolved, they were reheated and then allowed to cool to room temperature following the addition of a small amount of barium carbonate ($Ba_2CO_3$). The phenol–sulfuric acid method was employed to assess the total carbohydrate content, and the cooled solutions were subsequently filtered. An aliquot of 1 mL of the filtrate was transferred to a test tube, and 1 mL of 5% aqueous phenol was added. Immediately afterward, 5 mL of concentrated sulfuric acid was rapidly introduced to develop the characteristic color. The absorbance of the resulting-colored solution was then measured.

## Results

***Phytochemical screening***
The leaves (A1) and stems (A2) of *Viburnum tinus* were subjected to qualitative phytochemical analysis using both aqueous and alcoholic extracts. This analysis was conducted to detect various classes of secondary metabolites present in the plant. As shown in Tables 1 and 2, the phytochemical composition differs according to the plant part and the solvent used for extraction, revealing a wide range of bioactive compounds. Both stems and leaves exhibited a significant flavonoid presence (+++) in the aqueous extracts (Table 1). Additionally, the leaves had a high concentration (+++) of tannins, alkaloids, and saponins, while the stems had a moderate (++) or negligible (-) concentration. Anthraquinones were present in moderate amounts (++) in both extracts, while sterols/triterpenes were only marginally present (+). The alcoholic extracts had a different distribution (Table 2). The stems contained a high concentration of flavonoids and saponins (+++), but the leaves had a lower concentration. In contrast, the leaves contained a high concentration of tannins (+++), but the stems only had a small amount (+). Significantly, neither alcoholic extract contained alkaloids. Anthraquinones were found in moderate concentrations (++) in the leaves and in low concentrations (+) in the stems, but sterols/triterpenes were only found in the stems. In general, these findings support the notion that Viburnum tinus is a good source of bioactive substances, notably flavonoids, saponins, and tannins. The differences in phytochemical makeup between the extracts emphasize how crucial it is to choose the right solvent in order to isolate certain classes of compounds.

***Table 1. The phytochemical screening of aqueous extracts of the studied plant***

| Phytochemical screening test | A1 | A2 |
|---|---|---|
| Tannins | +++ | - |
| Alkaloid | +++ | ++ |
| Flavonoids | +++ | +++ |
| Anthraquinenes | ++ | ++ |


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| | | |
|---|---|---|
| Sterols and or Triterpiens | + | + |
| Saponins | +++ | ++ |

*A1: Viburnum tinus leafs A2: Viburnum tinus stems*

***Table 2. The phytochemical screening of Alcoholic extracts of the studied plant***

| **Phytochemical screening test** | **A1** | **A2** |
|---|---|---|
| Tannins | +++ | + |
| Alkaloid | _ | _ |
| Flavonoids | + | +++ |
| Anthraquinenes | ++ | + |
| Sterols and or Triterpiens | _ | + |
| Saponins | ++ | +++ |

*A1: Viburnum tinus leafs A2: Viburnum tinus stems*

***Total carbohydrate, Amino Acid, protein, and Metals***

***Total Carbohydrate Content***

(Table 3) shows the findings of the overall carbohydrate analysis performed on the leaf and stem extracts of *Viburnum tinus*. Significant variations in carbohydrate content between the two plant organs were seen in the analysis. The average carbohydrate concentration was much higher in the leaf extract (0. 492 mg/g) than in the stem extract (0. 346 mg/g). Under the tested conditions, the leaves of V. tinus have a higher total carbohydrate content than the stems.

***Table 3. The total carbohydrate contents of the studied plant extracts***

| Content / Sample | C1 | C2 | C3 | Average |
|---|---|---|---|---|
| *Viburnum tinus* leafs | 0.404 | 0.492 | 0.490 | 0.492 |
| *Viburnum tinus* Stems | 0.347 | 0.345 | 0.347 | 0.346 |

***Total Protein Content***

(Table 4) lists the findings of the overall protein content analysis performed on the leaf and stem extracts of *Viburnum tinus*. There was a noticeable difference between the two components of the plant. The protein content of the leaf extract, which was determined to be 0. 862 mg/g, was much greater on average. In contrast, the stem extract's average protein content was 0. 440 mg/g, which is about half that of the leaves. According to these data, the leaves of *Viburnum tinus* are the main storage place for proteins.

***Table 4. The contents of total protein of the studied plant extracts***.

| **Contents / Sample** | **$C_1$** | **$C_2$** | **$C_3$** |
|---|---|---|---|
| *Viburnum tinus* leafs | 0.860 | 0.864 | 0.861 |
| *Viburnum tinus* Stems | 0.440 | 0.439 | 0.441 |

***Total Amino Acid Content***

The entire free amino acid content of the Viburnum tinus leaf and stem extracts was measured, and the results are shown in (Table 5). The leaf extract was shown to be a higher source of amino acids, which is consistent with the patterns seen for proteins and carbohydrates. The average concentration in the leaves was determined to be 0. 656 mg/g. In contrast, the stem extract had an average concentration of only 0. 256 mg/g, which corresponds to less than one-half of the content found in the leaves.

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

| **Contents / Sample** | **C1** | **C2** | **C3** |
|---|---|---|---|
| *Viburnum tinus* leafs | 0.656 | 0.677 | 0.635 |
| *Viburnum tinus* Stems | 0.256 | 0.255 | 0.258 |

***The contents of Metals***

(Table 6) provides the minerals and metal concentrations of the flowers and stems of the studied plant, expressed in parts per million (ppm). The concentrations of elements in the tested plants varied within the following ranges: The study found that the concentrations of the metals on iron (Fe), nickel (Ni), and copper (Cu) were in the following ranges: (39. 15 – 60. 38 ppm) for iron (Fe), (1. 17 – 1. 57 ppm) for nickel (Ni), and (1. 89 – 5. 73 ppm) for copper (Cu). For both leaf and stems plant, there is a relative rise in Fe


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levels. The presence of Fe, Ni, and Cu in the studied plant, all of which are essential to human health, was documented in this investigation. Variations in the mineral composition of plants are complex traits influenced by the interaction between the plant's genetic makeup and, in part, various environmental factors such as soil type, climate, and agricultural practices [120].

***Table 6: Metal contents of the studied plants(µg/g).***

| Elements / Samples | Fe | Ni | Cu |
|---|---|---|---|
| A1 | 39.15 | 1.187 | 1.89 |
| A2 | 60.38 | 1.177 | 5.73 |

## Discussion

Using both aqueous and alcoholic solvents, the phytochemical analysis of Viburnum tinus leaf and stem extracts revealed a diverse and complicated array of secondary metabolites. The relevance of these findings, their relationship to the measured biological processes, and their comparison to the available literature are all covered in this section. The most notable outcome of the screening was the widespread and consistent presence of flavonoids and saponins in the majority of extracts (Tables 1 - 2). Flavonoids are a significant class of phenolic compounds with well-known antioxidant properties [121], and their high presence in both aqueous extracts and the alcoholic stem extract was evident (+++). Their abundance immediately explains the high total phenolic content (TPC) and total antioxidant capacity (TAC) seen in this study (Tables 4 & 5).

Flavonoids are important components of the plant's overall therapeutic and protective potential due to their well-known capacity for scavenging free radicals and donating hydrogen atoms. Likewise, the high concentration of tannins, notably in the aqueous and alcoholic leaf extracts, is noteworthy. Polyphenolic tannins are known for their antioxidant activity as well as their capacity to function as potent reducing and capping agents in the green synthesis of nanoparticles [122]. The leaf extract's high concentration of flavonoids and tannins probably aided in the effective production of the nanoparticles (A1), and this may have been responsible for their enhanced biological effects. The differential distribution of chemicals across solvents and plant components is also noteworthy. For example, the aqueous leaf extract had a high concentration of alkaloids (+++), but the alcoholic extracts had none. This shows the importance of solvent polarity in the extraction of phytochemicals, with water's higher polarity being more successful at extracting these nitrogenous compounds [123].

The alcoholic extracts' lack of alkaloids indicates that ethanol might be used to create an extract that is high in flavonoids and saponins while also having a low alkaloid concentration, which, in some cases, might be related to toxic responses. The moderate to low amounts of sterols/triterpenes and anthraquinones contribute to the chemical variety of the extracts. Other Viburnum species have been reported to contain triterpenoids, which have a wide range of bioactivities, including anti-inflammatory and anticancer properties [124]. Even small amounts of these compounds could synergistically enhance the overall effectiveness of the extracts and the nanoparticles made from them. Our results align with earlier phytochemical studies on the Viburnum genus, which have consistently shown it to be a good source of triterpenoids, saponins, and phenolic chemicals like flavonoids and tannins [125]. The high concentration of phenolic acids and flavonoids in Viburnum tinus was particularly emphasized in one study, which connected them to its notable antioxidant and neuroprotective capacity. This evidence backs up our quantitative findings for TPC and TAC. The disparity we saw between leaves and stems is likewise a typical occurrence that reflects the distinct physiological functions and metabolic processes of these organs [126]. The high antioxidant potential of *Viburnum tinus* and its usefulness to produce green nanoparticles are well explained by its phytochemical profile. The presence of flavonoids, tannins, and saponins acts as a natural mixture of reducing, capping, and stabilizing agents, while also giving the manufactured nanoparticles increased biological functionality.

The leaves of *Viburnum tinus* have a higher metabolic content than the stems, as shown by the quantitative examination of primary metabolites such as carbohydrates, proteins, and amino acids. The concentrations of carbohydrates (0. 492 mg/g), proteins (0. 862 mg/g), and amino acids (0. 656 mg/g) were all significantly greater in the leaves than in the stems (Tables 3-5). Botanical research has shown that this distribution is physiologically sensible. The main photosynthetic organs are leaves, which serve as source tissues for carbon fixation into carbohydrates. These carbs subsequently serve as the primary source of energy and carbon skeletons to produce other vital biomolecules, such as proteins and amino acids [127]. As the main sites of metabolic activity and growth regulation [128], these primary metabolites are predicted to accumulate more in the leaves.

In addition to their structural roles, the proteins and amino acids function as enzymes and precursors for a wide variety of secondary metabolites, which are directly related to the plant's ability to perform green synthesis [129]. The presence of these molecules, especially proteins, is known to aid in the capping and stabilization of nanoparticles during biogenic synthesis, which prevents their aggregation [130]. The tissue of Viburnum tinus was shown by elemental analysis to contain the vital microelements iron (Fe), copper


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(Cu), and nickel (Ni) (Table 6). Iron was the most prevalent element identified (60. 61–61. 38 µg/g), with nickel and copper present in far lesser amounts. These metals are essential because they serve as cofactors for many enzymes and are necessary for several metabolic processes in both plants and humans [131]. Iron, for example, is essential for cytochromes in the electron transport chain, whereas copper is necessary for enzymes including superoxide dismutase and polyphenol oxidase [132].
All discovered metals' concentrations were well inside the safe ranges for therapeutic plants established by the World Health Organization (WHO), demonstrating that the plant is safe for possible therapeutic applications without the danger of heavy metal poisoning [133]. The minor differences in mineral content between samples (A1 and A2) are to be expected and can be explained by a complex interaction between the plant's genetic makeup and a variety of environmental factors, such as soil composition, pH, and mineral bioavailability in the plant's habitat [134-136]. In addition to proving the plant's safety, this elemental profile emphasizes its potential nutritional worth as a source of vital trace minerals.

## Conclusion

According to the study, *Viburnum tinus* has a complex and varied phytochemical and biochemical makeup, which supports its potential as a source of biologically active chemicals. The findings revealed a considerable difference in the distribution of secondary metabolites between the leaves and stems, as well as between the aqueous and alcoholic extracts, underscoring the necessity of choosing the right solvent for extraction. In addition, it was determined that the leaves serve as the primary storage location for macronutrients like carbohydrates, proteins, and amino acids. Iron, copper, nickel, and other essential minerals necessary for human health were also found. The possibility of utilizing *Viburnum tinus* as a natural source of therapeutic chemicals and nutrients in the food and pharmaceutical industries is highly supported by these results. To ascertain the chemical makeup of the isolated substances and assess the biological activity of these extracts, more research is advised.

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***Conflict of interest***. Nil

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