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Qualitative Phytochemical Analysis of Roots, Stem, and Leaves Extracts of *Uritica Dioica*

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Abstract

Plants have found extensive use in both traditional and modern medicinal practices globally. Plant-derived remedies present a secure alternative, characterized by the absence of potential side effects commonly linked to pharmaceutical drugs. The comprehensive phytochemical analysis of medicinal plants has unveiled a diverse range of bioactive compounds within plants traditionally employed for medicinal purposes, thereby highlighting a broad spectrum of therapeutic properties. In the continuous investigation, a meticulous and thorough chemical identification process was undertaken for the Lebanese medicinal plant *Urtica dioica*. This rigorous approach not only substantiates the traditional applications of nettle but also emphasizes the considerable pharmaceutical value inherent in the plant. This study contributes significantly to our understanding of the plant's chemical composition, reinforcing its potential as a valuable resource in pharmaceutical research. The results obtained not only substantiate the traditional applications of nettle but also underscore the substantial pharmaceutical value inherent in the plant. Furthermore, this study has resulted in the successful isolation and characterization of different compounds from the distilled water, methanol and acetone extract of *Urtica dioica*. This comprehensive approach significantly contributes to enhancing our understanding of the plant's chemical composition, thereby reinforcing its potential as a valuable resource in pharmaceutical research.

KEYWORDS

Urtica dioica. Phytochemistry, phytochemical screening

1.0 INTRODUCTION

Urtica dioica, a dioecious herbaceous perennial, attains a height of 1-2 meters with extensively spread rhizomes and stolons exhibiting a vivid yellow hue, mirroring the perennial roots[1]. Its soft green leaves, ranging from 3 to 15 centimeters in length, are oppositely arranged along an erect, wiry green stem. These leaves feature a markedly serrated margin, a cordate base, and an acuminate tip with a terminal leaf tooth surpassing adjacent laterals in length. The plant produces numerous small greenish or brownish flowers densely arranged in axillary inflorescences [2]. The stem is erect, displaying a hollow to solid structure, characterized by fibrous toughness. It is mostly simple or branched, possessing a bluntly square shape with four pronounced vertical grooves. The stem measures 2-14 millimeters in thickness near the smooth naked reddish-purple base[3]. Stinging hairs, approximately 1 millimeter in length, are present on the stem. These hairs taper to a fine sharp point, and upon

contact, their tips disengage, transforming the hair into a needle-like structure. The released irritant comprises various chemicals, including acetylcholine, histamine, 5-HT (serotonin), moroidin, leukotrienes, and potentially formic acid[4], Upon contact with human skin, this irritant induces pain, wheals, or a persistent stinging sensation lasting for more than 12 hours.

The flowering and fruiting period spans from June to October. The flowers are monoecious, with individual flowers being either male or female, although both sexes coexist on the same plant[5]. The achene-type fruits are 1-seeded, measuring 1-1.5 millimeters in length, 0.7-0.9 millimeters in width, and 0.3 millimeters in thickness. They exhibit a smooth surface with a distinct dark marginal ridge and possess very thin walls. The tan seed completely fills the fruit[6].

Fig. 1: *Urtica dioica* plant

Urtica dioica, (**Fig. 1**) commonly known as Stinging nettle, is a thoroughly investigated medicinal plant belonging to the Urticaceae family. As a herbaceous perennial, it demonstrates adaptability to diverse tropical and temperate wasteland regions, highlighting its biological versatility[7]. Its name originates from the Latin verb "urere," signifying "to burn," accurately depicting the sensation caused by its stinging hairs. The dioica species, commonly encountered, indicates its tendency to bear either female or male flowers. The leaf morphology encompasses an oval shape, elongated structure, and toothed margins, with elongated petioles. The dioecious flowers produce diminutive achene fruits characterized by an oval shape and a greenish-yellow coloration[8].

The World Health Organization (WHO) has categorized the global landscape into 12 megabiodiversity nations, encompassing 91 traditional countries. Within these regions of significant biodiversity, approximately 20,000 plants of medicinal significance are found[9].Medicinal plants are acknowledged for their rich reservoir of biologically active compounds with therapeutic attributes. Dating back to ancient times, these botanical specimens have been extensively employed worldwide to address various health conditions, encompassing ailments related to the respiratory, gastrointestinal, and urinary systems [10-12].

The medicinal attributes of *Urtica dioica* encompass a range of positive physiological effects, including anti-inflammatory actions, mitigation of asthma symptoms, astringent properties, depurative effects, galactogogue activity, diuretic influence, nutritive qualities, and stimulant characteristics[13]. The extract from its powdered leaves traditionally serves as an anti-hemorrhagic agent, effectively addressing excessive menstrual flow and nosebleeds. The roots are used for benign prostate hyperplasia, and herbaceous parts tackle urinary tract disorders and rheumatic conditions. Fresh freeze-dried nettle leaves are employed for allergy management[14]. Numerous studies also highlight its analgesic properties, pain-alleviating abilities, antiaggregating effects, and positive impact on cardiovascular function and smooth muscle activity, positioning it as a valuable hypotensive agent[15].

Beyond medicinal uses, *Urtica dioica* find application in dye production, veterinary medicine, textile manufacturing, cosmeceutical formulations for hair loss, dandruff combat, and culinary practices for flavor enhancement [16]. The plant contains cyclic organic compounds, including steroids, and various phenolic compounds with antioxidant and anti-cancer properties. Phenols, in the form of glycosides, play a role influenced by their hydroxyl group count and position. Extracts from *Urtica dioica* leaves rich in these compounds show promise in antioxidant therapy and cancer treatment [17].

The leaves are also abundant in essential fatty acids, gaining significance for their role in regulating human

metabolism. These fatty acids, known for their antioxidant properties, contribute to cancer prevention, emphasizing the health-promoting potential of *Urtica dioica* leaves [18].

In this investigation, our objective is to assess the phytochemical composition and antimicrobial efficacy of diverse extracts obtained from the leaves, stem, and root of *Urtica dioica*, employing a range of established scientific methodologies.

2.0 MATERIALS AND METHODS

2.1. Plant material

A freshly mature specimen of the botanical subject *Urtica dioica* was collected from NathiaGali, located in District Abbottabad. Subsequent to collection, the plant materials underwent thorough washing with tap water and were subjected to a 21-day shade-drying process. For the analysis of macroscopic and anatomical features, fresh plant material was employed. The production of powdered drug material involved the utilization of an electrical grinder. The resulting powdered drug was meticulously stored in hermetically sealed containers to safeguard against mold, adverse climatic conditions, moisture, and potential insect infestation. This finely powdered substance was subsequently utilized for a range of biological and biochemical research investigations.

2.2. Preparation of extracts

A quantity of 10 grams of the powdered material underwent sequential extraction with 100 milliliters of acetone, methanol, and distilled water using a Soxhlet extractor over a period of 48 hours. Subsequently, all the obtained extracts were subjected to concentration and preservation in airtight bottles for future use [19].

2.3. Qualitative phytochemical analysis

The identification of phytochemicals in the methanol, distilled water, and acetone extracts of *Urtica dioica* was conducted using established standard procedures.

2.4. Alkaloids Detection Test

2.4.1. Mayer’s test

A small quantity of the filtrates was subjected to the addition of a drop of Mayer's reagent along the side of the test tube. The appearance of a creamy or white precipitate serves as an indicator of a positive test result[20].

2.4.2. Wagner's test

A small quantity of Wagner's reagent was introduced to a few drops of the plant extract, and the observation of a reddish-brown precipitate indicates the presence of alkaloids[21].

2.5. Flavonoids Detection Test

In the alkali reagent assay, a 2 ml solution of the plant’s extract underwent treatment with 1 ml of sodium hydroxide solution. The occurrence of yellow to red precipitates serves as an indication of the presence of flavonoids, in accordance with the methodology established [22].

2.6. Carbohydrates Detection Tests

2.6.1. Molisch’s Test

Alcoholic α-naphthol drops were added to the plant's extract, followed by the introduction of 0.2 ml of concentrated H_2SO_4 along the sides of the test. The confirmation of carbohydrates is inferred from the emergence of a purple to violet ring at the junction [23].

2.6.2. Benedict’s Test

Benedict's reagent, consisting of sodium carbonate, sodium citrate, and a solution of copper sulphate, is employed on the filtrate. The mixture is then heated to its boiling point for five minutes and subsequently allowed to cool. Carbohydrates are indicated by the formation of an orange-red precipitate [24].

2.6.3. Fehling’s Test

Equal volumes of Fehling’s A, containing Copper Sulphate in distilled water, and Fehling’s B, comprising Potassium tartrate and Sodium hydroxide in distilled water, were blended in a test tube. Upon introducing a minute quantity of the plant's extract and subsequent heating, the formation of brick-red precipitates of cuprous oxide signifies the presence of reducing sugars [25].

2.7. Saponins Detection Test

2.7.1. Foam test

To evaluate the presence of saponins, a 2 ml aliquot of the botanical specimen underwent homogenization with an equivalent volume of distilled water. Subsequently, the resulting mixture was subjected to centrifugation for a duration of fifteen (15) minutes. The identification of a discernible foamy layer on the upper surface of the centrifuged test tube serves as a reliable indicator for the existence of saponins, in accordance with the

methodology elucidated [26].

2.8. Tannins Detection Test

2.8.1. Lead Acetate Test

Upon the treatment of the test solution with a few drops of lead acetate solution (10%), the occurrence of a yellow precipitate is observed. This reaction is indicative of the presence of certain chemical constituents in the test solution. Lead acetate is commonly employed in qualitative analysis to detect the presence of sulfide ions, which form insoluble yellow lead sulfide precipitates. The appearance of this yellow precipitate confirms the specific reaction between lead acetate and the sulfide ions present in the test solution[27]

2.9.Phenol Detection Test

2.9.1. Ferric Chloride Test

A quantity of 10 mg of the extracts underwent treatment with a few drops of ferric chloride solution. The development of a bluish-black coloration following this reaction signifies the presence of phenolic compounds in the tested material[28].

2.10. Quinone Detection Test

2.10.1. H_2SO_4 test

In the process of detecting quinones, 2 ml of concentrated sulfuric acid was added to 2 ml of the plant's extract. The verification of quinone presence is indicated by the emergence of a red coloration [29].

2.11. Proteins Detection Tests

2.11.1. Xanthoproteic Test

The extracts were subjected to treatment with a small quantity of concentrated nitric acid solution. The development of a yellow coloration is indicative of the presence of proteins. This test relies on the reaction between nitric acid and proteins, resulting in the formation of a distinct yellow coloration [30].

2.11.2. Millon's Test

The sample extract was subjected to the addition of a few drops of Millon's reagent, followed by gentle heating. The observation of a reddish-brown coloration or precipitate serves as an indication of the presence of proteins residues [31].

2.12. Anthocyanin Test

To test for the presence of anthocyanin, a few drops of concentrated sulfuric acid were introduced to the 2 ml extract, resulting in the formation of a yellowish-orange color [32].

3.0. RESULTS AND DISCUSSION

Urtica dioica has emerged as the most frequently reported species, serving as a valuable reservoir of active principles for the development of innovative treatment strategies. Despite its ancient utilization across diverse cultures and regions for addressing various ailments, recent advancements highlight the pharmacological potentialities of *Urtica* species. These encompass noteworthy properties such as anti-inflammatory, anticancer, antioxidant, antidiabetic, antimicrobial, and antiviral effects. These effects align with both traditional uses and the presence of bioactive phytochemicals, including phenolic compounds and terpenoids. These findings underscore the potential application of *Urtica dioica* in preventive or therapeutic measures against communicable and noncommunicable diseases [33].

The quantitative analysis of phytochemicals in the root of *Urtica dioica* disclosed the presence of alkaloids, carbohydrates, saponins, tannins, anthocyanin, and proteins in the methanolic, aqueous, and acetone extracts of the plant. Notably, the methanolic extract did not manifest the presence of flavonoids and quinone, whereas the aqueous extract lacked phenols. A detailed summary of the outcomes is meticulously presented in **Table-1**.

Table-1. Phytochemical screening of three extracts of roots of *Urtica dioica*

Phytochemicals	Tests	Distilled water	Methanol	Acetone
Alkaloids	Wagner's test Mayer's test	- +	+ +	+ +
Carbohydrates	Molisch's test Fehling's test Benedict's Test	+ + -	+ + -	+ + +
Tannins	Lead acetate test	+	+	+
Saponins	Foam test	+	+	+
Proteins	Xanthoproteic test Millon's test	+ +	+ +	+ +
Flavonoids	Acid test	+	-	+
Phenol	$FeCl_2$ test	-	+	+
Quinone	H_2SO_4 test	+	-	-
Anthocyanin	Sulphuric acid test	+	+	+

In the ongoing investigation, an exhaustive examination of the phytochemical constituents in *Urtica dioica* was conducted, employing diverse solvents, specifically distilled water, methanol, and n-acetone. The quantitative analysis of phytochemicals in the leaves of *Urtica dioica* indicated the presence of alkaloids, carbohydrates,

saponins, quinone, and proteins in the methanolic, aqueous, and acetone extracts of the plant. Notably, the acetone extract did not demonstrate the presence of flavonoids and phenols. Furthermore, anthocyanins were not identified in any of the extracts. A detailed summary of the outcomes is scrupulously presented in **Table-2**.

Table-2. Phytochemical screening of three extracts of leaves of *Urtica dioica*

Phytochemicals	**Tests**	**Distilled water**	**Methanol**	**Acetone**
Alkaloids	Wagner's test Mayer's test	- +	- +	+ -
Carbohydrates	Molisch's test Fehling's test Benedict's Test	+ + +	+ + +	+ - -
Tannins	Lead acetate test	+	+	-
Saponins	Foam test	+	+	+
Proteins	Xanthoproteic test Millon's test	- +	+ +	+ +
Flavonoids	Acid test	+	+	-
Phenol	$FeCl_2$ test	+	+	-
Quinone	H_2SO_4 test	+	+	+
Anthocyanin	Sulphuric acid test	-	-	-

In the ongoing investigation, a comprehensive analysis of the phytochemical constituents in *Urtica dioica* was conducted, utilizing various solvents, specifically distilled water, methanol, and n-acetone. The quantitative assessment of phytochemicals in the stem of *Urtica dioica* revealed the presence of alkaloids, carbohydrates, flavonoids, tannins, phenols, and proteins in the methanolic, aqueous, and acetone extracts of the plant. Anthocyanins were not detected in any of the extracts. Saponins were absent in the methanolic extract, and quinones were not identified in the distilled water extract. A detailed summary of the outcomes is meticulously presented in **Table-3**.

Table-3. Phytochemical screening of three extracts of stem of *Urtica dioica*

Phytochemicals	**Tests**	**Distilled water**	**Methanol**	**Acetone**
Alkaloids	Wagner's test Mayer's test	+ +	- +	+ +
Carbohydrates	Molisch's test Fehling's test Benedict's Test	+ + +	+ + +	+ + +
Tannins	Lead acetate test	+	+	+
Saponins	Foam test	+	-	+
Proteins	Xanthoproteic test Millon's test	+ +	+ +	+ +
Flavonoids	Acid test	+	+	+
Phenol	$FeCl_2$ test	+	+	+
Quinone	H_2SO_4 test	-	+	+
Anthocyanin	Sulphuric acid test	-	-	-

4.0. CONCLUSION

The examination of phytochemical constituents in *Urtica dioica* has unveiled a diverse array of bioactive compounds within the five plant extracts, indicating their potential therapeutic implications. Notably, the isolation and identification of different bioactive compounds from the different extract of *Urtica dioica* underscore their antioxidant, anti-inflammatory, anti-bacterial, analgesic, and sedative activities. These findings robustly support the traditional medicinal applications of *Urtica dioica* and emphasize its potential as a reservoir for developing multi-resistant drugs in the future. This study offers valuable insights into the pharmacological potential of *Urtica dioica*, charting the course for further exploration in drug development.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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