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HISTOCHEMICAL ANALYSIS OF ROOTS OF RUMEX DENTATUS AND RUMEX PULCHER FLORA OF PALESTINE

***Dr. Khaled Taha Muhammad Abu Thaher**

Assistant Professor, College of Law and Forensic Sciences - Department of Forensic Sciences, Al-Istiqlal University (Palestinian Academy for Security Sciences), Jericho – Palestine.

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***Corresponding Author**
Dr. Khaled Taha Muhammad Abu Thaher
Assistant Professor, College of Law and Forensic Sciences - Department of Forensic Sciences, Al-Istiqlal University (Palestinian Academy for Security Sciences), Jericho – Palestine.

ABSTRACT

Secondary receptors are chemical molecules that are active in therapeutic plants and their accumulation sites within tissues can be found by histochemical analysis, which cannot be done using traditional phytochemical testing. In this research, the histochemistry of the roots of two types of sorrel (Rumex), namely Rumex pulcer and Rumex dentatus, from Palestinian plants, was studied. This study is considered the first of its kind, as the presence and location of phytochemical compounds was detected using a specific method of reagents. The results showed that these roots contain antraquinone compounds, phenolic compounds, and tannins. Anthraquinones have been found to accumulate in the conducting elements of the phloem, the surrounding epidermis, the third to fourth layers of the parenchyma, the developing vascular membranes, and the tracheal elements of the secondary xylem. While phenolic derivatives, especially (leucoanthocyanidins and catechins), accumulate in the outer skin layers and the three to four layers of cortical tissue that connect the tracheal elements in the secondary xylem to the membranes of the developing vessels, in addition to the phloem, sclera, and cambium elements in the primary elements and individual cambium, and secondary xylem vessels. As for tannins, they accumulate in the medullary ray cells and secondary phloem.

KEYWORDS: Rumex, Histochemistry, Active ingredients, Plants of Palestine.

1. INTRODUCTION

The identification of active ingredients in plants, particularly those with therapeutic properties, is one strategy for the advancement of the pharmaceutical sector. In this sense, studies on wild plants have been growing recently, and Palestine has a wide variety of wild plants, including the genus Rumex, which has a long history of usage in traditional folk medicine.[1-2]

There are 14 known species of sorrel in Palestine, several of which have not been thoroughly studied chemically. Therefore, the production of pharmaceuticals and herbal remedies requires research into the chemical composition and the creation of techniques for separating active and physiologically active ingredients.[3]

One way to determine the quality of active substances in plants and determine their locations of accumulation is through histochemical tests, which allow rapid detection of active substances and determination of their accumulation in plant tissues. One of their most important advantages is that they are preliminary and inexpensive tests.[4]

Histochemistry plays an important role in various sciences such as cell and tissue biology, and embryology.[5]

Histochemical analysis is related to detecting active substances in plants and determining their accumulation sites in plants and Plant tissues.[4]

Histochemistry is now more widely used than ever before in imaging and microscopic analysis.[6]

Furthermore, the outcomes of these histology analyses corroborate the findings of the remaining chemical analyses that scientists employed to identify and demonstrate the existence of active ingredients in plants.[7]

The purpose of the current study is to study the histochemistry of the roots of two types of sorrel (Rumex), namely Rumex pulcer and Rumex dentatus, from Palestinian plants, to detect the presence of active phytochemical substances and determine the places where they are collected.

Histochemical examination of roots of Rumex dentatus and Rumex pulcher, to our knowledge, quite limited. Therefore, the purpose of this study is to use histochemical tests to determine the phytochemical content and location of its accumulation in the roots of sorrel plants. This study is considered the first of its kind.

The active substances found in the sorrel plant (Rumex) are abundant and include tannins, polysaccharides, anthraquinones, flavonoids and their numerous derivatives, and phenolic compounds and their diverse derivatives.[8] These active ingredients are essential for the production of plant medicines[9], and have a wide range of medicinal and therapeutic applications.[10]

2. MATERIALS AND METHODS

In the world of plants, the genus Rumex, which includes roughly 150 species in tropical and subtropical regions, is extensively distributed. In Palestine, about fourteen different varieties of sorrel are grown. A modest amount of research has been done on the chemical makeup and pharmacological characteristics of these plants.[2-3]

In Palestinian traditional medicine, plants belonging to the genus Rumex are commonly employed as laxatives, astringents, hemostatic, anti- inflammatory, and dermatological agents.[1-2]

2.1. Plant Material

At the Faculty of Pharmacy at An-Najah National University in Nablus, Palestine, Professor Dr. Muhammad Jawad Fawzi Mismar identified the plant of Rumex pulcer and Rumex dentatus, which had been collected in July from the Tulkarm Governorate.

Since the histochemical analyses of these two sorrel species have not been studied before, we conducted this histological study to determine the active substances found in the roots of Rumex pulcher and Rumex dentatus, two types of sorrel that are considered plants of Palestine, as well as the locations where these substances are collected in the tissues of the roots of these two sorrel species.

2.2. METHODS

Root samples from the aforementioned species were examined, soaked, and preserved in an equal parts solution of glycerin, alcohol, and water. The samples were prepared in accordance with the American Pharmacopoeia and Herbal.[6]

The were stained in a few drops of reagent solution for 2 to 5 minutes, then washed carefully with water, and then covered with a special cover. The stained samples were observed under an MBI-6 microscope using zoom at 3, 5, 8, 20, 40 × 7 magnification and a FED 5B camera, they were examined and captured on Mikrat-200 and Mikrat-300 film as well as Kodak-200 color film.

2.3. Chemicals

Histochemical tests were performed using the following reagents.[8][11-13]

1. A 0.5% magnesium acetate solution for anthraquinone detection.
2. A 3% p-toluene sulfonic acid solution for the identification of catechins and leukoanthocyanidins.
3. A 1% vanillin solution in concentrated hydrochloric acid to identify catechins and leukoanthocyanidins.
4. A 1% ferrous ammonium alum solution for tannin detection.
5. The anthraquinone detection solution is 10% sodium hydroxide.
6. A concentrated hydrochloric acid solution to identify anthocyanins
7. A concentrated sulfuric acid solution to identify ellagic acid and anthraquinones.

The test findings were subjected to positive control by means of comparison with pertinent references from the literature.

A positive result for anthraquinones is indicated by the presence of a red color in tissues with a magnesium acetate solution or a sodium hydroxide solution. For phenolic derivatives, (leukoanthocyanidins- catechins), a positive result is indicated by the appearance of a yellow color with a toluene-sulfonic acid solution and the presence of a dark red color in the tissues when exposed to a vanillin solution in concentrated hydrochloric acid indicates. The appearance of a dark blue color in the tissues when exposed to an iron ammonium alum solution indicated a positive test for tannins.

3. Histochemical

Histochemical reactions give further information on the nature of the active compounds found in plant tissue, which is useful in determining the safety and viability of using plants as medicinal medicines. Additionally, they make it possible to identify the compounds found in tissues and cells, as well as the categories to which they belong among natural chemicals and the location of their concentration.[6-7]

3.1. - When added to the plant tissue we took from the Rumex, several reagents demonstrate the presence of a set of active compounds in the surrounding dermis and three to four layers of adjacent parenchyma. These substances are as follows.

A - The presence of leukoanthocyanidins and catechins is demonstrated by the previous sections turning reddish-brown when drops of a 1% solution of vanillin are introduced to concentrated hydrochloric acid.

B - The aforementioned components turn yellow-brown when drops of a 3% solution of p-toluene sulfonic acid are added; this is indicative of the existence of (leukoanthocyanidins and catechins).

C - The presence of anthraquinones is demonstrated by the formation of a red color when droplets of concentrated sulfuric acid are introduced. See Figure No.1.

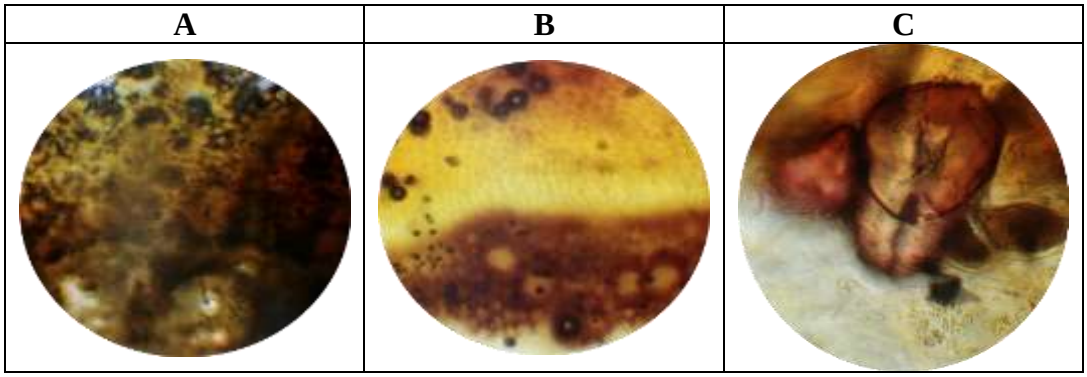

Figure 1: Histochemical characteristic of roots of Rumex dentatus and Rumex pulcher leukoanthocyanidins and catechins: A- reddish-brown with solution of vanillin are introduced to concentrated hydrochloric acid. B- yellow-brown with solution of p-toluene sulfonic acid. **Anthraquinones**: C- red color with concentrated sulfuric acid.

3.2. - When we added different reagents to the plant tissue we took from the Rumex, we were able to determine that the conductive parts of the secondary bark included a collection of active chemicals, as follows.

A- Dark red hue is produced when drops of a 10% sodium hydroxide solution are added, signifying the presence of (anthraquinones).

B- A yellow tint is produced when concentrated sulfuric acid drops are applied, indicating the presence of ellagic acid.

C - Sclereids turn pink and scarlet when a few drops of concentrated hydrochloric acid are added; this shows that anthocyanin is present in them; See Figure No. 2.

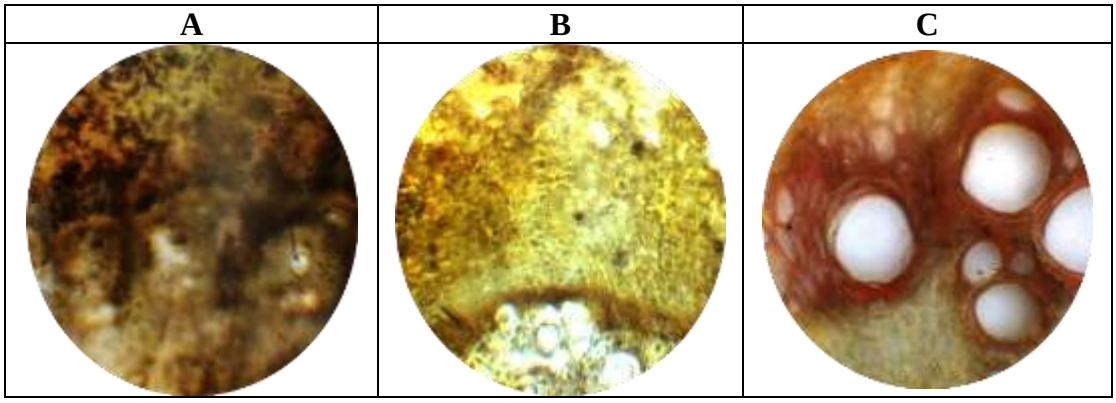

Figure 2: Histochemical characteristic of roots of Rumex dentatus and Rumex pulcher

Anthraquinones: A- Dark red hue with sodium hydroxide solution. **Ellagic acid** : B- yellow color with concentrated sulfuric acid. **Anthocyanin**: C- pink color with concentrated hydrochloric acid.

3.3. - Along with starch granules, crystalline and secretory cells are also visible in the bark and root wood; the latter can only be seen when a few drops of a 10% alcoholic sodium hydroxide solution are added, at which point a yellow hue occurs; See Figure No. 3.

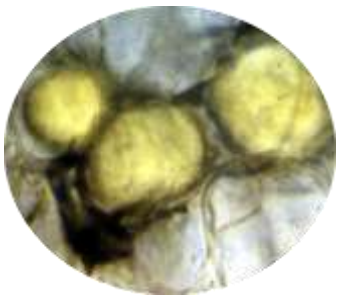

Figure 3. Histochemical characteristic of roots of Rumex dentatus and Rumex pulcher.

Starch granules: yellow colour with alcoholic sodium hydroxide.

3.4. – Cambium interacts favorably with a variety of chemicals, including:

A - It turns yellow-brown when a few drops of p-toluene sulfonic acid are added.

B- The presence of leukoanthocyanidins and catechins is shown by the red color that develops in a 1% solution of vanillin in concentrated hydrochloric acid, See Figure No. 4.

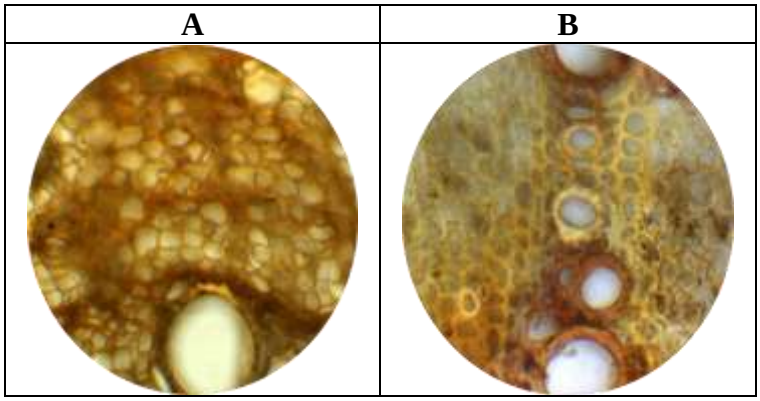

Figure 4: Histochemical characteristic of roots of Rumex dentatus and Rumex pulcher.

leukoanthocyanidins and catechins: A- yellow color with p-toluene sulfonic acid B- red color with solution of vanillin in concentrated hydrochloric acid.

3.5. – A class of active compounds is found in the tracheal elements of secondary xylem and the membranes of growing arteries, where:

A - The presence of anthocyanin is shown by the appearance of a pink and red color when concentrated hydrochloric acid is added.

B- The addition of a magnesium acetate solution causes a reddish-brown tint, which is indicative of the existence of anthraquinone derivatives.

C - The presence of catechins is demonstrated by the appearance of a yellow color when drops of P-toluene sulfonic acid solution are applied. See Figure No.5.

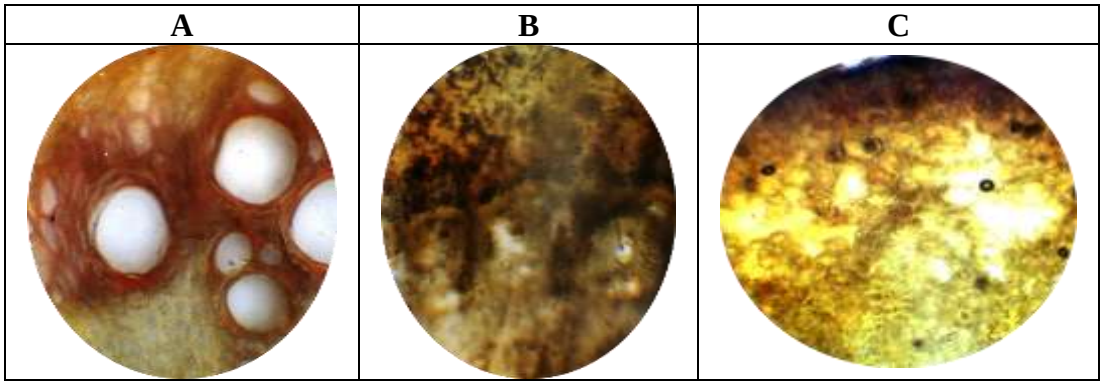

Figure 5: Histochemical characteristic of roots of Rumex dentatus and Rumex pulcher

Anthocyanin: A- pink and red color with hydrochloric acid**. anthraquinone:** B- reddish-brown with magnesium acetate solution**. Catechins:** C- yellow color with P-toluene sulfonic acid solution

3.6. – Upon the addition of specific reagents, a set of active chemicals was identified in the individual vessels of both primary and secondary xylem, including:

A- Yellow and brown contents build when drops of a toluene sulfonic acid solution are added.

B- A reddish-brown color occurs when vanillin reagent is added, which is thought to be evidence of the presence of leukoanthocyanidins and catechins; see Figure No. 6.

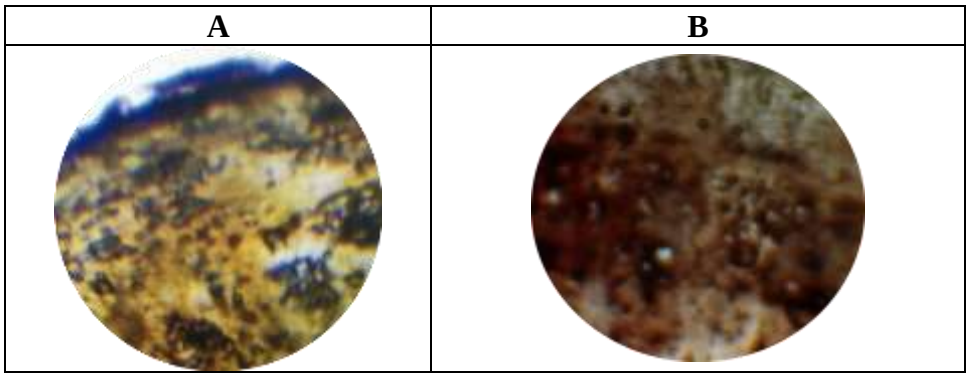

Figure 6: Histochemical characteristic of roots of Rumex dentatus and Rumex pulcher. leukoanthocyanidins and catechins. A- Yellow and brown with toluene sulfonic acid solution. B- A reddish-brown color with vanillin reagent

3.7. – When drops of a 1% solution of ferrous ammonium alum are introduced to working secondary phloem, (sieve tubes containing companion cells and phloem cells) and medullary ray cells, a dark blue color emerges, which is indicative of the accumulation of tannins, See Figure No. 7.

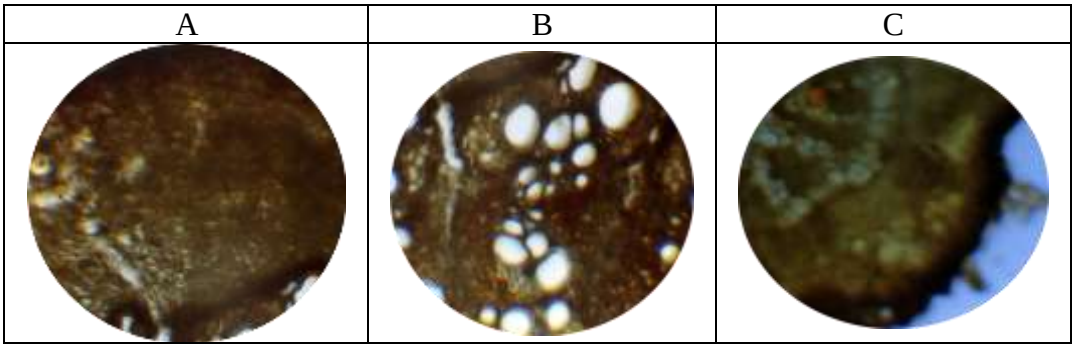

Figure 7: Histochemical characteristic of roots of Rumex dentatus and Rumex pulcher Tannins: A-B-C - dark blue color with solution of ferrous ammonium alum.

4. RESULTS AND DISCUSSION

A study using histochemical analysis was carried out for the first time on the roots of Rumex pulcer and Rumex dentatus from plants grown in Palestine. It was discovered that root tissues include a class of compounds known as leucoanthocyanidins, anthraquinones, catechins, and tannins. See table. No. 1.

Table 1: Results of histochemical analysis of roots (Rumex pulcher – Rumex dentatus).

No.	The reagent	The active substance	The color formed
1	0.5% solution of magnesium acetate	anthraquinones	Red color
2	3% solution of acid p-toluene sulfonic	Leukoanthocyanidins catechins	Yellow color
3	1% solution of vanillin in concentrated hydrochloric acid	leukoanthocyanidins catechins	Red brown
4	1% solution Ferrous ammonium alum	Tanins	Dark blue color
5	10% sodium hydroxide solution	anthraquinones	Red color
6	A concentrated solution of Sulphuric acid	Ellagic acid	Yellow color
		anthraquinones	Red color
7	A concentrated solution of hydrochloric acid	Anthocyanin	Reddish brown

A positive result for anthraquinones is indicated by the presence of a red color in tissues with a magnesium acetate solution or a sodium hydroxide solution. It have been found to accumulate in the conducting elements of the phloem, the surrounding epidermis, the third to fourth layers of the parenchyma, the developing vascular membranes, and the tracheal elements of the secondary xylem. See Figures No. 1 (C), No. 2 (A), and No. 5 (B).

For phenolic derivatives, (leukoanthocyanidins- catechins), a positive result is indicated by the appearance of a yellow color with a toluene-sulfonic acid solution, and the presence of a dark red color in the tissues when exposed to a vanillin solution in concentrated hydrochloric acid indicates, which accumulate in the outer skin layers and the three to four layers of cortical tissue that connect the tracheal elements in the secondary xylem to the membranes of the developing vessels, in addition to the phloem, sclera, and cambium elements in the primary elements and individual cambium secondary xylem vessels. See Figures No. 1 (A) (B), No. 4 (A) (B), No. 5 (C), and No. 6 (A) (B).

The appearance of a dark blue color in the tissues when exposed to an iron ammonium alum solution indicated a positive test for tannins, they accumulate in the medullary ray cells and secondary phloem. See Figure No. 7 (A) (B) (C).

The results of our current study are similar to the results of the following four studies.[4][14-16] that were conducted on different plants to determine the location of the accumulation of various active substances, such as phenol derivatives, anthraquinone derivatives, tannins, and flavanoid derivatives. However, our study differs from all previous studies in the type of chemical reagents used to detect the active substances, and it also differs by plant which was used in the study.

5. CONCLUSION

The results showed that the roots of Rumex pulcher and Rumex dentatus contain antraquinone compounds, phenolic compounds, and tannins.

A positive result for anthraquinones is demonstrated by the presence of a red color in tissues with either a sodium hydroxide or magnesium acetate solution. Phloem conducting elements, the surrounding epidermis, the third and fourth layers of parenchyma, the developing vascular membranes, and the tracheal elements of the secondary xylem have all been observed to accumulate with anthraquinones.

When exposed to a vanillin solution in concentrated hydrochloric acid, phenolic derivatives, or leukoanthocyanidins catechins, show a positive result by turning yellow in a toluene-sulfonic acid solution and producing a dark red color in the tissues. These compounds accumulate in the outer layers of the skin, the three to four layers of cortical tissue that connect the tracheal elements in the secondary xylem to the membranes of the developing vessels, as well as the phloem, sclera, and cambium elements in the primary elements and individual cambium,and xylem secondary vessels.

A positive test result for tannins was shown by the tissues' dark blue hue after being subjected to an iron ammonium alum solution. Tannins build up in the medullary ray cells and secondary phloem.

Histochemistry of the roots of sorrel plants (Rumex pulcher – Rumex dentatus) can be useful for identifying and determining the validity of this medicine in the herbal industry. The Researcher claims that histochemical analysis requires a fundamental understanding of chemical processes. The histochemical staining method is widely used in botanical research to locate the place of chemical component concentration, as well as to identify and define the active substances that plants contain. Histochemical analytical investigations are required because the active component in question in the plant tissue reacts with the specific reagent to generate a color.

6. The value of study

There have been few histochemical researches on sorrel plants to date. Consequently, this work, which uses histochemical testing to determine the phytochemical content in two

species of sorrel plants (Rumex pulcer and Rumex dentatus from Tulkarm Governorate in Palestine), is regarded as the first of its kind.

The study's significance seems to lie in its ability to identify, using specialized reagents, the active ingredients found in the plant tissue of sorrel roots and the locations from where they are harvested in two different varieties of sorrel plants.

7. Recommendations

In addition to offering scientific courses to enable professionals working in the field of plants to perform histochemical analytical research, all pharmacy colleges must have dedicated laboratories for studying the histochemical analysis of medicinal plants and the most up-to-date equipment required to accomplish this goal.

8. ACKNOWLEDGMENTS

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