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Phytochemical Analysis of Asphodelus aestivusBrot. in 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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ABSTRACT : This study aims to shed light on the plant (Asphodelus aestivus Brot.), belonging to the family (Asphodelaceae) in Palestine, in terms of the chemical composition of the roots and leaves .
As a result of the chemical study of various extracts of Asphodelus aestivus roots and leaves using specific chemical reactions and multiple types of chromatography, the presence of anthraquinone derivatives, flavonoid derivatives, tannins, hydroxycinnamic acids, coumarins , and amino acids was proven .
The biologically active compounds from Asphodelus aestivus roots and leaves were chemically examined using several types of chromatography and based on Rf values and dye color before and after treatment with special reagents, and by comparison with reliable samples for identification.
As a result of the chemical study of various extracts of Asphodelus aestivus roots and leaves , 15compounds for the first time were identified, which are the following: Anthraquinone derivatives (emodin, aloe-emodin, chrysophanol, physion, asphodelin) ,flavanoid derivatives (rutin, quercetin,) , hydroxycinnamic acids (caffeic acid, chlorogenic acid), and amino acids(Valine , Phenylalanine,Serine ,Proline,Glycin,alanine).
Keywords: Phytochemical analysis, Asphodelus aestivus, plants of Palestine.

I. INTRODUCTION

Within the Asphodelaceae family, Asphodelus L. is a genus that contains around twenty species. They are indigenous to Europe, North Africa, and Asia, particularly the Mediterranean region. First, to characterize it was Carl Linnaeus in 1753 (1).

Medicinal plants and their phytochemicals, especially secondary metabolites, are used to treat many diseases and are involved in the formulation of many medicinal drugs (2).

Asphodelusaestivus is consumed as food and utilized as a traditional remedy for hemorrhoids, gastrointestinal ailments, and eczema(3,4).There is currently an increasing interest in phytochemicals as possible new sources of natural antioxidants, with the intention of substituting synthetic antioxidants with them in food and medicinal preparations(5).

Various active compounds, including flavonoids, anthraquinones, amino acids, phenolic acids, fatty acids, triterpenoids, polysaccharides, gum, and esters, are present in all parts of the plant in Asphodelus species (2, 6).

For many years, asphodelus species have been utilized in traditional folk medicine to treat a wide range of illnesses, such as fungal infections, infections, burns, wounds, renal disease, stomach ulcers, and skin issues (2).

In rare cases, they have also been used to treat paralysis. They have also been employed as a diuretic and an anti-tumor agent(2, 6) .

They have also been used to treat Microbiological infections, psoriasis, jaundice, skin parasites, rheumatism, colds, eczema, and earaches (2, 7).

Palestinians use a variety of herbs, including Asphodelus species, in traditional medicine to treat a wide range of diseases. One species of Asphodelus, Asphodelus aestivus Brot, is an important wild medicinal plant that grows mostly in the Mediterranean regionFigure 1. The purpose of this study is to study the chemical composition of Asphodelus aestivus Brot. Leaves and roots that spread in Palestine for the first time.

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Fig.1.Asphodelus aestivusBrot

II. MATERIALS AND METHODS:

To eliminate any contaminants, the collected plant sample was cleaned three or four times using clean water. After washing, the leaves and roots of the Asphodelius plant are completely dried for approximately two months at a temperature 30°C. The plant sample dried completely after a month. To extract active substances from the roots and leaves, the sample is treated with organic solvents to obtain various extracts.

2.1. Identification of Plant: The plant was identified as Asphodelus aestivus by Dr. Rami Sami Al-Qaisi, from (Palestinian National Center for Agricultural Research) .

2.2. Plant Material

Rhizomes and leaves of Asphodelus aestivus were collected fromthe Wadi Al-Quff Protected Area (WAQPA), Hebron Governorate in the Occupied Palestinian Territories (OPT), in March , February respectively .

The Rhizomes and leaves were dried at a temperature of 30^0C in the shade. Next, use a mortar and pestle to pound it into a powder, and then strain it through a sieve with a 1 mm diameter .

Using a Soxhlet system at 60–80°C, the resultant powder was extracted using a range of solvents, including hexane, chloroform, acetone, and alcohol in different concentrations. After being concentrated in a rotating vacuum evaporator, these extracts were kept for later use at 4°C.

2.3. Methods

Phytochemical examination- Qualitative analysis:

Qualitative analysis of different bioactive components from leaves and root of Asphodelus aestivussuch as: anthraquinone derivatives, flavonoid derivatives, tannins, hydroxycinnamic acids, coumarins , and amino acids will be performed using standard protocols (8,9,10).

1. Anthraquinone Screening

• 10 ml of benzene was added to 20 ml of aqueous-alcoholic extract from the roots and leaves of Asphodelus aestivusand shaken for 3-4 minutes. In this case, the benzene layer turned yellow-orange. 5 ml of benzene extract was mixed with 5 ml of 10% ammonia solution. The ammonia layer acquired a red or cherry-red color, indicating the presence of anthracene derivatives in the raw material (10).

• 10 ml of the extract from the roots and leaves of Asphodelus aestivusobtained with 10% sodium hydroxide solution was acidified with diluted hydrochloric acid to a slightly acidic reaction and 10 ml of diethyl ether was added. The ethereal layer turned yellow. 5 ml of ether extract was shaken with 5 ml of ammonia solution. The ammonia layer was colored red, and the ether layer was yellow (10), which indicates the presence of anthraquinones in the raw material.

•Alcohol-water extracts from the roots and leaves of Asphodelus aestivus were chromatographed on “Silufol UV-366” plates and paper grades “B”, “S”, “M” in various solvent systems.

Chromatograms were studied in visible and UV light before and after treatment with chromogenic reagents, which were ammonia vapor and a 10% sodium hydroxide solution.The presence of the same composition of anthracene derivatives, which presumably can be classified as aglycones and glycosidic forms, was established.

2.Flavonoid Screening

• To 2 ml of alcohol-water extract from the roots and leaves of Asphodelus aestivus, 5-7 drops of concentrated hydrochloric acid and 0.1 g of metallic magnesium were added; after 3–5 minutes, a red-pink color was observed, indicating the presence of flavonoid compounds in the raw material (cyanidin reaction) (9,10).

•To 1 ml of alcohol-water extract from the roots and leaves of Asphodelus aestivus, 3-5 drops of a 2% solution of basic lead acetate were added; a yellow-orange color appeared - evidence of the presence of flavonoids(11).

• Chromatography of the studied samples was carried out using one-two-dimensional paper chromatography in various solvent systems.

The presence of this group of compounds in chromatograms was determined by development in

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UV light before and after treatment with ammonia vapor, 10% alcohol solution of sodium hydroxide, 1% solution of aluminum chloride(10,11).

3. TanninsScreening

The detection of tannins in extract from the roots and leaves of Asphodelus aestivuswas carried out using qualitative reactions of aqueous extracts (10).

A. Sedimentary reactions

•A 1% gelatin solution was added dropwise to 1 ml of aqueous extractfrom the roots and leaves of Asphodelus aestivus ; formation of an amorphous precipitate was observed.

• A solution of quinine chloride was added dropwise to 2 ml of the extract from the roots and leaves of Asphodelus aestivus; cloudiness of the solution was observed.

5ml of aqueous extract from the roots and leaves of Asphodelus aestivuswas mixed with 10 ml of 10% acetic acid and 5 ml of 10% lead acetate solution. Hydrolysable tannins precipitated. When a 1% solution of ferric chloride was added to the filtrate, the formation of a black-green color (condensed tannins) was observed.

B. Color reactions:

•To 2 ml of extract from the roots and leaves of Asphodelus aestivus, 2-3 drops of ferric ammonium alum solution were added; A black-blue color was observed.

The results of identification reactions of tannins in aqueous extracts fromthe roots of Asphodelus aestivus are positive.

4.Hydroxycinnamic acidsScreening

To detect this group of compounds, the obtained water and alcohol extractsfrom the roots and leaves of Asphodelus aestivus were chromatographed using two-dimensional chromatography on paper invarious solvent systems (9,10).

When chromatograms were processed with ammonia vapor, a 3% solution of iron (III) chloride and diazotized p-nitroaniline, at least 2 substances of hydroxycinnamic nature were found.

5.Coumarins Screening

Individually, a small amount of each extracts from the roots and leaves of Asphodelus aestivus fraction and crude sample was mixed with chloroform .

Then a few drops of 10% NaOH were incorporated. After leaving the Test-Tube for a while, ayellow colour appears, indicating the presence of coumarins(8,10).

6. Amino acids Screening

To detect free amino acids, 0.01 ml of aqueous extracts from the roots and leaves of Asphodelus aestivuswere chromatographedin various solvent systems.

The chromatograms were dried, treated with a 0.2% alcohol solution of ninhydrin and placed in a drying cabinet for 5 minutes at a temperature of 100-105^{0}C; red, red-violet and brown spots appeared, indicating the presence of amino acids (9,10).

III. RESULTS AND DISCUSSION:

All aqueous, alcoholic and organic extracts of the roots and leaves of the Asphodelus aestivus plant are included: anthraquinone derivatives, flavonoid derivatives, tannins, hydroxycinnamic acids, coumarins , and amino acids, as determined by qualitative reactions.The results of qualitative reactions in Table 1.

Table 1. Results of qualitative reactions to detect active substances in the root and leavesof the Asphodelus

N_0	Compound	Leaves	Roots
1	Anthraquinone derivatives	+	+
2	Flavonoid derivatives	+	_
3	Tannins	_	+
4	Hydroxycinnamic acids	+	+
5	Coumarins	+	-
6	Amino acids	+	+

From the results of qualitative tests, it was found that :

1 -The following active substances accumulate in the leaves of the plant :anthraquinone derivatives,Flavonoid derivatives,Hydroxycinnamic acids, coumarins and amino acids.

2- As for the roots, the following active substances accumulate:

anthraquinone derivatives,tannins, hydroxycinnamic acids, amino acids.

Anthraquinone derivatives : (chromatographic techniques)

Thin layer (TLC) and paperchromatography was utilized to analyze the anthracene derivatives in

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hexane, chloroform, acetone, and alcohol extractsfrom the roots and leaves of Asphodelus aestivus. They were chromatographed on "Silufol UV-366" plates and paper grades "B", "S", "M" in the mobile phase consisted of the following solvent systems:
in a thin layer of sorbent:
System A: toluene - acetone - 50% acetic acid (4:1:0.5)
System B: hexane - ethyl acetate - acetic acid (90:5:5)
 on paper
System C: n-butanol – acetic acid – water (4:1:2) – I
System D : 15% acetic acid – II

Chromatograms were studied in visible and UV light before and after treatment with chromogenic reagents, which were ammonia vapor and a 10% sodium hydroxide solution.The presence of the same composition of anthracene derivatives, which presumably can be classified as aglycones and glycosidic forms, was established.

The best results in the separation of anthracene derivatives in the studied extracts by chromatography in a thin layer of sorbent were shown by the solvent system B: hexane - ethyl acetate - acetic acid(90:5:5).
And thebest results in the identification of Asphodelin in the studied extracts by chromatography in a thin layer of sorbent were shown by the solvent System : petroleum ether - ethyl acetate (7:3).

The results of chromatographic studies showed the presence of anthracene derivatives in all extracts from the roots and leaves of Asphodelus aestivusin Table. 2.

Flavonoid derivatives: (chromatographic techniques)
Chromatography of the studied extracts from the roots and leaves of Asphodelus aestivus was carried out using one-two-dimensional paper chromatography in systems C and D.
System C: n-butanol – acetic acid – water (4:1:2) – I
System D : 15% acetic acid – II
The results of chromatographic studies showed the presence of this group of compounds in chromatograms , which was determined by development in UV light before and after treatment with ammonia vapor, 10% alcohol solution of sodium hydroxide, 1% solution of aluminum chloride ,in Table2.

Hydroxycinnamic acids: (chromatographic techniques)

To detect this group of compounds, the obtained water and alcohol extracts from the roots and leaves of Asphodelus aestivuswere chromatographed using two-dimensional chromatography on paper in solvent systems C, D and E.
System C: n-butanol – acetic acid – water (4:1:2) – I
System D : 15% acetic acid – II
System E: 2.5% acetic acid – II
When chromatograms were processed with ammonia vapor, a 3% solution of iron (III) chloride and diazotized p-nitroaniline , at least 2 substances of hydroxycinnamic nature were found.
The results of chromatographic analysis of hydroxycinnamic acid derivatives on paper are shownin Table.2.

Amino acids: (chromatographic techniques)

To detect free amino acids, 0.01 ml of aqueous extracts from the roots and leaves of Asphodelus aestivuswere chromatographed in system C.
System C: n-butanol – acetic acid – water (4:1:2)

The chromatograms were dried, treated with a 0.2% alcohol solution of ninhydrin and placed in a drying cabinet for 5 minutes at a temperature of 100-105^{0}C; red, red-violet and brown spots appeared, indicating the presence of amino acids, the results of chromatographic studies showed inTable.2.
In all chromatograms, compounds were detected by fluorescence in visible and UV light before and after treatment with various reagents:
1- 10% alcohol solution of sodium hydroxide ,
2- Ammonia vapor,
3- 1% solution of aluminum chloride,
4- % Alcohol solution of ninhydrin .

A group of active substances were identified and confirmed based on the use of different chromatographic techniques and comparing their values (Rf) with reliable samples and dye color before and after treatment with special reagents.Table 2.

The results of phytochemistry study were 15 compounds for the first time were identified from roots and leaves of Asphodelus aestivus, which are the following: Anthraquinone derivatives (emodin , aloe-emodin, chrysophanol, physion, asphodelin) , flavanoid derivatives (rutin, quercetin,) , hydroxycinnamic acids (caffeic acid,

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chlorogenic acid), and amino acids(Valine, Phenylalanine ,Serine ,Proline,Glycin, alanine)in table.2.

The results of our current study are similar to the results of studies (12-14)

However, our study differs from previous studies in the following points:

1 -For the first time, Asphodelus aestivus Brot. that grow in Palestine are being studied.

2 -For the first time, the leaves and roots of Asphodelus aestivus Brot. have been studied.

3- For the first time, 15 compounds from leaves and roots of theAsphodelus aestivusBrot. were identified.

IV. CONCLUSIONS

The study presents the results of phytochemical analyses conducted on a several extracts from Asphodelus aestivus Brot.in Palestaine.

The chemical analysis of several extracts from leaves and roots of the Asphodelus aestivus Brot. revealed a broad and varied group of active ingredients, the most significant of which are anthraquinone derivatives, flavonoids, tannins, hydroxycinnamic acids, coumarins , and amino acids.

As a result of the chemical study of various extracts of Asphodelus aestivus roots and leaves using specific chemical reactions and multiple types of chromatography and based on Rf values and dye color before and after treatment with special reagents, and by comparison with reliable samples for identification, 15compounds for the first time were identified from Asphodelus aestivus, which are the following: Anthraquinone derivatives (emodin , aloe-emodin, chrysophanol, physion, asphodelin) , flavanoid derivatives (rutin, quercetin,) , hydroxycinnamic acids (caffeic acid, chlorogenic acid), and amino acids(Valine, Phenylalanine ,Serine ,Proline ,Glycin, alanine).

Table 2.Chromatographic characterization of identified compounds

№	Compound	Rf value in solvent systems				Spot color			The detector
		A	B	C	D	Visible light	UV light before manifestation	UV light after manifestation	
1	Emodin	0.42	0.16			yellow	yellow	red	1
2	Aloe-emodin	0.35	0.14			yellow	yellow	red	1
3	Physion	0.84	0.42			yellow	yellow	red	1
4	Chrysophanol	0,86	0.46	-	-	yellow	Yellow	red	1
5	Asphodelin	System : petroleum ether - ethyl acetate (7:3) Rf = 0.74						red	1
6	Rutin	-	0,55	0,43	-	brown	Yellow	yellow-green	2,3
7	Quercetin	-	0,73	0,05	-	yellow	Yellow	yellow-green	2,3
8	Caffeic acid	-	0,75	0,50	0.26	-	Blue	bright blue	2
9	Chlorogenic acid	-	0,60	0,77	0.57	-	Blue	bright light green	2
10	Valine			0.44				purple	4
11	Phenylalanine			0.33				purple	4
12	Serine			0.16				purple	4
13	Proline			0.25				purple	4
14	Glycin			0.23				purple	4
15	Alanine			0.22				purple	4
Reagents: 1-10% alcohol solution of sodium hydroxide , 2- Ammonia vapor, 3- 1% solution of aluminum chloride,4- % Alcohol solution of ninhydrin .									

V. THE VALUE OF STUDY:

This research is considered important because it shows the results of a study of Asphodelus aestivus Brot. widespread in Palestine that has not been studied, in terms of the chemical composition of the leaves and roots.

VI. RECOMMENDATIONS:

Many wild medicinal plants, including Asphodelus aestivus Brot. (leaf and root), are used extensively in traditional folk medicine, although they are not employed in modern medicine or in the manufacturing of medications or pharmaceuticals. This explains why the chemical makeup and pharmacological activity of the beneficial chemicals discovered in the various portions of medicinal wild plants have not been determined through the requisite scientific analytical investigation.

Chemical researchers in general and botanists in particular must concentrate on looking for the active compounds in these plants, trying to isolate and identify them, and learning about their therapeutic benefits if they are to be useful in both traditional and modern medicine.

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