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PHYTOCHEMICAL ANALYSIS OF *ECBALLIUM ELATERIUM* (L.) A. RICH. FLORA OF PALESTINE

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ABSTRACT: This study aims to shed light on the plant *Ecballium elaterium* (L.) A. Rich., Flora of Palestine, which belongs to the *Cucurbitaceae* family, in terms of the chemical composition of its leaves, flowers, fruits, seeds, and roots. The chemical study of various extracts from leaves flowers, seeds, fruits and roots from *Ecballium elaterium* (L.) A. Rich. for biologically active compounds was carried out using multiple types of specific chemical reactions and multiple types of chromatographs . As a result of chemical examination of biologically active compounds from members of the *Ecballium elaterium* plant, which is widespread in Palestine, the presence of the following active substances was proven for the first time: triterpenoids, alkaloids, tannins, phenols, flavonoids, and amino acids.

INTRODUCTION: *Ecballium elaterium (L.) A. Rich.*, also known as wild cucumber (*family: Cucurbitaceae*), is a monotypic weedy species that grows by roadside and waste grounds from Northern Spain through Southern Europe, the Mediterranean region, Southwest Asia, and North Africa. It has a wide range of traditional uses [1]. It has been used in the traditional folk medicine of several Mediterranean countries, as Palestine [2] or Turkey [3]. Various plant components are employed in traditional medicine to treat a variety of conditions, including sinusitis, rhinitis, hepatitis, hemorrhoids, psoriasis, rheumatism, constipation, jaundice, otitis, chronic headache, malaria and epilepsy [4, 5–10].

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Several biological and pharmacological effects of *Ecballium elaterium* have been reported in the past and present [11], including antibacterial [12], anti-inflammatory [1, 13], anti-trypsin [8], anti-insect [14], antioxidant [1], antimalarial effects [4], cytotoxic [15] and hepatoprotective [16] properties. The majority of traditional medicine discusses using fruit juice of *Ecballium elaterium* as nasal drops to treat neonatal jaundice [17-18]. The plant's fruit has been shown through pharmacological studies to have a variety of benefits [17], including anticancer [19-20], antibacterial [21], antifungal [21], analgesic [22], antipyretic [22], anti-inflammatory [23], positive inotropic [24], hepatoprotective [25], blood bilirubin reducing [26], anti-leukemic [27], and anti-sinusitis [28-29].

Numerous bioactive substances, including as phenols, triterpenoids, flavonoids tannin and alkaloids, are found in the *Ecballium elaterium* plant [1, 2, 11, 16, 30-32]. The purpose of this research is to study the chemical composition of leaves, flowers, seeds, fruits and roots of *Ecballium elaterium* flora of Palestine for the first time **Fig. 1.**

FIG. 1: *ECBALLIUM ELATERIUM*

MATERIALS AND METHODS: *Ecballium elaterium* parts were all cleaned with cold distilled water, let to air dry, then ground into a powder using an electric grinder in order to prepare the extract. The powder was then kept in opaque plastic containers until it was needed.

Plant Material: Different parts of *Ecballium elaterium* (leaves, flowers, fruits, seeds, and roots) were collected from wild plants growing in the village of Izbet Shoofa in Tulkarm Governorate in the West Bank - Palestine, during July and August.

Methods:

Extraction: There were two techniques used in the extraction process:

A. The resulting powder was extracted using a variety of solvents, including hexane, chloroform, acetone, petroleum-ether and alcohol in varying concentrations, using a Soxhlet apparatus at 60–80°C. These extracts were concentrated in a revolving vacuum evaporator and stored at 4°C until needed.

B. Three days and three changes of a 90% ethanol solution were used to macerate the plant parts. A dry powder extract was produced by filtering and vacuum evaporating the resultant extract.

Phytochemical Examination- Qualitative Analysis: Qualitative analysis of the main chemical groups from different parts of *Ecballium elaterium* (leaves, flowers, fruits, seeds and roots) were identified using multiple types of specific chemical reactions [33-35].

Phytochemical Examination- Chromatographic Techniques: Through phytochemical analysis of plant parts, the main chemical groups were identified. In order to verify the results of chemical tests, we will use the TLC method on silica gel 60 F254 (Merck, Germany) and paper chromatography method in different systems to confirm the presence of active substances [36-38].

RESULTS AND DISCUSSION: This study employed multiple types of specific chemical reactions to chemically study extracts of leaves, flowers, fruits, seeds, and roots from (*Ecballium elaterium*), in order to identify biologically active compounds. The active substance was identified based on the dye's color change before and after treatment with special reagents **Table 1.**

TABLE 1: INITIAL QUALITATIVE ANALYSIS OF ACTIVE SUBSTANCES FROM *ECBALLIUM ELATERIUM*. PARTS

S. no.	Chemical composition	Qualitative interaction
1	Triterpenoids	Libermann-Burchard reaction
2	Anthraquinones	Borntrager's reaction
3	Flavonoids	Shibata's reaction (Shinoda reaction)
4	Tannins	Ferric chloride reaction
5	Coumarins	With 10% solution NaOH
6	Amino acids	Biuret reaction- Ninhydrin reaction
7	Alkaloids	Dragendorff‟s reaction , Wagner‟s reaction, Hager's reaction
8	Phenols	Lead acetate reaction

Phytochemical Examination- Qualitative Analysis: Analysis in qualitative terms of using a variety of particular chemical reactions, the major chemical groups found in *Ecballium elaterium's* leaves, flowers, fruits, seeds, and roots were determined.

Sterols and Triterpenoids Screening:

Libermann- Burchards Reaction: A few drops of acetic anhydride are added after the extracts from leaves, flowers, fruits, seeds, and roots of *Ecballium elaterium's* have been heated and cooled. Conc. sulfuric acid is then poured into the test tube

from the sides. Where two layers of the extract join together, there is a brown ring, and the top layer becomes green to indicate the presence of steroids. The development of a rich red hue indicates the presence of terpenoids.

Anthraquinone Screening:
Bortrager Reaction: After adding 1 ml of 25% NH_4OH to the ether extract, from leaves, flowers, fruits, seeds, and roots of *Ecballium elaterium's*, it was shaken; anthraquinone is indicated by a red hue.

Flavonoid Screening
Shibata's Reaction: After adding 0.1 g of metallic magnesium and 5-7 drops of concentrated hydrochloric acid to 2 ml of alcohol-water extract from leaves, flowers, fruits, seeds, and roots of *Ecballium elaterium's*, if a reddish-pink tint was seen after 3-5 minutes, signifying the existence of flavones aglycones in the raw material (Shinoda Test).

Tannins Screening: After diluting the water extract from leaves, flowers, fruits, seeds, and roots of *Ecballium elaterium's*, 1ml with 2 ml of water, 3 drops of a diluted ferric chloride $Fecl_3$ solution were added. There are tannins present when a blackish blue or blackish green color appears.

Coumarins Screening: Chloroform was combined with a little quantity of each extract from the fraction and crude sample separately. After that, a few drops of 10% NaOH were added. Following a period of time spent away from the test-tube, a yellow hue emerges, signifying the existence of coumarins.

Amino Acids screening:
Biuret test: A little amount of Biuret reagent was combined with a 2 ml filter. The color transitioned from light blue to violet/mauve, suggesting the presence of proteins or peptide connections.

Alkaloids Screening:
Dragendorff"s test: After being measured, 1 ml of the extract from leaves, flowers, fruits, seeds, and roots of *Ecballium elaterium's* was placed in a test tube. Next, 1 ml of Dragendorff's reagent was added and well combined. An orange-red deposit appeared as a sign of alkaloids.

Wagner"s test: Wagner's reagent and equal amounts of the extracts from leaves, flowers, fruits, seeds, and roots of *Ecballium elaterium's* were added to a dry, clean test tube and shaken. Alkaloids were present if a reddish-brown precipitate appeared.

Hager"s test: Using a saturated picric acid solution, the extracts from leaves, flowers, fruits, seeds, and roots of *Ecballium elaterium's* were processed. The formation of a yellow precipitate indicated the presence of alkaloids.

Phenols Screening:
Lead Acetate test: 2 ml of the extracts from leaves, flowers, fruits, seeds, and roots of *Ecballium elaterium's* were combined with 2 ml of 10% lead acetate after filtering.

The presence of bulky, white precipitate indicates the presence of phenolic and tannin compounds. The data obtained in the preliminary phytochemical screening were given in **Table 2.**

TABLE 2: CHEMICAL COMPOSITION OF THE EXTRACTS FROM DIFFERENT ORGANS OF *ECBALLIUM ELATERIUM* AND METHOD IDENTIFICATION

Organs / Chemical composition	Roots	Leaves	Flowers	Fruits	Seeds
Triterpenoids	+	+	+	+	
Anthraquinones					
Flavonoids		+	+	+	+
Tannins	+	+	+		
Coumarins					
Amino acids		+		+	+
Alkaloids	+	+	+	+	+
Phenols		+	+	+	+

Phytochemical Examination- Chromatographic Techniques: The primary chemical groups were determined by phytochemically analyzing various plant sections. We will employ the TLC method and the paper chromatography method in various systems to confirm the presence of active compounds in order to validate the findings of chemical testing.

Triterpenoids and Flavonoids- (Chromatographic Techniques):
Systems used: A-ethyl acetate: water: formic acid: acetic acid (100:26:11:11). B-chloroform: methanol: glacial acetic acid: water (64:12:32:8).

Anisaldehyde sulfuric acid was used as a reagent, and visualization was performed under 366 nm UV after approximately 10 min at 100 °C, brown spots evidence of existence triterpenoids and violet spots evidence of existence flavonoids.

Alkaloids- (Chromatographic Techniques):
Systems used:

C- toluene: acetone (8:2).
D-toluene: chloroform: acetone (40:35:25).
E- n-butanol: glacial acetic acid*:* water (50:10:40), showed alkaloids with florescent pink color.

Amino Acids- (Chromatographic Techniques): To detect free amino acids, 0.01 ml of aqueous extracts from leaves, flowers, fruits, seeds, and roots of *Ecballium elaterium's* were chromatographed in system F. F-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°C; red, red-violet and brown spots appeared, indicating the presence of amino acids. From the results of study as determined by qualitative reactions and chromatographic techniques, it was found that :the presence of the following active substances was proven for the first time- triterpenoids, alkaloids, tannins, phenols, flavonoids, and amino acids, in various extracts from leaves, flowers, seeds, fruits and roots of *Ecballium elaterium* flora of Palestaine **Table 2.** There are studies conducted on the plant (*Ecballium elaterium)* flora of Palestine, [2, 21]. However, our study differs from previous studies in the following points

1. For the first time, the chemical composition of the *Ecballium elaterium* flora of Palestaineis under study .
2. A chemical study was carried out on various extracts from leaves, flowers, seeds, fruits and roots of *Ecballium elaterium* .
3. For the first time, the existence of the following active substances has been proven: alkaloids, tannins, phenols, flavonoids, terpenoids, and amino acids, in the leaves, flowers, seeds, fruits and roots of *Ecballium elaterium*.

CONCLUSIONS: The goal of this study is to provide insight into the chemical makeup of the leaves, flowers, fruits, seeds, and roots of *Ecballium elaterium*, flora of Palestaine, a plant that is native to Palestine and is a member of the Cucurbitaceae family. Numerous types of particular chemical processes and numerous types of chromatography were used in the chemical research of diverse extracts from leaves, flowers, seeds, fruits, and roots in order to identify physiologically active chemicals. The existence of triterpenoids, alkaloids, tannins, phenols, flavonoids and amino acids was demonstrated for the first time by chemical analysis of physiologically active chemicals from organs of the *Ecballium elaterium* plant, which is widely distributed in Palestine.

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