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Research Article

DETERMINATION THE PRESENCE OF PHYTOMELIN IN ECBALLIUM ELATERIUM TO APPROVE ITS FOLK USES

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ABSTRACT

Purpose: this study aims to approve the folk uses for *Ecballium elaterium* [(L.) A. Rich.] for treatment of hemorrhoids, varicose veins also for nose bleeding in the West Bank-Palestine.

We search about phytochemicals, which improve elasticity of blood vessels. One of the most famous compounds with that effect is phytomelin.

Method: a qualitative and quantitative analysis of phytomelin from leaves, fruits, and flowers of Ecballium elaterium (L.) A. Rich (exploding cucumber) in Palestine. After drying the sample for 30 days at 60 ºC, phytomelin was extracted using methanol. The methanol extracts of these parts were quantified using reversed phase liquid chromatographic method. Isocratic elution was employed using a mixture of methanol, acetonitrile and a 0.28% (v/v) of acetic acid (5:10:35). The flow rate was 1 ml/min.

Results: calibration of the overall analytical procedure gave a linear signal(r>0.998) over a concentration range of 0.1 -0.6 mg/mL of phytomelin. The proposed method was successfully applied for quantitative determination of phytomelin in leaves, fruits, and flowers of Ecballium elaterium (L.) A. Rich Keywords: Exploding cucumber, Ecballium elaterium, HPLC analysis, glycoside, Phytomelin content.

Keywords: Phytomelin, *Ecballium Elaterium.*

INTRODUCTION

Ancient folk remedies in Palestine for *Ecballium elaterium (L.)* A. Rich (exploding cucumber) are for treatment of hemorrhoids, varicose veins, and nose bleeding so a fresh juice is applied locally to treat these diseases, also used for treatment of sinusitis in Turkey and a fresh juice applied directly into the nostrils for treatment of this disease [1]. Phytomelin also strengthens the capillaries, and, therefore, can reduce the symptoms of haemophilia [2] . The fresh juice also contains cucurbitacin B which has anti inflammatory effect.

Accordingly this plant may contain phytochemical compound called phytomelin. Recently, the increase in the residential and agricultural areas and the decrease in medical plants have triggered the interest in ethno-botanical studies around the world [3,20]

Ecballium elaterium (L.) A. Rich. also called the squirting cucumber or exploding cucumber, is a plant in the Cucurbitaceae family with the following classification which is reported in table 1.

It gets its unusual name from the fact that, when ripe, it squirts a stream of mucilaginous liquid containing its seeds, which can be seen with the naked eye. It is thus considered to have rapid plant movement. It is native to Europe, northern Africa, and temperate areas of Asia. It is grown as an ornamental plant elsewhere, and in some places it has naturalized.

It is suspected to provide food for the caterpillars of the tortrix moth *Phtheochroa rugosana*.

This plant, and especially its fruit, is poisonous. In the ancient world it saw use as an abortifacient.

In Turkey, the fresh fruit juice of this plant is directly applied into the nostrils for the treatment of sinusitis as a herbal/folk remedy. Clinical tests on voluntary patients revealed that the healing rate of double-high dosage application is higher than that of the single-low dosage (71.0 and 56.6%, respectively). In addition, total relief from the main symptoms is observed in 20% of the patients, although the sinuses are not completely drained off on x-ray examination[1]

Chemical Constituents of *Ecballium elaterium*

The fruit juice of *Ecballium elaterium* contains proteins, lipids, sugars, and minerals[4].

For many centuries dried sediment from the juice of the fruit of *Ecballium elaterium*, known as elaterium, was used in orthodox medicine. The chief component of elaterium was known as elaterin. In good quality elaterium there would be about 30% elaterin but if the quality is poor only 20% of this active ingredient. Elaterin was available in its pure form and was recommended in place of the crude extract in the 1911 edition of the British Pharmaceutical Codex[5]

From that we know that there are no previous studies about the phytomelin in this plant.

Elaterins are now known as cucurbitacins but were commonly referred to in many older articles as elaterin and occasionally as also momordicin. (Alpha-elaterin is synonymous with Cucurbitacin C & B; cucurbitacin D is synonymous with elatericin A; cucurbitacin I is synonymous with elatericin B & elaterin B.

Cucurbitacins are extremely bitter tasting plant sterols. They can be both abortifacient and anti-inflammatory in effect[6.18].

The active compounds of *Ecballium elaterium* liquid extract include cucurbitacins B, D, E, I, L, R and other cucurbitacin types as well as several phenolics and glycosylated compounds[4.7].

From all that we know, there was never a mention that this plant contains phytomelin.

Phytomelin, also called rutin, rutoside, quercetin-3- rutin oxide and sophorin, with anti-oxidant effect[19] ,is a citrus flavonoid glycoside found in buckwheat[8.9], the leaves and petroles of rheum species, and asparagus. Phytomelin is also found in the fruit rinds (especially citrus Brazill, fruits (orange, grapefruit, limes) and berries such as mulberry and cranberries. Its name comes from the name of Ruta graveolens, a plant that also contains phytomelin. Phytomelin is the glycoside between the flavonol quercetin and the disaccharide rutinose. Quercetin is a flavonoid with a wide range of biological activities. It mainly occurs in plants as glycosides, such as phytomelin (quercetin rutionside) in tea.

Phytomelin is a solid substance, pale yellow in appearance and only slightly soluble in water. It is, however, much more soluble in water than its aglycone quercetin. Phytomelin's molecular formula is $C_{27}H_{30}O_{16}.3H_2O$ with molecular weight of 664.6 Daltons.

Phytomelin may have antioxidant, anti-inflammatory, anticarcinogen, antithrombotic, cytoprotictive and vasoprotective activities[10.11.12.13]

Different extraction and determination methods have been used for the quantization of phytomelin in variety of plants and pharmaceutical preparations[14.15.16.17.] (. But none of the above methods reported a validated and economic analytical HPLC method for the phytomelin analysis and quantitative evaluation of phytomelin extract.

The aim of this study is to approve the folk use of *Ecballium elaterium* and to develop a new validated and economic HPLC method for the analysis and quantitative determination of phytomelin content in Exploding cucumber including the flowers, leaves and fruits.

MATERIAL AND METODS

Chemicals

Phytomelin trihydrate was purchased from MP Biomedicals (Solon, Ohio), acetic acid, methanol and acetonitrile were purchased from Labscan, Ireland and they were HPLC-grade and used as received. Double distilled high purity water was used Water for HPLC (Labscan, Ireland).

Extraction procedure

The cucumber plant was collected from different areas in Palestine as a whole plant including flowers, leaves and fruits. The plant was dried at room temperature for more than 30 days. Solid samples were milled by Udy-Tector mill, (Landskrona, Sweden), and 1.0 g of each sample was extracted with 10.0 ml of methanol in a water bath at 65 °C and shaking for 30 min.

HPLC Analysis

HPLC was performed on a model of Hitachi using EZchrom workstation with C18- Lichrospher-100, PR-18 Merck (125x4.6 mm, 5 μ m particle size) using a UV detector for the analysis of the extracts and standard. The mobile phase was methanol, acetonitrile and 0.28% (v/v) solution of acetic acid (5:10:35) at a flow rate of 1 ml/min. The injection volume was 20 µL and the elute was monitored at 360 nm.

Method Validation

This HPLC method was applied in the validation of the ambient extraction method. Linearity, precision, and phytomelin recovery were investigated. Table 2 shows these results. Validation of this analytical method was performed in order to evaluate its linearity, selectivity, stability, precision and accuracy. A calibration curve was constructed from different concentrations of the reference material of phytomelin trihydrate with assay of more than 95%. The calibration curve was linear in the range of 0.1 – 0.6 mg/ml with equation of y= 3 x 106 x - 8506 (R2 = 0.9989). The amount of phytomelin extracted from leaves, flowers and fruits were determined using this calibration curve and is shown in Fig. 2 and Table 2. In order to evaluate the accuracy of the extraction and determination method, each sample part was spiked with defined amount of 2.0 ml of 0.4 mg/ml of the standard. The amount of standard recovered was calculated by subtracting the amount determined for the sample from total. The results are shown in Table 2 and Fig. 3. Accuracy indicates the deviation between the mean value found and the true value. It is determined by applying the method to samples to which known amounts of analyte have been added. The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision is a measure of the reproducibility of the whole analytical method (including sampling, sample preparation and analysis) under normal operating circumstances. This is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions.

RESULT AND DISCUSSION

This study aims to approve the folk uses of *Ecballium elaterium* (L.) A. Rich for its effect for treatment of nose bleeding and sinusitis.

Other purpose of this study was to use a simple, low cost and fast method of separation and determination of phytomelin from different parts of Exploding cucumber plant.

The HPLC conditions described in the experimental section allowed good separations for the main flavonoid, phytomelin of the plant.

In Figure 1, a calibration curve was made for different concentrations of the phytomelin standard in the range of 0.1 to 0.6 mg/ml. As we see from the figure all the peaks were eluted at about 2.7 min. There was a small variation in retention time < ±0.2 min due to the variation in the concentrations and the instrument resolution.

The detection limits, based on three times the noise level of the lowest concentration of the reference materials of phytomelin.

A linear relationship was obtained between the phytomelin contents and the peaks height ($R = 0.9989$, $P< 0.001$). The coefficient of variation in the values of peak height obtained by repeated standard phytomelin injections was only 97% and the phytomelin contents of all parts of the sample of leaves, fruits and flowers analyzed were included within this calibration range and are summarized in Table 2. The results of Figure 2 and Table 2, are the average of three measurements of each part in the plant which gave us a good reproducibility with a very small variation as mentioned above. The second peak of the leaf chromatogram in Figure 2 that appears at 3.203 min was impurities from the vial and did not appear in the other parts of the plant.

Accuracy

In order to evaluate the accuracy of the extraction and determination method, each sample part were spiked with a defined amount of standard (2.0 ml of 0.4 mg/ml standard mixed with 2.0 ml of each part in the sample). All the procedure was repeated again and the total amounts of phytomelin were measured. The amount of standard recovered was calculated by subtracting the amount determined for the sample from total. The results are shown in Table 3.

Precision

The precision of the method was determined by analyzing the samples and standards 5 times in one day and four successive days. The mean coefficients of variation for within and between days were 2.11 and 2.53.

The results above showed, therefore, that Exploding cucumber is a rich source of phytomelin described here for the first time in the plant.

Finally, different techniques of sample preparation and extraction may lead to a better or more percent of phytomelin in the Exploding cucumber.

Limit of detection

LOD was experimentally determined by reading absorbance of dilute solutions. The LOD signal was considered as mean lack signal plus three times its standard deviation. So the limit of detection was 9.64×10^{-3} µg/ml.

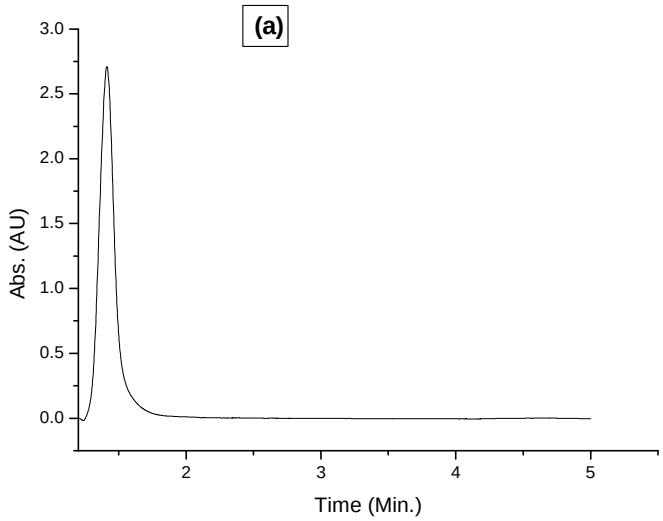

Fig. 1: HPLC Chromatograms for different concentrations of Rutin (a) = 0.1, (b) = 0.2, (c) = 0.4 and (d) = 0.6 mg/mL.

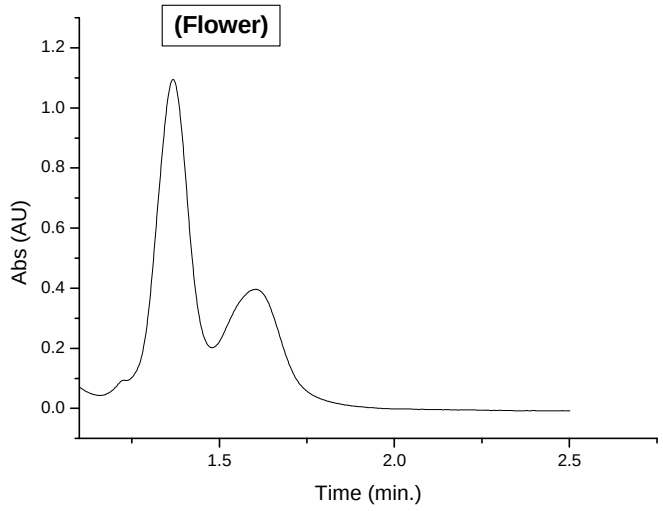

Fig. 2: HPLC chromatogram for rutin extracts of flowers, leaves and fruits of Exploding cucumber.

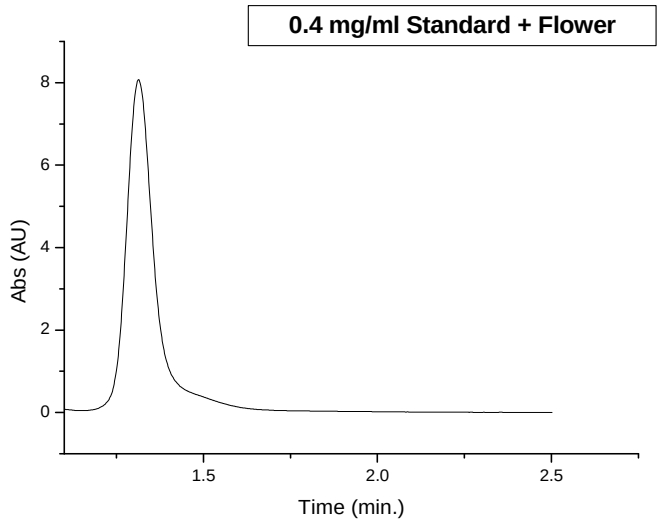

Fig. 3: HPLC chromatogram of 0.4% standard and the rutin from different parts in Exploding cucumber.

Table 1: Classification of *Ecballium elaterium* (L.) A. Rich

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Dilleniidae*
Order	*Violales*
Family	*Cucurbitaceae*
Genus	*Ecballium* A. Rich.
Species	elaterium (L.) A. Rich

Table 2: Amount of Phytomelin per 1.0 g of dry powder of flowers, fruits and leaves of Exploding cucumber (Results are the mean of three measurements)

Sample	Phytomelin (mg/mL)
Flower	1.59 ± 0.12
Fruit	1.84± 0.13
Leave	8.54 ± 0.56

Table 3: Recovery of the HPLC determination of extracted Phytomelin for the flowers, fruits and leaves using internal standard

Part	Standard added (mg/mL)	Standard recovered (µg/mL)	Error
Flowers	0.231	0.296	-0.064
Leaves	0.371	0.453	-0.081
Fruits	0.236	0.295	-0.058

CONCLUSION

The developed stability indicating HPLC method provides a simple, accurate and reproducible method for the determination of phytomelin in leaves, fruits and flowers of Exploding cucumber and to approve that this plant can be used for treatment sinusitis and nose bleeding.

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