

Phytochemical Study and Isolation of Scopolin from *Pimpinella anisum* Seeds Grown in Iraq

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Abstract

As widely known plants are divided into many categories, one of them is the herbs; which have been always used as effective remedies to treat many health issues. One of these remedies belongs to our plant under study *Pimpinella anisum* (anise), which belongs to Umbelliferae family, that has been characterized as an aromatic plant used in traditional medicine as carminative, disinfectant, and galactagogue. Anise dry seeds were collected from Iraqi herbal market to investigate their chemical constituents by using two methods: one of them hydrodistillation method using Clevenger apparatus in which oil sample was collected and then analyzed by GC/MS and revealed the presence of anethol as the major constituent with percentage of 82.91 % of the oil; the second method was alcoholic extraction by Soxhlet apparatus with ethanol till exhaustion then the extract was concentrated by evaporation under vacuum, after that partition with ethyl acetate was done, the ethanol layer was discarded while the ethyl acetate layer was concentrated under vacuum, the collected fraction were analyzed by TLC comparing it with standard Scopoletin .Phytochemical screening proved that the plant contain Scopoletin and was confirmed by comparing Rf value of the sample with the standard in three different solvent systems.

Keywords: *Pimpinella anisum*, GC/MS, Phytochemical screening, scopoletin.

Introduction

Worldwide surveys showed that there's an explosion of scientific information about crude plant extract, and various substances from plants as medicinal agents during last 20-30 years ¹. As known plants are divided into many categories, herbs are one of them, they have been always used as effective remedies to treat many health conditions², and this has begun since many years before the existence and development of medicine to treat those conditions ³. *Pimpinella anisum* (anise) is one of the most useful medicinal herbs which belong to the Umbelliferae (apiaceae) family; it described as delicate, white-flowered umbelliferous annual herb which grows to about 18 inches high, with secondary feather-like leaflets of bright green color ⁴. It has been stated to possess expectorant, antispasmodic, carminative and parasitic properties. Traditionally, it was used for bronchial catarrh, pertussis, spasmodic cough, flatulent colic ⁵. It's most specific use is for bronchitis, tracheitis with persistent cough ⁶ but its modern use is mainly

for the treatment of dyspeptic complaints and catarrh of the upper respiratory tract ⁷; also it has been reputed to increase milk secretion, promote menstruation and facilitate birth ⁸. Anise showed anti-inflammatory, anti-clotting and also revealed estrogenic effect due to the presence of the effective secondary metabolites like anethol ⁹.

Chemical Constituents

Anise contains many different chemical compounds that have important role in its effectiveness which presents in its fruit. It can be categorized into main compounds and minor compounds according to their presence and efficiency; Main compounds are:

1- Coumarins as Scopoletin (also known as Chrysochloric acid, Gelseminic acid is a 7-hydroxy 6-methoxy coumarin) ,Umbelliferone, Umbelliprenine; Bergapten (furanocoumarin) ¹⁰.

2- Flavonoids: Flavonol (quercetin) and flavone

(Apigenin, Luteolin)¹¹.

3- Glycosides like Quercetin-3-glucuronide, Rutin, Luteolin-7-glucoside, apigenin-7-glucoside, Isoorientin and Isovitexin (C-glucosides)¹².

4- Volatile oils with a percentage of 2–6%.

Major components are trans-Anethole (80– 95%), with smaller amounts of Estragole and Anise ketone (p-methoxyphenylacetone), While minor components including anisaldehyde and anisic acid (oxidation products of anethole), linalool, limonene, a-pinene, pseudoisoeugenol-2-methyl butyrate, acetaldehyde, p-cresol, cresol, hydroquinone, b-farnesene, a-, b- and g-himachalene, bisabolene, d-elemene, ar-curcumene and myristicin¹³. And also there's other constituents like carbohydrate (50%), lipids 16% (saturated and unsaturated), b-amyrin (triterpene), stigmasterol (phytosterol) and its palmitate and stearate salts.

Materials and Methods

Plant sample:

P. anisum dry seeds were brought from Iraqi local market in Baghdad province and later grinded to be used in the extraction methods.

Extraction Methods:

The sample undergone two extraction methods one of them to get the ethanolic extract which is done by using Soxhlet apparatus in which (25g) of dried *P. anisum* seeds was extracted with 200 ml of ethanol then this extract used in the phytochemical screening¹⁴; the other one was Hydrodistillation by using Clevenger apparatus in which 100 g of anise dried seeds soaked with 2 L of distil water to get the oil from the plant sample and investigate the important chemical compounds found in it by using gas chromatography technique.¹⁵

Method of Scopoletin Extraction

Dried anise seeds (25 g) were extracted by using Soxhlet apparatus with (200 ml) of hexane for 2 hours, then the extract was discarded (defatting process) and the marc was used for further extraction; it was extracted by Soxhlet apparatus with (200ml) ethanol for 3 hours, then the ethanolic extract was concentrated by evaporation under vacuum, after that three times partition with (15

ml) of ethyl acetate was made. The ethanol layer was discarded while the ethyl acetate layer was concentrated under vacuum. TLC analysis was made for Scopoletin compared with Scopoletin standard in three solvent systems (Ethyl acetate: Methanol: Water 10:1.65:1.35 ml respectively, Toluene: Ethyl acetate: Formic acid 5:4:1 respectively and Chloroform: Methanol 9:1 respectively). Then the preparative TLC was performed for Scopoletin standard as a marker.¹⁶

Phytochemical Screening

Chemical tests were carried out on the ethanolic extract after removing ethanol by rotary evaporator apparatus.

Test for saponin (Foam test)

The ethanolic extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 minutes no foam was presence indicate the absence of saponin¹⁷.

Test for tannins

Ethanolic extract (3 ml) was taken then 3 ml of FeCl₃ solution (5 % w/v) was added to it, the formation of a green precipitate was considered as a positive indication for the presence of tannins.

Test for flavonoids

Sodium hydroxide test: few milliliters of NaOH were added to few milliliters of ethanolic extract, the formation of yellow color which decolorized after an addition of diluted acid indicates the presence of flavonoids.

Test for coumarins

The ethanolic extract solution was placed in a test tube in the presence of DW. The tube was covered with filter paper soaked in sodium hydroxide solution (diluted and boiled), Yellow fluoroescence indicate the presence of coumarins after examination under ultra-violet.

Test for alkaloids

Dragendroff's test: 1 ml of ethanolic extract solution was added to 1 ml of dragendroff's reagents. Formation of orange or orange red precipitate indicates the presence of alkaloids.

Gas chromatography / mass spectroscopy (GC/MS)

It consists of two complementary mechanisms, the first one involved the separation of sample components, and then the separated components passed continuously according to their separation rate into the second part of the system (MS)¹⁸. This technique is performed to investigate the presence of different types of essential oils in the plant sample. This process was done at Ibn Al-bitar institution, Ministry of Industry and Minerals, Baghdad.

Infrared spectroscopy (IR)

Infrared spectroscopy is certainly one of the most important analytical techniques available for today's scientists. One of its great advantages is that virtually any sample in any state could be studied like liquids, solutions, pastes, powders, films, fibers, gases and surfaces all can be examined with a judicious choice of sampling technique.

Results and Discussion

The results showed that the percentage of essential oil extracted from anise plant was 0.5%, which is a

relatively low percentage that may indicate that Iraqi cultivation conditions is not the ideal condition for this plant according to the hot dry Iraqi weather¹⁹. As shown in Fig.1 the major peak occurs at time 12.939 which is related to Anethole with a percentage of 88.61%, this is an acceptable percentage of Anethole²⁰ and this result is comparable to the results in different countries. Anise oil also contains a trace amount of other volatile oil table (1).

The results of phytochemical screening revealed the presence of tannin, flavonoids, coumarin and alkaloids while absence of saponins. The presence of Scopoletin in Iraqi *P. anisum* was confirmed by TLC technique in which plant sample was compared with standard Scopoletin, by comparing R_f values in three solvent systems (table 2). Solvent system 1(S1) consist of Toluene: Ethyl acetate: Formic acid 5:4:1, (S2) consist of Ethyl acetate: Methanol: Water 10:1.65:1.35 and S3 which consist of Chloroform: Methanol 9:1. IR analysis for scopoletin comparing with standard scopoletin sample and the IR, cm⁻¹ spectrum of scopoletin revealed absorption bands as shown in (table 3). For further purification of Scopoletin a preparative TLC was done.

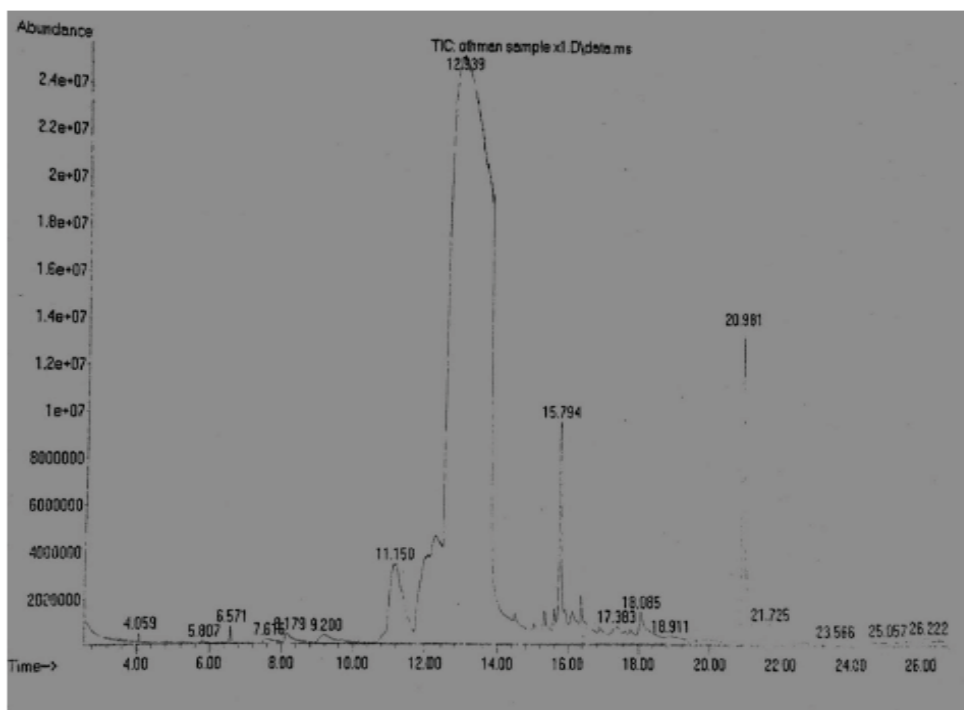


Figure (1) GC-Mass analysis of anise

Table 1: Volatile oil content of Anise

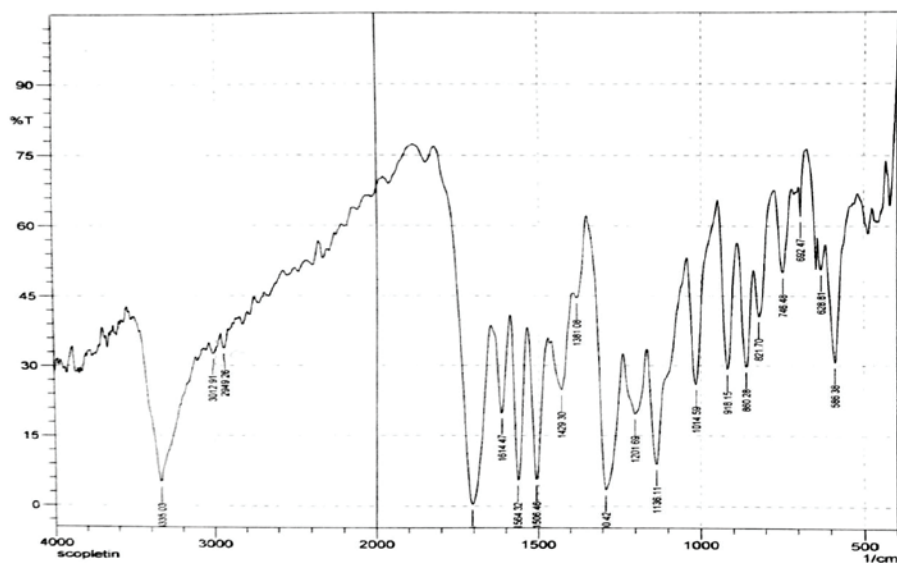
Chemical name	Retention time	% percentage
β - pinene	6.257	0.03
estragole	11.086	3.7
anethole	12.937	88.61
alloaromadendrene	15.746	1.67
caryophyllene	16.106	0.4
apiol	18.044	1.1
hexacosane	26.117	0.01

Table 2. Rf values of Scopoletin

Solvent system	Rf of standard Scopoletin	Rf of extracted Scopoletin (diluted)	Rf of extracted Scopoletin (concentrated)
S1	0.27	0.25	0.25
S2	0.45	0.44	0.44
S3	0.78	0.77	0.77

Infrared spectroscopy (IR) Results

IR analysis for scopoletin were as shown in (fig.2) comparing with standard scopoletin sample.

**Figure (2) IR Spectroscopy of isolated scopoletin**

The IR, cm^{-1} spectrum of scopoletin revealed absorption bands as shown in table 3.

Table 3 IR peaks for isolated scopoletin

Group frequency wave number	Functional Group
1720 cm^{-1}	Lacton group
1136 cm^{-1}	Ether group
2949 cm^{-1}	Hydroxyl group
1614 cm^{-1}	CH=CH group
3012 cm^{-1}	CH group
1564-1506 cm^{-1}	Aromatic benzene group

Conclusion

Plants consider as the origins of many drugs for centuries. Exploring the active compounds of these medicinal plants has a vital role in the identification of new drugs. *P. anisum* is one of the medicinal plants which have been used for the treatment of many diseases in conventional medicine. And this study confirmed the presence of an important active compound in the Iraqi anise which was scopoletin. So from the outcomes of this study and due to the broad spectrum of pharmacological effects of this plant, and very few clinical studies performed on this plant, more clinical trials are recommended to evaluate the beneficial effects of *P. anisum* in human models and further identification and isolation of active compounds of this plant that can lead to synthesis of new drugs in future.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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