RESEARCH ARTICLE

Iraqi *Hyacinthus orientalis* L. Flowers as the Source of Bioactive Compounds Especially Stigmasterol: Identification, Isolation and Characterization

Noor M. Shareef*, Thukaa Z. Abdul-Jalil

Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

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ABSTRACT

This research concentrated on the isolation and identification of stigmasterol from cultivated Iraqi *Hyacinthus orientalis* flowers part, as there are few studies that deal with these plant-specific flowers as e-research shows. Extraction were done for the flowers part of plant, by the soxhlet apparatus using 85% ethanol, followed by fractionation with petroleum ether to obtain the target of this study. Phytochemical screening was done by gas chromatography-mass spectrometry (GC-MS) for petroleum ether fraction qualitative and quantitative estimation and isolation of stigmasterol by reverse phase high-performance liquid chromatography (RP-HPLC), identification of isolated stigmasterol by Fourier transforms infrared (FTIR) spectroscopy, ultraviolet spectroscopy and liquid chromatography-mass spectroscopy (LC-MS). Announcing that the study considered the first to isolate stigmasterol from flowers part of Iraqi *H. orientalis.*

Keywords: Gas chromatography-mass spectrometry, *Hyacinthus orientalis* L., Reverse phase high-performance liquid chromatography, Stigmasterol.

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INTRODUCTION

Hyacinthus orientalis L. is an ornamental plant, also named common hyacinth, dutch hyacinth, flowering bulbs, and garden hyacinth, is a species of flowering plant in the family Hyacinthaceae, native to southwestern Asia, Turkey, Syria and Lebanon.[1,2] In the sixteenth century, it was brought to Europe. It is commonly cultivated throughout the temperate world for its fragrant flowers, which bloom quite regularly during Christmas time and appear unusually early in the season[3] as shown in Figure 1.

Only found in flowers, essential oils have been employed in cosmetics, toiletries, and soap fragrances. The flower oil from hyacinths has long been extracted commercially, and the extremely expensive hyacinth absolute has been utilized in high-end fragrances and other floral scents. Due to its limited availability on the global market, this raw material has recently lost some of its significance and has been substituted with synthetic hyacinth compounds. In the flower's essential oils, 56 different substances have been found.[4] Synthetic hyacinth compounds have been used in cosmetics, lipsticks, creams, powders, hair oils, brilliantines, and soaps, but mostly as components of sophisticated fragrances.[5]

In outmoded medicine, hemorrhoids, prostate illness, and wound healing were all treated using *H. orientalis* L.'s whole plant, leaves, and bulbs as hemostatics. Pharmacological chemical screening showed that presence of phenolic acids and flavonoids in the flowers of *H. orientalis* L. phenolic acids and flavonoids are well known for having antioxidant, anti-inflammatory, and anticancer effects. Additionally, substances isolated from this plant had strong inhibitory activity against -glucosidase, suggesting that they may one day be used to treat diabetes. Different studies showed that all parts of *H. orientalis* L. flowers, leaves and bulbs have anti cancers and immunomodulatory effects against different types of cancers such as breast cancer, lung cancer, esophagus cancers and others.[6]

Figure 1: *Hyacinthus orientalis* L. Plant

*Author for Correspondence: nour.mohammed1200m@copharm.uobaghdad.edu.iq

This study was designed for screening the phytochemicals of stigmasterol and their properties in (*H. orientalis*) plant flowers part that is cultivated in Iraq and isolation and identification by using different techniques like RP-HPLC, UV, LC/MS and FTIR since there is no phytochemical study had been done previously on stigmasterol in flowers of this plant.

MATERIAL AND METHODS

Plant Material Collection and Extraction

The flower part of *H. orientalis* was collected from the Baghdad nursery plantation in February 2021. Prof. Dr. Zainab Abd Aon, Department of Biology, College of Sciences, University of Baghdad, identified and verified the plant. The flower component was completely cleaned, dried in the shade, and processed into a fine powder using a machine. The plant's air-dried powder is weighed, after which it is extracted for 18 hours in a soxhlet with 85% ethanol. The extract is then mixed, dried by a rotary evaporator, and weighed again after which the extraction yield is determined. To achieve the goal of this investigation, the dry extract is suspended in water and then partitioned three times using petroleum ether, petroleum ether fraction, which was dried, weighted and prepared for this study.[7-9]

Phytochemical Screening by GC-MS

Phytochemical screening by GC-MS was carried out at the Ministry of Industry and Minerals, Ibn Al-Bitar Center using Agilent 19091S-433UI GC/MS equipment, the phytochemical screening of the petroleum ether fraction of flowers of Iraqi *H. orientalis* was determined. Helium was used as the carrier gas, the column HP-5ms Ultra Inert 30 m x 250 m x 0.25 m had its temperature increased from 80 to 265°C at a rate of 10°C/min, the injection volume was 1-m, the split ratio was 1:10, the inlet temperature was 250°C, and the ionizing energy was 70Ev, and the process took about 40 minutes.[10]

Identification, Isolation and Purification of Proposed Stigmasterol by Reverse Phase HPLC

At the Ministry of Science and Technology, Environmental and water research department, estimations of stigmasterol contained in the petroleum ether fraction of flowers were made using qualitative and quantitative methods. The qualitative identifications were made by contrasting the retention periods obtained under particular chromatographic settings of the tested samples with genuine standards. For quantification measurements, the calibration curve was displayed using the area under the curve AUC versus four concentration levels of the standards (designated by the x-axis). From this equation $y = m x + c$, the slope gradient and the intercept with the y-axis, m and c, were used to derive the analyst's concentration. Stigmasterol isolated from petroleum ether fraction of Iraqi *H. orientalis* flowers part of plant employing RP-HPLC with the Germany model SYKAMN With a C18-ODS (25 cm* 4.6 mm) column and acetonitrile: DW: acetic acid (60: 25: 5) as the mobile phase, the fraction collector model is FOXY R1 and the auto sampler model is S 5200. The separated ingredient was recrystallized with hot methanol for purification after the isocratic elution was performed for 10 minutes at a flow rate of 3 mL/min, injection volume of 200 L, and detection recorded using UV detector at 210 nm.[11]

Identification and Characterization of the Isolated Stigmasterol from the Petroleum Ether Fraction

The isolated stigmasterol was identified by using different identification methods:

Fourier transform infrared spectroscopy

Using a particular standard, the attenuated total reflection (ATR) technique and model for isolated Stigmasterol is SHIMADUZU1900, range 550 to 4000 nm. The standard, which was examined under comparable conditions and used as a reference, was examined after the isolated material.

Ultraviolet spectroscopy

UV computerized spectrophotometer model SHIMA-DUZU-1900/range 200 to 1000 nm was used to analyze the UV spectrum using a particular standard. The standard, which was examined under comparable conditions and used as a reference, was examined after the isolated stigmasterol.

Liquid chromatography-mass spectrometry

The isolated compound is normally dissolved in a solvent, commonly methanol, and pumped through a capillary inside a quartz tube that is not yet charged under the following circumstances: The injection volume is 1-µL, the run time is 3 minutes, the gas flow rate is 4 l/min, and the temperature is 300°C. mass spectra fill scan range 20 to 1020 m/z.

RESULTS AND DISCUSSIONS

The soxhlet apparatus is employed because it symbolizes a closed system, prevents plant material from coming into direct contact with the heat source, and only requires a small amount of solvent. It is best to employ a hot extraction procedure since heat will help the solvent penetrate the plant material by rupturing the plant tissue fibers. The percentage of yield of crude ethanolic extracts and weight of petroleum ether fraction of flowers part is illustrated in Table 1.

Phytochemical Screening of *H. orientalis* flowers via GC-MS

During GC-MS analysis, 11 bioactive phytochemical compounds were found in the petroleum ether fraction of Iraqi *H. orientalis* flowers. When it comes to phytochemical compound identification, we use retention times, peak areas, molecular weights, and molecular formulas. But, because there aren't enough reliable samples and associated molecular data in the library, many GC-MS peaks have remained unidentified. Figure 2 and Tables 2 and 3 show eleven chemicals found in Iraqi *H. orientalis* flowers petroleum ether fraction:

Purification of the Proposed Stigmasterol by Reverse Phase HPLC, Identification, and Isolation

The RP-HPLC chromatogram results showed that this method was effective for the qualitative and quantitative determination of stigmasterol in the petroleum ether fraction of Iraqi *H.*

Table 1: Percentage of yield of crude ethanolic extracts and weight of petroleum ether fraction of flowers plant part

Parts of plant	*%yield of crude ethanolic extracts*	*Weight of petroleum ether fraction*
Flowers	40%	2.5 gm

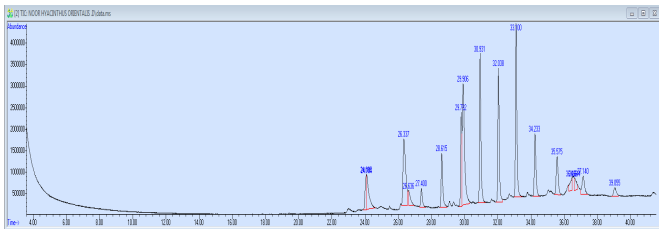


Figure 2: Petroleum ether fraction of *H. orientalis* flower GC/MS chromatogram

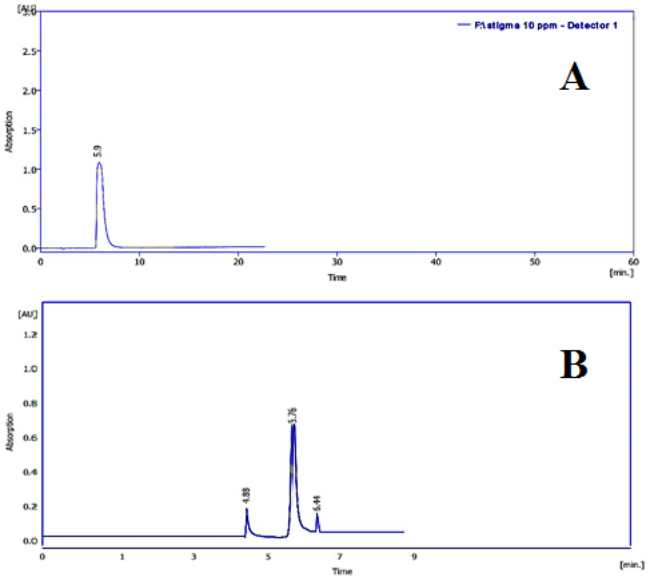


Figure 3: RP-HPLC chromatogram of A: Stigmasterol standard and B: Petroleum ether fraction of flowers of *H. orientalis*

orientalis flowers, in which stigmasterol standard and one compound (peak 2) of the three phytoconstituents have similar retention times (5.9 and 5.76 minutes for stigmasterol standard and petroleum ether fraction of flowers respectively) as depicted in Figure 3.

As shown in Figure 4 for a quantitative analysis, the calibration curve was plotted using area under the curve (AUC) versus the concentration of the standard stigmasterol, from which the concentration of the proposed stigmasterol in petroleum ether fractions of flowers was calculated using a straight line equation. The estimated concentration of suggested stigmasterol in the Iraqi *H. orientalis* flowers was equal 45 mcg/mL

Identification and Characterization of Isolated Stigmasterol

Further identification was performed to confirm the identity of the isolated stigmasterol by using the following techniques:

Fourier transform infrared spectroscopy

The most typical application of FTIR spectroscopy in phytochemical studies is as a fingerprinting tool for contrasting a natural with a synthetic reference standard. Therefore, when isolated stigmasterol was compared to ordinary stigmasterol, its IR spectra and distinctive IR absorption bands produced equivalent results, as shown in Figure 5 and Table 4.

Ultraviolet spectroscopy

Between 200 and 1100 nm of the UV spectrum were collected, and the results revealed that the highest absorbance of the isolated stigmasterol was identical to the maximum absorbance of the stigmasterol standard at the same wavelength, as shown in Figure 6.[12]

Liquid chromatography-mass Spectroscopy

Figure 7 exhibited a molecular ion peak at m/z 397 that corresponds to [M-H2O+H] +, [M-H2O] + at m/z 396, and [M-side chain -H^2O] + at m/z 255. Furthermore, by comparing the isolated stigmasterol's mass result with the stigmasterol standard's mass result.[13] From the above finding, all results are in favor of that compound is stigmasterol.[14-18]

Table 2: Identified chemicals from GC-MS data of *H. orientalis* flowers

Number of Peaks	*Peak Area %*	*Chemical Formula*	*Retention Time*	*Molecular Weight*	*Compound Name*
1	3.07	C17H34O2	24.085	270.45	Hexadecanoic acid Methyl ester
3	10.15	C19H36O2	26.337	296.5	Cis-13-octadecanoic acid, methyl ester
5	1.69	CH3(CH2)26CH3	27.4	394.77	Octacosane
6	4.89	C30H62	28.615	422.82	Triacontane
7	6.23	C24H50	29.792	338.7	Tetracosane
8	15.35	C18H28O3	29.906	292.4	Benzen propanoic acid3,4-bis Dimethyl ethyl-4-hydroxy-o Ctadecyl ester
11	13.79	C29H60	33.1	408.78	2-methyl octacosane
12	5.76	C4HF7O2	34.233	214	Heptafluorobutyroxydodecane
14	1.50	C34H62O2SI	36.46	530.94	Beta tocopherol-otert-butyldi Methyl silyl
16	2.72	C7H12O3	37.140	144.17	Valeric acid
17	1.34	C31H64	39.055	436.85	Hentriacontane

Table 3: Activity of major compounds identified in *H. orientalis* flowers by GC/MS

Number of Peak	*Retention Time*	*Class of Compounds*	*Name of Compounds*	*Uses*
1	24.085	Saturated fatty acid	Hexadecanoic acid methyl ester (Palmitic acid)	In production of soaps and cosmatics an industrial mold reiease agent anti-inflammatory hepatoprotective and hypocholestrolemic agent[12)]
3	26.337	Saturated Fatty acid	octadecanoic acid (stearic acid)	anti-inflammatory, insecticidesv antiandrogenic[13]
5	27.4	Alkane	Octacosane	Antimicrobial activity[14]
6	28.615	Alkane	Triacontane	pesticide[15]
7	29.792	Alkane	Tetracosan	cytotoxic activity
8	29.906	Alkyl benzene	benzene propanoic acid 3,5-bis(dimethyl ethyl)4hydroxy, Octadecyl ester	Antioxidant
11	33.1	Alkane	2-methyl octacosan	Antimicrobial and antioxidant activity[14]
12	34.233	Alkane	Dodecan	Use as vegetable oil and emollient in cosmatics[16]
14	36.466	Vitamin E metabolite	beta-tocopherol	Antioxidant[17]
16	37.140	Fatty acid	valeric acid	Intermediate in manufacture of flavors,perfumes,ester type lubricant ,plasticizer and vinyl stabilizers
17	39.055	Alkane	Hentriacontane	Antimicrobial,antioxidant,antiinfla mmatory,treatment of diabetes, ulcer,leucorrhea,urinary disease asthma,piles and gonorrhea[18]

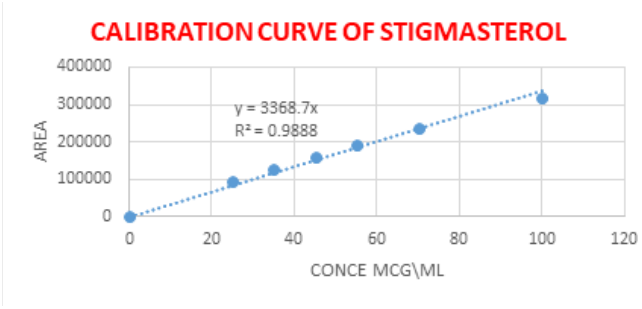


Figure 4: Calibration curve for stigmasterol

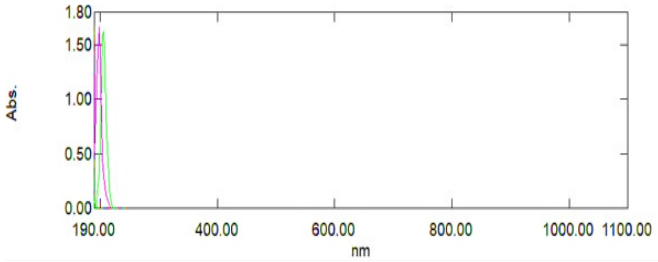


Figure 6: UV spectrum of the standard stigmasterol and the isolated stigmasterol from the petroleum ether fraction

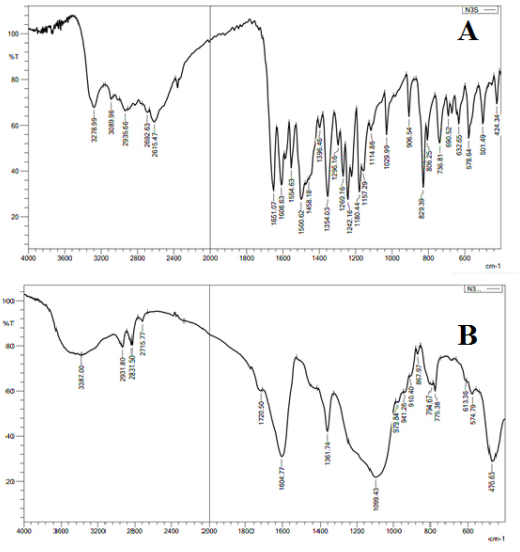
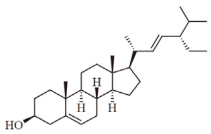


Figure 5: FTIR Spectra of A: stigmasterol standard and B: isolated stigmasterol

Table 4: Characteristic FTIR bands of absorption (cm^{-1}) of the isolated stigma sterol.[11]

The functional group	*Frequency Wave number (cm^{-1})*	*Assignment*
O-H	3387.00	O-H stretching vibration
C-H	2931.80 ,2831.50	Aliphatic C-H stretching
C=C	1604.77	C=C stretching
C-H	1458.18	Bending frequencies of cyclic (CH2)
CH2-CH3	1361.74	CH2(CH3)2 stretching
C-H	1099.43	Signifies cycloalkane
C-H	910.40,775.38, 613.36	C-H out plane bending vibration



Stigmasterol Structure

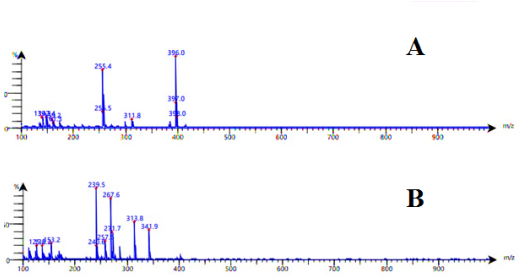


Figure 7: LC-MS of A: stigmasterol standard and B: isolated stigmasterol from petroleum ether fraction of flowers

CONCLUSION

This study used GC/MS and RP-HPLC to determine the presence of various types of saturated fatty acids, alkanes, vitamin E, and steroidal compounds in the Iraqi *H. orientalis* flower part as a novel natural source for various bioactive constituents. This is the first study conducted in Iraq and possibly the entire world about these substances. The findings show that the petroleum ether fraction of flowers has the highest concentrations of stigmasterol, equal 45 mcg/mL. The isolated stigmasterol from flowers of Iraqi *H. orientalis* will be identified by using different types of techniques like FTIR, UV and LC/MS. All of the result showed that the compound isolated is stigmasterol.

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