

GC/MS Analysis and Isolation of Stigmasterol from Viola Odorata Cultivated in Iraq

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Article Info:

Received Mar 2023

Revised May 2023

Accepted June 2023

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DOI : <https://doi.org/10.32947/ajps.v24i1.1002>

Abstract :

Viola odorata is a viola family flowering plant native to Europe and Asia. This is a small, hardy, herbaceous, perennial plant. It is also known as sweet violet and English violet. Numerous pharmacological properties of this flowering plant include anticancer, antibacterial, anti-inflammatory, antioxidant and antipyretic properties.

Previous literature review of different parts of *Viola odorata* resulted in detection of different chemical constituents such as flavonoids (Quercetin, kaempferol), glycosides (Rutin) and terpenoids (stigma sterol). Stigmasterol was detected using TLC and GC-MS in this study. It was isolated from *Viola odorata* using preparative layer chromatography. HPLC and FTIR analysis confirmed the isolated Stigmasterol.

Keywords: Viola, Stigmasterol, Detection, Isolation.

استخدام تقنية كروماتوغرافيا الغاز- مطياف الكتلة لتحليل وعزل الستكماستيرون من نبات ورد البنفسج المستزرع في العراق

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الخلاصة:

نبات ورد البنفسج نوع من النباتات المزهرة من العائلة البنفسجية، موطنها الاصلي في أوروبا و آسيا. هذا النبات هو نبات عشبي صغير دائم الخضرة. الاسم الشائع البنفسج الحلو والبنفسج الانكليزي. هذا النبات المزهرة له عدد من الفعاليات الدوائية مثل مضادات السرطان، والمضادات البكتيرية والفعالية المضادة للالتهابات ومضادات الأكسدة وفعالية خفض الحرارة. الدراسات الكيمونباتية لأجزاء مختلفة لنبات ورد البنفسج نتجت عن عزل مواد كيميائية مختلفة مثل الفلايفونويد (الكويرستين، الكامفيرول)، الكلايكوسايد (الروتين)، والتيربينويد (ستكماستيرون). ستكماستيرون من مشتقات ترايترين التي تتكون من 30 ذرة كاربون وتنتمي الى صنف الترايترين الرباعية الحلقة. ستكماستيرون يعتبر المادة الخام لصناعة البروجستيرون وكذلك صناعة فيتامين دي 3. ستكماستيرون كذلك له فعالية دوائية قوية مثل فعالية ضد السرطان وضد التهاب المفاصل ومضاد للالتهابات. في هذه الدراسة تم تشخيص ستكماستيرون عن طريق تقنية الفصل الكروماتوكرافي الطبقي الرقيق و الكروماتوكرافي الغازي وتحديد الكتلة. وتم عزله من نبات ورد البنفسج عن طريق تقنية الفصل الكروماتوغراف المحظور. طريقة تقنية الكروماتوكرافي السائل عالية الأداء والأشعة تحت الحمراء تم استخدامها لتشخيص ستكماستيرون المعزول.



الكلمات المفتاحية: البنفسج، سنكماستيرونول، كشف، عزل.

Introduction

Viola odorata is a flowering plant native to Europe and Asia in the viola family. This plant is a small hardy herbaceous perennial. It is also known as sweet violet and English violet ⁽¹⁾. The phytochemical studies of different parts of *Viola odorata* resulted in isolation of different chemical constituents such as Coumarins, caffeic acid, methyl salicylate, flavonoids (Quercetin, kaempferol), glycosides (Rutin) ⁽³⁾ and terpenoids (stigma sterol) ⁽⁴⁾. *Viola odorata* has antipyretic, antibacterial, diuretic, and laxative properties. It is used alone or in combination with other herbs for pulmonary problems. It is used internally to treat bronchitis, coughs, and asthma ⁽¹⁾. Due to its diuretic effects, seeds are used to treat urinary problems. Flower-syrup is used to treat coughing and hoarseness. The class of

tetracyclic triterpenes includes stigmasterol, a phytosterol derived from the triterpene C₃₀. The naturally occurring result of stigmasterol's C₂₂ desaturation, stigmasterol resembles cholesterol in structure. Stigmasterol is used as an intermediate in the manufacturing of estrogens, androgens, corticoids, and vitamin D₃, as well as a precursor for the production of progesterone. Stigmasterol also has potent pharmacological effects such as anticancer, anti-osteoarthritis, anti-inflammatory, anti-diabetic, immunomodulatory, antibacterial ⁽⁵⁾, antioxidant, and neuroprotective properties ⁽⁶⁾. The structure of Stigmasterol is illustrated in Fig (1). This study aims to detect Stigmasterol using TLC and GC-MS ⁽⁷⁾ and isolation from *Viola odorata* using preparative layer chromatography.

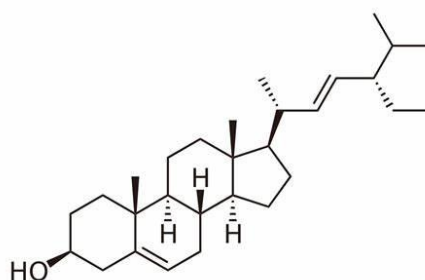


Fig (1): Stigmasterol structure

Material & Methods:

HPLC (SHIMADZU10AV-LCat), GC-MS, FTIR and ¹³C NMR. All chemicals and solvents are of analytical grade. Sigma Aldrich Company supplied standard Stigmasterol.

• Plant Material

Viola odorata aerial parts were collected from various nurseries in *Baghdad*. Dr.

Israa Abdul-Razaq authenticated the plant at the herbarium of the College of Science, University of *Baghdad*. The plant material was collected in *March* and *April* and dried in the shade at room temperature. The plant was then ground and weighed.

• Extraction of Stigmasterol from *Viola odorata* arial parts

Soxhlet apparatus was used to extract 100 gram of powdered plant arial parts with



1500 mL of hexane. The extracts were filtered and evaporated to dryness using a rotary evaporator under reduced pressure.

• Analytical TLC for detection of Stigmasterol

A spot from arial parts hexane extracts was applied by a capillary tube on a readymade TLC plate precoated with silica gel and developed in the following solvent system (S): -

S1: Toluene: ethyl acetate: chloroform (5:1:4) ⁽⁸⁾

S2: hexane: ethyl acetate (3.5:1) ⁽⁹⁾

S3: chloroform: acetone (9:1) ⁽¹⁰⁾

After drying, the TLC plate was sprayed with vanillin-sulphuric acid reagent. The visualizing agent was made as follows: To prepare solution I, 1 g vanillin was dissolved in 100 mL of ethanol, and 10 mL of concentrated H₂SO₄ was added dropwise to 90 mL of ethanol to prepare solution II. Plate was sprayed with solution I, then with solution II, and heated for about five to ten minutes at 110°C ⁽¹¹⁾.

• Detection of Stigmasterol by GC-MS

Hexane extract of arial parts was analyzed by GC-MS for detection of Stigmasterol, the conditions of GC-MS as shown in Table (1):

Table (1): GC-Mass analysis conditions of Stigmasterol

Instrument	Gas Chromatograph: Agilent (7820A) USA GC Mass Spectrometer
Analytical Column	Agilent HP-5ms Ultra inert (30 m length x 250 µm diameter x 0.25 µm inside diameter)
Injection volume	1 µl
Pressure	7.0699 psi
Temperature range	60 -325 °C
Injection Type	Split less

• Preparative TLC for isolation of Stigmasterol

hexane fraction was dissolved with hexane and subjected to preparative-glass plate using S1 mobile phase by glass pasture pipette 3-4 times on each plate while kept the spots drying between each application. The plate was then heated for 5 minutes at 110°C with monitoring and compared to standard, the detected bands were scraped off and extracted with acetone, and solvents were evaporated to collect the finished product. The detection of separated bands was carried out by spraying the side of the

plate with vanillin-sulphuric acid reagent (on the side of the plate) first solution I followed immediately by solution II, then the plate heated again until purple spot is appearing.

• Identification of Isolated Stigmasterol High Performance Liquid Chromatography (HPLC)

The test was carried out at the College of Pharmacy/University of Mustansyriah. The conditions of HPLC are illustrated in Table (2)

Table (2): HPLC Conditions for Identification and analysis of Isolated Stigmasterol from hexane fraction

Instrument	Sykam, Germany
Mobile phase	ACN: methanol (70:30) Mode: isocratic



Column	C18 (250 mm,4.6 mm,5 micro m)
Flow rate	0.8 min\ml
Volume of injection	20 microliters
Injection concentration	0.5mg\1ml
Wave length	210 nm
Solvent	Methanol
Standard	Stigmasterol

Fourier Transform Infrared Spectroscopy (FTIR): The isolated compound was analyzed using an FTIR spectrophotometer. It was carried out at Mustansiriyah University's College of Pharmacy using the SHIMADZU apparatus(SHIMADZU10AV-LCat).

Method of ¹³CNMR

The NMR spectra was performed at Pharmaceutical research Centre/Jordan University of science and technology Instrument Model:

Bruker 400 MHz-Avanc III. chemical shift expressed as part per million (ppm).

• Results and Discussion:

Thin-layer chromatography (TLC) analysis of an *Iraqi Viola odorata* hexane extract confirms the presence of Stigmasterol using Toluene, ethyl acetate, and chloroform (5:1:4) as mobile phase⁽⁸⁾, as shown in Fig (2) and R_f values are shown in Table (3)

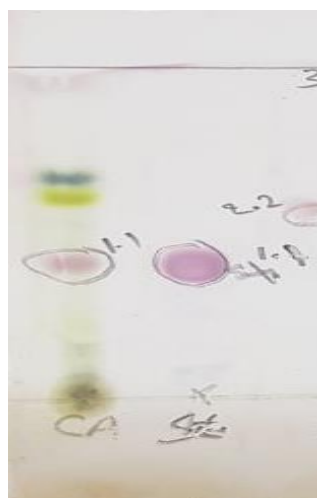


Fig (2): Analytical TLC showing hexane extract (CA) against Stigmasterol standard (st) using mobile phase S1: Toluene: ethyl acetate: chloroform (5:1:4)⁽⁸⁾

Table (3): R_f of Stigmasterol reference standard and hexane extract of aerial parts using the different solvent systems in TLC.

Mobile phase	R _f value of hexane extract	R _f value of Stigmasterol std
S5: chloroform: acetone (9:1) ⁽¹⁰⁾	0.6	0.62

S6: hexane :ethyl acetate (7:2) ⁽⁹⁾	0.58	0.52
S7: Toluene: ethyl acetate: chloroform (5:1:4) ⁽⁸⁾	0.35	0.40

The presence of Stigmasterol in thehexane extract of Iraqi Viola odorata was

confirmed by GC-MS at retention time (63.984) as shown in Fig (3):

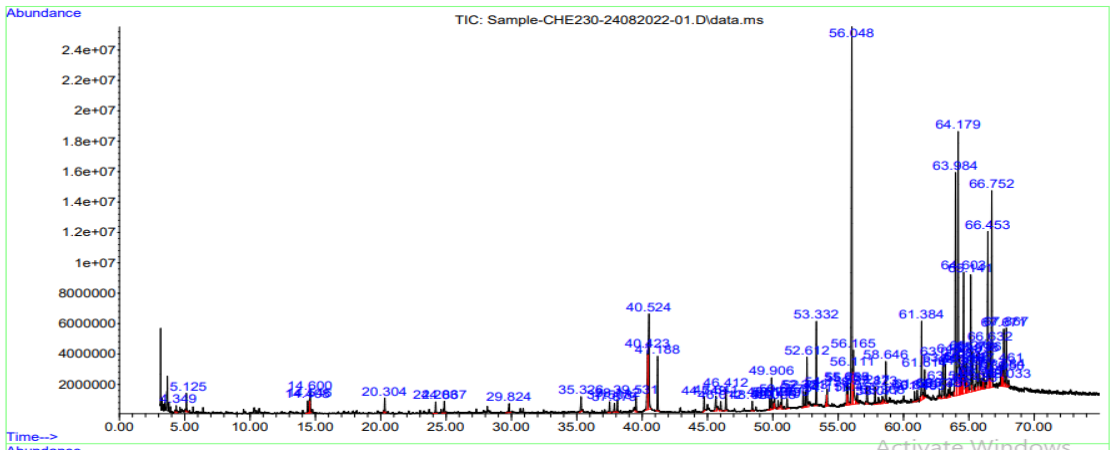


Fig (3): GC-MS chromatogram of hexane extract of arial part

In this study only the hexane extract was analyzed by GC/MS to confirm the presence of stigmasterol while the isolated stigmasterol was not analyzed by GC/MS

only by HPLC in comparison with stigmasterol standard.

Result of preparative PLC.



Fig (4): Isolation of stigmasterol by PLC.

The isolated stigmasterol was identified and characterized using chromatographic (HPLC) and spectroscopic (FTIR) methods. High Performance Liquid Chromatography (HPLC)

The pure isolated stigmasterol was also analyzed by HPLC for confirmation and its retention time (R_t) and standards' R_t were compared. The HPLC showed closely matched R_t data as illustrated in Fig (4) and Fig (5) and table (3)

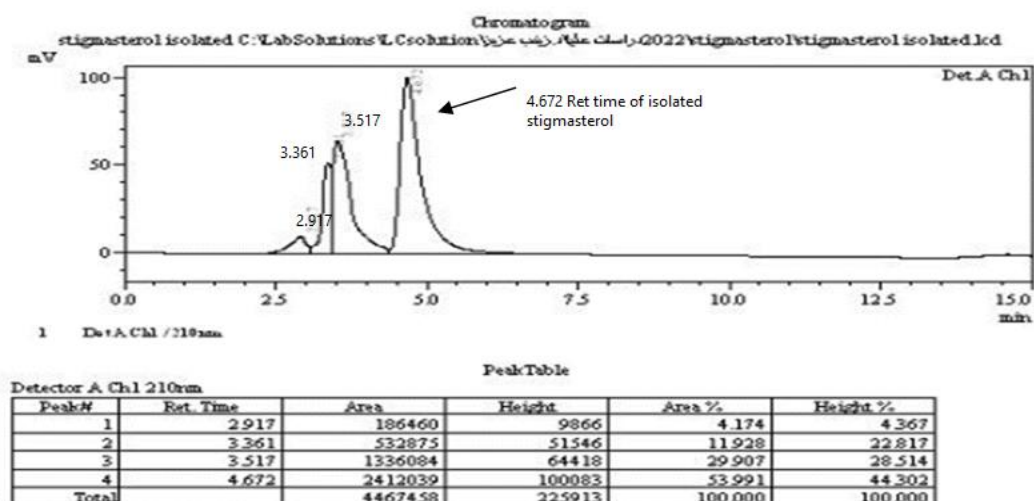


Fig (5): HPLC Chromatogram of isolated stigmasterol.

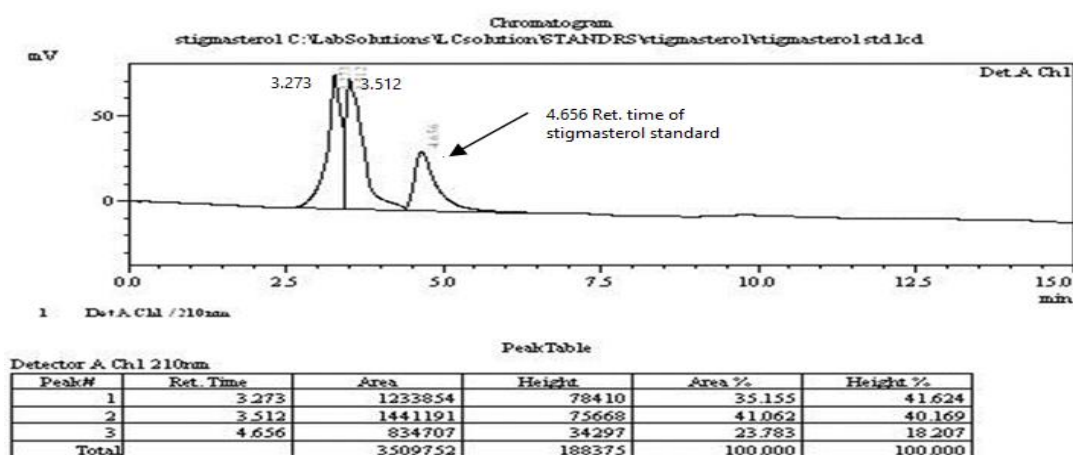


Fig (6): HPLC Chromatogram of stigmasterol standard.

Table (4): Retention time (R_t) of stigmasterol standard and isolated stigmasterol analyzed by HPLC

Standard	R_t of stigmasterol Standard	R_t of isolated stigmasterol
Stigmasterol	4.656	4.672

Fourier Transform Infrared Spectroscopy (FTIR):

The FTIR spectrum of isolated stigmasterol is shown in Fig (6):

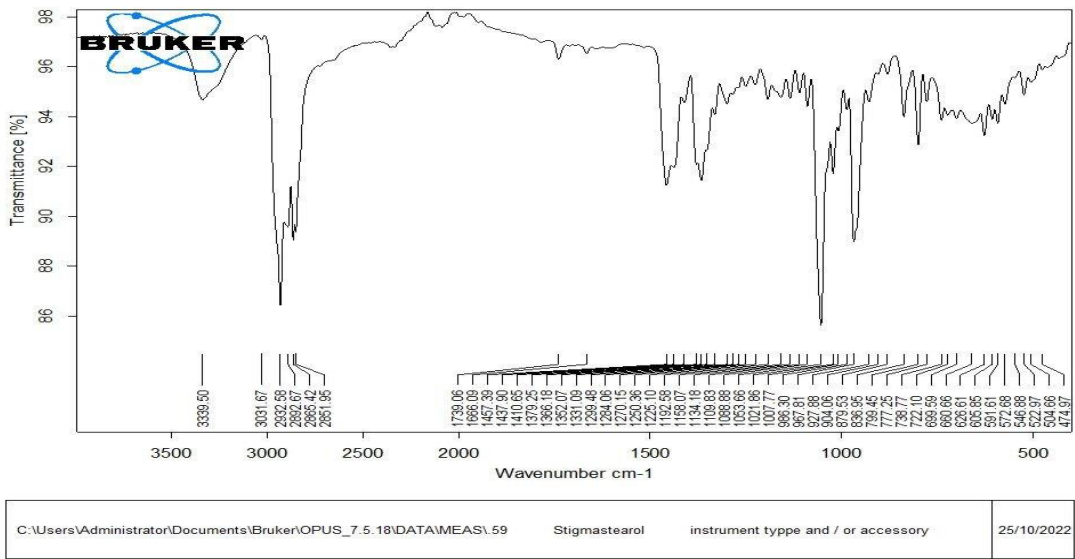
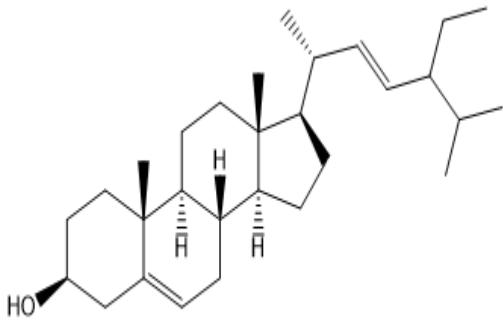


Fig (7): FTIR spectrum of isolated stigmasterol

Table (5):IR bands with their interpretations of isolated Stigmasterol

Compound	Bands (cm ⁻¹)	Interpretation
 Stigmasterol	3339.50	broad Stretching vibration of OH
	2932 & 2851	Stretching vibration of CH aliphatic (asymmetric and symmetric)
	1666-1457	Stretching vibration of C=C
	1462	Bending vibration of aliphatic.
	1056	Bending vibration of C-O of 2° alcohol



C9	28.9	C of CH ₂
C10	24.4	C of CH ₂
C11	56.62	C of CH
C12	42.09	C of C
C13	39.9	C of CH ₂
C14	21	C of CH ₂
C15	49.94	C of CH
C16	31.68	C of CH
C17	31.9	C of CH ₂
C18	121.48	C of CH
C19	140.54	C of C
C20	37.04	C of C
C21	37.2	C of CH ₂
C22	38.7	C of CH ₂
C23	71.6	C of CH
C24	41.5	C of CH ₂
C26	50.8	C of CH ₃
C27	11.83	C of CH ₃
C28	28.69	C of CH
C29	20.99	C of CH ₃
C30	20.95	C of CH ₃

Discussion

TLC and GC/MS confirm the presence of stigmasterol in the arial parts of *Iraqi V. odorata*, preparative thin layer chromatography method was used in this study for stigmasterol isolation and the structure of isolated stigmasterol was confirmed by FTIR and ¹³C NMR.

CONCLUSION

Stigmasterol, which is considered as a precursor for progesterone synthesis and functions as an intermediate in the manufacturing of estrogens, androgens, and corticoids as well as the creation of vitamin D₃, is confirmed to be present in *Iraqi V. odorata* arial parts by hexane extract. Stigmasterol presence in *Iraqi V. odorata* arial parts hexane extract was confirmed by GC/MS and TLC and isolated by PLC and the structure of the isolated stigmasterol

was confirmed by FTIR. *Iraqi* plants are rich in active constituents therefore further studies are needed to investigate phytochemical components of the plant and to study the biological activity of these components.

References:

- 1- Singh A., Dhaliwal S., Navneet. Traditional uses, Antimicrobial potential, Pharmacological properties and Phytochemistry of *Viola odorata*: A Mini Review. The Journal of Phytopharmacology, 2018; 7(1): 103-105.
- 2- Singh, Ajeet & Dhariwal, Shweta & Navneet. Traditional uses, Antimicrobial potential, Pharmacological properties and Phytochemistry of *Viola odorata*: A Mini Review. The Journal of



- Phytopharmacology, 2018; 7. 103-105. 10.31254/phyto.2018.7120.
- 3- Gautam, Shiv & Navneet & Kumar, Sanjay. Current Aspects on Phytochemistry and Bioactive Constituents of *Viola odorata* L. 2017; 5. 1-6.
 - 4- SHAIMAA FAKHRI JASIM, NOOR NIHAD BAQER*, ESAM ABD ALRAHEEM Ministry of Science and Technology, received: 15 December 2017, Revised and Accepted: 30 January 2018
 - 5- Ali, Zainab & Thamer, Mohamed & Alani, Widad. Antibacterial Activity of Chloroform Extract from *Tagetes Erecta* L. Flowers. Al Mustansiriyah Journal of Pharmaceutical Sciences. 2019; 19. 7-15. 10.32947/ajps. v19i4.627.
 - 6- Bakrim S, Benkhaira N, Bourais I, Benali T, Lee L-H, El Omari N, et al. Health Benefits and Pharmacological Properties of Stigmasterol. Antioxidants 2022; 11:1912. <https://doi.org/10.3390/antiox11101912>
 - 7- Kadhim NA, Al-Ani WMK, Al-Joboury IS. Detection of Lupeol in Calendula Officinalis Grown in Iraq by GC-MS Analysis. Al Mustansiriyah Journal of Pharmaceutical Sciences [Internet]. 2019 [cited 2022 Feb 11];19(4).
 - 8- Kuo HL, Chung KY, Chin AK and Ming DK. Solvent systems for improved isolation and separation of Teritrems A and B. Applied and environmental microbiology 1982; 44(4): 860-863.
 - 9- Asila ASA and Mohammad AH. Isolation, structure characterization and prediction of antioxidant activity of two new compounds from the leaves of *Dodonaea viscosa* native to the sultanate of Oman. Egyptian journal of basic and applied sciences 2018; 5: 157-164.
 - 10- Rand AA, Ibrahim SA and Enas JK. Isolation and characterization of β -sitosterol from *Elaeagnus angustifolia* cultivated in Iraq. Asian journal of pharmaceutical and clinical research 2018; 11(11): 442-446.
 - 11- V. Le A, E. Parks S, H. Nguyen M, D. Roach P. Improving the Vanillin-Sulphuric Acid Method for Quantifying Total Saponins. Technologies. 2018; 6(3):84. <https://doi.org/10.3390/technologies6030084>.

