



Phytochemical Study of Stigmasterol and β -sitosterol in *Viola odorata* Plant Cultivated in Iraq

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Abstract: *Viola odorata* L. (Violaceae) Cultivated in Iraq, it is an important folklore medicine for the treatment of various ailments as antioxidant and antibacteria. In the previous study two phytosterol (Stigmasterol and β -sitosterol) were detected and identified from the methanol extracts in roots, leaves, flowers and seeds by using chemical test, TLC and HPLC techniques. The results showed all the plant parts contain high amount of sterols the quantitative data revealed that the concentrations of Stigmasterol in *Viola odorata* were in roots(4.5mg/100g) ,in leaves(2mg/100g),in flowers(7.3mg/100g) and in seeds(2.4mg/100g). also the content of β -sitosterol in roots(9.2mg/100g),in leaves(3.2mg/100g),in flowers(5.3mg/100g) and in seeds (3mg/100g). Results showed high amount of Stigmasterol in flower while high amount of β -sitosterol in root.

Key words: *Viola odorata* L., stigmasterol and β -sitosterol, TLC,HPLC.

دراسة كيميائية للمركبات Stigmasterol و β -sitosterol في نبات البنفسج العطري المتواجد في العراق

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الخلاصة: درست نبتة البنفسج العطري (العائلة البنفسجية) المتواجدة في العراق لاهميتها في الاستخدامات الطبية كمضادات للاكسدة والبكتيريا اذ تم تحديد وتشخيص نوعين من المركبات الستيرويدية Stigmasterol و β -sitosterol في المستخلص الكحولي لاجزاء النبتة الجذر، الاوراق، الازهار و البذور وبأستخدام الكشوفات الكيميائية وتقنية TLC و HPLC، وقد اظهرت النتائج تواجد هذه الستيرويدات في جميع اجزاء النبتة اذ كانت تراكيز مركب Stigmasterol في الجذر (4.5mg/100g) والاوراق (2mg/100g) وفي الازهار (7.3mg/100g) ولللبذور (2.4mg/100g)، فيما كانت تراكيز β -sitosterol للجذر (9.2mg/100g) وللاوراق (3.2mg/100g) وفي الازهار (5.3mg/100g) ولللبذور (3mg/100g)، اذ بينت النتائج ان اعلى تركيز لمركب Stigmasterol موجود في الزهرة فيما كان اعلى تركيز لمركب β -sitosterol موجود في الجذر.

Introduction

Viola is a genus of flowering plants in the violet family Violaceae. It is the

largest genus in the family, containing between 525 and 600 species (1). Most species are found in the temperate Northern Hemisphere.

However some of these are also found in widely divergent areas such as Hawaii, Australia, and the Andes(2). Species of *V. odorata* is a native to Europe and Asia, but has also been begin to North America and Australia (3). It is frequently recognized as Wood Violet , Sweet Violet, English Violet, Common Violet, or Garden Violet(4) . It had been used as a medical herb because plant parts have activated compounds alkaloids, flavonoids, phenolic compounds and steroids (5) (6). Stigmasterol is an unsaturated plant sterol occurring in the plant fats or oils of many plants and medicinal herbs, used as a precursor in the manufacture of semisynthetic progesterone (7), available human hormone is an important physiological role in the regulatory and tissue rebuilding mechanisms that related with estrogen effects. as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D₃ and prevention of certain cancers such as ovarian, prostate, breast, and colon cancers, also phytoestersols may inhibit the absorption of cholesterol and lower serum cholesterol levels by competing for intestinal absorption. (8) Studies with laboratory animals fed stigmasterol found that both cholesterol and sitosterol absorption decreased 23% and 30%, respectively, over a 6-week period. Hypoglycemic and thyroid inhibiting properties (9), as well β -Sitosterol compound have the same medical effect. Chemical structures of this was similar to that of cholesterol. It cause reduction in blood levels of cholesterol, and sometimes using in treating hypercholesterolemia and inhibits cholesterol absorption in the intestine (10).The aims of this study is

detect and identify Phytosterols Compounds Stigmasterol and β -sitosterol in plant *Viola odorata* parts.

Materials and Methods

Viola Odorata L. plant which authenticated by the National Herbarium of Iraq Botany Directorate at Abu-Ghraib was collected in April and July .The plant was separated into four parts roots, leaves, flowers and seeds and each part dried at room temperature 25 c° in the shade for (10) days then each part of plant was crushed into powder by electric Grinder and weighted.

Preparation of Extracts

100 g of crushed powder of each part (roots, leaves, flowers and seeds) of *Viola Odorata* L. Plant was macerated for 36 hour with shaking at room temperature 25 c° in 1 Liter methanol and the resulting extract was filtered . Each part was filtered and the residue was re-extracted twice for complete exhaustion. The obtained filtrates were combined and concentrated by using rotary evaporator to get dry extract. Each dried part was dissolved in methanol and stored at 4°C in a refrigerator(3).

Chemical Identification of Steroids

Two types of reagent was used to identify Stigmasterol and β -sitosterol compounds.

- 1- Liebermann-Burchard test: 3ml of Extract were treated with chloroform, acetic anhydride and drops of sulphuric acid was added. The formation of dark pink color indicates the presence of steroids.

2- Salkowski reaction: 1gm of the extract dissolve in chloroform ,then some drops of concentrated sulphuric acid were added to the solution. A red color was seen in the upper chloroform layer(11).

Preliminary Identification of Sterols (stigmasterol and β -sitosterol) by using TLC

Silica gel Aluminum foil plates of GF 254(20x20)cm of 0.25 mm thickness were used which activated at 110c° for one hour, and using of different solvents systems to detect stigmasterol and β -sitosterol compounds obtained from plant parts. Solvent system (acetone : hexane 1:3) was prepared and placed in a glass tank (22.5 cm x 22 cm x 7cm) covered with a glass lid. The atmosphere of the glass tank should be saturated with the solvent vapors before running samples, so part of the inside of tank was lined with filter paper (Whatman No.2)to aid in this saturation process and allow to stand for 45 minutes before use (12), as well, standard reference of stigmasterol and β -sitosterol (obtained from sigma Aldrich/Germany) about 1mg dissolved in 1ml chloroform ,and 10 mg of each part of crude extract (flowers, leaves, roots and seeds) were dissolved in 10 ml methanol to make a concentration of 1mg/ml.

Each part of crude extract applied on silica gel Thin layer Chromatography (TLC) coated plates .Extract applied 1cm above the edge of the chromatographic plates along with the reference standards. By using capillary tubes in form of spots then developed

in tank already saturated with 150 ml of solvent systems and allowed to developed by the ascending technique .

Quantitative and qualitative estimation of (stigmasterol and β -sitosterol) compounds using HPLC.

High Performance Liquid Chromatography (HPLC) used for identification and qualitative of stigmasterol and β -sitosterol compounds . 10 mg extract of each part of *Viola odorata L.* were dissolved in 5 ml MeOH separately and used for HPLC analysis which, performed using a Shimadzu Model 2010 with mobile phase 6.5% De-ionized water solvent in acetonitrile, detection UV at 336 nm, flow rate 1.2/ml in 38c° .

Qualitative estimation of Stigmasterol and β -sitosterol of each part of plant were done by using (HPLC) which the equation below was used to calculate the percentage of the compound in the plant:

Concentration of compound in the part of plant=AUC of the sample /AUC of the standard \times conc.St \times DF.

Results and Discussion

Chemical tests were used to identify phytosterols compounds in *Viola odorata* and the results in table (1) showed Libermann-Burchard reaction and Salkowski reaction tests which proved presence of steroids in all parts of *v. odorata* (root, leaves, flowers and seeds).

Table (1) Chemical Identification of Sterols in Each Part of *Viola Odorata* Plant

Plant Parts	Libermann-Burchard reaction	Salkowski reaction
Roots	+	+
Leaves	+	+
Flowers	+	+
Seeds	+	+

+, - represent presence and absence of active constituents(sterols) respectively

Results in Table (2) showed Rf Values of Stigmasterol and β -Sitosterol compounds in root, leaves, flowers and seeds extract of *V. odorata* plant and Standards reference in Developing Solvent System in TLC. roots extract showed RF Values about 0.52 and 0.50 for Stigmasterol and β -sitosterol and

leaves extract about 0.54 and 0.53 as well, flowers extract about 0.52 and 0.50, seeds extract showed 0.51 and 0.50. while, standard showed RF values about 0.53 and 0.50 for Stigmasterol and β -sitosterol.

Table (2) - Rf Values of active constituents (Stigmasterol and β -Sitosterol) Obtained from Different Plant Parts and their Standards in Developing Solvent System in TLC. Visualization by vapour iodine (saturated iodine chamber)

Materials	RF value of steroid	
	Stigmasterol	β -sitosterol
Standard	0.53	0.50
roots extract	Upper spot 0.52	Lower spot 0.50
leaves extract	Upper spot 0.54	Lower spot 0.53
flowers extract	Upper spot 0.52	Lower spot 0.50
seeds extract	Upper spot 0.51	Lower spot 0.50

TLC of methanol extract from *Viola Odorata L.* parts showed the presence of stigmasterol and β -sitosterol in all parts of plant. The spots of the stigmasterol and β -sitosterol having the same color(brown to yellow) and Rf values as that of Stigmasterol and β -sitosterol standards on TLC plates after detected using visualizing reagents

(Figure 1) and TLC plats showed the approximate between stigmasterol and β -sitosterol in RF because of the similarity in structure for the tow compounds and only difference between them is the presence of C22=C23 double bond in stigmasterol and C22-C23 single bond in β -sitosterol in addition to the similarity in structures

,the molecular weight of Stigmasterol and β -sitosterol is also approximate (13).

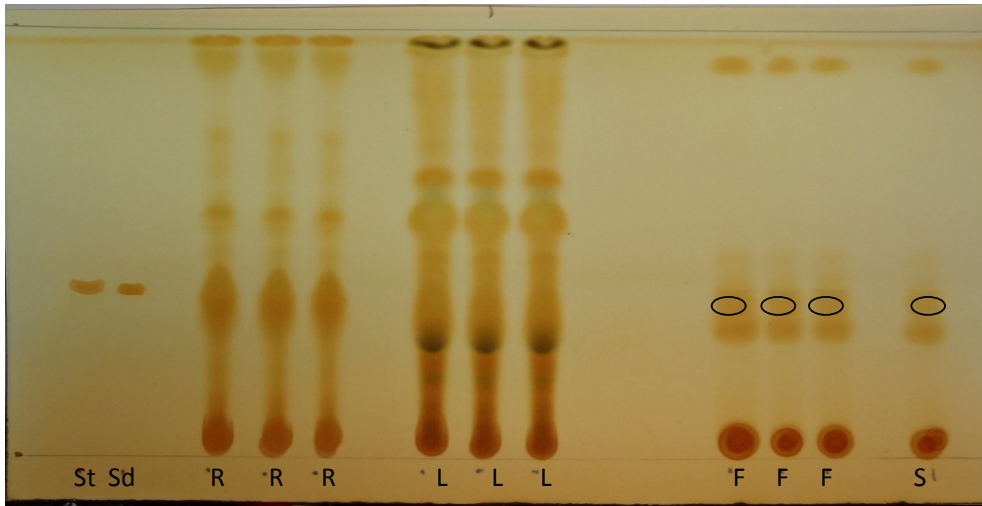
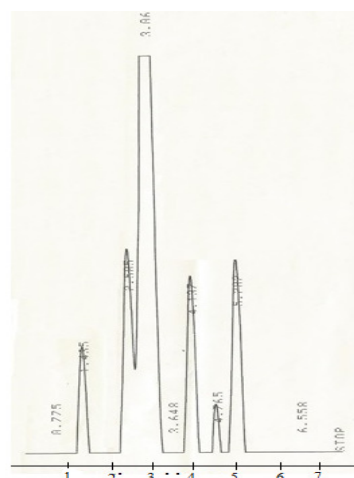
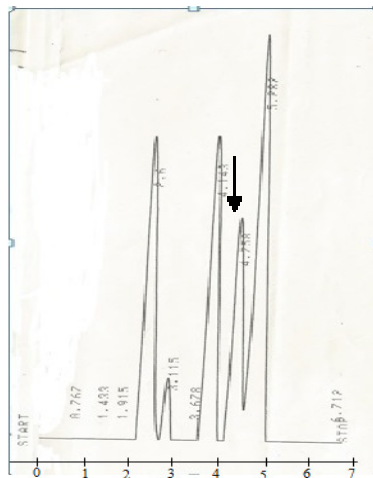


Figure (1): TLC of the different parts extracts of *Viola Odorata* showing.(St=stigmasterol and β -sitosterol standard, R=roots, L=leaves, F=flowers, S=seeds).Visulization by vapour iodine(saturated iodine chamber).



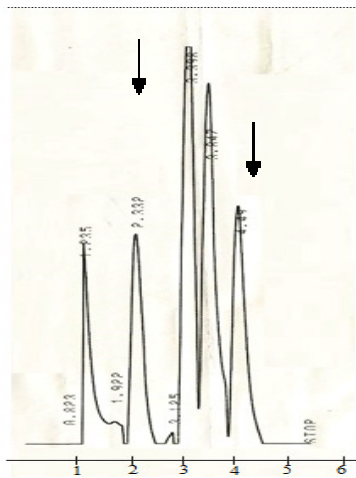


A

Phytosterol	Rt. (min)
Stigmasterol	3.67
β -sitosterol	4.75

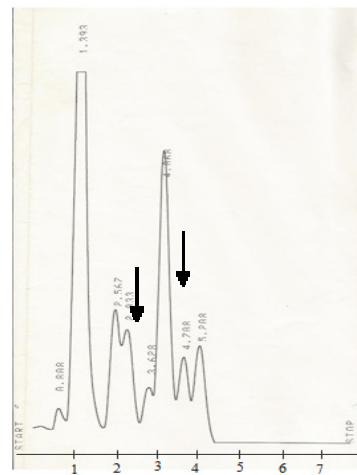
B

Phytosterol	Rt. (min)
Stigmasterol	3.64
β -sitosterol	4.76



C

Phytosterol	Rt. (min)
Stigmasterol	3.39
β -sitosterol	4.49



D

Phytosterol	Rt. (min)
Stigmasterol	3.62
β -sitosterol	4.70

Figure (2) HPLC analysis of qualitative estimation of Stigmasterol and β -sitosterol in different parts of *Viola odorata* L. Plant cultivated in Iraq. A=leaves,B=roots,C=flowers,D=seeds



Figure (3) HPLC analysis of Stigmasterol standard Rt = 3.72

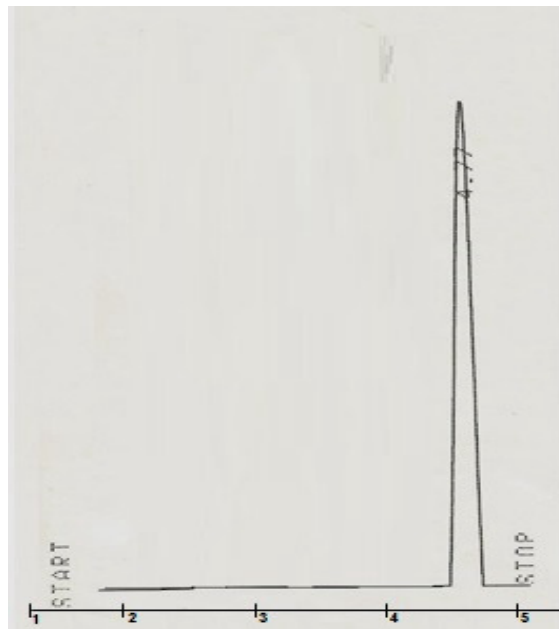


Figure (4) HPLC analysis of β -sitosterol standard Rt = 4.77

HPLC analysis

The results of HPLC qualitative analysis were made by the comparison of the retention times obtained at identical chromatographic conditions of analyzed samples and the authentic standards. Figures(2) show each the

identified compounds in each part of the plant has an identical retention time compared with their corresponding authentic standard in Figure (3) and (4). Results in Table (3) showed quantity of Stigmasterol and β -sitosterol in roots, leaves, flowers and seeds extract of *V. odorata* which are 4.5, 2, 7.3 and 2.4

mg\100g for Stigmasterol in root, leaves, flower and seeds, as well, 9.2, 3.2, 5.3 and 3 mg\100g for β -sitosterol.

Table (3) quantitative analysis of (stigmasterol and β -sitosterol) in different parts of *V. odorata* (mg\100g)

<i>V. odorata</i> extract	Phytosterols	
	Stigmasterol	β -sitosterol
Roots extract	4.5	9.2
Leaves extract	2	3.2
Flowers extract	7.3	5.3
Seeds extract	2.4	3

As shown on table (3) higher quantity percentage of Stigmasterol and β -sitosterol in root and flowers in crud extracts and moderate quantity in leaves and seeds, and this result correspond with (14) referred to presence of phytosterol (stigmasterol and β -sitosterol) in *Viola Odorata* L.[Violaceae] plant 330 ppm in all parts of plant. Furthermore the high quantity of Stigmasterol and sitosterol stimulated vegetative growth characteristics plant height, leaf area, plantfresh and dry weight both typical (Sitosterol and Sigmasterol) and atypical sterols play a regulatory function in plant development 15.

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