Detection of Cholesterol in Suaeda Baccata (Chenopodiaceae)

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Abstract

This study detects the presence of cholesterol in an Iraqi plant named Suaeda baccata Forsk of the family Chenopodiacae, wildly and widely grown in Iraq. The absence of any publication concerning the sterol content of this Suaeda specie, and the industrial importance of cholesterol depending on its role as a precursor in the synthesis of some hormones, like progesterone, acquired this study its value. The investigations revealed the presence of cholesterol that was proved by TLC together with the standard compound cholesterol, and anisaldehyde spray reagent using three different solvent systems, then authenticated by HPLC, in which the retention time of both the standard cholesterol and the plant extract cholesterol were identical.

Key Words: Cholesterol, Suaeda Baccat and Chenopodiaceae

الخلاصة

هذه الدراسة تختص بالكشف عن مادة الكولستيرول في نبات عراقي مسمى بـ Suaeda baccata الذي ينموبريا و بشكل واسع في العراق .ان عدم وجود اي مجلة علمية او دورية تتناول المحتوى (Chenopodiaceae) الستيرويدي لهذا الصنف من النبات وأهمية الكولستيرول للصناعة الدوائية بكونة المادة الابتدائية لتصنيع بعض الهرمونات مثل البروجستيرون يبين قيمة هذة الدراسة. بينت التجارب البحثية وجود مادة الكولستيرول والذي اكد بواسطة استخدام تقنية كروماتوغرافيا الطبقة الرقيقة TLC بواسطة ثلاثة أنواع من المحاليل الناقلة .كما تم إجراء الفحص التأكيدي لمادة الكولستيرول في المستخلص النباتي بواسطةHPLC والذي طابق الكولستيرول القياسي.

Introduction

The Chenopodiaceae (goosefoot family) is a large family including about 102 genera, and 1400 species of low growing plants. [1]

Members of this family including Suaeda baccata mostly grow naturally in soils containing much salt (halophytes) [2].

Suaeda baccata specie is distributed in Iraq, in south of Jazira District, Southern Desert District, Western Desert Central Alluvial Plain District and Eastern Alluvial District [3].the photo of this plant is demonstrated bellow

Literature survey indicated that different species of the genus Suaeda contain several different compounds, including sterols [4, 5], thus it was deemed desirable to find out the sterol content of this plant. Steroids constitute a natural product class of compounds that is widely distributed throughout nature. [6] The chemical structure of steroids is base on (a) perhydrocyclopenta phenanthrene nucleus. The steroidal nucleus is derived from isopentyl pyrophosphate as follows:-

Acetate ⟶ mevalonate ⟶ isopentenyl pyrophosphate ⟶ squalene ⟶ cholesterol pathway .[7,8]

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Figure (1) [photography of Suaeda accata]

The first steroids isolated from nature were a series of C27 –C29 alcohols that were found in lipid fraction of many tissues. These compounds were solids and therefore named sterols from the **Greek *stereos***, meaning solid. The most widely occurring sterol is cholesterol, (Greek Word, chole, meaning bile). It was first isolated from human gallstones. Until recently cholesterol was thought to be restricted to the animal kingdom; however, it has now been identified in plants.[7, 9, 10].

There is a widespread belief among the public and even among the chemists that plants do not contain cholesterol and this is an error, further more even some references refer to this mistake [11, 12]. The fact is that the cholesterol widespread in the plant kingdom (although other related sterols, such as β-sitosterol generally occur in higher quantity) [13, 14]. Cholesterol occurs in both free and esterified. It occurs as a component of plant membranes and as part of the surface lipids of leaves where it is sometimes the major sterol. The quantity of cholesterol is generally small when expressed a percent of total lipid. While cholesterol averages perhaps 50 mg/kg total lipid in plants, it can be as high as 5 g/kg (or more) in animals. [15].

The quantities of cholesterol in a number of vegetable (plant) oils are given in the following table.

Table (1) Cholesterol content of some plant oils

	Source	Cholsterol (mg/kg)	References
1	Palm oil	20	16
2	Palm kernel	17	17
3	Coconut oil	14	17
4	Cotton seed oil	45	17
5	Soybean oil	29	17
6	Corn oil	55	17
7	Peanut oil	24	17
8	Sun flower oil	14	17
9	Canola oil	53	17
10	Avogadro oil	< 30	18
11	Olive oil	0.5-2	19,20
12	.Sesame oil	Ca.1	19,20

Cholesterol and its esters are important constituents of plant membranes.
The following table represents some data on the sterol fraction of some plant organelles.

Table (2) Sub-cellular distribution of cholesterol in plant

Source	Free Choles - terol%	Chole sterol - ester%	Refer ence
1. Green bean leaves a. Whole	1	1	21
b. Chloroplasts	24	33	
c. Mitochondria	---	---	
d. Microsomes	1	28	
2.Etiolated bean leaves a. Whole	6	23	21
b. Chloroplasts	27	26	
c. Mitochondria	---	---	
d. Microsomes	6	34	
3. Organelles of 21-day maize shoots a. Nuclei	22	76	22
b. Chloroplasts	2	52	
c. Mitochondria	1	32	
d. Microsomes	1	32	

While cholesterol is usually a minor constituent of the sterol fraction in plants, it is the major constituent of some plant surface as shown in table (3)

Table (3) Sterol content of Rape (Canola) [23]

Source	Cholesterol %	Sitosterol %
1. Leaves a. Surface	71.5	0.6
b. Intracellular	15	30
2. Seeds a. Surface	7.2	62
b. Intracellular	0.7	67
3. Seedpods a. Surface	35	21

The proportion of cholesterol in the sterol fraction of Liliaceae, Solanaceae and Scrophulariaceae families is especially large. [24,25]

Cholesterol ($C_{27}H_{46}O$), **3β-chole…..sta-5-en-3ol,** mol.wt. 386.66, is practically insoluble in water, slightly soluble in alcohol, more soluble in hot alcohol, one gram dissolves in 2.8ml of ether, in 4.5ml of chloroform, In 1.5ml of pyridine, also it is soluble in petroleum ether, benzene[26].

Cholesterol structure is represented bellow. [26]

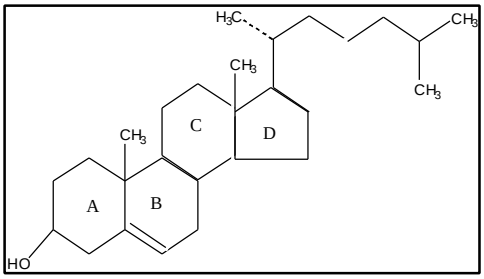

Figure (2) cholesterol structure

Although total synthesis of some medicinal steroids is employed commercially, there is also a great demand for natural products which will serve as a starting material for their partial synthesis [2]

Accordingly, cholesterol has serve as a precursor for the synthesis of progesterone [27] as represented in the following diagram, and this acquired this study its importance.

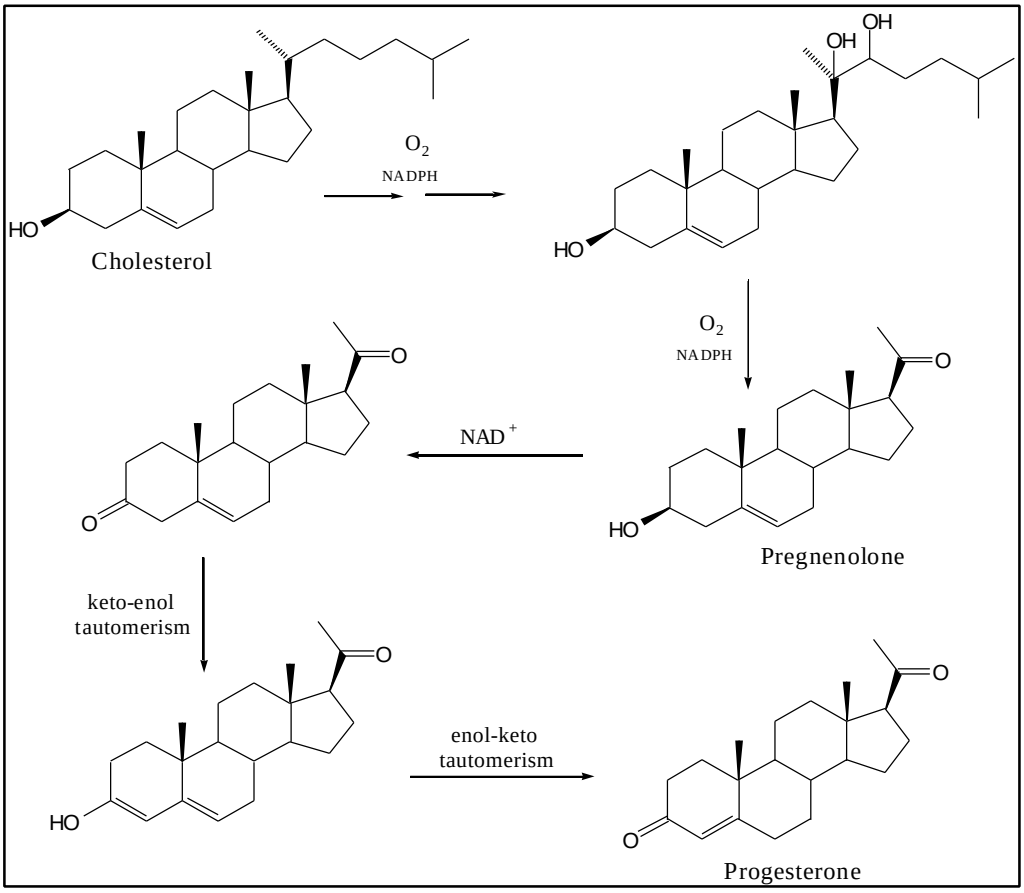

Figure (3) :conversion of cholesterol to progesterone [27]

Material and Methods

The plant material (aerial part) was collected during months of July, August, and September 2005. From the high ways of Baghdad city. The plant was identified by the Department of Pharmacognosy , college of Pharmacy/University of Baghdad ; and authenticated by the National Herbarium of Iraq. Botany Directorate at Abu-Ghraib, Iraq.

Forty grams of the dried aerial parts were first macerated with 500 ml of n-hexane for 24 hours. The residual plant part then was dried at room temperature, soaked in water for 24 hours, dried , and then refluxed with 2 NHCl solution for two hours. After filtration by Buchner funnel, 5% ammonia solution was added to the residual plant part, and then washed by distilled water several times, until neutral. This plant part after drying over night will be extracted again with 250 ml of petroleum ether (60^0C -80^0C) for ten hours by the use of soxhlet apparatus. The petroleum ether filtrate will then be evaporated to dryness to be ready for the identification of steroid by TLC. [28] The following diagram represents the extraction procedure of steroids from Suaeda baccata .

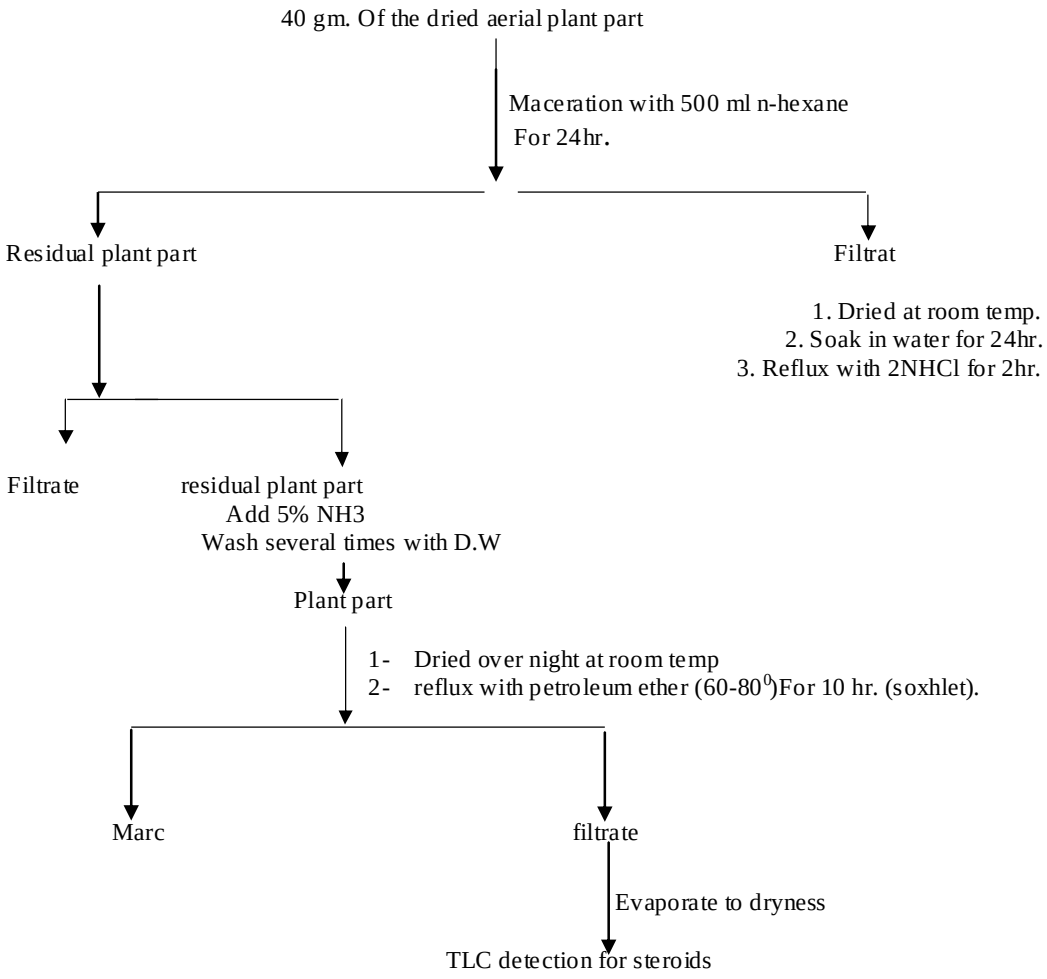

Figure (4)Schematic procedure for the extraction of steroids from Suaeda baccata

Identification of the Steroid (Cholesterol)

Identification was performed first by TLC, using silica gel G, anisaldehyde spray reagent [29,30][0.5ml Anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85ml methanol and 5 ml concentrated sulphuric acid in that order. the TLC plates are sprayed with 10ml, heated at 110^0 for 5-10 minutes.], standard cholesterol, and different solvent systems that were: [31,32]

Solvent (1): Toluene: ethyl acetate (90:10)

Solvent (2): Benzen: acetone (90:10)

Solvent (3): Petroleum ether (60^0-80^0): ethyl acetate (75:25)

Then this identification was authenticated by HPLC

Result and Discussions

Cholesterol can be found in plant either in the free state or conjugated as simple glycoside[33] therefore the extraction procedure included the use of water and acid, necessary for the cleavage of the glycosidic linkage and the release of the aglycone part (cholesterol), and the possible sugar site attachment is C3- atom in its structure. As the cholesterol is soluble in petroleum ether, therefore this solvent was used in its extraction.

The identification of cholesterol was performed by TLC using three different solvent systems S1, S2, S3. As represented in the following diagrams:-

A B

Figure (5): TLC plate of the aerial plant extract, and standard, using S1 mobile phase

A=Standard cholesterol
B=aerial plant extract

B A

Figure (6) :TLC plate of the aerial plant extract, and standard, using S2 mobile phase)

A=Standard cholesterol
B=aerial plant extract

B A

Figure (7): TLC plate of the aerial plant extract, and standard, using S3 mobile phase

A=Standard cholesterol
B=aerial plant extract

The Rf. value of each solvent system of the standard cholesterol and the plant extract cholesterol, are represented in the following table.

Table (4) [Rf values of both, the aerial plant part extract and standard cholesterol]

Solvent system	Rf. of standard cholesterol	Rf. of the plant extract
S1(Toluene: ethyl acetate (90:10)	0.739	0.730
S2(Benzen:acetone (90:10)	0.192	0.192
S3(Petroleum ether (60^0-80^0): ethyl acetate (75:25)	0.513	0.521

Further identification to the cholesterol in the plant extract was performed by HPLC. In which the retention time of both the standard cholesterol and the plant extract cholesterol were identical as represented in the chart bellow.

Table (5) :Table of the HLPC conditions

Conditions of cholesterol HPLC (19)	
Mobile phase	Acetonitrile:methanol 50:50 containing 3% D.W
Column	C18
Detector	210
Flow rate	1ml/min
Injection Volume	

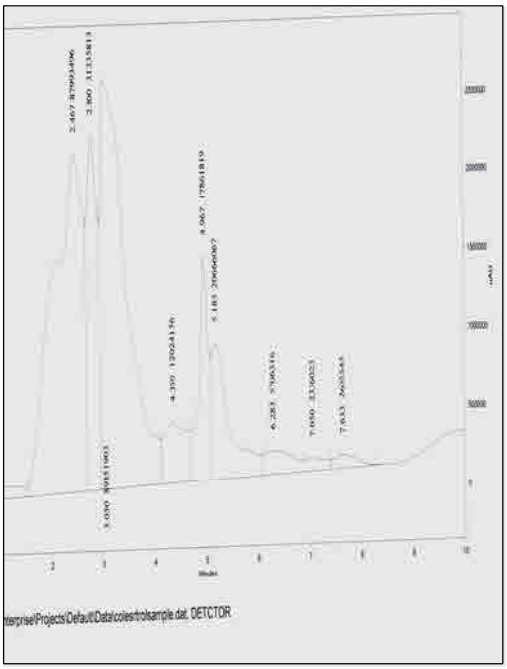

Figure (8) :HPLCchart of plant extract

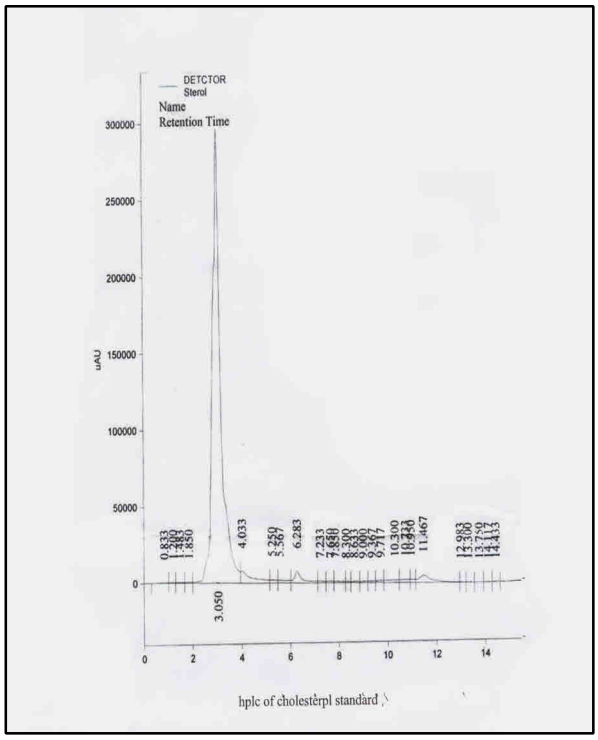

Figure (9) :HPLC chart of standard cholesterol

Conclusion

Suaeda baccata Forsk (Chenopodiacae), a wild Iraqi plant, contains cholesterol as one of its constituents.

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