Phytochemical Study of Steroidal Sapogenin "Tigogenin" Present in the Leaves of *Agave americana* Cultivated in Iraq

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Abstract

The presence of the most important steroidal sapogenin "Tigogenin" in the leaves of *Agave americana* cultivated in Iraq was detected. The absence of any study concerning the Tigogenin content of this medicinal plant in Iraq, and the industrial importance of Tigogenin depending on its role as a precursor in the synthesis of some steroidal drugs, acquired this study its value. This search include extraction, isolation, purification and dentification of Tigogenin from the leaves of *Agave americana*. Extraction of this compound was carried out using two methods. Identification of this compound is carried out by using thin layer chromatography (TLC) where three different mobile phase have been used. Detection is done by using Libermann – Burchard reagent .High performance liquid chromatography (HPLC) is also used for further identification of tigogenin and then this steroidal saponin was isolated and purified. Isolated Tigogenin is identified by using Thin layer chromatography (TLC), melting point (M.P.), Infrared spectroscopy (IR) and High performance liquid chromatography (HPLC) . This study confirms the presence of Tigogenin in the leaves of *Agave americana* cultivated in Iraq. Also the result of this study showed that the second extraction method is better, because the amount of both tigogenin and extract obtained from method No.2 are more than one extraction method.

Key words : *Agave americana* , Steroidal saponin , Tigogenin.

دراسة كيموأحيائية للستيرويد الصابوني التيكوجينين الموجود في اوراق نبات الأغاف المزروع في العراق

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الخلاصة

هذه الدراسة تختص بالكشف عن وجود اهم مادة ستيرويدية صابونية موجودة في اوراق نبات الأغاف المزروع في العراق وهي مادة التيكوجينين . ان عدم وجود اي دراسة في العراق تتناول محتوى هذا النبات من مادة التيكوجينين المهمة للصناعات الدوائية بكونها المادة الابتدائية لتصنيع بعض الادوية الستيرويدية المهمة مثل الكورتزون والهورمونات الجنسية يبين قيمة هذه الدراسة . في هذه الدراسة تم استخلاص وكشف وفصل وتنقية مادة التيكوجينين الموجودة في اوراق نبات الأغاف. حيث تم استخلاص هذه المادةباستخدام طريقين للفصل وتم الكشف عنها بوساطة طريقة كروماتوكرافيا الطبقة الرقيقة , باستخدام مذيبات مختلفة كوسيط ناقل والكشف عنها باستخدام كاشف ليبرمان بيرجارد . وكذلك بوساطة تقنية كروماتوكرافيا الاداء العالي السائل وبعدها تمت عملية الفصل والتنقية . وقد استخدمت مجموعة من التقنيات للتحقيق من نوعية المركب المفصول ودرجة نقاوته والتي شملت : درجة الانصهار , كروماتوكرافيا الطبقة الرقيقة , مطياف الاشعة تحت الحمراء وكذلك تقنية كروماتوكرافيا الاداء العالي السائل. اثبتت هذه الدراسة وجود مادة التيكوجينين في اوراق نبات الأغاف المزروع في العراق . كما اظهرت هذه الدراسة بان الطريقة الثانية للاستخلاص هي الافضل لكون كمية المستخلص ومحتواه من هذه المادة كان اعلى مماعطته الطريقة الاولى للاستخلاص.

الكلمات المفتاحية: نبات الأغاف،الموادالستيرويديةالصابونية، مادة التيكوجينين.

Introduction

Agave americana is succulent plants that are present in abundance in Swaziland especially in the Middleveld and Lowveld . Agave plant is a monocotyledon that refer to the Agavaceae family. It is a native of Mexico and other parts of tropical America. It is described by rigid, fleshy and hard-surfaced leaf grow directly out from the central stock to form a dense rosette. Common names include American aloe , maguey, or century plant [(1)] .

A.americana crude extract have been report to possess insecticidal and molluscicidal properties [(2)] and also have antisporulant activity versus *Sclerospora graminicola* [(3)]. The leaves of this plant possess angiotensin converting enzymes that are represent a powerful medicine for hypertension treatement. In addition, the leaves is a rich source of steroidal sapogenins like sarsasapogenin ,hecogenin and tigogenin

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which can be ustilized in the production of semisynthetic corticosteroids. Active constituent of *A. americana* such as tigogenin is used as starting material for the synthesis of other steroids [2] . Antibacterial activities of *A. americana* leaf extract is attributed to the presence of saponins [4]. Saponins have immune-stimulating activity , hemolytic, anti-inflammatory and expectorative. In addition, saponins displayed antimicrobial activity particularly against bacteria, fungi and protozoa. The antifungal activity of steroidal sapogenin has been demonstrated particularly versus agricultural pathogens [1] and other activities for this type of compounds include hypoglycemic, antitumor, immunoregulatory[5].

Figure (1):*Agave americana* plant cultivated in Iraq

Material and Methods

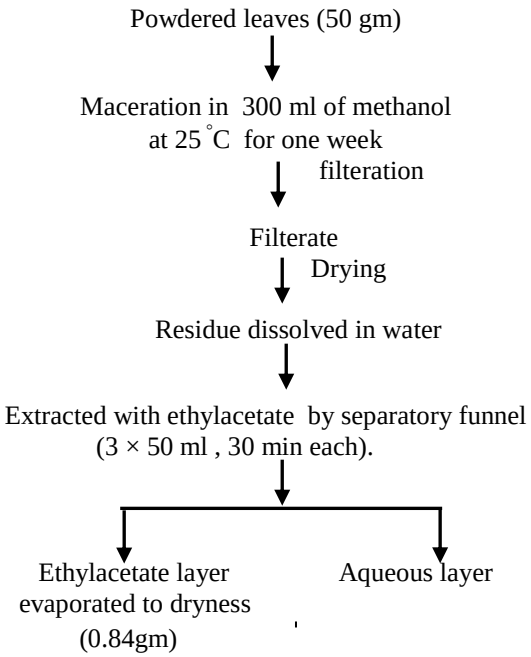
Plant materials

The leaves of *Agave americana* L. were collected from garden of College of Pharmacy of Baghdad University during November (2014). They were cleaned and dried in oven at a temperature between (30 – 40) for (16) hours then these plant materials were coarsely powdered by mechanical grinder and weighed. 50 gm of dried powdered plant materials were extracted by using two methods.

Extraction

Extraction method No.1 [6]:

50 gm of powdered leaves of *A. americana* L. were macerated with 300 ml of methanol 99.8% at 25 °c for one week. After evaporation of the methanol under vacuum, the extract is dissolved in 50 ml of H_2O and then extracted with ethyl acetate by separatory funnel (3 × 50 ml , 30 min each), the organic phase is evaporated to dryness under vacuum, and then subjected to identification as shown in figuer (2).

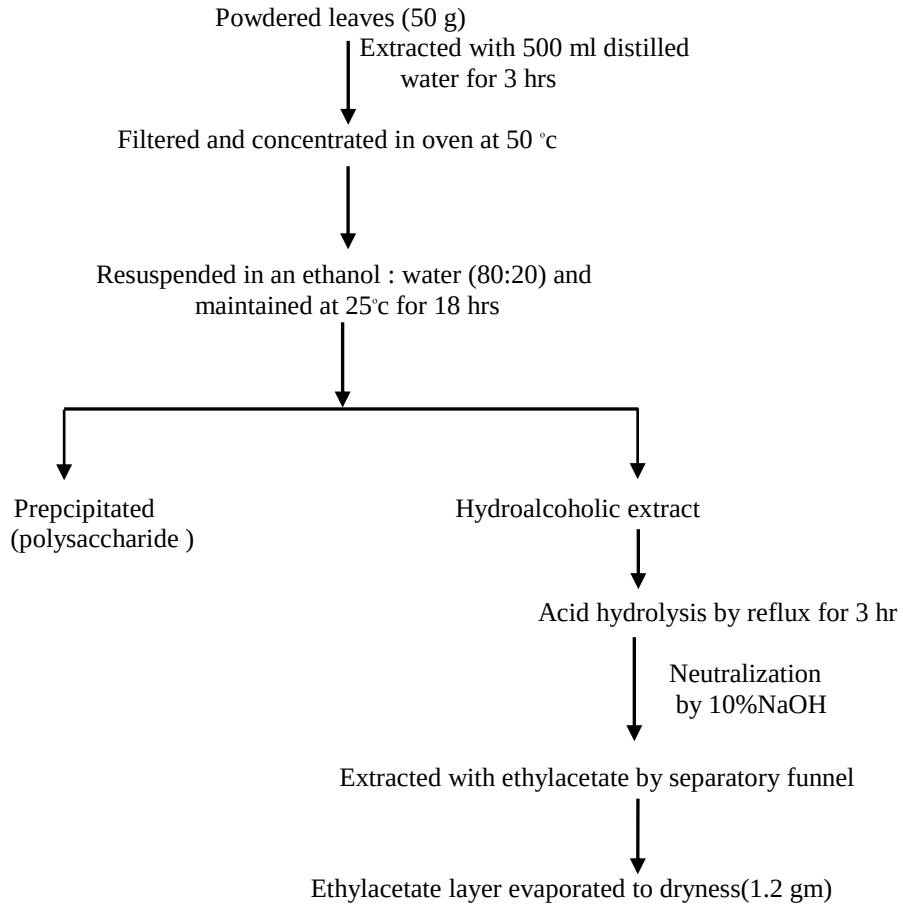


Figuer (2): General scheme for method No.1 for extraction of steroidal sapogenins from the leaves of *Agave americana L.*

Extraction method No.2 [7]

50 gm of dried powdered leaves was refluxed in water (500 ml) for 3 hrs.The aqueous extract is filtered then concentrated in oven at 50 °c then resuspended in an ethanol :water (80:20) solution and maintained at 25°c for 18 hrs to separated polysaccharides.Then the precipitated polysaccharides were separated by filteration and the hydroalcoholic extract was concentrated.

The hydroalcoholic extracts was subjected to a hydrolysis reaction under reflux for 3 hrs in a hydroethanol solution 80% (30 ml) containing 5 ml of Conc. HCl.The reaction product was neutralized with 10% NaOH and then extracted with ethylacetate to yield the sapogenins, after removal of the solvent using a rotary evaporator . The extract was evaporated to dryness under vacuum, and then subjected to identification , shown in figure (3).



Figure(3):General scheme for method No.2 for extraction of steroidal sapogenin from the leaves of *Agave americana* L.

Identification of the steroidal sapogenin "Tigogenin":

The preliminary identification of steroidal sapogenin (tigogenin)was performed by using:

1- Thin layer chromatography (TLC):

In this qualitative identification:- used a ready-made aluminum plates of silica gel GF_{254} and detection is done by Libermann – Burchard reagent [8] in comparison with three different developing solvent systems that were [9 ,10] :-

Solvent 1 (S1): hexan :ethylacetate (1:1)
Solvent 2 (S2): Chloroform:Methanol (95:5)
Solvent 3 (S3): benzene:Acetone (90:10).

2- Preperative high performance liquid chromatography (PHPLC):-

Qualitative estimations of Tigogenin component in crude extract obtained by extraction methods was carried out by using preperative high performance liquid chromatography (PHPLC). The qualitative estimations are performed by comparing retention time of Tigogenin component in the crude extracts with that of authentic standard at identical chromatographic conditions. HPLC analysis was done by using the following conditions[11] .

1. Mobile phase : Methanol 100%
2. Column:Phenomenex C18 250 mm x 4.6 mm, 5 µm particle size.
3. Column temperature: Ambient
4. Flow rate : 10 ml / min
5. Injection volume : 2 ml
6. Injection concentration: 1 mg /ml
7. Detection: UVDetector at λ 254 nm**.**

Isolation and purification of Tigogenin

Isolation and purification of Tigogenin was done by using the following steps:

1.Using preparative TLC plates

Isolation of Tigogenin compound is carried out using preparative TLC . The crude extracts obtained by extraction method No.2 was dissolved in ethylacetate then apply by using capillary tube in a row of spots as a concentrated solution five times on each plate (the spots must dry before other application) . The thickness of silica gel on the plate was 0.75 mm. The solvent system used was S3 (benzene : acetone (90:10)) .

The detection was done using Libermann – Burchard reagent in one side of the plate . The band is scraped out that corresponding to the tigogenin standard and put in a beaker, added chloroform: methanol (95:5) with stirring then left a side for hour, and filtered. After evaporation of the solvent, the final filtrate gives yellowish precipitate .

2.Purification of Tigogenin by recrystalization

The final solid product that has been isolated by preparative TLC was dissolved by heating insufficient quantity of methanol and decolorizing charcoal(small amount) is added , with stirring to the hot methanol solution until supernatant liquid is almost colorless. Then the hot solution is filtered and then the methanol is evaporate to obtain powder [(12)].

Results and Discussion

Extraction methods

Two methods of extraction of steroidal sapogenin Tigogenin were tried to select the best one. Results showed that the method No.2 was better, because the yield of crude extract was higher than obtained from method No.1. In addition quantitative estimation by using HPLC analysis showed that the amount of Tigogenin obtained by method No.2 was much more compared with that obtained by method No.1 as showed in table (1). So, we select method No. 2 as an extraction procedure in our work.

Table (1): Quantitative of crud extracts and Tigogenin obtained from extraction methods.

Extraction method	Yield of crude extract (g)	% yield of crude extract	% yield of Tigogenin
Method No.1	0.84gm	1.68%	0.23%
Method No.2	1.2gm	2.4%	0.43%

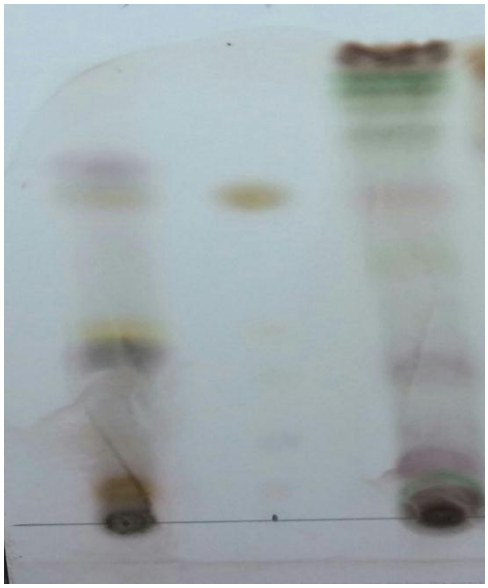
Identification of Tigogenin by TLC:

TLC for the crude extracts from *Agave americana* leaves obtained by using the extraction method No.1 and No.2, confirms the presence of Tigogenin in these extracts in comparison with tigogenin standard . As presented in table (2) and figures (4 ,5 and 6).

Table(2): Rf values of Tigogenin from *Agave americana* L. leaves extract obtained by extraction methods No.1 and No.2 and its standard in three different mobile phases in TLC.

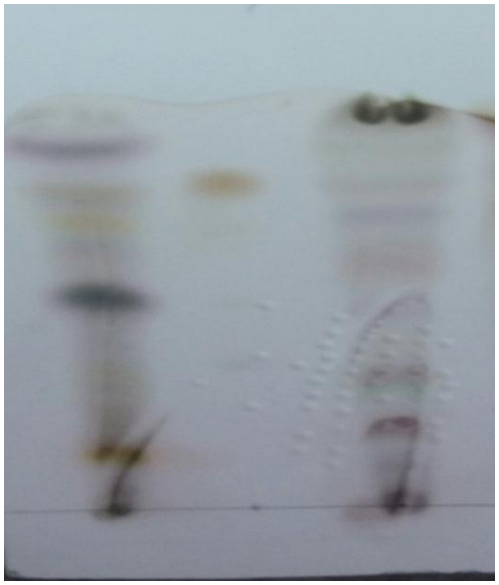
Solvent system	E1	E2	S
S1	0.671	0.685	0.671
S2	0.746	0.733	0.746
S3	0.446	0.43	0.43

S_1: hexan : ethylacetate (1:1)
S_2: Chloroform : Methanol (95:5)
S_3: benzene : Acetone (90:10)
E_1 :crude. extra ct of method No.1
E_2: crude extract of method No.2
S: tigogenin standard



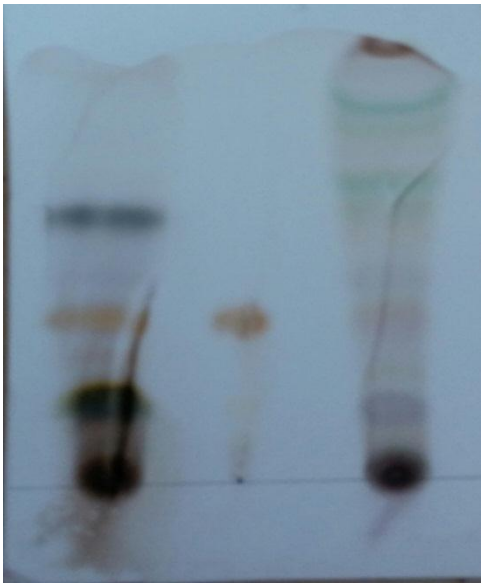
E2 T E1

Figure(4): TLC for *Agave americana* leaves extract obtained by extraction methods No.1 and No.2 using silica gel GF_{254} as adsorbent and (S_1) as solvent system . Visualization by Liebermann-Burcrhard spray reagent.
T: Tigogenin standard
E1: extraction method No.1
E2: extraction method No.2



E2 T E1

Figure (5) : TLC for *Agave americana* leaves extract obtained by extraction methods No.1 and No.2 using silica gel GF_{254} as adsorbent and (S_2) as solvent system . Visualization by Liebermann-Burcrhard spray reagent.
T: Tigogenin standard
E1: extraction method No.1
E2: extraction method No.2



E2 T E1

Figure (6): TLC for *Agave americana* leaves extract obtained by extraction method No.1 and No.2 using silica gel GF_{254} as adsorbent and (S_3) as solvent system . Visualization by Liebermann-Burcrhard spray reagent.
T: Tigogenin standard
E1: extraction method No.1
E2: extraction method No.2

Identification and Characterization of the isolated Tigogenin

1. Analytical TLC

Isolated compound (Tigognin) appeared as a single spot having the same color and Rf value to that of standard .

2. Melting point

The purified tigogenin is characterized from its sharp melting point of 200 - 202 ºC compared to standard tigogenin melting point 202-204 ºC

3. FTIR

The IR spectra of isolated Tigogenin was gave identical results with that of tigogenin standard, as showed in table (4) and figure (7).

4. HPLC analysis

The retention time for the isolated tigogenin was identical to the main peak of the standard reference as showed in figures (8 and 9) .

Table (4):): The most significant group frequencies from FT-IR spectrum of isolated tigogenin .

Functional group	Group frequency wave number (Cm^{-1})	Assignment
Free O-H	3533	Free O-H stretching of alcohol
O-H	Broad band (3431-3238)	Broad O-H stretching band indicate hydrogen bonding
C–H	2926,2848 2962,2875	Asymmetric and symmetric stretching of CH_2 and CH_3 groups
C-H	1456,1367	C-H bending of CH_2 and CH_3
C-O	1242-1041	C-O stretching of aliphatic ether

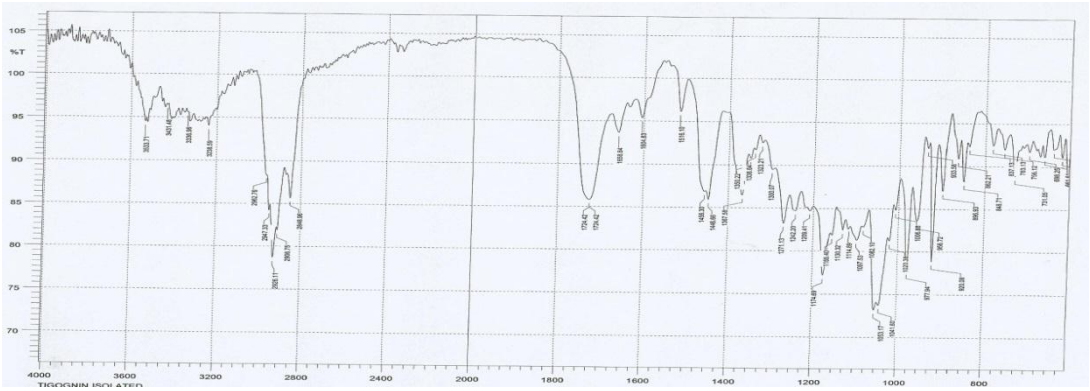


Figure (7): IR spectrum of isolated tigogenin

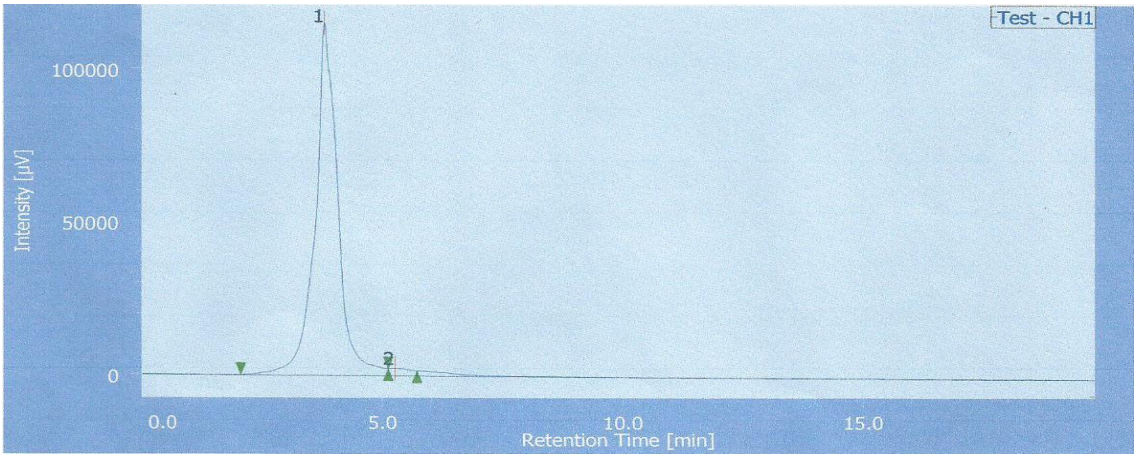


Figure (8):- HPLC analysis of isolated and purified Tigogenin.

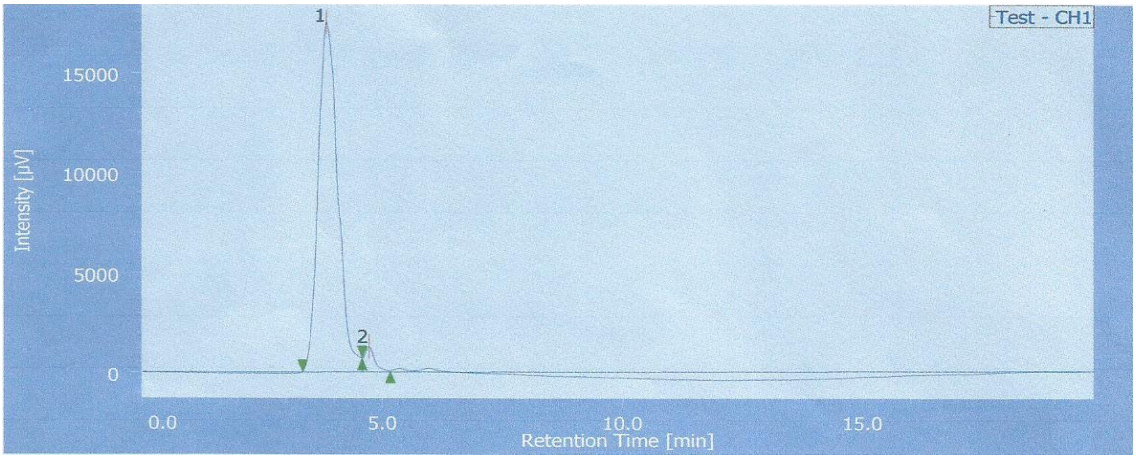


Figure (9) :- HPLC analysis of Tigogenin standard.

Conclusions

Phytochemical investigation of *Agave americana* leaves, cultivated in Iraq confirm the presence of "Tigogenin" steroidal sapogenin as potent medicinal natural product . Tigogenin was extracted by using two extraction methods, and identified by using TLC and HPLC method . Isolation and purification Tigogenin compound was made by using the following steps: preparative TLC plates, and purification by using charcoal material. The isolated Tigogenin is identified by using Thin layer chromatography , melting point , infrared spectroscopy and HPLC.

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