

## Phytochemical Study of Steroidal Saponin "Tigogenin" Present in the Leaves of *Yucca aloifolia* Cultivated in Iraq

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### Abstract

This study detects the presence of the most important steroidal saponin "Tigogenin" in the leaves of *Yucca aloifolia* widely cultivated in Iraq. 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 study concerned with extraction, identification, isolation, and purification of Tigogenin from the leaves of *Yucca aloifolia*. Extraction of this compound was carried out using two methods. Identification of this compound was done using thin layer chromatography (TLC) where different solvent systems had been tried. Libermann – Burchard reagent was used for detection. This identification was further augmented by using high performance liquid chromatography (HPLC) and then this steroidal saponin was isolated and purified. The identification of isolated Tigogenin was carried out using melting point (M.P.), Thin layer chromatography (TLC), infrared spectroscopy (IR) and High performance liquid chromatography (HPLC). This study confirms the presence of Tigogenin in the leaves of *Yucca aloifolia* cultivated in Iraq. Also the result of this study showed that the second extraction method was the best, because the amount of both extract and Tigogenin were higher than one extraction method.

**Key words :** *Yucca aloifolia* , Steroidal saponin , "Tigogenin"

### دراسة كيموأحيائية للستيرويد الصابوني التيكوجينين الموجود في اوراق نبات اليوكا

المزروع في العراق *Yucca aloifolia*  
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### الخلاصة

هذه الدراسة تختص بالكشف عن وجود اهم مادة ستيرويدية صابونية موجودة في اوراق نبات اليوكا 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 الكورتيزون والهورمونات الجنسية يبين قيمة هذه الدراسة . في هذه الدراسة تم استخلاص وكشف وفصل وتنقية مادة التيكوجينين الموجودة في اوراق نبات اليوكا . حيث تم استخلاص هذه المادة باستخدام طريقتين للفصل وتم الكشف عنها بوساطة طريقة كروماتوغرافيا الطبقة الرقيقة , باستخدام مذيبات مختلفة كوسيط ناقل والكشف عنها باستخدام كاشف ليبرمان – بيرجارد . وكذلك بوساطة تقنية كروماتوغرافيا الاداء العالي السائل وبعدها تمت عملية الفصل والتنقية . وقد استخدمت مجموعة من التقنيات للتحقيق من نوعية المركب المفصول ودرجة نقاوته والتي شملت : درجة الانصهار , كروماتوغرافيا الطبقة الرقيقة , مطياف الاشعة تحت الحمراء وكذلك تقنية كروماتوغرافيا الاداء العالي السائل . اثبتت هذه الدراسة وجود مادة التيكوجينين في اوراق نبات اليوكا المزروع في العراق . كما اظهرت هذه الدراسة بان الطريقة الثانية للاستخلاص هي الافضل لكون كمية المستخلص ومحتواه من هذه المادة كان اعلى مما عطته الطريقة الاولى للاستخلاص .

الكلمات المفتاحية: نبات اليوكا، الموادستيرويديةالصابونية، مادة التيكوجينين.

### Introduction

*Yucca aloifolia* is a shrub or tree – like plant of the Agavaceae family<sup>(1)</sup>, native to the deserts of the south – western United States and northern Mexico<sup>(2)</sup> (Figure 1). This plant contains several physiologically active phytochemicals. It is a rich source of steroidal saponin like sarsasapogenin and Tigogenin and is used commercially as a steroidal saponin source<sup>(3)</sup>. These saponin are important starting materials for the semi

synthesis of steroidal drugs especially the cortisone compound and steroidal hormones<sup>(4)</sup>. The saponins from *Yucca* are the main medicinal agents in the plant that elevate the body's production of cortisone possibly the herb's ability to aid in arthritic pains<sup>(5)</sup>. Saponins also provide anti – inflammatory relief as well as the ability to break up inorganic mineral obstructions and deposit<sup>(6)</sup>.

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*Yucca* also has laxative properties and is also used to establish a flora balance in the GI tract. It is also speculated that yucca saponin block release of toxin from the intestines, which inhibit normal formation of cartilage<sup>(7)</sup>. Both leaves and roots, function well as diuretic and emetics and has hair strengthening<sup>(8)</sup>.



Figure (1): *Yucca aloifolia* plant

## Material and Methods

### Plant materials

The plant materials (leaves) of *Yucca aloifolia* L. were collected from private garden during the months of September and October (2010), they were cleaned and dried in oven at a temperature between (30 – 40)° C for (4 – 5) hours then these plant materials were coarsely powdered by mechanical grinder and weighed. A 50 gm of dried powdered plant materials were extracted by using two methods.

### Extraction

#### Extraction method No 1<sup>(9)</sup>:

A 50 gm of dried powdered plant materials (leaves) were extracted in a soxhlet apparatus with 500ml dichloroethane for 20 – 24 hours. After that the plant material was dried, then refluxed with 500ml of 4N H<sub>2</sub>SO<sub>4</sub> for 3 hours. After cooling the mixture was filtered, the residue on the filter was washed with water, neutralized with 250ml 5% sodium bicarbonate solution to pH 7.5 and dried at 100 – 105°C. the dry hydrolysate ( the plant material ) was extracted with petroleum ether (b.p 60 – 80°C) in soxhlet apparatus for 10 hours. The extract was condensed to small volume under vacuum and then subjected to identification (Figure 2).

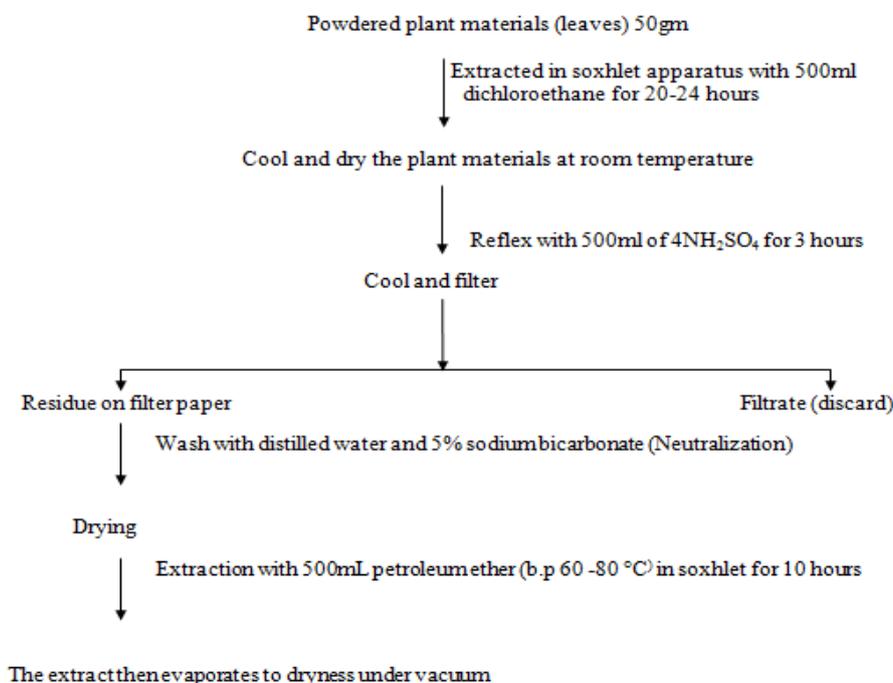
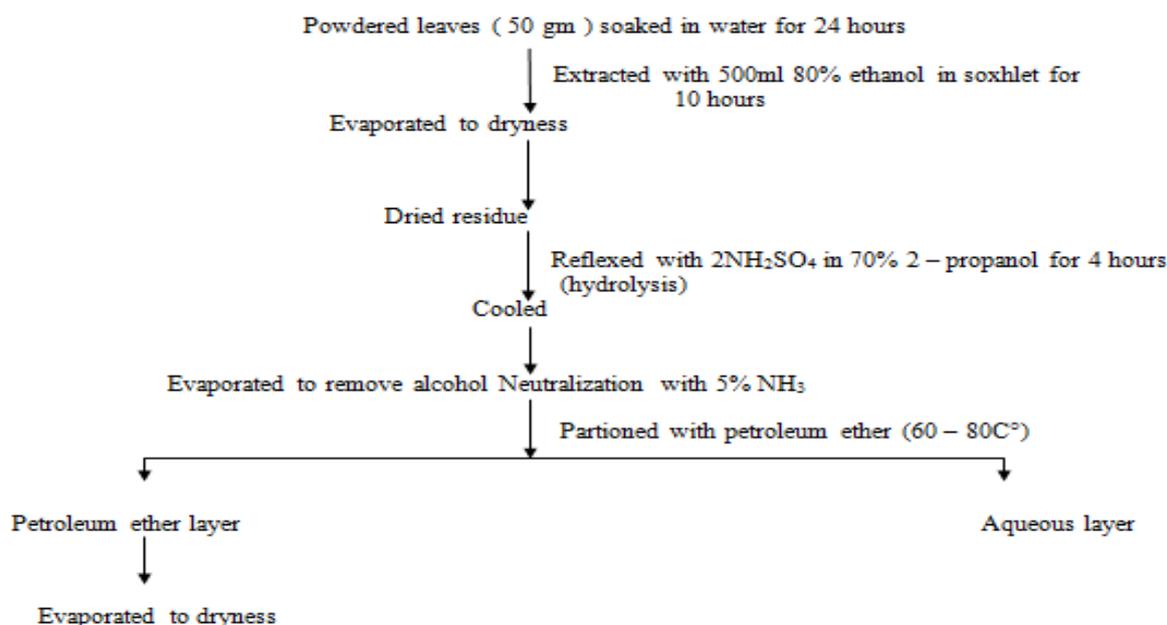


Figure (2): General scheme for method No.1 for extraction of steroidal sapogenin tigogenin from the leaves of *Yucca aloifolia*.

**Extraction method No.2<sup>(10)</sup>:**

A 50gm of dried plant material (leaves) was soaked in water for 24 hours and then extracted with 80% ethanol (500ml) in soxhlet extractor for 10 hours. Then the residue was evaporated to dryness under vacuum. The dried residue was refluxed with 2N H<sub>2</sub>SO<sub>4</sub> in water containing 70% 2 – propanol (500ml) at 100°C for 4 hours. The mixture was cooled

and evaporated under vacuum to remove the alcohol completely, and then the mixture was neutralized with 5% NH<sub>3</sub> then partitioned with equal volume of petroleum ether (b.p 60 – 80°C) using separatory funnel to give two layers, aqueous layer and the petroleum ether layer. The petroleum ether layer was taken and evaporated to dryness under vacuum and then subjected to identification (Figure 3).



**Figure (3):** General scheme for method No.2 for extraction of steroidal “sapogenin tigogenin” from the leaves of *Yucca aloifolia*.

**Identification of the steroidal sapogenin “Tigogenin”:**

C.1: Thin layer chromatography (TLC):

In this qualitative identification:- using a ready-made aluminum plates of silica gel GF254, one detection method by using Libermann – Burchard reagen<sup>(11)</sup> in comparison with three different developing solvent systems that were<sup>(12-13)</sup>:-

Solvent 1 (S1):

Chloroform:Methanol (95:5)

Solvent 2 (S2):

Chloroform:Petroleum ether: Methanol (85:10:5)

Solvent 3 (S3):

Chloroform:Acetone (80:20)

C.2: High performance liquid chromatography (HPLC):-

Qualitative and quantitative estimations of Tigogenin component in the crude extract obtained by extraction methods was carried out by using high performance liquid chromatography (HPLC). The identifications were made by comparison of retention time of

Tigogenin component in the crud extracts with that of authentic standard at identical chromatographic conditions. HPLC analysis was done by using the following conditions:

- 1) Mobile Phase: Acetonitrile 100%
  - 2) Column: Phenomenex ODS 250mmX4.6mm, 5µm particle size.
  - 3) Column temperature: Ambient
  - 4) Flow rate: 1ml/min
  - 5) Injection volume: 5µL
  - 6) Injection concentration: 1mg/ml
- Detection: UV Detector at λ 209nm.

**Isolation and purification of Tigogenin:**

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

**1: Fractionation by column chromatography:**

The final residue obtained from extraction method No2 (best method) was subjected to column chromatography using glass column (80cm X 5cm) packed with silica gel (0.063 – 0.200 mm) slurry in (250 ml) CHCl<sub>3</sub>, in a ratio of 20gm of silica gel to each 1gm of the residue. A dry loading of the sample (residue) was used by dissolving it in small volume of

Chloroform and adsorbing it on small amount of silica gel of the same grade used for packing the column, then dried, grinded and applied to the top of column in order to prevent clogging. The column was eluted by gradient elution technique using  $\text{CHCl}_3$ : methanol with an increasing percentage of methanol from zero to 100% (the ratios of  $\text{CHCl}_3$ : methanol used were 100:0, 95: 5, 90:10, 85:15, 80:20, 70:30, 60:40, 50:50, 40:60, 25:75 and  $\text{CHCl}_3$ : methanol 0:100). The column developed by adding 50 ml of each eluent with collecting 5ml fractions, then monitored by TLC with solvent system (S1): Chloroform: Methanol (95:5). A total number of 100 fractions were obtained. Those consecutive fractions, which have the same number of spots with the same  $R_f$  values, were combined and concentrated to dryness to get major fractions. After that, the major fractions were subjected to thin layer chromatography with tigogenin reference standard and solvent system S1 Chloroform : Methanol (95:5). the results showed that the tigogenin compound was found in the major fraction No.5.

### 2: Using preparative TLC plates:

Isolation of Tigogenin compound is carried out using preparative TLC which was performed by using readymade plates of silica gel GF254 ( 20 x 20cm ) of 1mm thickness (Merck). The major fraction (F 5) residue obtained from column chromatography applied as a concentrated solution in a row of spots using capillary tube four times on each plate (the spots should dry before the next application). The solvent system used was S1 (Chloroform : Methanol (95:5)) . The detection was done using Libermann – Burchard reagent in one side of the plate . The band corresponding to the tigogenin standard was scraped out and collected in a beaker, mixed with chloroform: methanol (95:5), stirred and left a side for one hour, then filtered. After evaporation of the solvent, the final filtrate gives yellowish precipitate .

### 3.Purification of Tigogenin by using charcoal material:

The final solid product that has been isolated by preparative TLC was dissolved by heating insufficient quantity of methanol and a small amount of decolorizing charcoal was added, with stirring to the hot methanol solution until the supernatant liquid was almost colorless. Then the hot solution was poured through filter paper into another flask. Then the solvent was evaporated to give solid product <sup>(14)</sup>.

### Identification and Characterization of the Isolated steroidal sapogenin Tigogenin:

**1. TLC:** Analytical TLC was performed by using ready made plate. The purified sapogenin Tigogenin was applied on silica gel plate as one spot by using capillary tube along with its standard, using the solvent system (S1) Chloroform : Methanol (95:5) . The detection was done by using Liebermann – burchard reagent .

**2. Melting point:** The melting point of the purified sapogenin Tigogenin was done and compared with that of the available standard tigogenin.

**3. FTIR:** Infrared spectra were carried out by using KBr disc for both purified sapogenin Tigogenin and its standard.

**4. HPLC analysis:** HPLC analysis was made by comparison of retention times obtained at identical chromatographic conditions of analyzed purified sapogenin Tigogenin and its standard. HPLC conditions as mentioned previously

## 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 methods	Crud extract (mg)	Tigogenin %
Method No.1	2.9	3.86
Method No. 2	3.5	5

### Identification of Tigogenin by TLC:

TLC of the crud extracts obtained from the leaves of *Yucca aloifolia* 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).

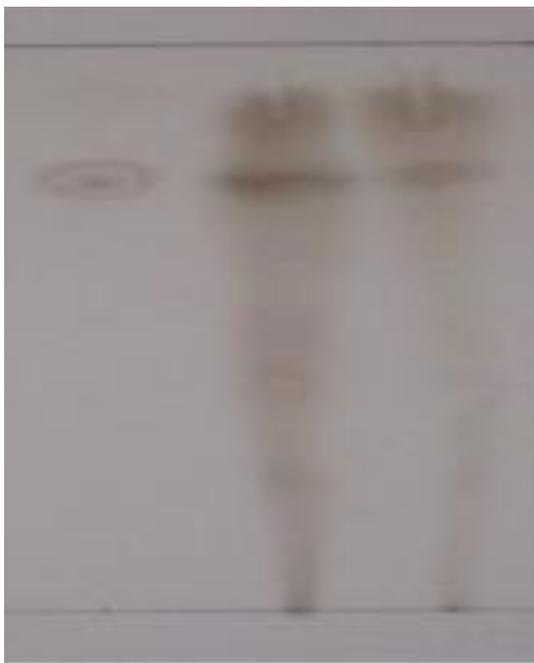
**Table(2): R<sub>f</sub> values of Tigogenin from *Yucca aloifolia* leaves extract obtained by extraction methods No.1 and No.2 and its standard in different developing solvent systems in TLC**

Solvent system	S1	S2	S3
R <sub>f</sub> value of standard Tigogenin	0.55	0.74	0.80
R <sub>f</sub> value of Tigogenin in crud extract of method No1	0.54	0.75	0.80
R <sub>f</sub> value of Tigogenin in crud extract of method No2	0.56	0.78	0.81

S1: Chloroform:Methanol (95:5)

S2: Chloroform:Petroleum ether: Methanol (85:10:5)

S3: Chloroform:Acetone (80:20)



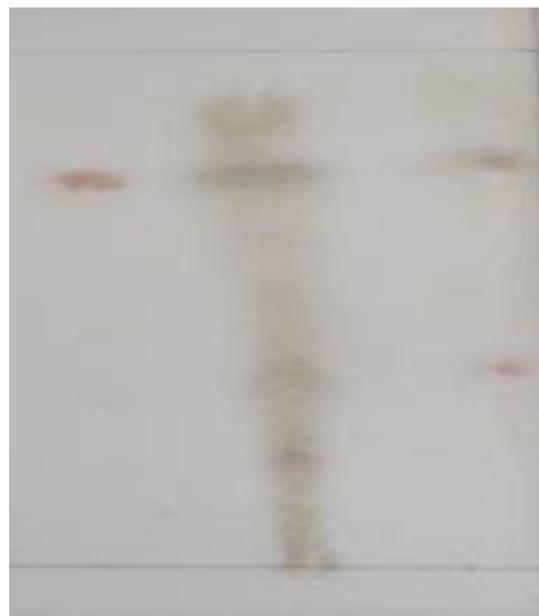
T E1 E2

**Figure(4): TLC for *Yucca aloifolia* leaves extract obtained by extraction methods No.1 and No.2 using silica gel GF 254 as adsorbent and (S1) as solvent system . Visualization by Liebermann-Burcrhard spray reagent.**

**T: Tigogenin standard**

**E1: extraction method No.1**

**E2: extraction method No. 2**



T E1 E2

**Figure (5) : TLC for *Yucca aloifolia* leaves extract obtained by extraction methods No.1 and No.2 using silica gel GF 254as adsorbent and (S2) as solvent system . Visualization by Liebermann-Burcrhard spray reagent.**

**T: Tigogenin standard**

**E1: extraction method No.1**

**E1: extraction method No. 2**



T E1 E2

**Figure (6): TLC for *Yucca aloifolia* leaves extract obtained by extraction method No.1 and No.2 using silica gel G<sub>254</sub> as adsorbent and (S3) as solvent system . Visualization by Liebermann-Burcrhard spray reagent.**

**T: Tigogenin standard**

**E1: extraction method No.1**

**E2: extraction method No.2**

**Isolation and purification of Tigogenin;**

One hundred fraction obtained from column chromatography were monitored by TLC. The Consecutive fractions that have the same number of spots of the same  $R_f$  values were combined to get 8 major fractions, which were concentrated to dryness and subjected to identification, as listed in table (3). In the first 70 fractions, fraction 1-10 gave one spot in TLC and were collected to give fraction one (F1). Fraction (11-20) gave one spot were collected to give fraction two (F2), while fraction 21-30 gave One spot and were collected to give fraction three (F3), fraction 31-40 gave one spot and were collected to give the fraction four(F4). Fraction 41-52 gave two spots and were collected to give the major fraction five (F5). Fraction 53- 54 gave one spot and were collected to give the fraction six (F6). Fraction 55-57 gave two spots and were collected to give the fraction seven (F7). Fraction 58-70 gave the fraction eight (F8). In the last 30 fractions no spots were appear in TLC examination. The results showed that the tigogenin is found in the fraction five (F5) therefore we selected this fraction to isolation this compound.

**Table (3): Major fractions obtained from column chromatography**

Major Fractions	No. of collections 5 ml each	No. of spots
F1	1-10	1
F2	11-20	1
F3	21-30	1
F4	31-40	1
F5	41-52	2
F6	54-53	1
F7	55-57	2
F8	58-70	4
F9	70-100	Negative

**Identification and Characterization of the isolated Tigogenin****1. Analytical TLC**

Isolated compound ( Tigognin) appeared as a single spot having the  $R_f$  value as that of reference standard.

**2. Measuring melting point**

The isolated tigogenin was identified from its sharp melting point of 200 - 202 °C compared to standard tigogenin melting point 202-204 °C.

**3. FTIR**

The IR spectra of isolated Tigogenin was gave identical results with that of tigogenin standard, as showed in table (4) and figures(7 and 8).

**Table (4): The characteristic IR absorption bands (in  $\text{cm}^{-1}$ ) of the isolated tigogenin in comparison with that of tigogenin as reference standard (120)**

Functional group	Isolated tigogenin	Tigogenin standard	Assignment
Free O-H	3524	3522	Free O-H stretching of alcohol
O-H	Broad band (3390-3263)	Broad band (3389-3269)	Broad O-H stretching band indicate hydrogen bonding
C-H	2929,2848	2941,2874	Asymmetric and symmetric stretching of CH <sub>3</sub> and CH <sub>2</sub> groups
C-H	1456,1373	1456,1373	C-H bending of CH <sub>2</sub> and CH <sub>3</sub>
C-O	1242-1049	1242-1049	C-O stretching of aliphatic ether

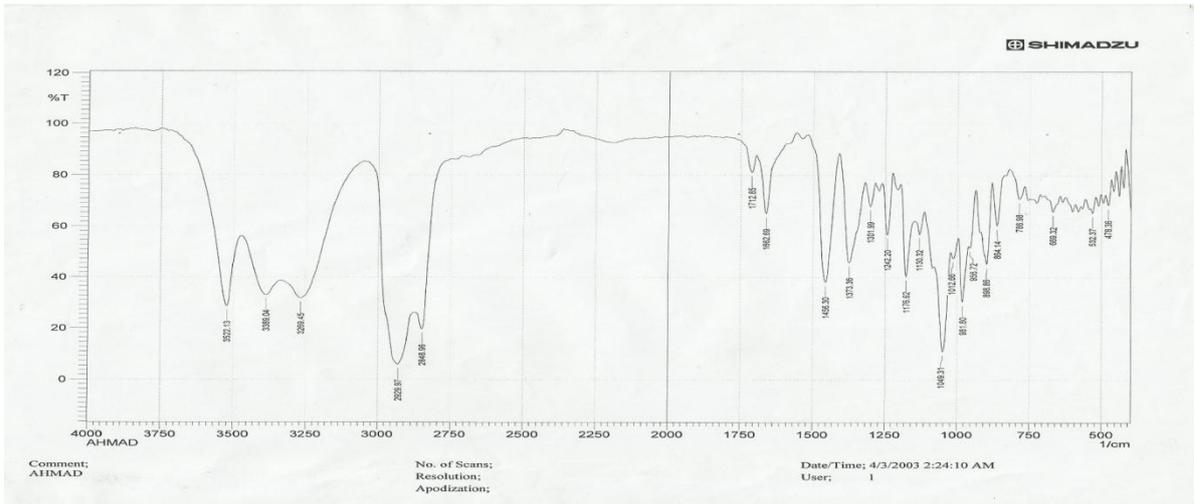


Figure (7): IR spectrum of tigogenin standard

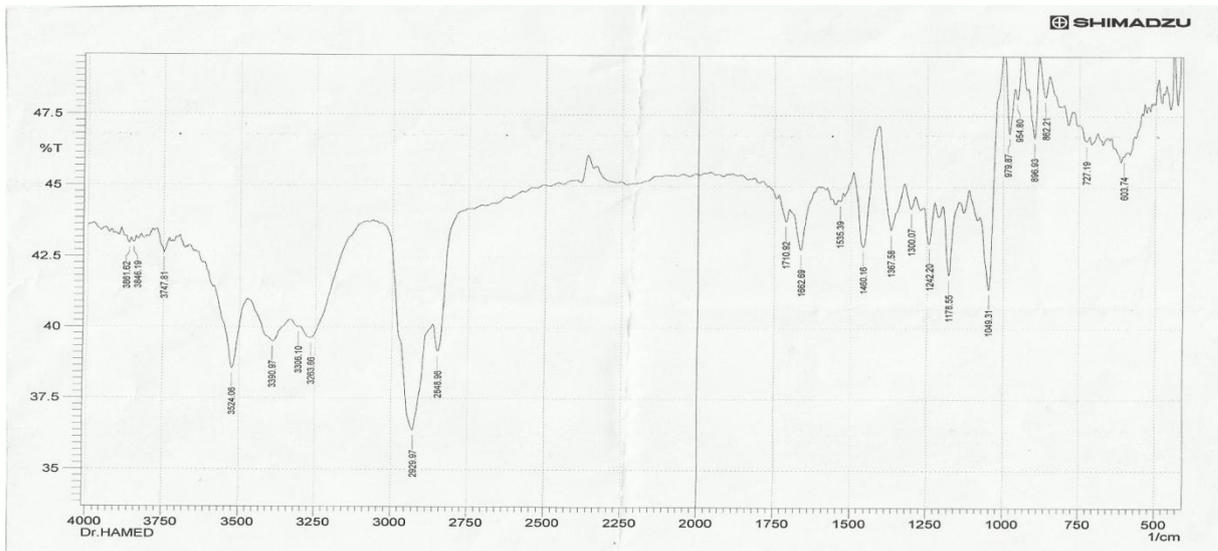


Figure (8): IR spectrum of isolated and purified tigogenin

4. HPLC analysis

The retention time for the isolated tigogenin was identical to the main peak of the standard reference as showed in figures ( 9 and 10 ).

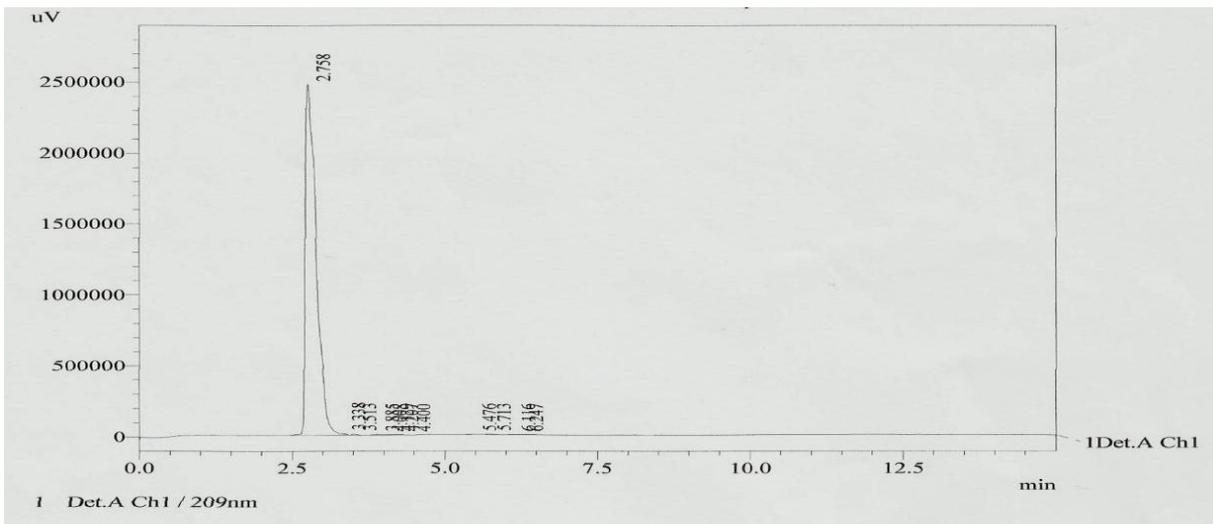


Figure (9):- HPLC analysis of isolated and purified Tigogenin.

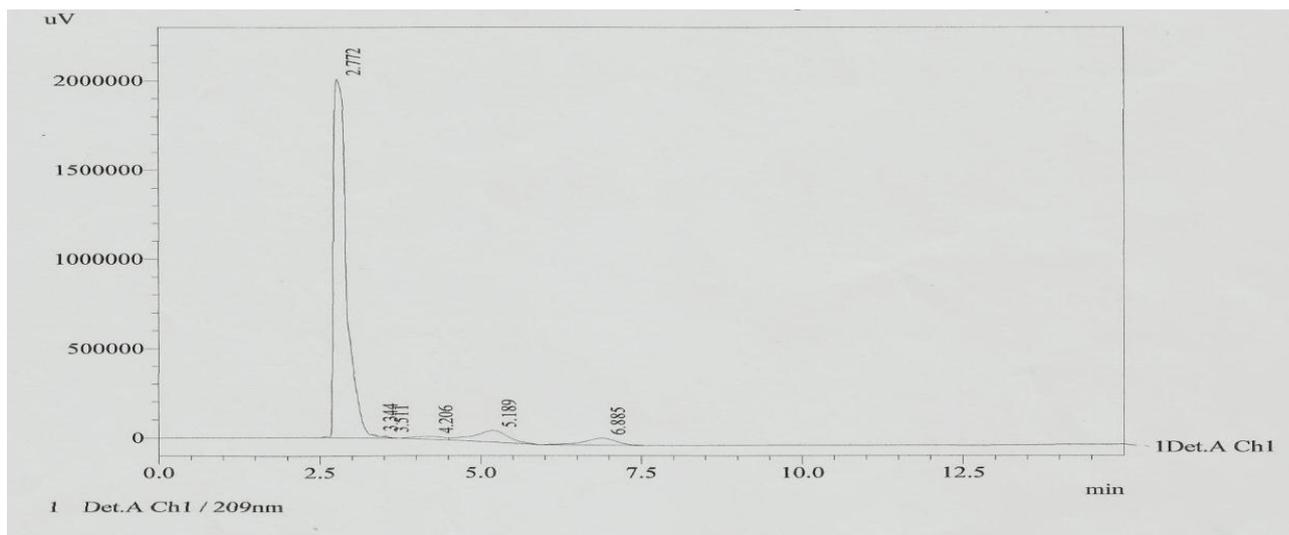


Figure (10) :- HPLC analysis of Tigogenin standard.

### Conclusions:

Phytochemical investigation of *Yucca aloifolia* leaves, cultivated in Iraq revealed the presence of important medicinal natural product “Tigogenin” belong to steroidal saponin. 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: Fractionation by column chromatography, preparative TLC plates, and purification by using charcoal material. The identification of isolated Tigogenin was carried out using melting point, Thin layer chromatography, infrared spectroscopy and HPLC.

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