

Isolation and Characterization of Iridoid Glycoside (Gardenoside) Present in the Leaves of *Gardenia jasminoides* J.Ellis Cultivated in Iraq

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Abstract

Iridoid glycosides are a group of naturally occurring chemical compounds. They are a large family of compounds biosynthesized by plants, they often have pharmacological effects. The aim of this study is to isolate and identified iridoid glycoside in a newly studied, cultivated in Iraq named *Gardenia jasminoides*. The medicinal importance of iridoid glycoside, on one hand and absence of phytochemical investigation on leaves of *Gardenia* on the other hand, acquired this study its importance. Many compounds were isolated from leaves plant part one of these compounds was identified by different chemical analysis like: melting point (MP), thin layer chromatography (TLC), Fourier transforms infrared spectra (FTIR) and high performance liquid chromatography (HPLC).

Keywords: *Gardenia jasminoides* , Gardenoside, Geniposide, Genipin.

فصل وتوصيف الأريديويد كلايكوسايد (الكاردينوسايد) الموجود في أوراق نبات الكاردينيا المستزرع في العراق

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الخلاصة

الأريديويد كلايكوسايد هي مجموعة من المركبات التي تصنع من قبل الكثير من النباتات وتلعب دورا مهما في معالجة وتنظيم الكثير من الأمراض. ان الهدف من هذه الدراسة هو فصل وتشخيص الكلايكوسايدات الموجودة في النبات المستزرع في العراق اسمه (*Gardenia*).

وللاهمية الطبية للأريديويد كلايكوسايد وعدم وجود دراسته علميه تتناول المكونات الكيميائية لأوراق نبات الكاردينيا المستزرع في العراق، اكتسبت هذه الدراسة أهميتها حيث تم فصل أكثر من مركب من الأريديويد كلايكوسايد (الكاردينوسايد والجينيبيوسايد) من أوراق النبات والتعرف على التركيب الكيميائي لواحد منها بواسطة قياس (M.P, TLC, FTIR and HPLC).
الكلمات المفتاحية : الكاردينيا ، الكاردينوسايد ، الجينيبيوسايد ، جنين.

Introduction

Rubiaceae are an easily recognizable family characterized by opposite leaves that are simple and entire, with interpetiolar stipules, tubular sympetalous corollas and an inferior ovary⁽¹⁾. Exceptionally, there are some plants that have only a single leaf at each node, alternating from one side to the other. In these cases, the alternate leaf arrangement is produced through the suppression of one leaf at each node⁽²⁾. A wide variety of growth forms are present in the Rubiaceae. While shrubs are most common, members of the family can also be trees, lianas or herbs. The flowers, which are usually bisexual, have a 4–5 lobed calyx and generally a 4–5 lobed corolla, 4 or 5 stamens and two carpels⁽²⁾.

Gardenia was named by Linnaeus after Dr. Alexander Garden (1730–1791), a Scottish physician who immigrated to South Carolina and corresponded with Linnaeus about American plants; *G. jasminoides* is botanical Latin for 'jasmine-like'. *Gardenias* are evergreen shrubs and small trees growing 1–5 m tall. The leaves are opposite or in whorls of

3 or 4 cm long and 3–25 cm broad, dark green and glossy with a leathery texture⁽³⁾. The flowers are solitary or in small clusters, white, or pale yellow, with a tubular-based corolla with 5–12 petals from 5–12 cm in diameter. Many species are strongly scented. The flowers are produced on or at the ends of branches⁽⁴⁾. Cultivated forms often have double rose-like flowers that open from large buds with a distinctive whorl of petals. Fleshy or leathery berries then follow. *Gardenias* will persist in a wide range of conditions, but if they are not perfectly content, they will tend to look quite awful. They seem to do best in a protected corner of the garden with some morning sun – they don't like the full glare of the afternoon sun⁽⁴⁾. There are over 200 species of *gardenias*, but most of them are hybrid varieties. Apart from *G. jasminoides*, the other most common types of *gardenia* are⁽⁵⁾:

- *Gardenia augusta*;
- *Gardenia thunbergia*, also known as Star *Gardenia*; this can be a shrub or a small tree and grows to about 1.2–1.5 m tall;

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- *Gardenia nitida*, a sturdy plant that can reach almost 1.5 m when taken care of properly and well maintained;
- *Gardenia radicans*, a dwarf variety that grows to about 45 cm, and produces double blooms.

Gardenias originated in China and Japan, and are now found in Africa, Asia and Australasia. They are attractive landscape subjects in warm climates, and make good container plants. The flowers of some species are used to perfume tea, and others are used to treat influenza and colds in modern Chinese herbalism. A yellow dye was made from the fruits⁽⁵⁾.

Gardenias tend to leach trace elements from the soil. Patterning yellow of the leaves may indicate manganese or magnesium deficiencies, and these can be corrected by the addition of appropriate trace elements, or using an enriched fertilizer; but it is quite usual for the lower leaves of healthy plants to turn yellow and fall off as new growth is made at the head of the branches. The root system is shallow and sensitive, so a thick layer of mulch to control weeds is better than cultivating⁽⁵⁾.

G. jasminoides is a smooth, unarmed shrub 1 to 2 meters high. Leaves are opposite, elliptic-ovate, 2 to 6 centimeters long, narrowed and pointed at both ends, shining and short petioled, and stipulate. Flowers are large and very fragrant, occurring singly in the upper axil of the leaves. Calyx is green, with funnel-shaped tube and about 1.5 centimeters long, 5-angled, or winged and divided into linear lobes about as long as the tube⁽⁶⁾. Corolla is usually double, white but soon turning yellowish, and 5 to 8 centimeters wide. Stamens are as many as the corolla lobes. Anthers are linear, sessile. Ovary is 1-celled; style stout, clavate, fusiform, or 2-cleft, ovules numerous on parietal placentas. Fruits are ovoid or ellipsoid, 2.5 to 4.5 centimeters long, 1.5 to 2 centimeters in diameter, yellow, with 5 to 9 longitudinal ridges⁽⁷⁾, as show in figure (1).



Figure (1):- Photo of *Gardenia jasminoides*

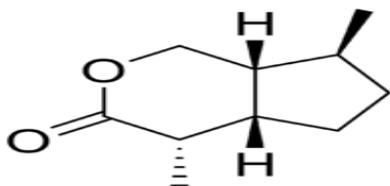
G. jasminoides is an evergreen flowering plant originated in Asia. It is most commonly found growing wild in Vietnam, Southern China, Taiwan, Japan, Myanmar and India distributed in broad-leaved forests at low to medium elevations⁽⁸⁾. With its shiny green leaves and heavily fragrant white summer flowers, it is widely used in gardens in warm temperate and subtropical climates and as a houseplant in temperate regions⁽⁹⁾.

- Roots used for fever with delirium.
- Decoction of roots used for flatulence, dyspepsia, and nervous disorders due to dentition.
- Decoction of leaves and flowers used for dyspepsia, flatulences, nervous disorders and abdominal pains.
- Decoction of bark used for menorrhagia and uterine problems.
- Decoction of flowers used as wash for inflamed eyes.
- Poultice of leaves for swollen breasts; may be mixed with violeta and other herbs.
- Antioxidant / Crocin: - Crocin is a water soluble carotenoid found in the fruits of gardenia (*Gardenia jasminoides*) and seems to possess moderately strong antioxidant activity⁽¹⁰⁾.
- Diabetes / Genipin:- Study discovered "genipin" from the Gardenia extract. Genipin blocks the UCP2 enzyme (uncoupling protein 2) that inhibits pancreatic insulin secretion. It suggests a potential for genipin-related compounds⁽¹¹⁾.
- Antiangiogenic Activity: The n-butanol fraction of the ethanol extract of gardenia fruit was found to be most effective in the anti angiogenic assay⁽¹²⁾.
- Anti-Cerulein Pancreatitis Protective Activity: A Study showed that *Gardenia jasminoides* pretreatment ameliorated the severity of cerulein-induced acute pancreatitis in rats⁽¹³⁾.
- Sandostatin and Gardenia Combo / Pancreatitis: Study showed a combination of sandostatin and *Gardenia jasminoides* can protect pancreatic mitochondria injury in severe acute pancreatitis⁽¹⁴⁾.

Iridoids Glycosides

Iridoids are a class of secondary metabolites found in a wide variety of plants and in some animals. They are monoterpenes biosynthesized from isoprene and they are often intermediates in the biosynthesis of alkaloids. Chemically, the iridoids usually consist of acyclopentane ring fused to a six-membered oxygen heterocycle. The chemical structure is exemplified by iridomyrmecin, a defensive chemical produced by

the *Iridomyrmex* genus, for which iridoids are named as show in Figure (1-3).



Chemical structure of iridomyrmecin

Figure (2):- The basic structure of Iridoid (a glycon of glycoside).

Materials and methods

Plant materials

The leaves of *Gardenia jasminoides* plant Family (*Rubiaceae*) was collected from the

garden of college of pharmacy, Baghdad University during the November, March and April (2013-2014). The plant leaves were cleaned and dried in oven at a temperature (40-50 °C) for (15-20) mints then these leaves were coarsely powered by mechanical grinder and weight.

Extraction methods of Iridoid glycosides:- Extraction method NO.1⁽¹⁵⁾

A 20gm of the dried powdered leaves of *Gardenia jasminoides* was extracted with three times by reflex with volume (200ml) of 50% Ethanol for three hours. After filtration the extract was combined and evaporated to dryness by rotary evaporator at 60 °C and then subjected to identification, as shown in figure (3):-

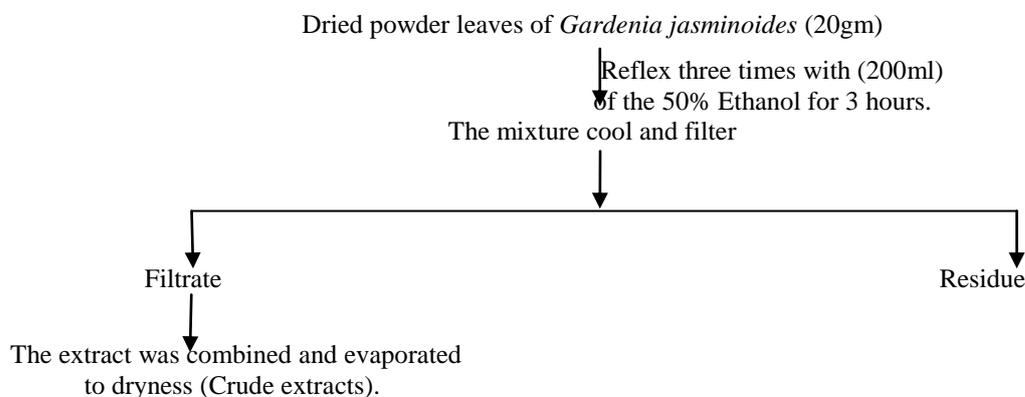


Figure (3):- General scheme for method NO.1 for extraction Iridoid glycosides from the leaves of *Gardenia jasminoides*⁽¹⁵⁾.

Extraction method NO.2⁽¹⁶⁾

A 20gm of the dried powdered leaves of *Gardenia jasminoides* was extracted (maceration) with (200ml) of the 90% Ethanol for (3-4) days at room temperature, after filtration off the solid parts and evaporation the

green solution. The residue is partitioned with water (50ml) and ether (250ml) and separated in a funnel. The aqueous extract was evaporated dryness and then subjected to identification, as shown in figure (4):-

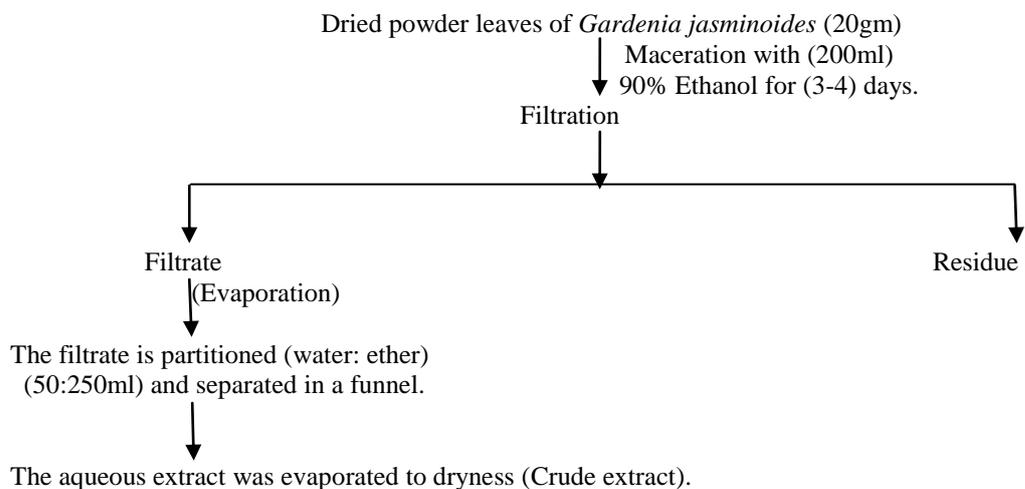


Figure (4):- General scheme for method NO.2 for extraction Iridoid glycosides from the leaves of *Gardenia jasminoides*⁽¹⁶⁾.

Preliminary identification of Iridoid glycosides

The preliminary identification of Iridoid glycosides of crude extracts of powdered leaves obtained from the extraction method NO.1 in result were performed by using thin Layer chromatography (TLC) was carried out using the following requirements. Ready made plates of silica gel GF 254 (20×20 cm) of 0.25 mm thickness (MERCK) were used, and then the plates were activated at 110 °C for 10 min. before used. Volume of 100 ml of solvent system was placed in a glass tank (22.5 cm × 22 cm × 7cm), and covered with glass lid and allowed to stand for 45 min. for saturation before use different solvent systems were used for development of Iridoid glycosides (gardenoside) ⁽¹⁷⁻¹⁹⁾.

S1= Water: Acetic Acid: N-Butanol
(50:10:40).

S2= Water: N-Propanol: N-Butanol
(60:20:40).

S3= Water: Ethanol: N-Butanol (20:20:80).

S4= Water: N-Butanol (50:50).

Reagent used for detection:-

- Liebermann-Burchard reagentis used in this study and prepared as follow ⁽²⁰⁾ add carefully 5 ml of acetic anhydride and 5 ml of concentrated sulfuric acid in to 50 ml of absolute ethanol, while cooling in ice. Spray the developed plate and heat it at 100 °C for 5–10 mints.

- Vanillin-Sulphuric acid reagent (VS)

Solution I: - 5% Ethanolic Sulphuric acid.

Solution II: - 1% Ethanolic Vanillin.

The plate is sprayed vigorously with (10 ml) of the (Solution I). Followed immediately by (5-10 ml) of the (Solution II), after heating at 110 °C for (5-10 min) under observation.

Isolation and purification of Gardenoside

The dry crude extract obtained from extraction method NO.1 of iridoid glycoside was used for isolation and purification of gardenoside and performed as following:

Fractionation by column chromatography

The final residue obtained from the leaves by extraction method NO.1 was subjected to column chromatography by using glass column (100 cm x 5 cm) packed with silica gel (0.063-0.200 mm) slurry in (250ml) chloroform, in a ratio of 20 gm of silica gel to each 1 gm of the residue. A dry loading of the sample (residue) was used by dissolving it in small volume of methanol 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 column in order to prevent clogging. The column was eluted by gradient elution technique using (chloroform: methanol) with an increasing gradually

percentage of methanol from zero to 100% and the ratios of (chloroform: methanol) was used (100:0, 90:10, 85:15, 80:20, 70:30, 60:40 and so on till chloroform: methanol 0:100).The column developed by adding 50 ml of each eluent with collecting 5 ml fractions, then monitored by TLC using S3 as mobile phase. A total number of 76 fractions were obtained. Those consecutive fractions, which have the same number of spots with the same R_f values, were combined and evaporated to dryness to get four major fractions.

Table (1): Major fractions obtained from column chromatography.

| Major fraction | No. of collections 5ml each | No. of spots |
|----------------|--------------------------------|--------------|
| F1 | 22–36 | 1 |
| F2 | 37–45 | 2 |
| F3 | 46–63 | 3 |
| F4 | 64–76 | 3 |

Preparative TLC plates

Isolation of Iridoid glycosides is carried out by using preparative TLC which was performed by using ready made plates of 20x20cm, which are coated by silica gel GF 254 layers of 1mm thickness, (Merck).The major fraction (F₃) obtained by column chromatography was 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 systems (S1, S2, S3 and S4) was each placed in a glass tank (22.5 cm X 22 cm X 7 cm), and covered with a glass lid and allowed to stand for 45 minutes before use for saturation. The best solvent used from these four solvent is the S3 because in S3 separation is better than other solvent systems (S1,S2 and S4) in preparative TLC plate. The band corresponding to the (gardenoside) standard was scraped out and collected in a beaker, mixed with methanol, stirred and left a side for one hour, then filtered. After evaporation of the solvent, the obtained residue was subjected to chromatography with the available reference standard of (gardenoside) using different mobile phases for identification.

Qualitative and quantitative estimation of Gardenoside using HPLC analysis ⁽²¹⁾:-

Qualitative and quantitative estimations of (gardenoside) component in the crude extract obtained by all extraction methods was carried out using high performance liquid chromatography (HPLC). The identifications was made by comparism the retention time of (gardenoside) (obtained from crude extract with that of authentic standard at identical chromatographic conditions.

HPLC conditions of Gardenoside ⁽²²⁻²⁴⁾

1. Mobile phase: Acetonitrile: 0.1% Phosphoric acid in Water (30:70).
2. Column Type: Thermo BDS Hypersil [(C18) 2.4 μ m].
3. Column Dimensions: 100 x 4.6 mm. ID.
4. Column temperature: Ambient.
5. Flow rate: 1.3 ml/min.
6. Injection volume: 20 μ L.
7. Injection concentration: 50 μ g/ml.
8. Detection mode and setting: UV Detector at λ 238 nm.

Results

Two methods of extraction of iridoids glycoside (gardenoside) from dried leaves of *G.jasminoides* that method NO.1 was better, because the percentage yield of crude extract was higher than that obtained from method NO.2. In addition quantitative examination by using HPLC analysis showed that the amount of gardenoside and obtained by method NO.1 was much more compared with that obtained by method NO.2 as showed in table (2).

Table 2: Percentage yield of crude extracts obtained from extraction methods NO.1 and NO.2.

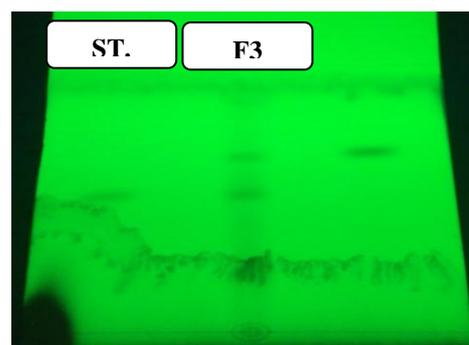
| Extraction method | %yield of crude extract | %yield of Gardenoside |
|-------------------|-------------------------|-----------------------|
| Method NO.1 | 6.32 | 2.75 |
| Method NO.2 | 4.91 | 1.34 |

Identification of iridoid glycoside (Gardenoside) by TLC:-

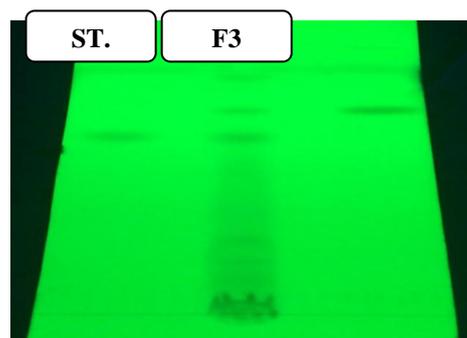
Gardenoside appeared as a single spot in different developing solvent systems (S1, S2, S3, and S4) against gardenoside reference standard and it has the same color and R_f values as that of gardenoside reference standard on the TLC plates after visualization by Liebermann-Burchard spray reagent, as shown in figure (5). Results showed that S3 is the best and more efficient for qualitative and quantitative analysis.

Identification and characterization of isolated Gardenoside**Analytical TLC**

The TLC plates of the gardenoside showed that after the initial isolation purification using silica gel GF₂₅₄ plate's detection under UV light at a wave length of 254 or by spraying with reagent gave two spots using the developing solvent system (S3). The spots have the color and R_f values to these of gardenoside.



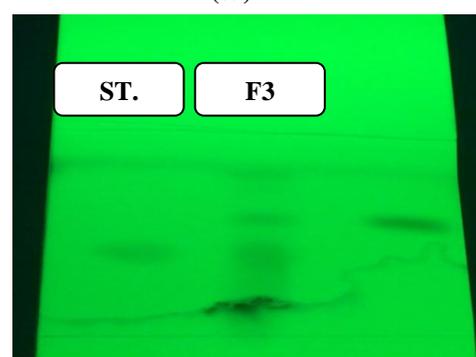
(s1)



(s2)



(s3)



(s4)

Figure (5):-TLC of gardenoside (ST.) and the major fraction (F3) was using four solvent systems (S1, S2, S3 and S4) as developing solvent systems, visualization under UV₂₅₄.

Melting point

The crystals of the isolated samples which were obtained from methanol showed a melting point (117-120 °C) of the isolated gardenoside compared to melting point of (118-120 °C) of the gardenoside standard.

HPLC (high performance liquid chromatography)

The retention time for the isolated gardenoside was identical to the main peak of the crude extract and standard reference; moreover the peaks isolated gardenoside and the standard reference were super imposable as shown in figures (6 and 7).

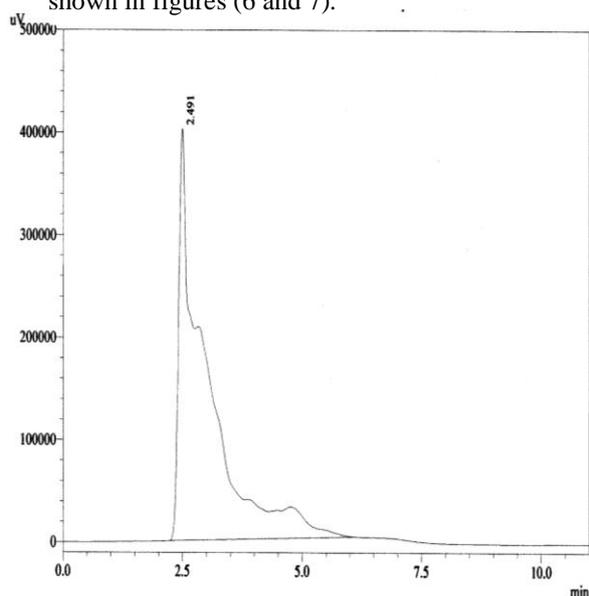


Figure (6):- HPLC analysis of gardenoside standard

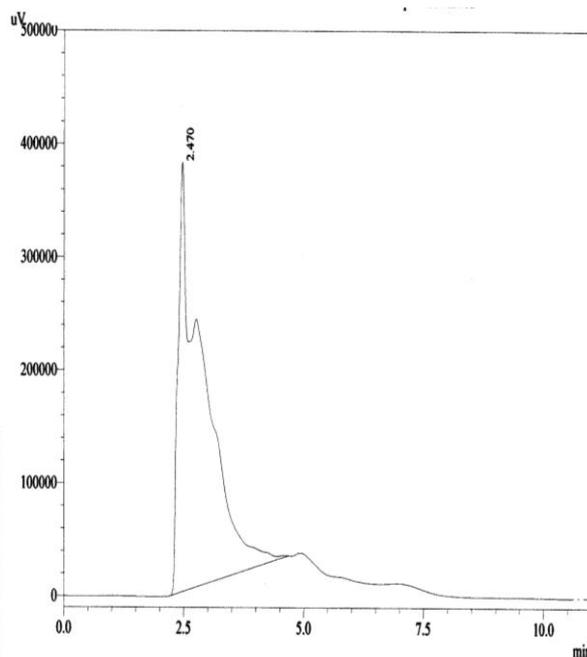


Figure (7):- HPLC analysis of isolated gardenoside.

FTIR

The FTIR spectrum of the isolated sample material and its gardenoside standard reference were identical which confirm that the isolated compounds are gardenoside as shown in figures (3-19) to (3-22). The IR spectra of the isolated gardenoside and its standard reference material⁽²⁵⁾, as showed the following absorption bands at cm^{-1} in table (3) and as shown in figures (8 and 9).

Table (3):- The characteristic IR absorption bands (in cm^{-1}) of the isolated gardenoside in comparison with that of gardenoside as reference standard

| Functional group | Isolated Gardenoside | Gardenoside standard | Assignment |
|------------------|----------------------|----------------------|---|
| O-H | 3367 | 3371 | Broad O-H stretching band of alcohol indicate hydrogen bonding |
| C-H | 2908 | 2909 | Stretching of CH_3 and CH_2 groups |
| C=O | 1689 | 1688 | C=O stretching of lactone (cyclic ester) |
| C=C | 1631 | 1631 | Stretching of C=C bond |
| C-H | 1438,1373 | 1442,1377 | C-H bending of CH_2 and CH_3 , also O-H bending |
| C-O | 1292 | 1311 | C-O stretching of ether |

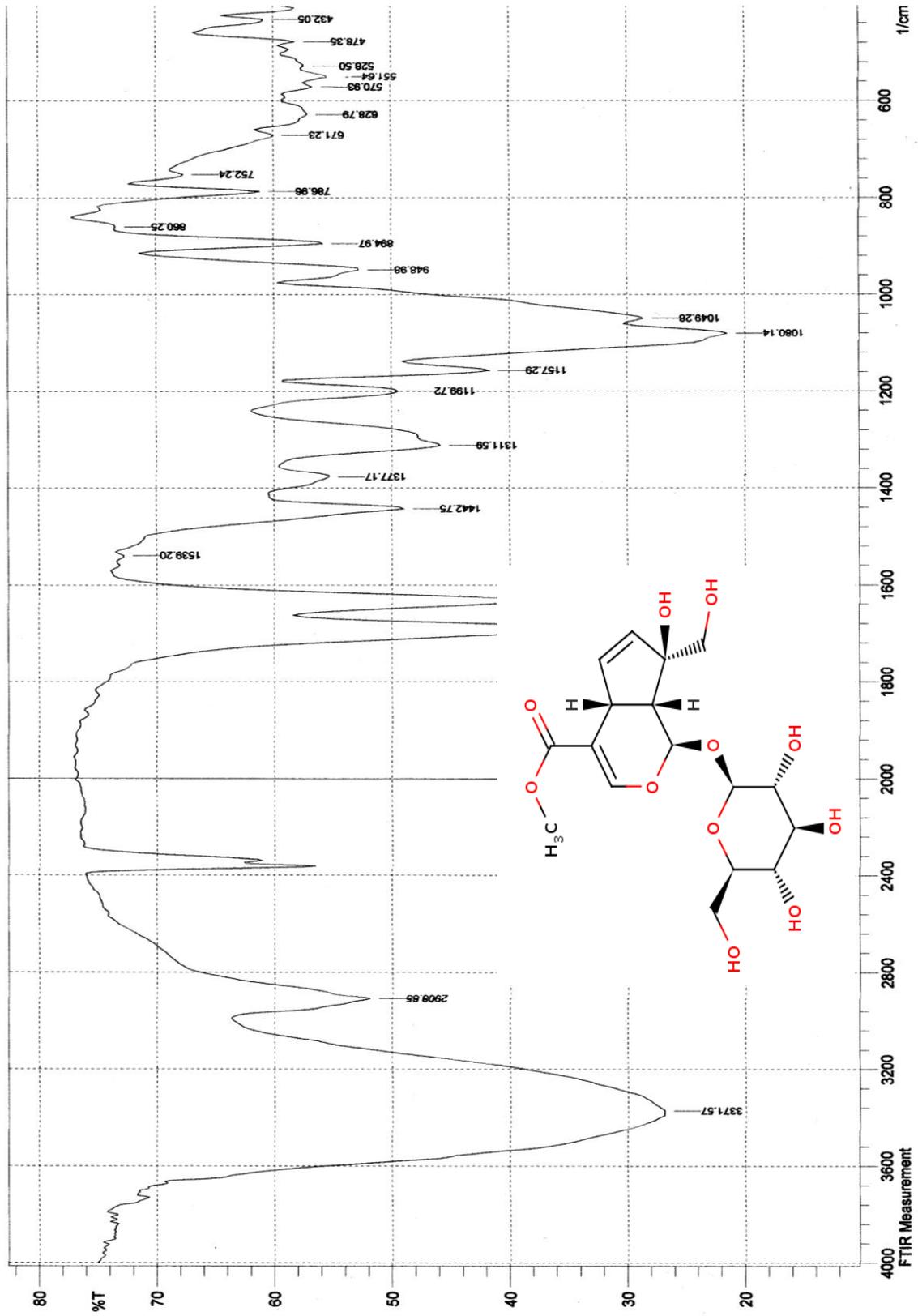


Figure (8):- FTIR spectrum of gardenoside standard.

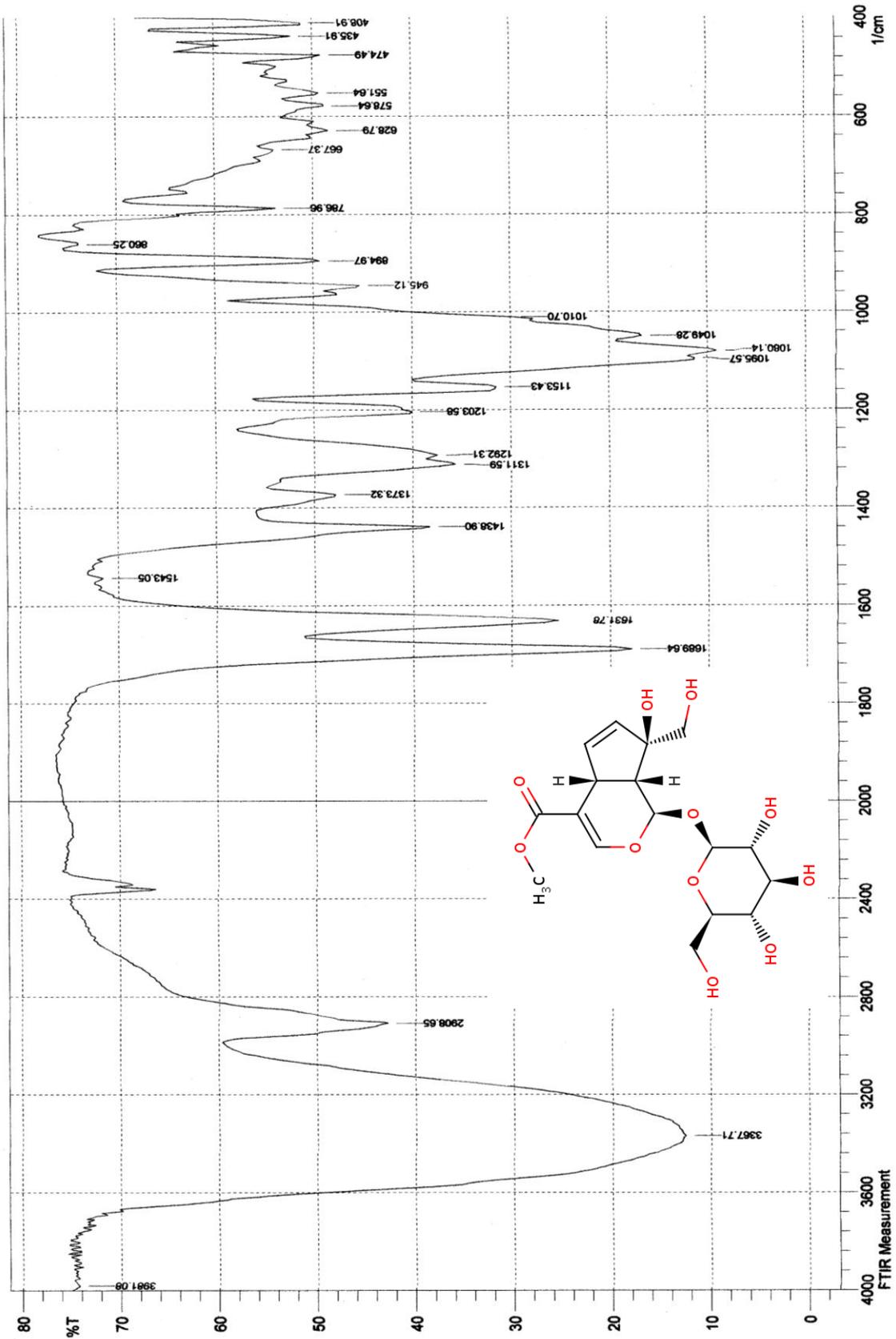


Figure (9):- FTIR spectrum of isolated gardenoside.

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