Phytochemical investigation of the Aerial Part of Iraqi *Convolvulus arvensis*

Mustafa H. Alwan *,1 and Maha N. Hamad *

**Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Baghdad, Iraq**

Abstract

*Convolvulus arvensis* is a species of bindweed that is rhizomatous and is in the morning glory family (*Convolvulaceae*) native to Europe and Asia. The plant is naturally grown in Iraq. The plant was reported to be used in traditional medicine from as early as 1730s.

The aerial parts of *Convolvulus arvensis* were macerated in 80% ethanol for 6 days. The concentrated extract was partitioned with n-hexane, chloroform, ethyl acetate- and n-butanol successively. The n-hexane and ethyl acetate, fractions were examined for the presence of phytochemicals by thin layer chromatography and high performance liquid chromatography and its steroid and flavonoid contents were investigated. Stigmasterol was isolated from n-hexane fraction and identified by liquid chromatography/mass spectroscopy. Rutin was isolated from the ethyl acetate fraction and identified by liquid chromatography/mass spectroscopy. The aim is to examine the phytochemical constituents of the aerial parts of *Convolvulus arvensis*, literature survey available so far revealed that there were no studies about the phytochemical investigation for *Convolvulus arvensis* in Iraq.

Different chromatographic techniques like Thin Layer Chromatography and mass spectroscopy were used and the presence of Stigmasterol and Rutin in aerial parts of *Convolvulus arvensis* was indicated.

Keywords: *Convolvulus Arvensis*, Phytochemical analysis, Steroids, Flavonoids.

**Introduction**

*Convolvulus arvensis* is a species of bindweed that is rhizomatous and is in the morning glory family (*Convolvulaceae*) native to Europe and Asia(1).

The genus *Convolvulus* (*Convolvulaceae*) has a cosmopolitan, though largely temperate distribution and comprises approximately 200 species worldwide. More than half of the species occur in the Mediterranean region, Macaronisia and Western Asia(2). The species *Convolvulus arvensis* is a perennial plant that are woody at the base, with trailing or scrambling unarmed stems, petiolate leaves that are truncate or rounded at the base, flowers borne in axillary cymes, conspicuous peduncles that are generally shorter than the subtending bracts, and corollas that are blue, yellow or white (Figure 1). (3)

1Corresponding author E-mail: Mustafa.7san@yahoo.com
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Aerial parts of *Convolvulus arvensis* was used as laxative, wound healing, anti-spasmodic anti-hemorrhagic, anti-angiogenetic and for the treatment of parasites and jaundice\(^{(4-6)}\). In addition it was used as diuretic and in skin disorders such as anti-furunculosis, antidandruff and in spider bites\(^{(7)}\). Traditionally *Convolvulus arvensis* was used as decoction in cough and flu, to treat the painful joints, inflammation and swelling\(^{(8)}\). A purified water extract of leaves of bindweed is used to inhibit the growth of tumor cells, growth of blood vessels and enhance immune function\(^{(9)}\). *Convolvulus arvensis* reported to contain major compounds which are steroids, flavonoids, phenols, Lipids and coumarins\(^{(10)}\). Campesterol, stigma-sterol and \(\beta\)-sitosterol were considered to be the most abundant steroid compounds in *Convolvulus arvensis*, they exist in the aerial parts of the plant. Campesterol has Antiangiogenic activity and reduces cholesterol level\(^{(11)}\), while stigma sterol has Anti-fungal, potent antioxidant, hypoglycemic and thyroid inhibiting properties, laxative properties, reduce cholesterol level and anticancer\(^{(12,13)}\), \(\beta\)-sitosterol has Hypocholesterolemia activity, anti-diabetic effects, for benign prostatic hyperplasia (BPH), and chemo preventive effect\(^{(14,15)}\). Quercetin, rutin and kaempferol are the most abundant flavonoids in the aerial parts of *Convolvulus arvensis*, they have anti-oxidant, anti-inflammatory and anti-cancer effects\(^{(16,17)}\). The most abundant phenols in *Convolvulus arvensis* are Chlorogenic acid, caffeic acid and ferulic acid which exist in the Aerial parts of the plant. They possess anti-oxidant, anti-inflammatory anti-cancer effects and anti-diabetic effects\(^{(18-20)}\).

**The aim:** to examine the phytochemical constituents of the aerial parts of *Convolvulus arvensis* using different chromatographic (TLC, HPTLC) and mass spectroscopy. Literature survey available so far revealed that there were no studies about the phytochemical investigation for *Convolvulus arvensis* in Iraq.

**Experimental Section**

**Plant material**

The Aerial parts of *Convolvulus arvensis* were obtained from the farm of College of Pharmacy/ University of Baghdad. The plant was identified and authenticated by Dr. Khansaa Rasheed/Iraq Natural History Research Center and Museum /Plant and Environment Department.

**Extraction**

Powdered plant material 400 grams were soaked in 1600ml ethanol (80%) with occasional shaking, at room temperature. After 2 days, the extract was filtered. The same process was repeated twice on the retained part. The filtrate (aqueous ethanol) from the three times was collected and evaporated to dryness under vacuum using rotary evaporator. A dark greenish residue was obtained. Water 500ml was added to the residue and partitioned successively with n-hexane, Chloroform, ethyl acetate, and n-butanol (3x500 ml) for each fraction.

The first three fractions were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness.

**Preliminary qualitative phytochemical analysis of crude extracts:**

1. **Chemical tests**

The following tests were carried out on each extract which were obtained from the previously mentioned methods\(^{(21)}\).

- **Test for flavonoids:** few milligrams of each extract were placed in test tube suspended in few milliliters of ethanol and few drops of 5% alcoholic KOH were added and noticed for the formation of yellow color which will disappear upon the addition of dilute hydrochloric acid.

- **Test for alkaloids:** two tests were used for detection of alkaloids:
  - **A. Dragendroff's reagent:** Dragendroff reagent (3drops) were added to 2 ml of ethanolic extract then observe of orange brown precipitate.
  - **B. Mayer's reagent:** Mayer s reagent (4 drops) were added to 2 ml of ethanolic extract and observe of white creamy precipitate.

- **Test for phenols:** 2 ml of the 5% ferric chloride was added to 2 ml of ethanolic extract the formation of green to deep blue indicates the presence of phenols.

- **Test for flavonoids:** ethanolic KOH was used for the detection of flavonoid which give yellow color.

**Test for Saponin**

About (2 gm) of the powdered sample from the leaves and seeds was boiled in (10 ml) of distilled water in a water bath and filtered. (5 ml) of the filtrate was mixed with (5 ml) of distilled water in a test tube and shaken vigorously. The formation of froth that persists for 15 minutes indicates the presence of saponins.

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**Figure 1. Photo of Convolvulus arvensis**

Aerial parts of *Convolvulus arvensis* was used as laxative, wound healing, anti-spasmodic anti-hemorrhagic, anti-angiogenetic and for the treatment of parasites and jaundice\(^{(4-6)}\). In addition it was used as diuretic and in skin disorders such as anti-furunculosis, antidandruff and in spider bites\(^{(7)}\). Traditionally *Convolvulus arvensis* was used as decoction in cough and flu, to treat the painful joints, inflammation and swelling\(^{(8)}\). A purified water extract of leaves of bindweed is used to inhibit the growth of tumor cells, growth of blood vessels and enhance immune function\(^{(9)}\). *Convolvulus arvensis* reported to contain major compounds which are steroids, flavonoids, phenols, Lipids and coumarins\(^{(10)}\). Campesterol, stigma-sterol and \(\beta\)-sitosterol were considered to be the most abundant steroid compounds in *Convolvulus arvensis*, they exist in the aerial parts of the plant. Campesterol has Antiangiogenic activity and reduces cholesterol level\(^{(11)}\), while stigma sterol has Anti-fungal, potent antioxidant, hypoglycemic and thyroid inhibiting properties, laxative properties, reduce cholesterol level and anticancer\(^{(12,13)}\), \(\beta\)-sitosterol has Hypocholesterolemia activity, anti-diabetic effects, for benign prostatic hyperplasia (BPH), and chemo preventive effect\(^{(14,15)}\). Quercetin, rutin and kaempferol are the most abundant flavonoids in the aerial parts of *Convolvulus arvensis*, they have anti-oxidant, anti-inflammatory and anti-cancer effects\(^{(16,17)}\). The most abundant phenols in *Convolvulus arvensis* are Chlorogenic acid, caffeic acid and ferulic acid which exist in the Aerial parts of the plant. They possess anti-oxidant, anti-inflammatory anti-cancer effects and anti-diabetic effects\(^{(18-20)}\).
Tests for steroids

**Liebermann-Burchard test:** Extract (3ml) was treated with chloroform, 5ml of acetic anhydride and drops of sulphuric acid was added. The formation of dark pink or red color indicates the presence of steroids.

2. **Analytical Thin layer chromatography.**
A- using ready-made plate aluminum coated TLC sheet G/UV254, 0.20 mm for n-hexane stationary phase used was silica gel, using Libermann Burchard spray reagent to detect the spot. The mobile phase was n-hexane: ethyl acetate (10: 4) [22].
B- using ready-made plate aluminum coated TLC sheet G/UV254, 0.20 mm ethyl acetate fraction stationary phase used was silica gel, using UV lamp for detection of the spot. mobile phase: chloroform: methanol: formic acid (15: 4: 1) [23].

3. **High Performance Thin Layer Chromatography Analysis**
A- hexane fraction was analyzed also for its steroids contents utilizing HPTLC (Eike Reich/CAMAG-Laboratory, Switzerland, the chromatogram was developed in a mobile phase composed of ethyl acetate: hexane (50:50) examined at 254 nm wavelength. The number. of tracks were 3, injection volume was 100 µl, dosage speed 150 nl/s. the band length 8.0 mm.
B- ethyl acetate fraction was analyzed also for its phytochemicals contents utilizing HPTLC (Eike Reich/CAMAG-Laboratory, Switzerland, the chromatogram was developed in a mobile phase composed of ethyl acetate: water (84: 4: 4: 10) examined at 254 nm wavelength. The number. of tracks were 16, injection volume was 100 µl, dosage speed 150 nl/s. the band length 8.0 mm.

**Isolation of phytochemicals by Preparative thin layer chromatography (TLC):**
A-Isolation of steroids from hexane fraction using the same stationary and mobile phase in the analytical thin layer chromatography.
B- Isolation of rutin from ethyl acetate fraction using the same stationary and mobile phase in the analytical thin layer chromatography.

**Identification of isolated phytochemicals by LC/MS:**
The isolated flavonoid and steroid were recognized as rutin and stegmasterol respectively by Liquid chromatography /mass spectrometry (LC/MS):
Analytical LC-MS was performed using a Agilent 6410 QQQ System under the following conditions:

<table>
<thead>
<tr>
<th>Number</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mobile phase , acetonitrile and water</td>
</tr>
<tr>
<td>2.</td>
<td>Flow rate 1 mL/min</td>
</tr>
<tr>
<td>3.</td>
<td>Column size 0.19 mm</td>
</tr>
<tr>
<td>4.</td>
<td>C18 with 5µm particle size</td>
</tr>
<tr>
<td>5.</td>
<td>m/z range was 250 to 1000, 200K</td>
</tr>
</tbody>
</table>

**Table 2.** LC mass condition for steroids

<table>
<thead>
<tr>
<th>Number</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>mobile phase, solvent A: 0.05% TFA in water, solvent B 0.05% TFA in methanol (pH 2.5).</td>
</tr>
<tr>
<td>2.</td>
<td>C18 RP with 5µm particle size</td>
</tr>
<tr>
<td>3.</td>
<td>Flow rate 1 mL/min</td>
</tr>
<tr>
<td>4.</td>
<td>Column size 0.19 mm</td>
</tr>
</tbody>
</table>

**Results and Discussion**

1- Preliminary phytochemical investigation like chemical tests were carried on the arial parts of *Convolvulus arvensis* and showed the following results as shown in Table 2.

**Table 3: Phytochemical analysis of the aerial parts of Convolvulus arvensis extract.**

<table>
<thead>
<tr>
<th>Phytochemical components</th>
<th>Extract of C. arvensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
</tbody>
</table>

The table shows the presence of flavonoids, phenols and steroids. And the absence of alkaloids.

2- **Analytical Thin Layer Chromatography analysis for the hexane extract**
A-TLC test was carried on hexane fraction using a mobile phase of Hexane: ethyl acetate (10: 4) and revealed the presence of steroid in the hexane fraction which was sprayed by Liebermann-Burchard reagent and gave deep violet colour as compared with standard of stigmasterol as shown in (Figure 2).

**Figure 2.** Thin layer chromotography for hexane fraction, A: Beta-sitosterol (standard), B: Hexane fraction, C: Stigma-sterol (standard), mobile phase: Hexane: ethyl acetate (10: 4).

B-TLC for flavonoids was carried on ethyl acetate fraction of *Convolvulus arvensis* using a mobile phase: chloroform: methanol: formic acid (15: 4: 1), the spot gave florescence under UV light 245 as compared with standard of rutin as shown in figure 3.
Fig. 3. TLC for ethyl acetate fraction, A: ethyl acetate fraction, B: Rutin (standard), mobile phase: chloroform: methanol: formic acid (15: 4: 1).

3- HPTLC Analysis:
A- HPTLC test was carried on hexane fraction of Convolvulus arvensis using a mobile phase of hexane: ethyl acetate (50: 50) and revealed the presence of steroids as compared retardation factor (RF) with standard of stigma-sterol as shown in Figure 4.

B- HPTLC was carried on ethyl acetate fraction using mobile phase: Ethyl acetate: formic acid: acetic acid: water (84:4:4:10) and revealed the presence of rutin as compared the retardation factor (RF) with standard of rutin as shown in figures 5-7.

Figure 4. HPTLC for hexane fraction, A: Beta-sitosterol (standard), B: Stigma-sterol, C: Hexane fraction, mobile phase: Hexane: ethyl acetate (50: 50).

Figure 5. HPTLC diagram for Rutin standard

Figure 6. HPTLC diagram for isolated Rutin
Isolation of phytochemicals by Preparative Thin Layer Chromatography

A preparative TLC test was performed on hexane fraction to isolate the steroid for further analysis using mobile phase: Hexane: ethyl acetate (10: 4) as shown in (Figure 8).

A preparative TLC test was performed on ethyl acetate fraction to isolate the rutin for further analysis using mobile phase: chloroform: methanol: formic acid (15: 4: 1) as shown in (Figure 9).
Identification of Isolated Compounds by LC/MS analysis

A- LC/MS test for steroid was performed to confirm the structure of the isolated steroids. The fragmentation pattern and molecular ion peak (412) indicated that the isolated steroid is stigmasterol as shown in (Figure 10).

![Figure 10. LC/MS for Stigma-sterol in hexane fraction](image)

Fragmentation pattern was complained with that reported in the literature for stigmasterol from an earlier study shown in figure 11.

![Figure 11: molecular ion and fragments of stigmasterol in hexane fraction](image)

B- LC/MS for the isolated rutin confirmed the structure of the isolated rutin since fragmentation pattern and molecular ion peak (611) indicate that the isolated compound is rutin as shown in figure 12.
Phytochemical investigation of *Convolvulus arvensis*

**Conclusion**

The phytochemical investigation of the Iraqi plant revealed the presence of stigma-sterol in hexane fraction and rutin in the ethyl acetate fraction of *Convolvulus arvensis* aerial part of the plant.

**References**


