RESEARCH ARTICLE

Phytochemical Investigation of Some Active Components in Iraqi *Conyza canadensis* (Syn. *Erigeron canadensis*)

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ABSTRACT

This study aimed to explore and separate the phytochemicals of the whole plant *Conyza canadensis*, a naturally growing plant in Iraq, since no phytochemical research was done previously in Iraq. The whole plant of *C. canadensis* was defatted by maceration in hexane for 24 hours. The defatted plant materials were extracted using Soxhlet apparatus, the aqueous ethanol 85% as a solvent extraction for 9 hours, and fractionated by petroleum ether, chloroform, ethyl acetate, and n-butanol. The petroleum ether, chloroform, and ethyl acetate fractions were analyzed by high-performance liquid chromatography (HPLC) for their steroids, alkaloids, and polyphenolic (phenolic acids and flavonoids) contents. One alkaloid was isolated from chloroform fraction by HPLC, and three polyphenolics compounds were isolated from ethyl acetate fraction by preparative thin-layer chromatography (TLC), then identified by HPLC and high-performance thin-layer chromatography (HPTLC).

The different chromatographic and spectroscopic results revealed stigmasterol, β-sitosterol in petroleum ether fraction, harmine alkaloid in chloroform fraction, quercetin, quercitrin, apigenin, p-coumaric acid, and caffeic acid in ethyl acetate fraction of *C. canadensis*. Three polyphenolics compounds (p-coumaric acid, caffeic acid, apigenin), and harmine alkaloid were isolated from the whole plant and matched their retention time with the related standards by HPLC and by measuring their max Rf values by HPTLC analysis.

Keywords: *Conyza canadensis*, Steroid, Alkaloid, Polyphenolics, High-performance liquid chromatography, High-performance thin-layer chromatography.

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INTRODUCTION

The genus Conyza, which belongs to a vital plant family called Asteraceae, involves about 50–80 species allocated in the temperate and subtropical areas of the world.[1,2] On the other hand, the plant *Conyza canadensis* arose in North America and is widely distributed in different countries globally, including China, Iran, Pakistan, Japan, Turkey, Vietnam, South Africa, Tunisia, Poland, and Australia.[3,4] In Iraq, *C.canadensis* is commonly known as the we altheb that is naturally grown and widely dispersed in North of Baghdad, Baquba, Kut, Rustam, Mosul, Abu Ghriab, Rowanduz, Za'franiya, Pushtashan, Qerna Qaw valley, North-east of Zakho, 50 km from Basra to Nassiriya.[5,6] *Conyza canadensis* is an annual herb (grows in summer and winter), taprooted and reaching a height of 5–100 cm (2–40 inches). Leaves are numerous, thin, and distributed around the stem in alternate arrangements. The stem is unbranched covered with long white hair.[7] The flower is small, characterized by smaller yellow flowers in the center with outer tiny erect white ray florets having no scent. The seeds are light in weight, so distributed by wind.[8]

In 1753, Linnaeus was the first scientist who describes the *C. canadensis* using the term Erigeron canadensis, which is considered an older name but still valid and widely used, which then transferred to the Conyza genus by Cronquist in 1943.[3]

C. canadensis has been used as traditional medicine to treat wounds, swellings, pain caused by arthritis, and various pathological conditions, including inflammation, diarrhea, microbial infections like urinary infections, respiratory tract infections, etc is used locally as a sweetening agent.[9,10] In Iraq, a decoction of the whole plant has been used for metrorrhagia and as a sedative agent.[5] Several studies have demonstrated that the extracts (ethanolic, methanolic) and the fractions (chloroform, ethyl acetate) of the plant have shown a substantial antibacterial effect against both gram-positive and gram-negative bacteria with high susceptibility against *Escherichia col*i, *P. aeruginosa*, and *Staphylococcus aureus.* Also, the ethyl acetate fraction exerted an excellent antifungal effect against *Canada albicans* fungus with high inhibition zone equal to 45 mm.[11,12] Furthermore, the plant possessed an important pharmacological activity includes

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anti-inflammatory,[13] anticoagulant,[14] Anti-gastric ulcers,[15] anti-diabetic,[16] antioxidant, anti-cancer,[17] and mutagenic effects.[18,19]

In general, the phytochemical investigation performed on the whole plant pointed out important secondary metabolites like terpenes, flavonoids, tannins, phenolic acids, alkaloids, essential oils, and sterols. Also, the plant contains fatty acids and sphingolipids.[20] The study aims to carry on a phytochemical investigation of Iraqi *C. canadensis* with the objectives to isolate some of the crucial phytochemicals detected in the plant-like phenolic acid, flavonoid, and alkaloid.

MATERIALS AND METHODS

Plant Materials

The whole plant of *C. canadensis* was collected from Baghdad city on the College of Pharmacy, University of Baghdad farm during July (2020). The plant was authenticated at the Iraq Natural History research center and Museum Plant and Environment Department by Dr. Khansaa Rasheed and Dr. Zainab Abid Aun. The aerial parts and roots were washed thoroughly, dried under shade, chopped, pulverized by hand milling into a coarse powder, and then weighed.

Extraction of Plant Material[21]

About 300 grams of shade-dried pulverized plant materials were first defatted by maceration with 2L hexane for 24 hours. The defatted plant materials were extracted using Soxhlet apparatus in which the pulverized plant packed in the thimbles and boiling chips were added to round bottom flask 1 L then extracted with 4.25 L of 85% ethanol as a solvent extraction for 9 hours. The extract was filtered to remove boiling chips, and the solvent was evaporated under reduced pressure using a rotary evaporator to get 65 gm of a dry dark-greenish crude extract.

Fractionation of Different Active Constituents[12]

Thirty-five grams of the dried residue of the whole plant were suspended in 200 mL water and partitioned successively with petroleum ether (P.E (B.P. 40–60°C), chloroform, ethyl acetate (EA), and n-butanol using (3 × 200 mL) for each one. All the fractions were dried over anhydrous sodium sulfate except n-butanol fraction, then filtered and evaporated to dryness.

Preliminary Qualitative Phytochemical Analysis of Crude Extracts and Different Fractions

A-Chemical Tests[22]

Test for alkaloids:

- Dragendorff's test: To 2 mL of alcoholic extract, 1–2 drops of Dragendorff's reagent were added. Observation of orange precipitate indicates a positive result for alkaloids.
- Mayer's test: To 2 mL of alcoholic extract, 1 to 2 drops of Mayer's reagent were added. Observation of creamy white precipitate indicates a positive result for alkaloids.

Test for coumarins: A 2 mL of alcoholic extract was taken, and 3 mL of 10% sodium hydroxide was added. The yellow color points to the presence of coumarins.

Test for flavonoids: Lead acetate test: 1-mL of 10% Lead acetate solution was added to 5 mL of alcoholic extract, the formation of a yellowish-white precipitate was taken as a positive test for flavonoids.

Tests for steroids: Liebermann-Burchard test: To 1-mL of ethanolic extract, 1 mL of chloroform, 2–3 mL of acetic anhydride, and two drops of concentrated H_2SO4 were added (concentrated H_2SO4 was added carefully along the side of the test tube). Dark green coloration gives a positive result.

Test for phenolic compounds: Ethanolic extract (10 mg) in 10 mL distilled water was filtered, then the 3 mL of the filtrate and 3 mL of $FeCl_3$ solution (5%w/v) were mixed. The formation of a dark green or blue-black precipitate was considered a positive result.

Test for terpenoids: Salkowski test: five mL of plant extract were mixed with two ml of chloroform followed by the careful addition of three ml concentrated H_2SO4. A layer of reddish-brown coloration was formed at the interface, thus indicating a positive result for the presence of terpenoids.

B- HPLC Analysis

The HPLC analysis (SYKAM, Germany) was carried out to identify phytosterols, alkaloids, polyphenolics present in P.E, chloroform, E.A. fractions, respectively, using Column C18-ODS (25 cm x 4.6 mm) and specific experimental condition for each fraction that listed in The Table 1.

Table 1: HPLC experimental condition.

Phytochemicals	*Mobile phase*	*Elution*	*Detector*	*Injection volume*	*Flow rate*
Phytosterols	Acetonitrile: D.W: acetic acid (60:25:5) modified23	Isocratic elution	UV-280 nm	25 µL	1 mL/min
alkaloids	Phosphate buffer: acetonitrile (70:30) modified24	Isocratic elution	UV-330 nm	100 µL	1.2 mL/ min
Polyphenolic compounds (phenolic acids and flavonoids)	Solvent A: methanol: D.W: formic acid (70:25:5) Solvent B: methanol: D.W: buffer (60: 10: 30) modified25,26	Gradient elution from (0–5)min A=60%, from (5–20) min A=20%	UV-280 nm	70 µL	1.3 mL/min

Isolation and Purification of Different Active Constituents Found in *Conyza Canadensis*

A- Isolation of Alkaloid by High-Performance Liquid Chromatography (HPLC)

The HPLC was used for the isolation of harmine alkaloid, depending upon the retention time match with the standard harmine through dissolving 80 mg from the fraction in a minimum quantity of methanol (HPLC grade), then 200 µL of the sample was injected four times using the same conditions for HPLC analysis mentioned above. A fraction collector was used to collect the target peak from its appearance until its disappearance, which was between 8.6–9.8 minutes.

B- Isolation of Phenolic Acids and Flavonoids by Preparative Layer Chromatography PLC from the Ethyl Acetate Fraction

- To readymade silica gel GF254 (20x20 cm) plates with a layer thickness of 0.5 mm, about 0.5 gm from the fraction was dissolved in a minimum quantity of absolute ethanol and applied in the form of a streak on the preparative layer using a capillary tube, then introduced in the saturated jar containing chloroform: acetone: formic acid (75:16.5:8.5)[27] as a mobile phase for the isolation of phenolic acids (*p*-coumaric acid and caffeic acid). After development, the bands were detected under U.V. light at 254 and 365 nm.[28]
- For the isolation of apigenin flavonoid from ethyl acetate fraction, use toluene: ethyl acetate: formic acid (36:12:6) modified as a mobile phase and used the same procedure for phenolic acids isolation.[29]

Identification and Characterization of the Isolated Compounds

Identification of the isolated compounds (harmine, *p*-coumaric acid, caffeic acid, and apigenin) through using advanced chromatographic techniques listed below:

A. *HPLC:* As mentioned before.
B. *HPTLC:* the four isolated compounds were analyzed by utilizing HPTLC (Eike Reich/CAMAG Laboratory, Switzerland), using silica gel GF254 plates (20.0x10.0 cm dimension) developed in a mobile phase composed of chloroform: acetone: formic acid (75:16.5:8.5) for polyphenolics compounds (*p*-coumaric acid, caffeic acid, and apigenin) and developed in a mobile phase composed of toluene: ethyl acetate: formic acid: acetic acid (2:1:1:0.75) for harmine alkaloid.[30] The application volume of the sample was 3 µL, and the spots were detected by U.V. light of 254 and 366 nm.

RESULTS AND DISCUSSION

Preliminary Qualitative Phytochemical Analysis

The preliminary qualitative phytochemical analysis of the crude extract and different fractions through chemical tests are given in Table 2.

Most of the important secondary metabolites were detected in crude ethanolic extract of the Iraqi *C. canadensis* plant. The fractionation of crude extract of the plant was done with different solvents from low polar to high polar solvent (PE, chloroform, EA, and n-butanol); therefore, the phytochemicals were detected in each fraction according to polarity difference. In PE and chloroform fractions showed alkaloids, coumarins, steroids, and terpenoids, with the absence of flavonoids and phenols. E.A and butanol fractions revealed coumarins, flavonoids, phenols, and terpenoids with the lack of alkaloids and steroids.

The HPLC results of the PE fraction are given in Table 3. The identification of the expected steroids by HPLC is usually performed by comparing their retention time with the authentic standards (stigmasterol and β-sitosterol). Their chromatograms are shown in Figure 1.

HPLC analysis confirms the presence of β-sitosterol and stigmasterol in the PE fraction of the Iraqi *C. canadensis* plant as the retention time of peaks matches the standard steroids used, and stigmasterol has the high area among the other steroids present in the P.E fraction of *C. canadensis* plant.

The HPLC chromatogram of chloroform fraction revealed more than five major peaks in this fraction with different retention times, and the expected alkaloid was identified by comparing the retention time with the standard harmine, as shown in Figure 2.

The above results give an idea about the presence of more than one peak in chloroform fraction, representing a different type of alkaloid in the Iraqi *C. canadensis* plant. The retention time of the peak in chloroform fraction (9.24 minutes) matches with the standard used (8.99 minutes), which confirms the presence of harmine alkaloid in the Iraqi *C. canadensis* plant. The HPLC results of ethyl acetate fraction are given in Table 4. The identification of the expected flavonoids and phenolic acids

Table 2: Preliminary qualitative phytochemical analysis of the crude ethanolic extract and different fractions (petroleum ether, chloroform, ethyl acetate, n-butanol).

Phytochemicals	*Crude ethanolic extract*	*Petroleum ether*	*Chloroform*	*Ethyl acetate*	*n-butanol*
Alkaloids	+	+	+	–	–
Coumarins	+	+	+	+	+
Flavonoids	+	–	–	+	+
Steroids	+	+	+	-	–
Phenolic compounds	+	-	–	+	+
Terpenoids	+	+	+	+	+

(+), (–) represent the presence or absence of phytochemicals, respectively.

by HPLC is usually performed by comparing their retention time with the standard compounds (quercitrin, apigenin, caffeic acid, and *p*-coumaric acid, and quercetin). Their chromatograms are shown in Figures 3 and 4.

As shown from the figures, HPLC analysis confirms the presence of quercitrin, quercetin, apigenin, *p*-coumaric acid, caffeic acid since their retention time matches the standards used with the presence of other peaks related to other unidentified polyphenolics compounds in ethyl acetate fraction.

Isolation and Purification of Different Active Constituents from Iraqi *Conyza canadensis*

A. HPLC chromatogram of chloroform fraction demonstrates six different compounds with different retention times, the fifth peak with retention time equal to 8.6–9.8 minutes match with the harmine standard retention time (8.99 minutes), the marked peak was collected by the fraction collector.

B. Preparative TLC was used to isolate and purify three polyphenolic compounds from ethyl acetate fractions. Through using chloroform: acetone: formic acid (75:16.5:8.5), two bands were matched with the related standards (*p*-coumaric acid and caffeic acid), which

Table 3: Retention times in minutes of steroids in PE fraction.

Compounds	*The retention time of standards*	*Retention time in PE fraction*
β-sitosterol	3.20	3.48
Stigmasterol	5.27	5.15

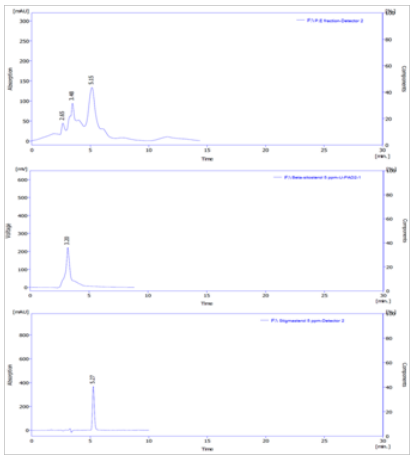

Figure 1: HPLC chromatogram of P.E fraction, β-sitosterol, and stigmasterol standards.

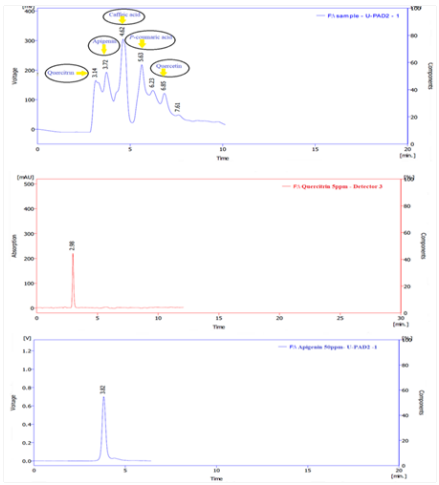

Figure 3: HPLC chromatogram of E.A fraction, quercitrin, and apigenin standards.

Table 4: Retention times in minutes of flavonoids and phenolic acids in EA fraction.

Compounds	*The retention time of standards*	*Retention time in EA fraction*
Quercitrin	2.98	3.14
Apigenin	3.82	3.72
Caffeic acid	4.79	4.62
p-coumaric acid	5.62	5.63
Quercetin	6.78	6.85

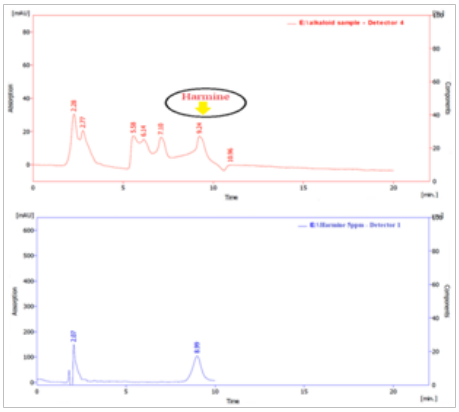

Figure 2: HPLC chromatogram of chloroform fraction and harmine standard.

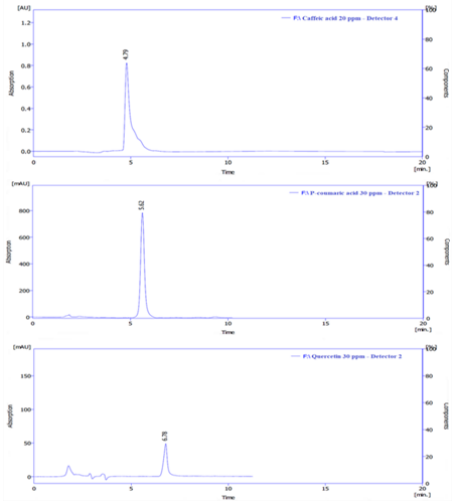

Figure 4: HPLC chromatogram of caffeic acid, p-coumaric acid, and quercetin standards.

symbolized as A and B respectively, then scraped off, eluted with E.A, filtered, and dried. The detection was performed at 254 and 365 nm, as shown in Figure 5.

Another solvent system was used for apigenin isolation, composed of toluene: ethyl acetate: formic acid (36:12:6) modified. The marked band (C) was matched with the related standard (apigenin), scraped off, eluted with E.A, filtered, and dried. The detection was performed at 254 and 365 nm, as shown in Figure 6.

Characterization and Identification of the Isolated Compounds

A- HPLC Analysis

The isolated harmine alkaloid was identified by measuring its retention time (8.99 minutes) which matches the retention time of harmine standard (8.99 minutes).

Three compounds were isolated from E.A fraction of the *C. canadensis* plant was identified by measuring their retention time compared with related standards by HPLC analysis. The retention time of the isolated compounds (A 5.61, B 4.82, and C 3.72) matched the retention time of the related standards (*p*-coumaric acid 5.62, caffeic acid 4.79, and apigenin 3.82), respectively, which demonstrated in Figures 7 and 8.

B- HPTLC Analysis

HPTLC was used to further identify the isolated alkaloid by measuring the max R_f values of the isolated compound (0.29), and related standard (0.28) obtained from the UV spectrum and the detection performed by UV light of 254 and 366 nm. The results obtained were summarized in Figure 9.

HPTLC was used to further identify the isolated polyphenolics compounds by measuring the max R_f values of the isolated compounds (0.52, 0.34, and 0.55) and related standards (*p*-coumaric acid 0.51, caffeic acid 0.34, and apigenin 0.53), respectively. The results obtained were summarized in Figure 10.

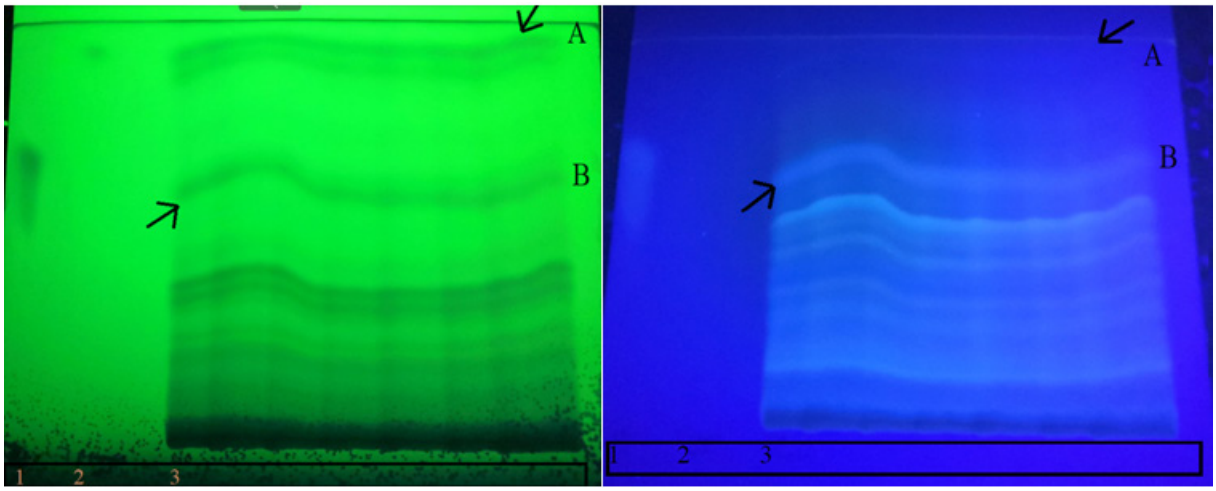

Figure 5: Preparative thin-layer chromatography plate of caffeic acid and p-coumaric acid isolation was observed at 254 nm and 365 nm. Where 1: caffeic acid standard, 2: p-coumaric acid standard, and 3: E.A fraction.

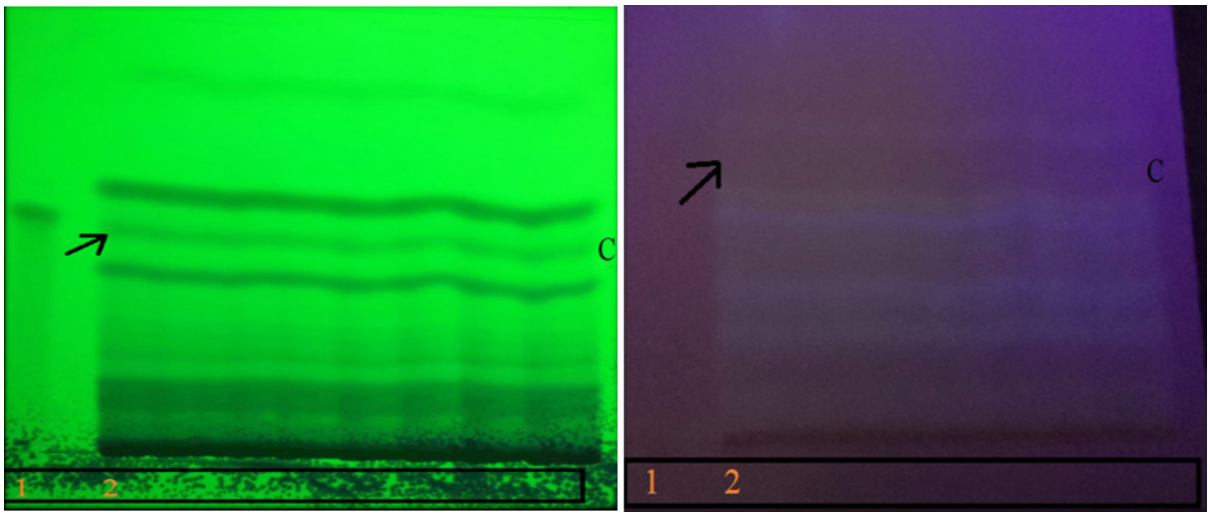

Figure 6: Preparative thin-layer chromatography plate of apigenin isolation was observed at 254 nm and 365 nm, where 1: apigenin standard and 2: E.A fraction.

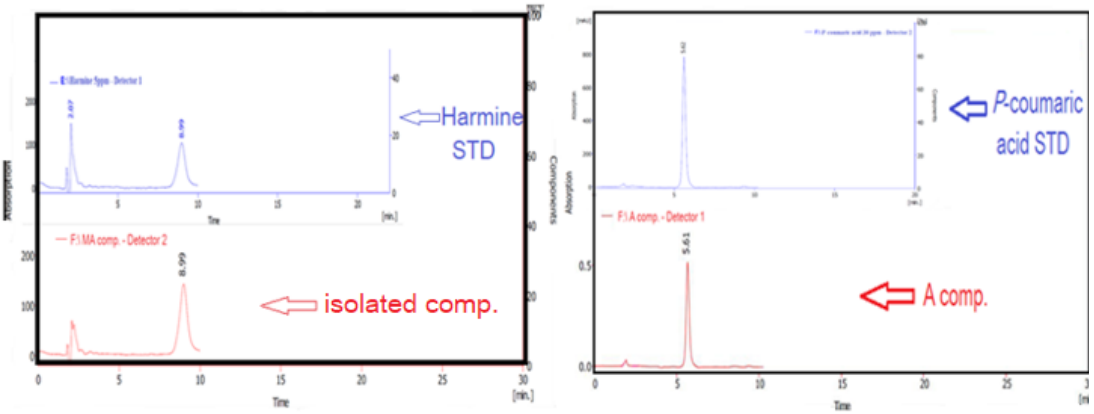

Figure 7: HPLC chromatogram of the isolated harmine alkaloid and harmine standard (Harmine STD) and the isolated A compound and p-coumaric acid standard.

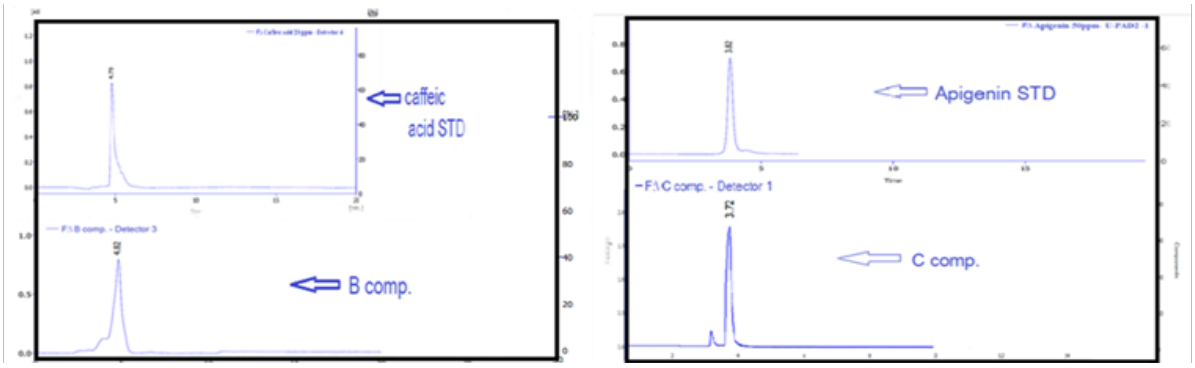

Figure 8: HPLC chromatogram of the isolated B compound and caffeic acid standard and the isolated C compound and apigenin standard.

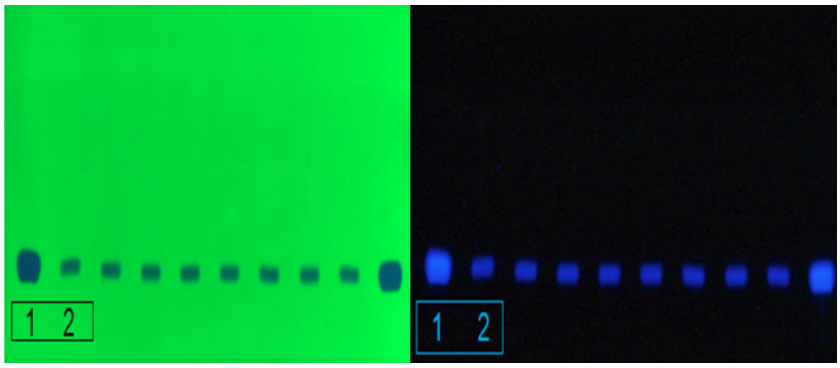

Figure 9: HPTLC plate for harmine standard (1) and isolated compound (2), developed in toluene: ethyl acetate: formic acid: acetic acid (2:1:1:0.75) as a mobile phase and observed at 254 and 366 nm wavelength.

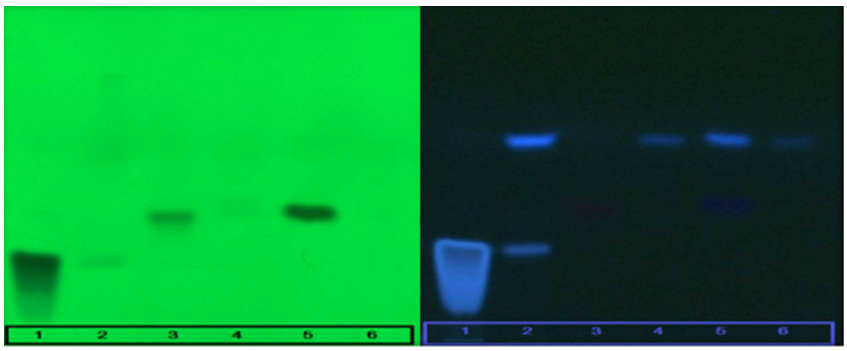

Figure 10: HPTLC plate for isolated compounds (2, 4, and 6) and related standards (caffeic acid, apigenin, and *p*-coumaric acid) developed in chloroform: acetone: formic acid (75:16.5:8.5) solvent system and observed at 254 nm and 366 nm wavelength.

CONCLUSION

- The current study results showed steroids such as stigmasterol and β- sitosterol in the petroleum ether fraction and the presence of harmine alkaloid in the chloroform fraction of Iraqi *C. canadensis* plant. Also, the ethyl acetate fraction of the whole plant contained important polyphenolics compounds such as *p*-coumaric acid, caffeic acid, apigenin, quercetin, and quercitrin.
- The current study results showed the isolation of phenolic acids (*p*-coumaric acid and caffeic acid), and flavonoid (apigenin) from the ethyl acetate fraction by preparative TLC. They showed the isolation of harmine alkaloid by HPLC from the chloroform fraction.

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