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Research Article

Flavonoid Separation from different extracts of aerial parts of *Convolvulus arvensis* by HPLC and Screening of Anthelmintic Activity

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ABSTRACT

Objective: *Convolvulus arvensis* is one of the world's top noxious weeds and is commonly known as field bindweed. The objective of research work was separation and identification of flavonoids and to check the anthelmintic activity of this plant.

Methods: The flavonoids were analyzed by High-Performance Liquid Chromatography (HPLC) method from alcoholic, hydroalcoholic and aqueous extract of aerial parts of *Convolvulus arvensis*. Acetonitrile:water (containing 0.05% Trifluoroacetic Acid (TFA) each) was used as mobile phase and isolation was done by using RP-18 column with UV variable wavelength detector (254 nm). Chromatogram of standards was compared with sample. Anthelmintic activity also was performed on prepared extracts of various concentrations.

Results: The presence of flavonoids like rutin, kaemferol, quercetin and quercetrin in crude extract was revealed. Significant anthelmintic activity in ethanolic extract was also reported.

Keywords: Flavonoids, HPLC, Anthelmintic activity, *Convolvulus arvensis*, Kaemferol

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INTRODUCTION

Convolvulus arvensis is a weed (Whitson TD, *et al.*, 2006) and it is found in 32 different crops in 54 countries (Holm LG, *et al.*, 1977). *Convolvulus arvensis* is native to Europe and Asia, and it grows in temperate, tropical and Mediterranean climates (Lyons KE, 1998; Gubanov I, *et al.*, 2004).

A perennial vine (0.4-2 inches in height), field bindweed has deep, persistent roots that spread widely. Possibly reflecting soil moisture and fertility variability, this species has extremely variable leaves. Petals are generally white to very pale pink. This plant has whitish roots and fleshy, cord-like rhizomes. Taproots form from the primary root, which can penetrate soil to depths of 2-10 feet. At the stem base, adventitious buds and taproot buds form lateral roots. (Arora M and Malhotra M, 2011; Mehrafarin A, *et al.*, 2009).

According to an analysis of its phytochemical constituents, *Convolvulus arvensis* contains alkaloids, phenolic compounds, flavonoids, sugars, carbohydrates, mucilage, sterols, tannins, unsaturated sterols, triterpenes, lactones, and proteins (Krzaczek T, *et al.*, 2004; Hilal SH, *et al.*, 1983).

Historically, the plant has been used as a medicine since the 1730s. *Convolvulus arvensis* aerial parts were used as laxatives, wound healers, anti-spasmodic, anti-haemorrhagic, antiangiogenetic, and for treatment of parasites and jaundice (Alkofahi A, *et al.*, 1996). Additionally, it was used to treat skin disorders such as furunculosis, dandruff, and spider bites (Leporatti ML, *et al.*, 2003). In addition, *C. arvensis* was traditionally used for pain relief, inflammation, and swelling in coughs and flu (Ali M, *et al.*, 2013).

MATERIALS AND METHODS

Plant materials

The aerial parts of the plant were collected from the waste land of Amritsar region in the month of October-November and authenticated by Dr. B.K. Kapahi, Taxonomist, Department of Botany, IIIM, Jammu. A voucher specimen was retained and deposited at the crude drug repository of the herbarium of IIIM, Jammu. (Vide CDR accession No. 21583).

Preparation of extracts

The collected aerial parts were shade dried, coarsely powdered and the powder was exhaustively extracted with both ethanol (95%), ethanol (50%) separately using percolator for 16 hours. The extract was drained, filtered and solvent was removed using rotary evaporator. Moreover the hydro alcoholic was lyophilized. To prepare the stock solutions, 20 mg of each extract was dissolved in respective concentration of Dimethyl Sulfoxide (DMSO) and 100 µl Roswell Park Memorial Institute (RPMI)-1640 media was added. pH was adjusted to 7.2, penicillin was added and sterilized by filtering through 0.2 µ microfilter and kept in refrigerator (2°C-8°C).

Standards and chemicals

HPLC grade methanol and other chemicals (Acetonitrile) of analytical reagent grade were procured from Merck. The authentic standards of the studied flavonoids were taken from of IIIM, Jammu.

Development of protocol for HPLC method using the extracts of Convolvulus arvensis

Preparation of standards: 1.2 mg of each standard (rutin, kaempferol, quercetin and quercitrin) was taken in 5 ml of methanol (HPLC grade). From which 5, 10, 15, 20, 25 µl were injected in HPLC system for making standard curve.

Preparation of extract solution: 20 mg of dry alcoholic, hydro-alcoholic and aqueous extract was dissolved in 10 ml extraction solvent (HPLC grade) to get 2 mg/ml solution, centrifuged and filtered through 0.45 µm and was injected to HPLC (water) system.

Quantification

The compounds exhibited linear responses in the calibration curves, which were prepared by using the multipoint calibration curve method. Working solutions as such and after mixing were

injected in different amounts. Calibration curves were obtained for rutin, quercetin and kaempferol. Calibration curves were determined on the basis of fine amount of each standard.

Calibration plots

Calibration plots were prepared in order to find out the range of marker concentration, which shows a linear relation with respect to response of analytical technique. Once this range of response was established, concentrations of all test samples were so adjusted as to give the established linear range (*Figure 1*).

Standardization and calculation

The chromatograms were acquired using the analyst software and the data was processed by peak area method. The unknown concentration of the samples were calculated from the following equation using regression analysis of spiked calibration standard with the reciprocal of the ratio of the extract to the Internal Standard (IS), concentration as a weighing factor (1/concentration^2)

y=mx+b

Where, y is peak area ratio of the extract over IS, m is slope of the calibration curve, x is concentration ratio of extract over IS, b is y-axis intercept of the calibration curve

Anthelmintic activity

While studying the literature review of plant it was found that ethanolic extract of plant possess various pharmacological activities and most of its chemical constituents are soluble in ethanol. So, ethanolic extract of *Convolvulus arvensis* was selected for the activity and percentage yield of that extract was found to be 18.6%. Further the prepared ethanol extract was evaluated by anthelmintic activity against *fetida* worms (diseases/infections caused by helminths) at three concentrations (50, 100 and 150 mg/ml) (*Figure 2*). The study showed that ethanolic extract of *Convolvulus arvensis* was effective as anthelmintic. Results interpret dose dependent reduced motility of worms with reference to the standard (Albendazole). Therefore, current investigation leads to conclusion that the roots of *Convolvulus arvensis* have potent anthelmintic activity when compared with the conventionally used drugs and hence can be treatment of helminthic infections.

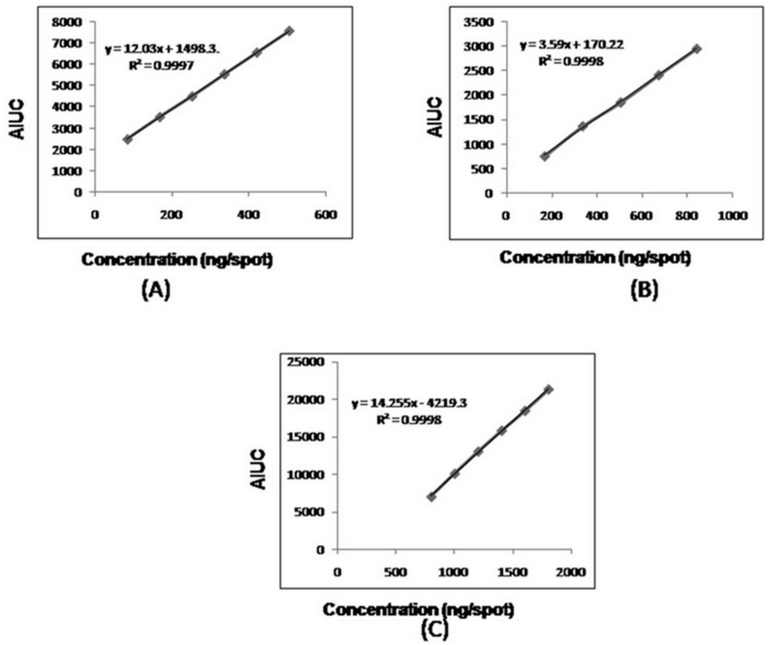

Figure 1: Calibration plots (A): Quercetrin; (B): Kaempferol and (C): Rutin

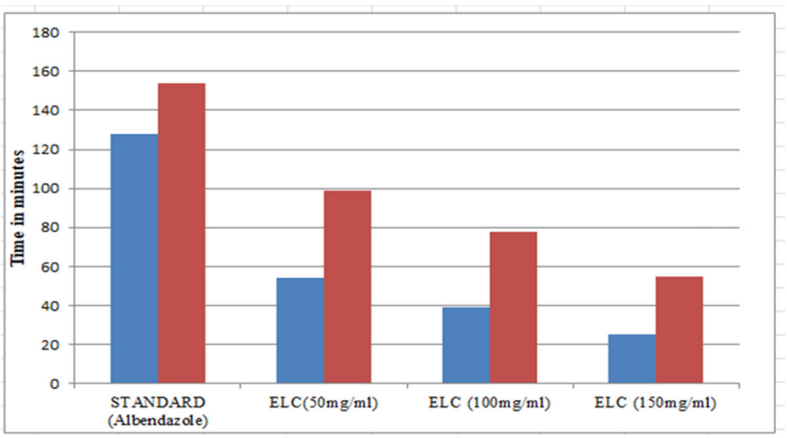

Figure 2: Graph for anthelmintic activity of ethanolic extract of *Convolvulus arvensis*
Note: (■): Series 1; (■): Series 2

RESULTS AND DISCUSSION

The RP-HPLC behavior of the flavonoids on the reversed phase column was tested sequentially varying the proportion of the acetonitrile-water elution mixture. The separation was achieved by modifying the mobile phase with small amount of trifluoroacetic acid. HPLC fingerprints of alcoholic extract of *Convolvulus arvensis* given in *Figure 3* and their retention times and amounts were calculated as shown in *Table 1*.

Results of HPLC analysis of *Convolvulus arvensis* alcoholic, hydroalcoholic and aqueous extract at 254 nm had shown the presence of abundant constituents as evidenced by chromatogram obtained at various retention times shown in *Figures 4, 5 and 6* respectively. Kaempferol had shown the highest retention time followed by quercetin. These constituents detected were present in ng amounts. Rutin was present in very high amount as compared to other constituents. The retention time of standards rutin, quercetrin, quercetin and kaempferol appeared at 17.644, 24.471, 38.193 and 39.533 as shown in *Table 1*.

On the basis of quantitative HPLC for flavonoids content (*Table 2*), it was found that maximum percentage of flavonoids was present in alcoholic and aqueous extracts as compared to 50% hydro-alcoholic of aerial parts of the *Convolvulus arvensis*. On the basis of above experiment performed, the ethanolic extract of *Convolvulus arvensis* with a concentration of 50 mg/ml shows significant anthelmintic activity (*Table 3*).

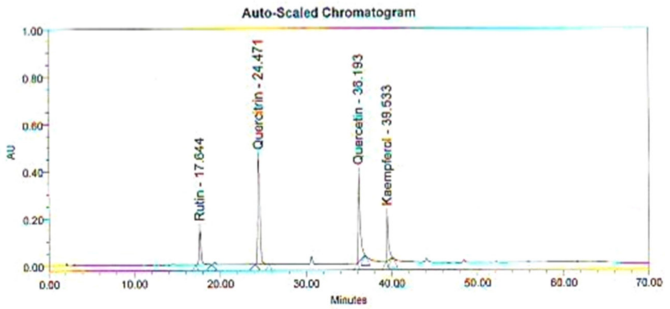

Figure 3: Retention time of standards rutin, kaempferol, quercetrin and quercetin

Table 1: Retention time and amount calculated of all standards

S. no	Peak name	RT	Area (µV sec)	% Area	Amount (ng)
1	Rutin	17.644	2329005	13	1875
2	Quercetrin	24.471	7566882	42.25	1500
3	Quercetin	38.193	5500590	30.71	1500
4	Kaempferol	39.533	2512380	14.03	1500

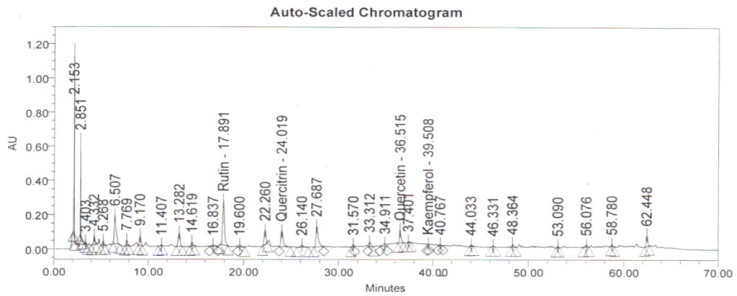

Figure 4: Chromatogram for the alcoholic extract of *Convolvulus arvensis*

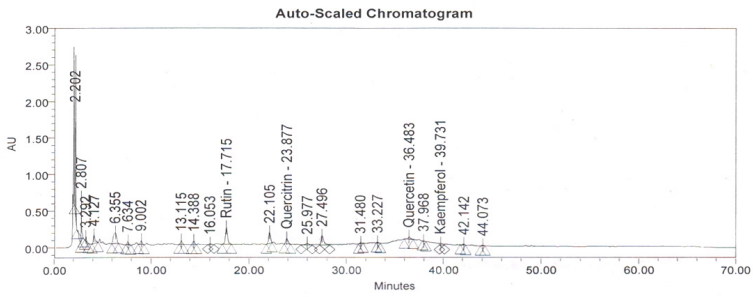

Figure 5: Chromatogram for 50% alcoholic extract of *Convolvulus arvensis*

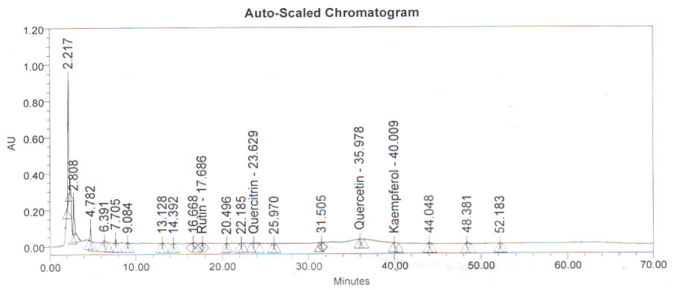

Figure 6: Chromatogram for the aqueous extract of *Convolvulus arvensis*

Table 2: Percentage amount of some flavonoids present in *Convolvulus arvensis* extract

Flavonoid	Alcoholic extract	50% alcoholic extract	Aqueous extract
	% of flavonoids		
Rutin	1.63	1.29	1.63
Quercetrin	1.3	1.03	1.3
Quercetin	1.3	1.03	1.3
Kaempferol	1.3	1.03	1.3

Table 3: Anthelmintic potency of ethanolic extract of *Convolvulus arvensis*

Extract	Concentration (mg/ml)	Fetida	
		Paralysis time	Death time
Standard (Albendazole)	20 mg/ml	128 min	154 min
Ethanolic extract	50 mg/mg	54 min	99 min
	100 mg/ml	39 min	78 min
	150 mg/ml	25 min	55 min

CONCLUSION

Based on HPLC studies of different extracts of *C. arvensis*, it is concluded that rutin is present in higher concentration in all extracts, although other flavonoids like kaemferol, quercetin and quercetrin are also present in extracts. These flavonoids are responsible for various therapeutic activities of the plant. The ethanolic extract of this plant exhibits promising anthelmintic activity, which can treat infections of animals with parasitic worms.

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