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Research Article

Quantification of Gallic Acid, Rutin and Quercetin in Hydro-Ethanolic Extract of Holarrhena Antidysenterica using High Performance Thin Layer Chromatography (HPTLC)

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Abstract

Holarrhena antidysenterica possesses antidiarrhoeal and antibacterial activity. The present study was focused to detect bioactive compounds by high-performance liquid chromatography (HPLC). HPLC is a tool to separate mixture of compounds in analytical chemistry (identify, quantify, purify) but cannot be used to establish biological activity. HPLC was performed to separate mixture of compounds with varying molecular sizes like gallic acid, quercetin and rutin in hydro-ethanolic extracts of seeds of *Holarrhena antidysenterica.* These markers were detected, quantified and compared with standards at 200 - 450 nm wavelength using mobile phase Toulene: Ethyl Acetate: Formic Acid: Methanol (3:3:0.3:0.2) with CAMAG Scanner III system. The hydro-ethanolic seeds extracts of *Holarrhena antidysenterica* showed peak *Rf* values which were coinciding with standard *Rf* value of gallic acid, quercetin and rutin. Comparable *Rf* values were found for gallic acid, quercetin and rutin in sample and reference standard. This study confirmed the presence of gallic acid, quercetin and rutin in *Holarrhena antidysenterica* seeds hydro-ethanolic extract might be important for preventing diarrhoea. In conclusion, the hydro-ethanolic extract of seeds of *Holarrhena antidysenterica* could be a potential source of antidiarrhoeal, antibacterial, astringent and anti-inflammatory drugs.

Keywords: Gallic Acid; *Holarrhena Antidysenterica*; Hptlc; Rutin; Quercetin

Introduction

Diarrhoea in ruminants can be greatly treated with medicinal plants because of better assimilation, rumen microbe friendliness and fewer side effects in their complex stomach. Now a days interest in herbal remedies for the treatment of such ailments is on rising. Numerus plants possess antidiarrhoeal and antibacterial activity acts by reducing the gastrointestinal motility and or the secretions. Although these plants have gained importance for the treatment of diarrhea, many remain to be evaluated scientifically, even though they have been traditionally used in the treatment of diarrhoea in India. Some of the most common plant possessing the antidiarrhoeal and antibacterial activity is *Holarrhena antidysenterica* [1]. Seeds of *Holarrhena antidysenterica* (Wall) have astringent and styptic property justifying its use in the treatment of diarrhoea and dysentery [2]. Crude aqueous and alcoholic extracts of *Holarrhena antidysenterica* stem bark also possess anti-bacterial effect against known enteric pathogens [3,4]. Kurchicin is an active principle of Kutaj (*Holarrhena antidysenterica*) is highly effective against causative micro-organisms of diarrhoea, dysentery i.e., especially amoebic type. At present, very limited literatures are available on phytochemical evaluation of seeds of *Holarrhena antidysenterica*. Hence, seeds of *Holarrhena antidysenterica* was selected to undertake phytochemical studies. Several alkaloids, glycosides, flavonoids, tannins, saponins, sterols and turpenoids [3,5] phytoconstituents isolated and identified from different parts of the plants.

Phytochemical evaluation for quality assessment includes preliminary phytochemical screening, chemo profiling and analysis of markers using modern analytical techniques. High performance thin layer chromatography (HPTLC) is an important method used for qualitative and quantitative phytochemical analysis [6]. This includes TLC fingerprint profiles and estimation of chemical markers and bio-markers [7]. Very limited literature is available on quantitative phytochemical evaluation of seeds of *Holarrhena antidysenterica*. This study was carried out for quantitative analysis (HPTLC) of phytochemicals with active bioactive compounds having antidiarrhoeal and antibacterial activity in this plant. In the present study Gallic acid, rutin and quercetin were estimated by High performance thin layer chromatography (HPTLC).

Materials and Methods

Preparation of cold extract

Holarrhena antidysenterica seeds were collected, identified and authenticated from botanist, Department of Botany, Shri Shivaji Science College, Akola (M.S). The seeds of *Holarrhena antidysenterica* after shade drying was powdered using pulverizing machine. 25g of freshly prepared powder was soaked in hydro-ethanolic solution (40% distilled water + 60% ethanol) in a flask plugged with cotton and mounted on orbital shaker for 48 hours and 150 rpm at room temperature and then filtered through Whatman filter paper No. 1. Final filtrate was evaporated and per cent extractability was measured. Airtight screw cap vials used to store the extracts which were kept in the desiccator until further use [15].

Methodology of high-performance thin layer chromatography [HPTLC]

Current study was performed on the hydro-ethanolic extracts of *Holarrhena antidysenterica* seeds for the quantitative estimation of biomarkers gallic acid, rutin and quercetin.

Instrumentation

CAMAG HPTLC system with a sample applicator Linomat V, 100 ml syringe (Hamilton, Bonaduz, Switzerland), twin trough development chamber (20 cm x10 cm) size, TLC Scanner III linked to WinCats software (CAMAG), pre-coated with 0.2 mm thickness silica gel 60 F254 (Merck) were used.

Reagents and chemicals

Toluene, Ethyl acetate, Methanol, Formic acid of analytical grade were procured and gallic acid, quercetin and rutin were procured from the Natural Remedies Ltd, Bangalore. TLC aluminium sheets precoated with silica gel 60F254 (10 x10 cm, 0.2 mm thick) were procured from E. MERCK KGaA.

Procedure

20 cm × 10 cm HPTLC silica gel G60 F254 plates with fluorescent indicator was used for analysis. HPTLC plates were cleaned by predevelopment with methanol by ascending method before start of analysis and then immersed in a solvent system consisting of CAMAG glass chamber (20 cm × 10 cm) filled with 30 ml methanol (HPLC grade) covered with glass lid and left till development of the plate to the top with methanol. The plate was removed from TLC glass chamber and dried in an oven after complete development. Three spots of 10 µl of standard preparation in the form of band were applied along with three spots of 10 µl of sample preparation on the same plate by means of a CAMAG Linomat 5 which is an automated spray-on applicator equipped with a 100 µl syringe operated with settings of 6 mm band length, 15 mm distance between bands, from the plate side edge and bottom of the plate [8,16].

Chromatographic conditions: TLC development and scanning

Extracts sample and standard gallic acid and rutin were spotted as 8 mm wide band width on a TLC aluminium sheets precoated with silica gel 60 F254 (10 x 10cm, 0.2 mm thickness) using automatic TLC applicator Linomat V,10 mm from the bottom. Toulene: Ethyl Acetate: Formic Acid: Methanol (3:3:0.3:0.2) for gallic acid, quercetin and rutin was used as mobile phase. Saturation was done by keeping plates for 15 min. in twin trough chamber then plates were dried in oven after development and gallic acid, quercetin and rutin were scanned at 200, 250, 300, 350, 400, 450 nm with CAMAG Scanner III.

Calibration curve for standards

TLC plate applied with the standard solutions was further developed and scanned as per the above-mentioned chromatographic conditions and recording of peak areas were done. Calibration curve of gallic acid, quercetin and rut in was obtained by plotting peak area against concentration of applied standards.

Results and Discussion

High performance thin layer chromatography (HPTLC) is an important analytical tool for qualitative and quantitative estimation of phytochemicals in plant [8]. HPTLC was carried out in the current research to identify specific biomarkers like gallic acid, quercetin

and rutin in *Holarrhena antidysenterica* seeds hydro-ethanolic extract. gallic acid, quercetin and rutin were detected and quantified at wavelength of 200, 250, 300, 350, 400 and 450 nm. Wavelength of 200 to 450 nm with reference to gallic acid, rutin as rutin trihydrate and quercetin as quercetin dihydrate as standards in mobile phase Toulene: Ethyl Acetate: Formic Acid: Methanol (3:3:0.3:0.2) used to measure given samples.

The HPTLC profile for hydro-ethanolic seeds extracts of *Holarrhena antidysenterica* is presented in Table 9. The *Rf* value of standard gallic acid found to be 0.42, 0.41, 0.40, 0.43, 0.42 and 0.44 at 200, 250, 300, 350, 400 and 450 nm respectively. The average *Rf* value of gallic acid showed peak of 0.42. The peak number 3 at wavelength 200nm, 250nm, 300nm and 350nm found in average 93.18% area in gallic acid standard i.e., more pure form of gallic acid at Rf 0.42 and at wavelength 400 and 450nm found Rf 0.42 shows 100% area in peak number 1. It confirms that gallic acid is present in the sample.

The *Rf* value of standard rutin were found to be 0.035, 0.03, 0.06, 0.03 and 0.03 at 200, 250, 300, 350 and 400 nm respectively. The average *Rf* value of rutin was 0.03, showed peak of rutin. In rutin trihydrate standard peak number 2 and 1 at wavelength 200 to 400 nm found in an average 82.23% area i.e., more pure form of rutin at Rf 0.03. No peak was seen at wavelength 450 nm.

The *Rf* value of standard quercetin were found to be 0.59, 0.58, 0.54, 0.55, 0.55 and 0.58 at 200, 250, 300, 350, 400 and 450 nm respectively. The average *Rf* value of quercetin showed peak of 0.56. In quercetin dihydrate standard peak no. 3 at 200 to 350 nm, peak no. 4 at 300nm and peak no. 2 at 400 and 450nm found highest purity i.e., in average 81.99% area and Rf 0.56 confirming presence of quercetin dihydrate in sample.

At wavelength 200 to 350 nm compound was found at Rf 0.40 in track 5 of *Holarrhena antidysenterica* which was very close to standard gallic acid peak and coinciding with standard *Rf* value of gallic acid. There was no peak of gallic acid at wavelength 450 nm. quercetin was found at wavelength 250 to 350nm with Rf 0.55 i.e., equal to standard quercetin and at wavelength 200, 400 and 450nm found Rf 0.62 i.e., nearer to standard quercetin. It showed presence of quercetin. At wavelength 300 and 400nm a compound rutin was found with Rf 0.06. Peak *Rf* values of *Holarrhena antidysenterica* were coinciding with *Rf* value of rutin standard. It confirms the presence of rutin. Comparable *Rf* values were found for gallic acid, quercetin and rutin in sample and reference standard. These findings confirmed the presence of gallic acid, quercetin and rutin in *Holarrhena antidysenterica* seeds hydro-ethanolic extract

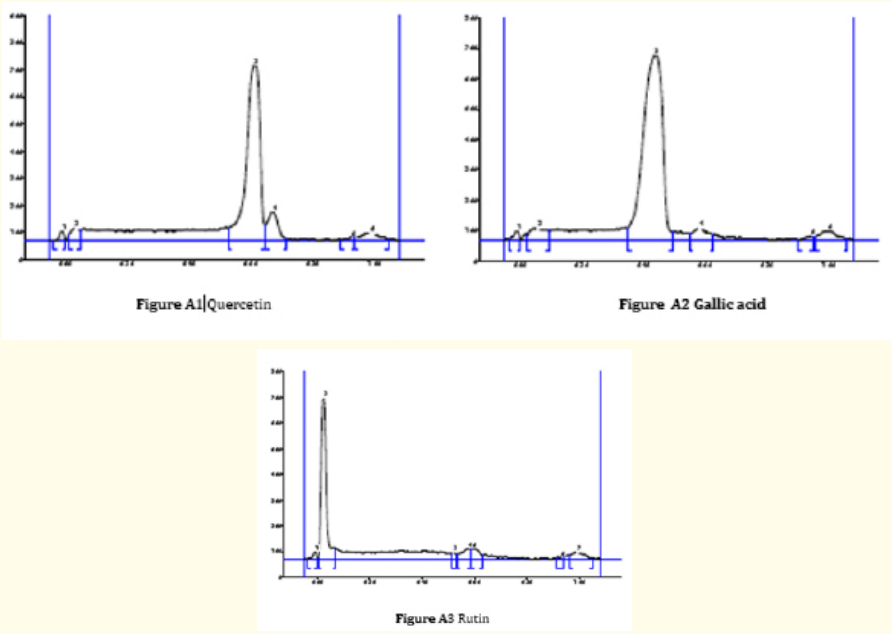

Figure 1: HPTLC Chromatogram of Quercetin (A1), Gallic Acid (A2) and Rutin (A3).

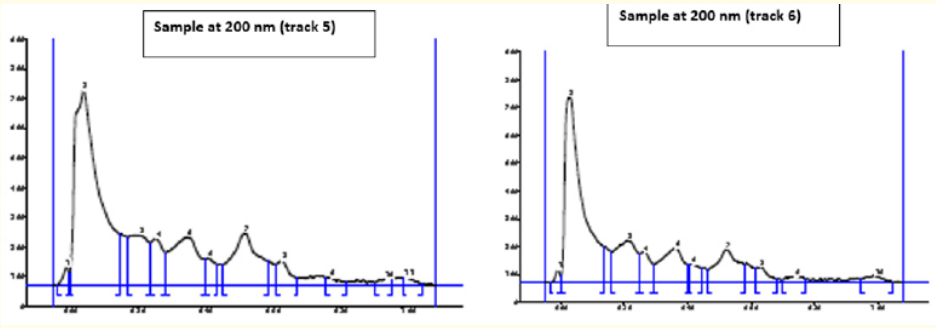

Figure 2: HPTLC Chromatogram of hydro-ethanolic extract of *Holarrhena antidysenterica* at 200 nm.

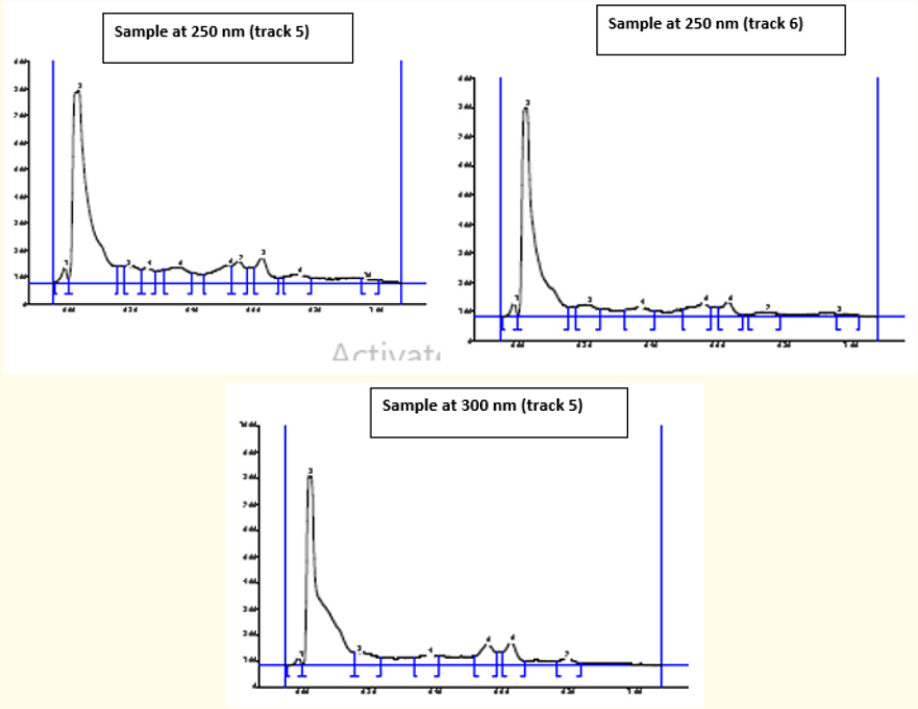

Figure 3: HPTLC Chromatogram of hydro-ethanolic extract of *Holarrhena antidysenterica* at 250 nm and 300 nm.

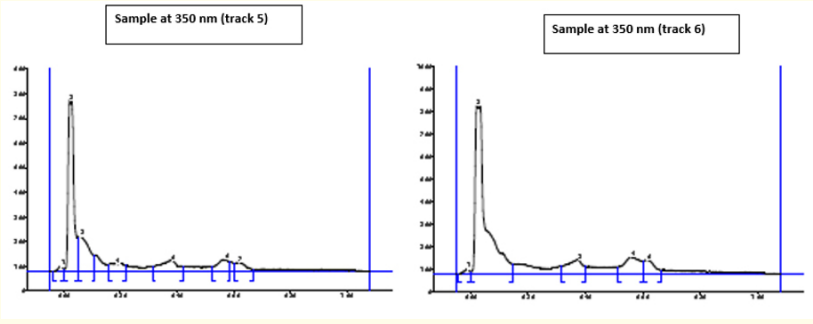

Figure 4: HPTLC Chromatogram of hydro-ethanolic extract of Holarrhena antidysenterica at 350 nm.

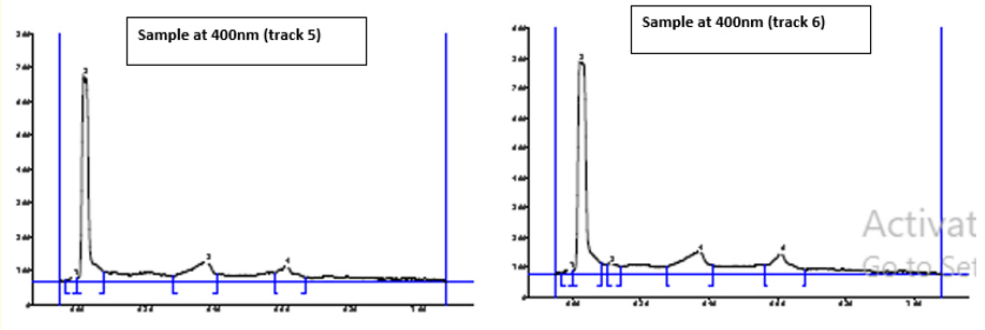

Figure 5: HPTLC Chromatogram of hydro-ethanolic extract of *Holarrhena antidysenterica* at 400 nm.

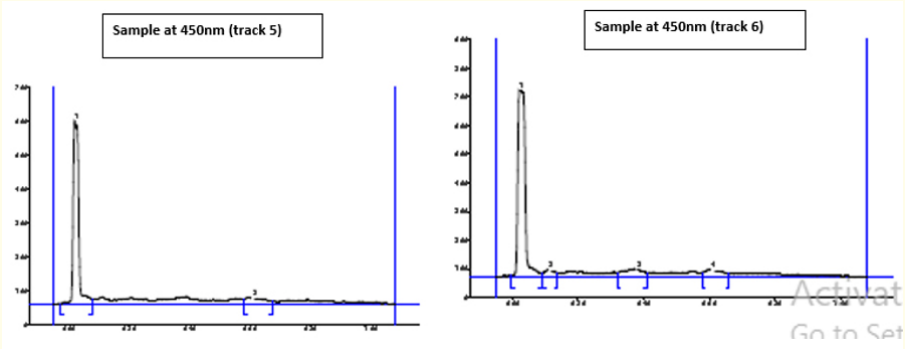

Figure 6: HPTLC Chromatogram of hydro-ethanolic extract of *Holarrhena antidysenterica* at 450 nm.

Sr. No	Name of sample	Wavelength (in nm)						Observation	Inference
		200	250	300	350	400	450		
1	Gallic acid std	Rf – 0.42 % Area –85.80 Peak no.- 3	Rf -0.41 % Area – 96.86 Peak no.- 3	Rf – 0.40 % Area -97.71 Peak no.- 3	Rf -0.43 % Area -92.27 Peak no. 3	Rf -0.42 % Area -100 Peak no. 1	Rf -0.44 % Area -100 Peak no. 1	At Rf 0.42 shows peak of Gallic acid	Gallic acid present
2	Rutin trihydrate std	Rf - 0.035 % Area – 70.75 Peak no. 2	Rf – 0.03 % Area – 88.61 Peak no.- 2	Rf -0.06 % Area – 76.70 Peak no. 2	Rf -0.03 % Area -85.51 Peak no. 2	Rf -0.03 % Area -89.77 Peak no. 1	No peak	At Rf 0.03 shows peak of Rutin trihydrates.	Rutin trihydrate present
3	Quercetin dihydrate std	Rf -0.59 % Area - 79.14 Peak no.- 3	Rf -0.58 % Area -83.27 Peak no. 3	Rf – 0.54 % Area- 81.20 Peak no. 4	Rf -0.55 % Area- 80.80 Peak no. 3	Rf -0.55 % Area -82.23 Peak no. 2	Rf – 0.58 % Area -51.5 Peak no. 2	At Rf 0.56 shows peak of Quercetin dihydrate.	Quercentine dehydrate present.

4	Sample - *Holarrhena antidysenterica* Track 5	Rf -0.42 Rf- 0.62	Rf- 0.39 Rf- 0.54	Rf- 0.08 Rf- 0.37 Rf-0.56	Rf-0.37 Rf- 0.56	Rf- 0.04 Rf- 0.62	Rf- 0.65		Gallic acid and Quercetin may be present
	Track 6	Rf -0.42 Rf- 0.64	Rf -0.37 Rf- 0.57		Rf -0.07 Rf- 0.36 Rf- 0.56	Rf -0.04 Rf- 0.35 Rf- 0.65	Rf -0.62		All are present

Table 1: HPTLC profile for standard biomarkers and hydroethanolic extract of *holarrhena antidysenterica* seeds.

Various herbal formulations can be tested by comparing the fingerprints with standard biomarkers. Flavonoïds (e.g., quercetin), glycosides (e.g., rutin) and simple phenols (e.g., gallic acid) are mainly found [3,12]. Flavonoids is a widely distributed group of polyphenol compounds throughout the plant tissues. It is present in the plant tissue in a free state, (quercetin: 5, 7, 3', 4'-tetrahydroxy flavonol), or in glycosides (rutin: 5, 7, 3, 4, tetrahydroxy flavonol-3-rhamnoglucoside. The most common flavonoïds found in plant tissues are quercetin and rutin [9]. The antibacterial property of *Holarrhena antidysenterica* might be due to the presence of tannin and flavoinoids as flavoinoids are known for antibacterial activity [10].

The simple phenol compounds (3, 4, 5- trihydroxybenzoates) found commonly in plant tissues is gallic acid. It possesses an astringent activity. Therefore, the gallic acid is well represented in chromatogram during quantitative estimation. In general Tannins and flavonoids have antidiarrhoeal activity by reducing intestinal motility, antimicrobial and antisecretory action [11].

Current study represents quantitative analysis of three biomarkers by the HPTLC and confirmed gallic acid as most abundant biomarker in the extract based on chromatographical representation. HPTLC study of *Holarrhena antidysenterica* seeds extracts showed presence of gallic acid, rutin and quercetine responsible for its antidiarrhoeal and antimicrobial activity [14]. This study support the antidiarrhoeal, antibacterial, astringent and anti-inflammatory activity of the said plant extract [11,13].

Conflicts of Interest

There are no conflicts of interest.

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