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Research Article

CHARACTERIZATION OF PHENOLIC COMPOUNDS IN *PSEUDARTHRIA VISCIDA* ROOT EXTRACT BY HPLC AND FT-IR ANALYSIS

THINAGARAN RAJAN[1] AND SURIYAVATHANA MUTHUKRISHNANA[2]

[1]Department of Biochemistry, Muthayammal College of Arts and Science, Rasipuram, Tamilnadu, India.,[2]Department of Biochemistry, Periyar University, Salem, Tamilnadu, India. E-mail:drrajan2012@gmail.com

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ABSTRACT

Gallic acid, Caffeic acid, Rutin, Quercetin and Ferulic acid were analyzed simultaneously by HPLC with UV detection at 280nm. The separation was carried out by C18 reverse phase column (Ø4.6x250mm) packed with 5µm diameter particles. Solvent gradients were performed by dual pumping system, with varied proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B [methanol]. Solvent B was increased to 80% in 10 minutes at a flow rate of 1.0ml/min. Presence of gallic acid, caffiec acid, rutin, quercetin and ferulic acid in the root of *Pseudarthria viscida* was identified by comparison of its retention time (t_R) with the standards. The contents of gallic acid, caffeic acid, rutin, quercetin and ferulic acid were successfully determined at 5.6, 9.3, 10.2, 12.3 and 23.8 minutes respectively. Three fractions were collected at the retention time (t_R) of 5.6, 10.2 and 12.3 minutes were subjected to FT-IR studies to confirm the presence of polyphenols in *Pseudarthria viscida* root extract. In FT-IR studies, the first fraction showed the groups specific for gallic acid, the second fraction confirms the presence of rutin while the third fraction gave the peaks relevant to quercetin.

Keywords: Gallic acid, Quercetin, Ferulic acid, *Pseudarthria viscida*

INTRODUCTION

Phytochemicals are non-nutritive plant chemicals that have protective (or) diseases preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. Plant and plant products are being used as a source of medicine since long. India has about 45,000 plant species and among them many have been claimed to possess medicinal properties. The need for scientific validation of these useful medicinal plants is very essential. The importance of ethno pharmacological investigation in the discovery of new therapeutic agents from plants has been discussed extensively [(1)].

Pseudarthria viscida (L) wight & Arn (Fabaceae) is a semi-erect diffuse under shrub, distributed throughout South India. The extract from the leaf, root, stem and callus of *Pseudarthria viscida* showed anti-fungal property [(2)]. A potential anti-oxidant activity has been reported from the stem and root extracts [(3)]. Literature survey revealed, the extract of this plant has been showed to exert anti-diabetic [(4)],anti-diarrhoeal [(5)] and anti-cancer effect [(6)]. But no data has been reported for the separation of phenol compounds from the root extract of *Pseudarthria viscida*. The objective of this work was to analyze the phenolic content present in the root of *Pseudarthria viscida* using HPLC and FT-IR techniques.

Gallic acid, Caffeic acid, Rutin, Quercetin and Ferulic acid are phenolic compounds. Structurally they have phenol groups which serve as a source of readily available hydrogen atoms such that the subsequent radicals produced can be delocalized over the phenol structure [(7, 8)]. The interest of these compounds is due to their pharmacological activity as radical scavengers [(9, 10)]. They have proved to have potential preventive and therapeutic effects in many diseases. These five phenols are widely distributed in the plant kingdom. In this study, HPLC with UV detector was used for determination of Gallic acid, Caffeic acid, Rutin, Quercetin and Ferulic acid in the root extract of *Pseudarthria viscida*, and FT-IR analysis was carried out for the three fractions of HPLC.

MATERIALS AND METHODS

Sample preparation

The fresh roots of *Pseudarthria viscida* were washed and dried at 60˚C.Then, dried roots were ground to fine powder. Extraction was carried out using 2g of powdered root with 50ml of 95% ethanol under 80KHZ, 45˚C in ultrasonic extraction device for 30 minutes, repeated twice. The extract was collected and filtered; the filtrate was dried at 50˚C under reduced pressure in rotatory evaporator. The dried crude extract was dissolved in the 100 ml mobile phase. After filtering through a filter paper and a 0.45µm membrane filter (Millipore), the extract was injected into HPLC by auto sampler.

High Performance Liquid Chromatography

Qualitative and Quantitative analysis of phenols was performed through HPLC by using Shimadzu (Japan) equipped with C18 reverse-phase column (Ø4.6mmX250mm) packed with 5µm diameter particles. The column oven [CTO 10 AS_{VP}] temperature was set at 40°C. The HPLC system consisted of LC-10 AVP series pumping system, SIL-6A automatic injector furnished with the 20µl loop and SPD-10AVP UV detector at 280nm. Data was integrated by Shimadzu class VP series software and results were obtained by comparison with standards. Results are the mean values of three replicates of the same samples.

Fourier Transform Infrared Spectroscopy-FT-IR

FT-IR spectra were recorded with a Perkin Elmer-Spectrum RxI Spectrometer equipped with a Mullard I-alanine doped triglycine sulfate (DTGS) detector. The spectrometer was continuously purged with dry nitrogen to eliminate atmospheric water vapour and carbon dioxide. Fractions obtained from HPLC were scanned at room temperature (25° ± 1° C) in the 4000-400 cm^{-1} spectral range. To improve the signal to noise ratio for each spectrum, 100 interferograms with a spectral resolution of ±4 cm^{-1} were averaged. Background spectra, which were collected under identical conditions, were subtracted from the sample spectra automatically. The frequencies for all sharp bands were accurate to 0.001 cm^{-1}. Each sample was scanned under the same condition for five times. These replicates were averaged and then used. Transmittance percentage of the peaks was calculated with base-line method. The spectrum was analyzed using origin 6.1 software.

RESULTS AND DISCUSSION

HPLC analysis

The five phenol compounds viz., Gallic acid, Caffeic acid, Rutin, Quercetin and Ferulic acid are polar molecules. In the beginning various proportions of either methanol-water (or) acetonitrile-water system were used as mobile phase but separation was not satisfactory. The presence of acid in a mobile phase system gave a

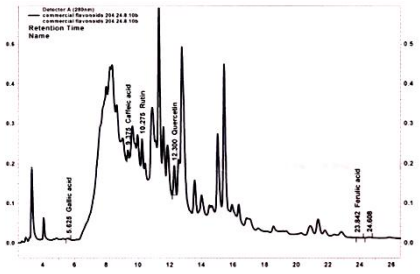
much better separation. The gradient elution of a solvent A [water-acetic acid (25:1, V/V)] and solvent B [methanol] had a significant effect on the resolution of a compounds. As a result, solvent gradients were using dual pumping system, by varying the proportion of solvent A [water-acetic acid (25:1, V/V)] to solvent B [methanol]. Solvent B was increased to 50% in 4 mins and subsequently increased to 80% in 10 mins at a flow rate of 1.0 ml / min. Detection wavelength was 280nm.

Standard mixture solutions of two concentrations, at high and low concentrations, were injected five times to determine the reproducibility to peak areas and retention times under the optimum conditions. The extract of Pseudarthria viscida was analyzed under the proposed condition. The content of each phenol compound was calculated by comparison to calibration curve derived from standards. From the calibration curve results, the amount of Gallic acid, Caffeic acid, Rutin, Quercetin and Ferulic acid was calculated as 8, 110, 40, 852 and 5.0µg/gm sample respectively. Fig: 1 represents good separation which can be achieved with in 26 minutes. The elution order and the retention time (t_R) for Gallic acid, Caffeic acid, Rutin, Quercetin and Ferulic acid were 5.6, 9.3, 10.2, 12.3 and 23.8 min respectively.

FT-IR Studies

Using fraction collector in HPLC, three fractions are collected at the retention time (t_R) 5.6, 10.2 and 12.3 minutes were subjected to FT-IR analysis and the functional groups associated were determined (Fig 2 to 4). The FT-IR spectrum of first fraction (t_R=5.6 min) contains nine peaks (Fig. 2). Of that, five peaks are characteristics of Gallic acid includes 3409.38 cm^{-1}(polymeric OH stretch), 1639.45 cm^{-1}(C=O stretch), 1389.62 cm^{-1}(Phenol (or) tertiary alcohol, OH bend), 1276.15 cm^{-1}(C-O stretch) and 1016.36 cm^{-1}(C-C stretch). The FT-IR spectrum of second fraction (t_R=10.2 min) recorded 11 peaks (Fig. 3). Out of that, six peaks confirm the presence of functional groups of rutin. They are 3436.50 cm^{-1} (polymeric OH stretch), 2966.13 cm^{-1} (C-H stretch (or) C-H group of aromatic ring), 1643.62 cm^{-1} (C=O stretch), 1397.33 cm^{-1} (phenol (or) tertiary alcohol, OH bend) 1276.67 cm^{-1} (C-O stretch) and 1018.85 cm^{-1} (C-C stretch). In FT-IR analysis, the third fraction (t_R=12.3 min) gave 12 peaks (Fig. 4). In that, seven peaks confirmed the presence of functional groups to Quercetin. It includes 3395.32 cm^{-1} (polymeric OH stretch), 2955.51 cm^{-1} (C-H group in aromatic ring), 1645.94 cm^{-1}(C=O stretch), 1399.96 cm^{-1}(phenol (or) tertiary alcohol, OH bend), 1275.97 cm^{-1} (C-O stretch), 1110.11 cm^{-1} (C=C stretch) and 1019.45 cm^{-1} (C-C stretch). The above infrared functional group characteristics were cited in literature [11-14].

By HPLC studies, the presence of five phenolic compounds viz. Gallic acid, Caffeic acid, Rutin, Quercetin and Ferulic acid were analyzed both qualitatively and quantitatively in the root extract of Pseudarthria viscida. The existence of Gallic acid, Rutin and Quercetin were further confirmed by the FT-IR analysis of HPLC fractions.



Time (min)

Figure1: HPLC chromatogram of *Pseudarthria Viscida*

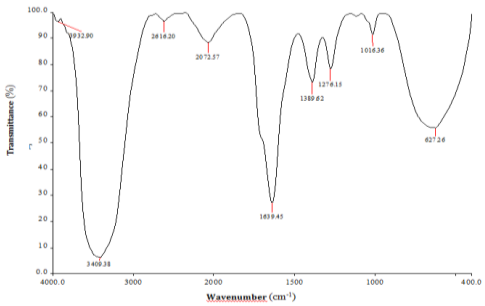


Figure 2: FT-IR Spectrum of *Pseudarthria Viscida* (HPLC Fraction 1,t_R=5.6min)

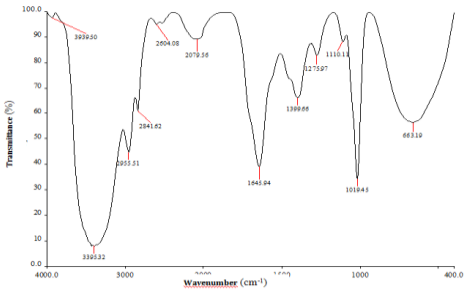


Figure 3: FT-IR Spectrum of *Pseudarthria Viscida* (HPLC Fraction 2,t_R=10.2min)

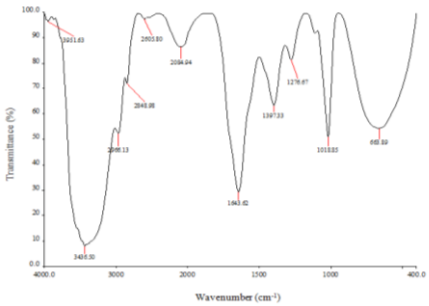


Figure 4: FT-IR Spectrum of *Pseudarthria Viscida* (HPLC Fraction 3,t_R=12.3min)

CONCLUSION

Simultaneous analysis of Gallic acid, Caffeic acid, Rutin, Quercetin and Ferulic acid by HPLC method has been developed. This HPLC procedure provides an excellent identification and quantification tool for these five phenolic compounds present in *Pseudarthria viscida* root with a short analysis time of 26 minutes. The experimental result indicated that *Pseudarthria viscida* root extract contained high concentration of Quercetin followed by Caffeic acid, Rutin, Gallic acid and Ferulic acid in order. The three fractions collected from HPLC at standard retention time of Gallic acid, Rutin and Quercetin were analyzed by FT-IR studies. This confirmed the presence of three phenolic compounds in the root extract of *Pseudarthria viscida*. Since the phenolic compounds have been of interest of health benefits, the present analytical study could be a potential application to identify and quantify the phenolic compounds in this plant.

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