



**HPLC FINGER PRINTS OF PHENOLIC COMPOUNDS PRESENT IN *ACTINIOPTERIS RADIATA*
(SWARTZ) LINK.**

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ABSTRACT

The present investigation was carried out to analyze the phenolic or flavonoid components from the whole plant of *Actiniopteris radiata* (Pteridaceae) using HPLC technique. The results clearly showed the presence of five phenolic compounds such as Gallic acid (GA), Caffeic acid(CA), Rutin(RU), Quercetin(QU) and Ferulic acid(FA). The five peaks were observed at 280nm and these five fractions were collected at different retention time (Rt), peak area, height and concentration. The HPLC chromatogram of the five peaks of the flavonoid compounds detected and the results based on the retention time such as Gallic acid (R_t-5.967), Caffeic acid (R_t-9.600), Rutin (R_t-10.525), Quercetin (R_t- 12.292) and Ferulic acid (R_t-23.917) in *A. radiata* were found to be in the concentration of 0.5 µg/gm, 0.90 µg/gm, 2.0 µg/gm, 2.0 µg/gm, and 0.1 µg/gm respectively. The obtained values were compared with the chromatographic separations of standards. The Individual flavonoid content for all the flavonoids were calculated from the corresponding calibration curve, plotted and presented as validation data. The biological activities of phenolic or flavonoid compounds were found to be effective antimicrobial substances against a wide range of microorganisms. The HPLC fingerprints of the compounds could be serve the purpose of established benchmarks for future plant research.

KEYWORDS: *Actiniopteris radiata*, HPLC analysis, phenolic components, flavonoids, standards.

INTRODUCTION

Phytochemistry is one of the more fashionable and rapidly expanding areas of plant taxonomy (chemosystematics) which utilizes chemical information to improve the classification of plants. The phytochemical characters could be used as markers to identify and differentiate the species. Phytochemical analysis of ferns makes the basis for the investigations on medicinal uses of the plants. ^[1] All the living and extinct organisms in the World are made up of organic and inorganic chemicals. Autotrophic plants synthesize the organic foods and heterotrophs utilize them. Apart from synthesizing food materials, plants synthesize a variety of chemicals for additional purposes. Such chemicals are called secondary metabolites. ^[2]

Secondary metabolites are more valuable chemicals for chemotaxonomy due to their restricted occurrence among plants. Since ferns and fern allies have survived from Paleozoic times, they have adapted with many more various changes of environment than the other primitive vascular plants. ^[3] Therefore, ferns are expected to have many useful secondary metabolites than other plants. Ferns were reported to have many useful phytochemicals

such as flavonoids, steroids, alkaloids, phenols, triterpenoid compounds, varieties of amino acids and fatty acids. The ferns and their allies appear to have relatively diverse polyphenolic profiles, including a variety of flavonoids and xanthenes. Currently twelve flavonoid types have been documented for the ferns and their allies. ^[4]

Current information indicates that the various orders comprising in these ferns, contain a characteristic group of polyphenolics. Thus it is very easy to trace out the evolutionary pathways based on these chemicals. Phytochemical studies on such chemo taxonomically valuable group of chemicals i.e. polyphenolics have been done on a good number of Indian ferns with the report of several new compounds. They also have some unique secondary metabolites which have not been discovered in higher plants. ^[5] Polyphenols are useful phytochemicals which provide health benefits such as antioxidant activity. It is generally recognized to reduce the risk factors of chronic disease. They can be beneficial to the human body by removing pathogens and old proteins. ^[6-]

Due to an increased concern about human health, longevity and eco-friendly life style, the health supplement markets are expanding rapidly. Synthetic compounds were popular due to their cheap price and quick efficacy in the past. However many studies reported their side effects, such as carcinogenesis.^[8] The preference of natural substances has increased rapidly worldwide. In these circumstances, human interests of ferns and fern allies will be not only for ornamental but also used for medicinal purposes.^[9]

To facilitate the health benefits and minimize the risk factors of chronic disease, the isolation of secondary metabolites from an important medicinal fern *A. radiata* was done by performing High Performance Liquid Chromatography (HPLC). It is a fast and reliable method for identification of plant phenolics. It can be employed for the routine analysis of natural and synthetic compounds in pharmaceutical formulations and in bulk drug preparations as well as for the quality assurance of related extracts and market samples. The main objective of the present study is to determine the chromatograms of standard phenolic chemical compounds which are found in this experimental plant *A. radiata* by HPLC.

MATERIALS AND METHODS

Collection and Preparation of the extract

A. radiata is commonly grown in hilly areas or foot hills of the Western Ghats which is one of the most fertile places in the state of Tamil Nadu. It was collected from crevices of rock in Sothuparai dam site at Palani hills, Theni district, Tamil Nadu, India. The collected whole plant was thoroughly washed, shade dried and then powdered with the help of a blender. The powdered whole plant material was used for extraction. The extraction was carried out using 2ml of fermented broth with 50ml of 95% ethanol under 80 KHz, 45°C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50°C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in the 100ml mobile phase. After filtering through a filter paper and a 0.45µm membrane filter (Millipore), the extract was injected into HPLC instrument.

Preparation of standards

5mg each of gallic acid, caffeic acid, rutin, quercetin and ferulic acid were accurately weighed into a 25ml of volumetric flask and dissolved in 3ml of methanol. Each of them was then sonicated for 5 min. and the final volume was made up to 5ml with the same solvent to obtain stock solutions of 1mg/ml. The different concentrations of standard solutions were prepared such as 2, 4, 6, 8 and 10µg/ml. All the standard solutions were filtered through 0.45µm membrane filter (Millipore) and injected by an auto sampler.

HPLC Analysis^[10]

Plant phenolics or flavonoids were analysed using a HPLC method, Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD-10ATVp UV VIS detector and a loop injector with a loop size of 20µl. The peak area was calculated with CLASS-VP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6mm, particle size 5µm, Luna 5µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, 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 min. and subsequently increased to 80% in 10 min at a flow rate of 1.0 ml/min. Detection wavelength was 280 nm. Gallic acid (GA), Caffeic acid (CA), Rutin (RU), Ferulic acid (FA) and Quercetin (QU) was used as internal and external standards. Phenolic acids present in each sample were identified by comparing chromatographic peaks with the retention time (R_t) of individual standards and further confirmed by co-injection with isolated standards. The amount of each phenolic acid is expressed as micrograms per gram of fresh weight unless otherwise stated.

RESULTS

The studies on the phenolics or flavonoids of *A. radiata* by HPLC analysis clearly showed the presence of five phenolic or flavonoid compounds such as Gallic acid, Caffeic acid, Rutin, Quercetin and Ferulic acid. The five peaks were observed at 280nm and these five fractions were collected at different retention time (R_t), peak area, height and concentration.

The chromatographic separations of standards based on Retention time (R_t) such as GA (R_t -5.750), CA (R_t -9.450), RU (R_t -10.517), QU (R_t -12.400) and FA (R_t -24.175) were showed in Figure-1. The Individual flavonoid content for all the flavonoids were calculated from the corresponding calibration curve, plotted and presented as validation data as shown in Table-1.

The results based on the Retention time (R_t) such as Gallic acid (R_t -5.967), Caffeic acid (R_t -9.600), Rutin (R_t -10.525), Quercetin (R_t -12.292) and Ferulic acid (R_t -23.917) in *A. radiata* were found to be in the concentration of 0.5 µg/gm, 0.90 µg/gm, 2.0 µg/gm, 2.0 µg/gm, and 0.1 µg/gm respectively (Table-2). The obtained values were compared with the standards. The HPLC chromatogram of the five peaks of the flavonoid compounds detected were shown in Figure- 2.

Table-1: HPLC Validation data for flavonoid standards

SPD10Avp (280nm)								
Pk #	Retention Time	Area	Height	Area Percent	Height Percent	concentration	Units	Name
1	5.750	56744802	2757981	42.84	29.72	10.000	µg/ml	Gallic acid
2	9.450	17443471	1882880	13.17	20.29	10.000	µg/ml	Caffeic acid
3	10.517	42056735	3198304	31.75	34.47	10.000	µg/ml	Rutin
4	12.400	13396467	1402866	10.11	15.12	10.000	µg/ml	Quercetin
5	24.175	2810655	36358	2.12	0.39	10.000	µg/ml	Ferulic acid

Table -2: HPLC Validation data for whole plant of *A. radiata* (Swartz) Link.

SPD10Avp (280nm)								
Pk #	Retention Time	Area	Height	Area Percent	Height Percent	concentration	Units	Name
1	5.967	25932	981	41.30	1.40	0.5	µg/ml	Gallic acid
2	9.600	15597	1111	2.81	2.48	0.90	µg/ml	Caffeic acid
3	10.525	7850	118	1.31	0.14	2.0	µg/ml	Rutin
4	12.292	2093	0	0.5	0	2.0	µg/ml	Quercetin
5	23.917	117	0	0.06	0	0.1	µg/ml	Ferulic acid

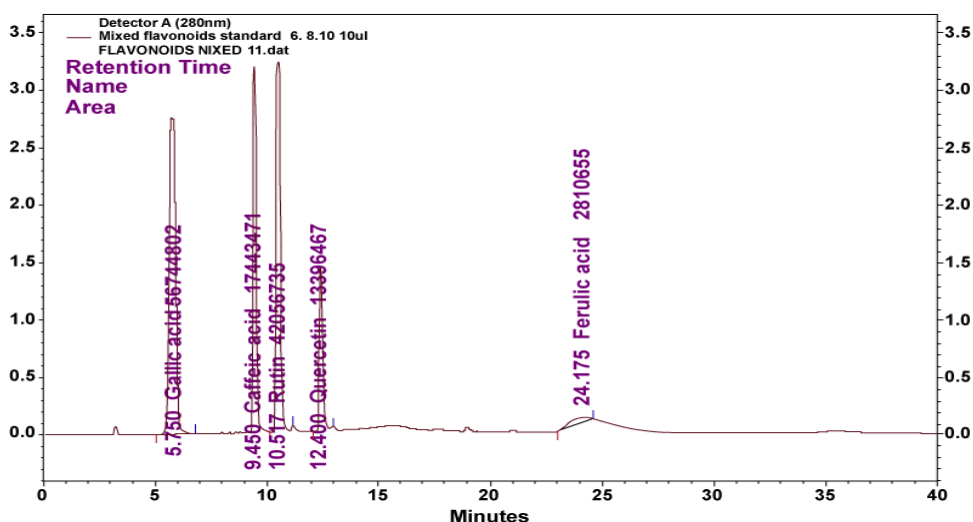


Fig -1: HPLC Chromatogram of Flavonoid Standards

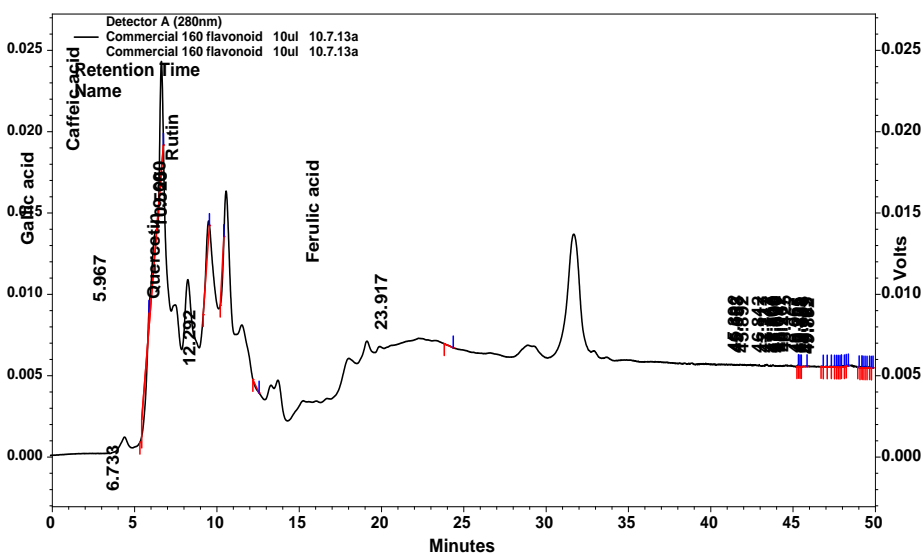


Fig-2: HPLC Chromatogram of the whole plant of *Actiniopteris radiata* (Swartz) Link.

DISCUSSION

The results of HPLC analysis was supported by many authors. They estimated the quantitative determination of flavonoid compound such as Quercetin in the seeds of ethanolic extract of *Elaeocarpus ganitrus* by using high performance thin layer chromatography.^[11] The concentration of quercetin in *Elaeocarpus ganitrus* seed was calculated based on calibration curve and the types of flavonoids such as astragalin, kaempferol glucoside and kaempferol rutinoid have been reported in *C. parasitica*.^[12] Diterpenoids isolated from the extract of *Pteris semipinnata* by HPLC- APCI- MS.^[13]

The bioactive flavonoids, namely Rutin and Quercetin may be extracted from the macrophyte aquatic fern *Azolla microphylla* in an inexpensive route and concluded that the plant may be a potential source for the isolation of these bioactive flavonoids that would be useful to prepare plant-based pharmaceutical preparation to treat various complications linked with human diseases. For bio-evaluation studies, Rutin is used for the treatment of various conditions related to capillary bleeding and increased capillary fragility and permeability.^[14]

The phenolic or flavonoid compounds were used as major ingredients in plant based pharmaceutical preparations such as Gallic acid (GA) is used to treat albuminuria, psoriasis, diabetes and external haemorrhoids. Caffeic acid (CA) is a promising compound for dermal diseases. Rutin (RU) is an important compound to prevent blood clots, heart attacks and strokes. Quercetin (QU) is an effective bronchodilator and acts against allergic or inflammatory chemicals in the body and prevent or treat cancer cells in humans. Ferulic acid (FA) was found to have antitumour activity against breast and liver cancer.^[15]

The combination of flavonoids such as Rutin and Quercetin has been frequently used in the allergic conditions. The Quercetin alone exerts cytotoxic effects against the human cancer cell line.^[16] Additionally, flavonoids are also widely used in the food industry for the preservation of food to elongate the shelf life by preventing or delaying the oxidation process.^[17] The three flavonoids were isolated from the ethyl acetate fraction of dried fronds of *Adiantum capillus-veneris* and identified as quercetin, quercetin-3-O-glucoside and quercetin-3-O-rutinoside (rutin). These isolated compounds showed an anti-inflammatory activity.^[18]

The HPLC fingerprints of these standard phenolic compounds would serve the purpose of established benchmarks for future plant research. The qualitative and quantitative analysis of the actual phenolic compounds present in any unknown plant sample would be facilitated by means of comparison with such standard chromatograms, enabling identification and confirmation of presence of these phenolic compounds in the experimental plant *A. radiata*.

CONCLUSION

It could be concluded that, HPLC analysis is the first step towards understanding the nature of active principles in the folklore medicine prevails in the tribal community which become a practical application in future. The present study such as HPLC profiles on *A. radiata* revealed the presence of five phenolic or flavonoid compounds. The biological activities of flavonoid compounds were found to be effective antimicrobial substances against a wide range of microorganisms. The presence of various bioactive compounds justifies that the whole plant is used for various ailments by traditional practitioner especially tribal people. So it is recommended as a plant of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

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