Estimation of quercetin in different tissues and genotypes of *Asparagus racemosus* using RP-HPLC-DAD

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Abstract:

Asparagus racemosus is one of the most extensively used traditional medicinal plant in India. The therapeutic properties of *A. racemosus* are due to the presence of secondary metabolites like quercetins. This study aims to measure the levels of quercetins in several *Asparagus racemosus* genotypes' tissues, including the roots, shoots, and leaves. HPLC-DAD method was optimized for the quantification of quercetins. In roots, shoots, and leaves, quercetin levels ranged from 0.043 mg/ml to 0.276 mg/ml, 0.033 mg/ml to 0.257 mg/ml, and 0.046 mg/ml to 0.293 mg/ml, respectively. For quercetin, the LOD and LOQ values were 0.048 and 0.146, respectively. Insights on the variety of quercetin content across distinct genotypes and plant parts are provided by the current study. Additionally, it was shown that leaves contain more quercetins than roots and shoots.

Keywords: *Asparagus racemosus*, Secondary metabolite, Quercetin, HPLC

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1. Introduction:

The investigation of phytochemicals and their potential to improve human health has seen a considerable increase recently. This recent development focuses on researching numerous plant-derived substances and their potential to enhance wellbeing. These phytochemicals have demonstrated promising effects in areas such as anti-aging, and cosmetics, as well as in the treatment and management of diseases such as cancer, cardiovascular disorders, and neurological conditions.[1] The pharmacological effects observed in plants can be attributed to the presence of metabolites, secondary metabolites, and secretory products within their composition.[2] Organic substances known as secondary metabolites are produced by bacteria, fungus, and plants that are indirect contributors to the host organism's regular development, growth, or reproduction. Despite this, these compounds have applications throughout industry, agriculture, and medicine and play essential roles in ecological interactions.[3] *Asparagus racemosus*, commonly known as Shatavari, is a plant prominently known for its richness of

secondary metabolites.[4] The word "Shatavari" means "who possesses a hundred husbands or acceptable to many," emphasizing its essential contributions to enhancing the health of female reproduction. A renowned scientist Galen called it a "cleansing and healing agent" and gave it the title of "Queen of Herbs" for its capacity to foster adoration and devotion.[5,6] Secondary metabolites such as steroidal saponins, flavonoids, alkaloids, and essential oils are highly concentrated in shatavari. A class of pigments known as flavonoids, which gives plants their vivid hues, includes quercetin. Quercetin and other flavonoids have various important roles in plants, including pigmentation. One of these is UV defence, since these substances serve as natural sunscreens, protecting plant tissues from the damaging effects of ultraviolet light. Quercetin performs crucial roles in plant physiology, including UV defence, pollinator attraction, and herbivore defence. These substances also have anti-inflammatory, anti-oxidative, and anticancer effects that might be advantageous for human health.[7,8]

Understanding the effects of cultivation region and plant genetics on the profile of secondary metabolites in plant extracts has received more attention in recent years.[9] High-Performance Liquid Chromatography (HPLC) has become a valuable method for characterizing these secondary metabolites. HPLC enables accurate analysis by isolating the constituents of a complicated mixture and identifying them based on their retention times and spectrum properties. Additionally, it makes it possible to quantify each ingredient in the mixture, which is essential for quality assurance during the production of natural goods. The extraordinary sensitivity of HPLC, which allows for the identification of even minute levels of chemicals in a sample, is one of its most significant benefits.[10] Since secondary metabolites frequently occur in trace amounts in plant and microbial extracts, this property is beneficial for the investigation of secondary metabolites. Researchers can precisely test and assess these metabolites because of HPLC's great sensitivity, providing information on their existence and possible biological actions. The primary objective of the current study was to evaluate and compare the levels of quercetin in 42 genotypes collected from various geographical regions, namely in the tissues of roots, shoots, and leaves. This initiative aimed to evaluate the changes in secondary metabolite composition between these different tissues and regions.

Overall, a thorough understanding of secondary metabolite profile in plants is provided by combining HPLC with research on cultivation area and plant genetics. This study advances our understanding of how genetic variants affect the levels of a particular chemical, quercetin, in distinct plant tissues derived from different geographical locations.

2. Materials and Methods:

2.1 Plant Materials:

A comprehensive collection of 42 genotypes of *Asparagus racemosus* was acquired for the study, with 32 genotypes obtained from CCSHAU, Hisar, and an additional 10 genotypes procured from NBPGR, New Delhi.

2.2 Extract Preparation:

In *Asparagus racemosus*, quercetin was extracted using maceration extraction method from roots, shoots and leaves of all genotypes by maceration method described by Marsoul et. al., (2020) with minute modifications.[11] Roots, shoots and leaves from different genotypes were shade dried at room temperature and then ground to a fine powder. This powder is then subjected to methanol extraction for further HPLC analysis of quercetin. In 100ml of 100% methanol, 10 grams of each sample was dissolved and incubated for 24 hours in a shaking incubator. The residue obtained after filtration through Whatman filter paper (3.1) was extracted again using fresh solvent under the same conditions. Quercetin was quantified by high performance liquid chromatography-diode array detector (HPLC-DAD) according to the method described by Huang et. al., (2022).[12]

The combined methanolic extract was used for HPLC analysis of quercetin. *Asparagus racemosus* extract samples were precisely weighed and diluted in a 1 mg/ml final concentration. HPLC grade water was used to dissolve a suitable amount of analyte. Before being injected into the HPLC, the dissolved samples were sonicated in an ultrasonic water bath and filtered using 0.45 m syringe filters. Quercetin content in plant extract was determined by calculating the peak area of standard quercetin and extracted samples following the completion of the run. Plotting the peak area against the relevant standard quercetin concentration led to the creation of calibration curves.

2.3 HPLC analysis:

All the solvents used in HPLC analysis were of HPLC grade and purchased from Merck, Darmstadt, Germany. Quantitative estimation of quercetin in different samples was done by using a high-performance liquid chromatography-diode array detector (HPLC-DAD) from Agilent technology containing reverse-phase chromatographic C18 column.

A standard graph was prepared from known concentrations of pure quercetin ranging from 0.1 mg/ml to 0.5 mg/ml purchased from MP Biomedicals. The presence and quantification of

quercetin in methanol extracts prepared earlier was done in the HPLC at wavelength of 368 nm with 1.0 ml/min flow rate at injection volume of 10 µl at 30 °C column temperature. The mobile phase consists of water: acetonitrile: methanol in ratio of 45:15:40.[14]

2.4 Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The Limit of Detection (LOD) refers to the lowest detectable amount of an analyte using a specific analytical method, ensuring reliable detection. On the other hand, the Limit of Quantitation (LOQ) denotes the lowest measurable amount of an analyte with reasonable accuracy and precision using a specific analytical method. LOD and LOQ are crucial parameters for evaluating the sensitivity of the High-Performance Liquid Chromatography (HPLC) analytical method. They play a vital role in establishing the detection and quantification limits of the method, allowing for proper evaluation of its suitability for the intended application.

3. Results and Discussion:

3.1. Extract preparation:

Extracts were prepared from all forty-two genotypes of *A. racemosus* and used for further analysis by HPLC-DAD.

3.2 HPLC Analysis:

3.2.1 System Suitability

Quantity and quality of quercetin extracted from forty-two genotypes of *Asparagus racemosus* plant were done by High-performance liquid chromatography. Quercetin was analysed by the method already described by Huang et al. (2022)[12].

3.2.2 Linearity

A linear correlation was observed in the concentration range of 0.1-0.5mg/ml (0.1,0.2,0.3,0.4 and 0.5 mg/ml) of quercetin (Figure 1) as indicated by the plotted graph that shows the concentration (mg/ml). The obtained regression equation for quercetin was y= 5E+08x - 2E+06 (r^2=0.9951). By calculating the correlation coefficient, the degree of association between two variables, represented by the square value denoted as r^2, was determined. The resulting value indicates that the graph plotting method employed exhibits a linear relationship between the two variables under analysis: peak area and solution concentration. This linear relationship holds true across the range of concentrations that were examined. The chromatograms obtained for standard quercetin and methanolic extracts in different tissues of *A. racemosus* are

represented in figure 2. Representative C-18 RP-HPLC chromatogram of standard quercetin is presented in figure 2(a), while figures 2(b), 2(c), and 2(d) show RP-HPLC chromatograms of methanolic extracts of quercetin in roots, shoots and leaves of GP-HAR- 12 genotype respectively. The retention time observed for standard quercetin is 3.1±0.5 min.

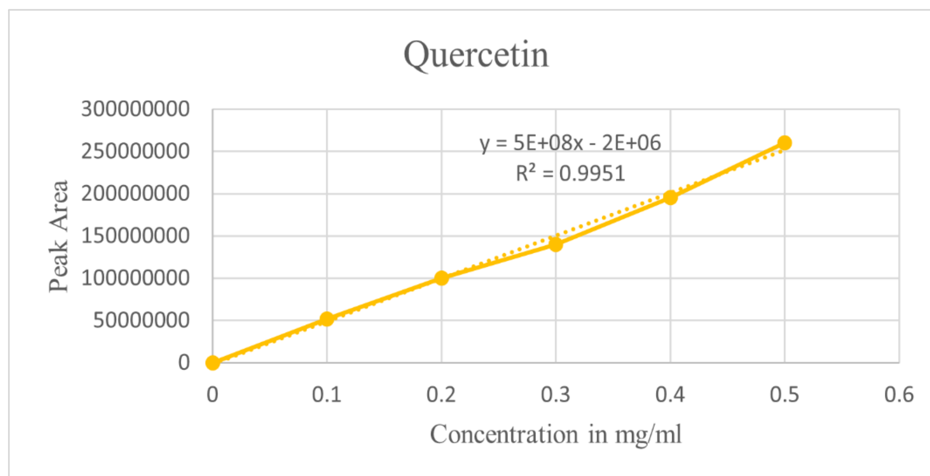

Figure 1. Calibration curve using the peak area versus corresponding concentration of the standard quercetin.

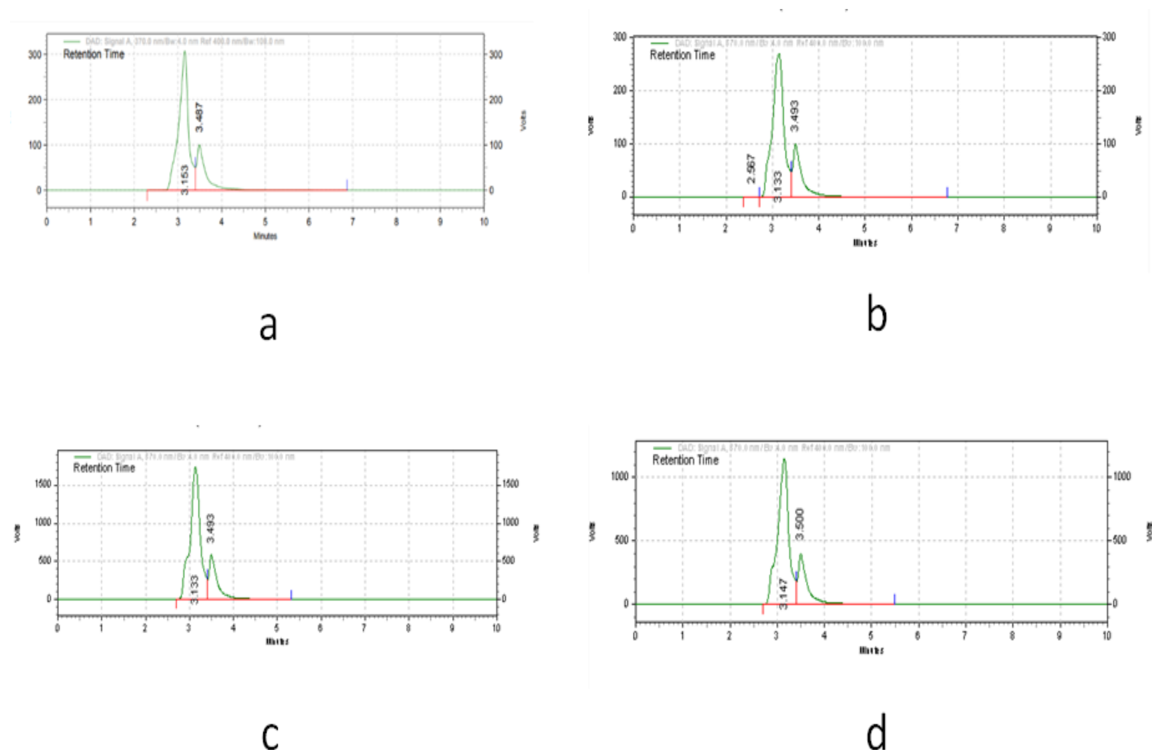

Figure 2. RepresentingC-18 RP-HPLC chromatograms of standard quercetin (a) and methanolic quercetin extract of roots (b), shoots (c) and leaves (d) in GP-HAR- 12

3.2.3 Quantification

The concentration of quercetin in the roots of all the studied genotypes was found to be in the range of 0.043mg/ml to 0.276mg/ml, whereas, in the shoots, the range varied from 0.033mg/ml to 0.257mg/ml. On the other hand, the leaves exhibited a range of 0.046mg/ml to 0.293mg/ml quercetin concentration. By taking the average values of quercetin concentration across all the genotypes, it was observed that the roots had an average concentration of 0.113mg/ml. In comparison, the shoots and leaves had average concentrations of 0.99mg/ml and 0.125mg/ml, respectively (Figure 3). The concentration of quercetin in roots, shoots and leaves of all the genotypes is represented in Table 1. These results clearly indicate that the leaves of the collected genotypes had a higher concentration of quercetin as compared to the shoots and roots. However, there was a noticeable variation in quercetin concentration within each plant part, with some genotypes exhibiting substantially higher or lower concentrations than others. The findings are in correlation with the study conducted by Mondal (2014)[13], which confirmed the presence of flavonoids in methanolic leaf extracts of *A. racemosus*. The total flavonoid content in ethanolic root extracts was 183.3mg/g of quercetin equivalent, which showed antimicrobial properties in a study conducted by Roy et al. (2014)[14]. These findings highlight the potential of plant leaves as a promising source of quercetin, a compound that has been linked to numerous health benefits.

Table 1. Concentration of quercetin in roots, shoots and leaves of 42 *A. racemosus* genotypes.

SAMPLE NAME	Quercetin Concentration (mg/ml)			SAMPLE NAME	Quercetin Concentration (mg/ml)			SAMPLE NAME	Quercetin Concentration (mg/ml)		
	Roots	Shoots	Leaves		Roots	Shoots	Leaves		Roots	Shoots	Leaves
GP-HAR-1	0.213	0.196	0.219	GP-HAR-15	0.168	0.145	0.174	GP-HAR-29	0.208	0.235	0.238
GP-HAR-2	0.176	0.165	0.186	GP-HAR-16	0.138	0.120	0.150	GP-HAR-30	0.183	0.178	0.219
GP-HAR-3	0.144	0.132	0.163	GP-HAR-17	0.174	0.157	0.193	GP-HAR-31	0.045	0.040	0.058
GP-HAR-4	0.202	0.187	0.228	GP-HAR-18	0.192	0.177	0.206	GP-HAR-32	0.054	0.027	0.065
GP-HAR-5	0.059	0.050	0.066	GP-HAR-19	0.157	0.127	0.189	IC471896	0.072	0.063	0.081
GP-HAR-6	0.068	0.054	0.083	GP-HAR-20	0.062	0.073	0.088	IC471897	0.276	0.257	0.293
GP-HAR-7	0.117	0.108	0.138	GP-HAR-21	0.078	0.070	0.091	IC471898	0.259	0.219	0.275
GP-HAR-8	0.125	0.114	0.145	GP-HAR-22	0.084	0.088	0.103	IC471899	0.195	0.183	0.211
GP-HAR-9	0.049	0.039	0.062	GP-HAR-23	0.119	0.106	0.126	IC471900	0.223	0.206	0.266
GP-HAR-10	0.040	0.027	0.054	GP-HAR-24	0.130	0.092	0.149	IC471901	0.162	0.151	0.192

GP-HAR-11	0.066	0.041	0.073	GP-HAR-25	0.142	0.125	0.151	IC471902	0.130	0.113	0.138
GP-HAR-12	0.043	0.033	0.046	GP-HAR-26	0.152	0.133	0.163	IC471903	0.132	0.106	0.159
GP-HAR-13	0.096	0.084	0.109	GP-HAR-27	0.242	0.223	0.267	IC471904	0.051	0.049	0.066
GP-HAR-14	0.108	0.093	0.112	GP-HAR-28	0.199	0.174	0.214	IC471905	0.068	0.065	0.072

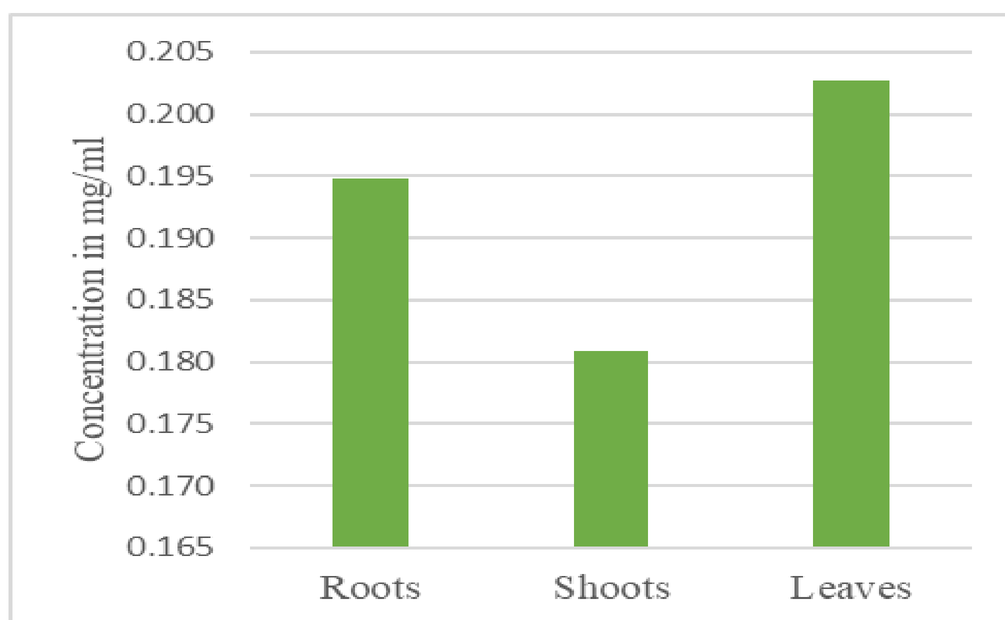

Figure 3. Graph representing average values of quercetin in different tissues of *A. racemosus.*

3.2.4 Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ values for quercetin 0.048 and 0.146. A low LOD and LOQ are desirable as they indicate high sensitivity and the ability to accurately detect and quantify very low levels of the analyte. However, achieving low LOD and LOQ values can be challenging and may require optimization of the HPLC method, including sample preparation, column selection, and detection parameters. Due to its high sensitivity, accuracy, and efficient and uncomplicated procedures, RP-HPLC is increasingly used to detect secondary metabolites in various plants. 1.As suggested by Negi et al. (2011)[15], better separation of secondary metabolites can be achieved by using diverse combinations of stationary and mobile phases. Phytoconstituents like flavonoids and saponins present in *A. racemosus* are responsible for itsvarious therapeutic properties. Flavonoids are present in the leaves, roots, flowers and fruits ofthe plant.[5] Quercetin is characterized by HPLC and exhibits antioxidant properties in root extracts of *A. racemosus,* as studied by Potduang et al. (2008).[16] Quercetin is present in aerial parts of *A. racemosus* is

confirmed by Ashraf (2021)[17] using HPTLC method and total flavonoid content expressed as quercetin/g is found more in methanol extract compared to n- hexane, chloroform and ethyl acetate extracts.

The levels of quercetin can vary significantly due to several factors, including the type of tissue used for extraction, differences in genotype, environmental conditions of the sampling site, age, and physiological state of the plants, as well as the techniques employed for extraction.[18,19] Genotypic variation plays a crucial role in determining the content of secondary metabolites in different plant species or cultivars. Environmental conditions, such as temperature, humidity, and soil composition, can also impact the production of secondary metabolites. For instance, plants grown in regions with high levels of ultraviolet radiation may produce higher amounts of secondary metabolites as a protective mechanism. The age of the plant and its physiological state can also affect the quercetin content. Therefore, to obtain accurate and reliable data on quercetin content, it is necessary to consider all these factors and standardize the extraction procedures.

Ethics approval and consent to participate:

This research did not involve using animal or human data or tissue.

Author contributions:

VC and CM conceived the current research work. CM collected sample plant material and performed HPLC. CM, AK and RK analyzed the data obtained. CM prepared the original draft, which was further reviewed and edited by VC, AK and RK. The whole study was done under the supervision of VC. All authors approved the final draft of the manuscript after they had reviewed the findings.

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Conflict of interest:

The authors declare no conflict of interest relating to financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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