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Original Article

Comparative Study of Quercetin Content in Marketed Seeds of *Linum usitatissimum* L. (Flax), *Salvia hispanica* L. (Chia), and *Helianthus annuus* L. (Sunflower) and their Microgreens Using HPTLC from West Bengal

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ABSTRACT

Background: Microgreens, the early developmental stage of edible plants, have gained prominence for their dense nutrient composition. Yet the variations in quercetin content among different microgreen species remain insufficiently explored. **Aim:** The aim of this research was to use HPTLC to compare the quercetin content of commercial flax, chia, and sunflower seeds as well as the comparable microgreens. **Materials and Methods:** Toluene, ethyl acetate, and formic acid were used as the mobile phase at a ratio of 5:4:0.2 (%v/v/v) for the chromatographic analysis, which was conducted using aluminum-backed silica gel 60F_{254} plates. To ensure that the results were precise, accurate, and reproducible, rigorous technique validation processes were carried out. **Results:** Total Flavonoid content in the three seeds and microgreens studied, and the highest value was 38.92±0.4 and 76.36±0.4gm QE/100g. Well separated and compact spots (R_f) of quercetin (0.41±0.03) were detected. The regression equation obtained was y=0.0002x + 0.0001, with a correlation coefficient (R^2) of 0.9833. The linearity range (µg/ spots) was 20-100. The LOD/ LOQ (ng/spot) were 37.66/114.15. *Salvia hispanica* L Seeds and microgreens (0.84±0.01 and 0.88±0.005% W/W) contained maximum amount of quercetin compared to *Linus usitatissimum* L. and *Helianthus annus* L. in ethanolic extract. **Conclusion:** The study revealed that the *Salvia hispanica* L. microgreen possess the highest quercetin content among the studied seeds and microgreens, while microgreens of all three plants are promising sources of quercetin, showcasing a remarkable increase compared to their seeds. Highlighting their potential as dietary sources rich in quercetin.

Keywords: Microgreens, Quercetin, Comparative nutritional value, *Linum usitatissimum* L. (Flax), *Salvia hispanica* L. (Chia), *Helianthus annuus* L. (Sunflower).

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INTRODUCTION

The key element of good health is nutritious diet. The foundation of a long-term healthy lifestyle composed of good diet, regular exercise, and enough sleep.[1] Micronutrient Malnutrition (MNM) is a significant socioeconomic issue on a global scale. It has a significant impact on third-world countries; particularly for children, adolescents, pregnant and nursing mother. In comparison with recommended levels, more people worldwide consistently consume less nutritious foods. Currently, living a healthy lifestyle is becoming more important in society, which aligns with the food industry's goal in supplying the more demanding nutritional needs of customers. Consequently, novel nutraceuticals with therapeutic and medical characteristics are getting the spot light. Food For Specified Health Use (FOSHU), refers to these items as functional foods.[1,2]

Plant-based raw materials play a crucial role in both preventing and treating a variety of modern ailments. Dietary patterns based on larger intake of plant-based goods are becoming more popular as a result of the rising frequency of disorders linked to diet. Indeed, health professionals advise switching to a dietary pattern focused on increasing intake of plant-based items rather than the Western diet, which has a reputation for eating a lot of animal products, saturated fats, and processed carbohydrates.[3] The Mediterranean Diet (*MedDiet*) is gaining attention nowadays in this situation and is now a recognized and suggested eating pattern that the general community is turning to more frequently. *MedDiet* is linked with overall health and bodily function that have been supported by science.[4] Microgreens (micro leaves) are particularly valuable, common, and age-old component of the diet in this regard. Their

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consumption can significantly contribute to diet diversity and nutritional enrichment for health. The soft young greens known as microgreens, known as "vegetable confetti," are cultivated from the seeds of legumes, herbs, and vegetables.[3] They include a lot of bioactive substances, microgreens are regarded as "super foods".[5] They have a wide range of textures, colors, and potent flavors in addition to having a high nutritional content.[6] Additionally, due to their generally higher quantities of phytochemicals than their mature counterparts, microgreens have been promoted as healthful foods,[7] According to a recent assessment, microgreens are a new diet for the twenty-first century and may have anti-carcinogenic, anti-inflammatory, anti-atherosclerotic and anti-obesogenic, properties.[8]

Because of this, new applications for various kinds of seeds and microgreens are being discovered every day. Literature shows that, Seeds of *Linum usitatissimum* L. (Flax), *Salvia hispanica* L. (Chia), and *Helianthus annuus* L. (Sunflower) and their microgreens show promising Pharmacological effect due to the presence of unique bioactive compounds like ascorbic acid, tocopherol, carotenoids, folate, tocotrienols, anthocyanins, Flavonoids, glucosinolates and phylloquinones etc.

More than 6000 different flavonoids' structures have been identified globally. The microgreens contain a variety of flavonoids in variable amounts.[9] Quercetin is a flavanonol glycoside that is widely distributed in microgreens. This bioflavonoid has anticancer,[10] antimutagenic, antioxidative,[11] anti-inflammatory,[12] neuroprotective,[13] antihypertensive,[14] and blood sugar-lowering properties.[15]

The aim of the present study is to comparative quercetin content analysis among the seeds and microgreens, using a precise HPTLC analytical method.

MATERIALS AND METHODS

Seeds and Microgreens

In the present study, *Linum usitatissimum* L. (Flax), *Salvia hispanica* L. (Chia), and *Helianthus annuus* L. (Sunflower) seeds are collected from the local market, West Bengal and grown as microgreens in the kitchen garden.

Chemicals

From Loba Chemie Pvt. Ltd., standard quercetin was purchased. The utilized chemicals were of analytical reagent grade and solvents of HPLC grade.

Sample preparation for analysis of quercetin in ethanolic extract of seeds and their microgreens

The air-dried three different microgreens and seeds were individually coarsely ground, macerated in methanol for 7 days, and thoroughly extracted after that. Each of the leftovers was separately dissolved in methanol in 50 mL volumetric flasks after the solvent was evaporated to dryness under reduced pressure using a rotary vacuum evaporator.

Determination of Total flavonoids Content

Preparation of standard quercetin for quercetin calibration curve

A colorimetric aluminum chloride assay was used to determine the extracts' total flavonoid concentration (TFC).[16] 1 mL of methanol was used to dissolve 100 mg of quercetin to create a stock solution (100 mg/mL). In order to create solutions with concentrations of 20, 40, 60, 80, and 100 µg/mL, this standard solution was serially diluted. In the test tube, combine this with 0.3 mL of 5% $NaNO_2$ and 4 mL of distilled water. After 5 min, combine with 0.3mL of 10% $AlCl_3$. After 6 min, 1 M NaOH was added to the mixture. The combination was immediately diluted to a concentration of 10 mL with distilled water. The absorbance at 510 nm was measured with a spectrophotometer.

Preparation of sample for Total Flavonoid Content

The extracts were first made into 100 mg/mL stock solutions in methanol, which were then diluted to make 0.5 mg/mL concentration solutions. Using a method similar to quercetin analysis, the absorbance was measured using a spectrophotometer at 510 nm. Through the use of the average absorbance from three measurements, the total flavonoid content was calculated. The calibration curve was used to generate a linear equation that converted the flavonoid content into mg QE/100 g of quercetin equivalent.

Chromatographic condition

A 10x10 cm aluminium-packed TLC plate with a 0.2 mm layer of silica gel $60F_{254}$ (E. Merck Ltd, Darmstadt, Germany) was used to capture the chromatogram, which was then dried and stored in a desiccator. Application was carried out using a Linomat V applicator and a Hamilton micro syringe (Switzerland). Spotting was carried out on a TLC plate with an ascending development and migration distance of 80 mm (distance to the bottom edge was 10 mm) in a Camag chamber that had been pre-saturated for 20 min with ethyl acetate: toluene: formic acid (5:4:0.2 V/V/V%) as the mobile phase. At a spraying rate of 15s µL-1, 8 mm broad band's representing the sample concentration (6 µL) was applied on three tracks. Following development, the plate was dried in a hot air oven for 2 min at 60°C. Then, using a Camag TLC Scanner 3 equipped with win CATS Software Version 1.3.0, densitometric scanning was done at max 254 nm and 366 nm using Deuterium light sources, with slit size of 6.00 X 0.45 mm, and at max 620 nm using Deuterium and tungsten lights source. Chromatograms were recorded.[17]

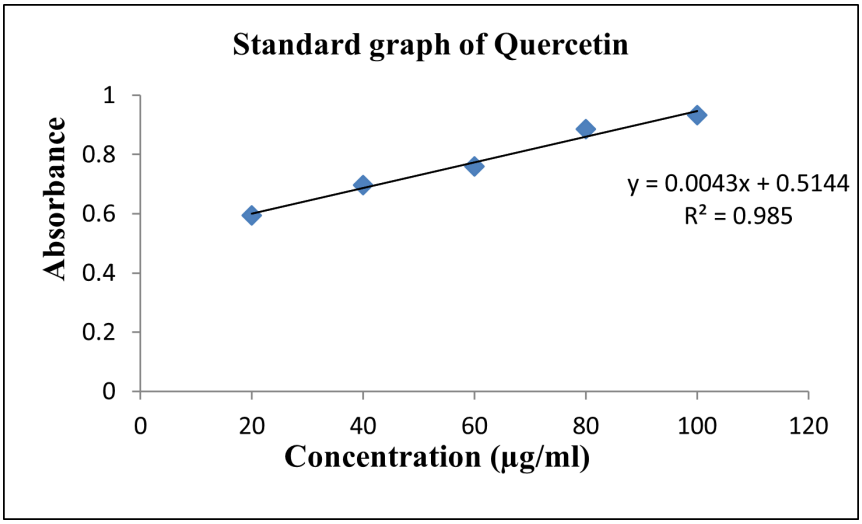

Figure 1: Calibration curve on Standard Quercetin.

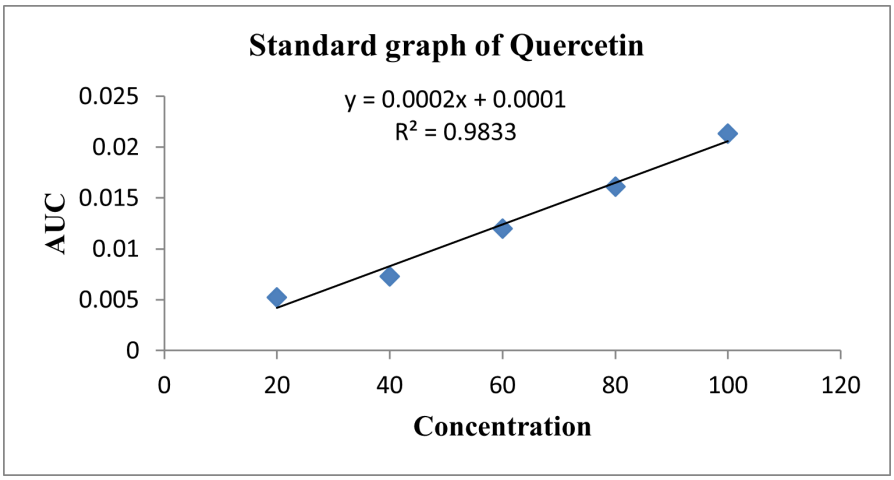

Figure 2: Regression curve of standard quercetin.

Method Validation

Accuracy

Utilizing the recovery study at three levels allows for an evaluation of the method's accuracy. Adding 50, 100, and 150% of the standard drug to the pre-analyzed formulations is how recovery tests are carried out. The resulting mixture is then reanalyzed six times.[18]

Precision

The intra-day and inter-day precisions are used to evaluate precision. Analyzing sample solutions of analyte from formulations at three levels covering low, medium, and higher concentrations of the calibration curve for five times on the same day allows for the determination of intraday precision. Analyzing sample solutions of the analyte at three levels covering low, medium, and higher concentrations over a period of seven days allows for the determination of inter-day precision. The resulting peak areas are utilized to compute the mean and percent RSD (relative SD) values.[18]

Specificity

Analyzing the sample solutions in respect to interferences from formulation constituents establishes the specificity of the suggested approach. By comparing the spot's Retardation factor (R) values to those of the standard, the spot for the sample is verified.[18]

Limit of Detection and Limit of Quantification

The acceptable ranges for the signal-to-noise ratios for the Limits of Detection (LOD) and Quantification (LOQ) were found to be 3:1 and 10:1, respectively.

Quantification of quercetin in different varieties of methanolic extract of seeds and microgreens

Using the exact same conditions as for the analysis of standard quercetin, the test samples were applied to the plates and

chromatograms were obtained. The region of the peak that corresponded to the quercetin standard's R_f value was noted, and the amount present was determined using the graph.

RESULTS

Total Flavonoid Content Determination (TFC)

The total flavonoid content of the methanolic extract of the three seeds and microgreen was reported as mg Quercetin equivalents/g of extract (Figure 1). Three copies of each sample were examined. The range of total flavonoid content from seeds 19.82±0.3 to 38.92±0.4 mg and microgreen this range is 33.7±0.4 to 76.36±0.4 mg QE/g dry weight (Table 1).

Method Validation

Calibration Curve

The calibration curve in Figure 2 shows that the response is linearly related to the quercetin concentration range of 20-100 µg/mL, regression equation was, y=0.0002x+0.0001 The slope, intercept, correlation coefficient, SE of intercept and SD of intercept were 0.0002, 0.000608, 0.9833, 0.00102086 and 0.002283 respectively.

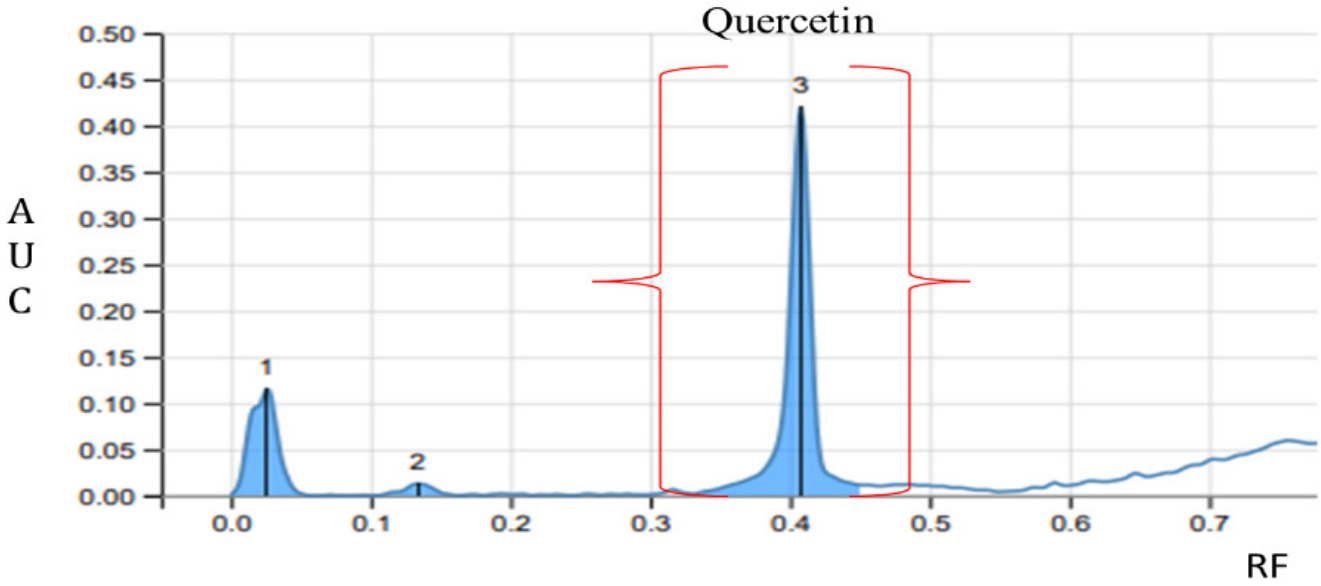

Figure 3: HPTLC Chromatogram of Standard quercetin.

Table 1: Total Flavonoid Content of three different types of seeds and microgreens.

Plant name	Parts	Total Flavonoid Content (Mean±SD; QE/g Dry Weight)
Helianthus annus L.	Seeds	19.82±0.3
	Microgreens	33.7±0.4
Salvia hispanica L.	Seeds	38.92±0.4
	Microgreens	76.36±0.4
Linus usitatissimum L.	Seeds	28.26±0.5
	Microgreens	50.14±0.4

Table 2: Precision of the proposed method (*n*=3).

Repeatability (Intra- Day Precision)				Intermediate Precision (Inter-Day)		
Conc. (µg/mL)	ARE±SD	Std. Error	%RSD	ARE±SD	Std. Error	%RSD
100	0.01205±0.00055	0.00032	5.6122	0.0247±0.001031	0.000595	4.174
100	0.01491±0.0002050	0.00011	1.3749	0.02478±0.000459	0.000265	1.852
100	0.01429±0.0002029	0.00011	1.1498	0.02607±0.00041	0.00024	1.596

Precision

Table 2 displays the repeatability and intermediate precision measurement results as SD (%). For repeatability and intermediate precision, the RSD was in the range of 1.14 to 5.62 and 1.59 to 4.17, respectively. These low results confirmed the method's accuracy.

Accuracy

Results from recovery studies, given in Table 3, showed good accuracy of the procedure and were within acceptable limits (92.46 to 96.58%).

Limit of Detection and Limit of Quantification

The LOD and LOQ of the proposed strategy were found to be 37.66 and 114.15 ng/spot, respectively, for quercetin, indicating that the method can be effectively applied in a variety of situations for quercetin detection and quantification.

Method Development

To develop an acceptable and precise densitometric HPTLC method for quercetin analysis, the mobile phase composition was improved. Using the mobile phase Toluene-Ethyl acetate-Formic acid 5:4:0.2 (%v/v/v), a robust, symmetrical, and well-resolved peak at R_f value of (0.41±0.05) was obtained (Figure 3).

Table 3: Accuracy study of proposed method (*n*=3).

Excess drug added to analyte (%)	Concentration found (µg±SD)	% Recovery	% RSD
50	48.29±0.76	96.58	1.58
100	94.99±0.50	94.99	0.52
150	138.69±0.49	92.46	0.35

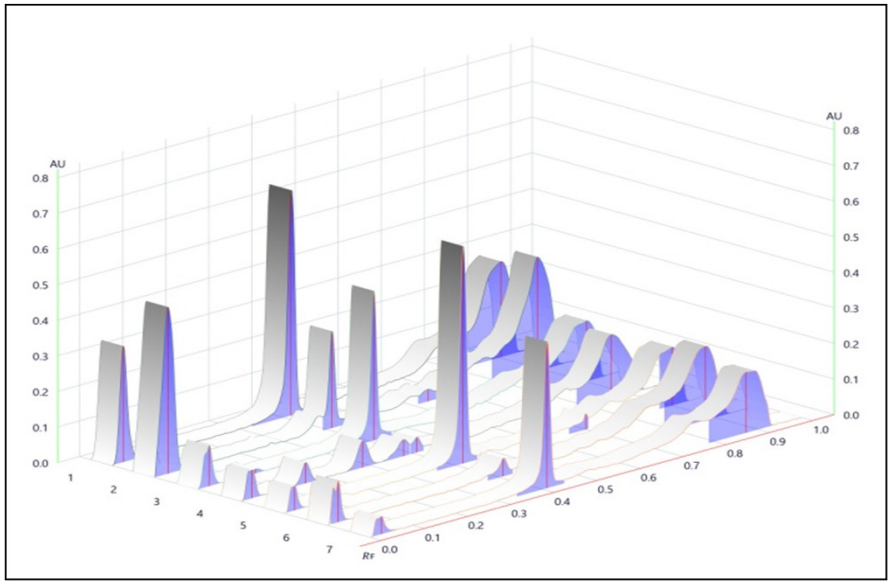

Figure 4: HPTLC Densitometric spectra of methanolic extract of three Seeds and microgreens.

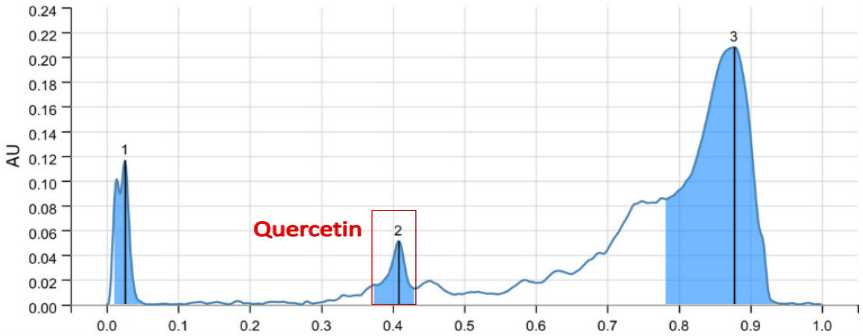

Figure 5A: HPTLC Chromatogram of methanolic extract of *Linum usitatissimum* L. (Flax) Seeds.

Quantification of quercetin in different varieties of methanolic extract of seeds and microgreens

By comparing the single spot at R_f=0.41±0.05 (Figure 4, 5A-5F) with those obtained by chromatography of the standard under the same circumstances, quercetin peaks from the methanolic extracts of three different types of seeds and microgreens were recognized. The quercetin content of three distinct types of seeds and microgreens was determined shown in Table 4.

DISCUSSION

HPTLC is a valuable tool to analyze the quality of chemical compounds that can be found in different seeds, green vegetables, microgreens, and plants. The TLC process was improved. R_f = 0.41±0.05 for quercetin was the value for the mobile phase, toluene, ethyl acetate, and formic acid in the ratio (5:4:0.2% v/v/v) (Figure 3). The HPTLC technique created for quercetin quantification in the methanolic extracts of Seeds of *Linum usitatissimum* L. (Flax), *Salvia hispanica* L. (Chia), and *Helianthus annuus* L. (Sunflower) and their microgreens. The results of phenolic metabolomics showed that there were significant variations in all three classes of phenolic profiles between seeds and microgreens, and that most phenolic chemicals were significantly increased after germination.

The maximum amount of Total Flavonoid Content from seeds was 38.92±0.4 mg QE/g dry weight in *Salvia hispanica* L. followed by *Linum usitatissimum* L. (28.26±0.4mg QE/g dry weight) and *Helianthus annuus* L.(19.82±0.3mg QE/g dry weight) as well as

Table 4: Quantity of Quercetin in three selected Seeds and microgreens.

Plant Name	Parts	Quercetin Content (Mean±SD/100g Dry Weight)
Helianthus annus L.	Seeds	0.36±0.005
	Microgreens	0.58±0.003
Salvia hispanica L.	Seeds	0.84±0.01
	Microgreens	0.88±0.005
Linus usitatissimum L.	Seeds	0.78±0.002
	Microgreens	0.85±0.005

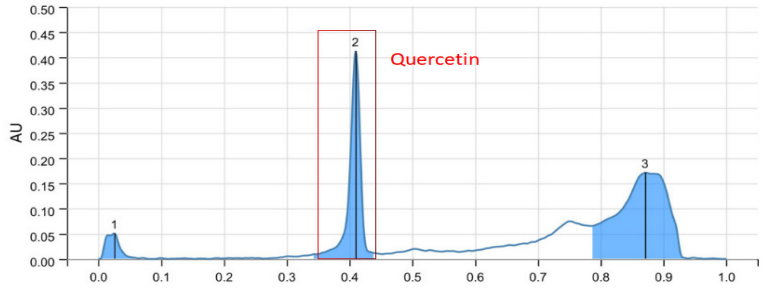

Figure 5B: HPTLC Chromatogram of methanolic extract of *Linum usitatissimum* L. (Flax) Microgreens.

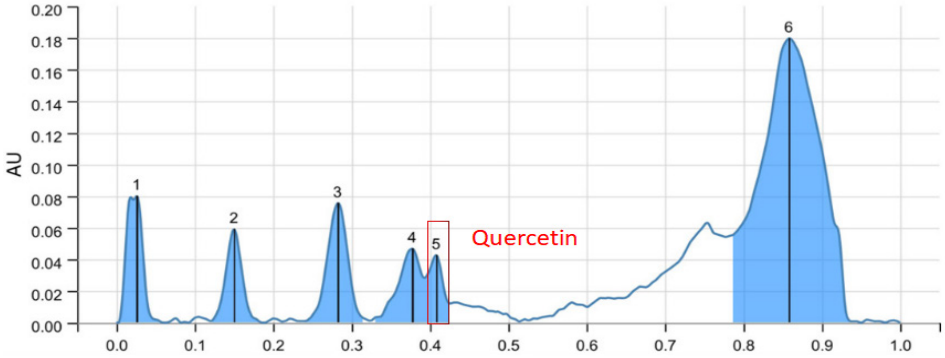

Figure 5C: HPTLC Chromatogram of methanolic extract of *Salvia hispanica* L. (Chia), Seeds.

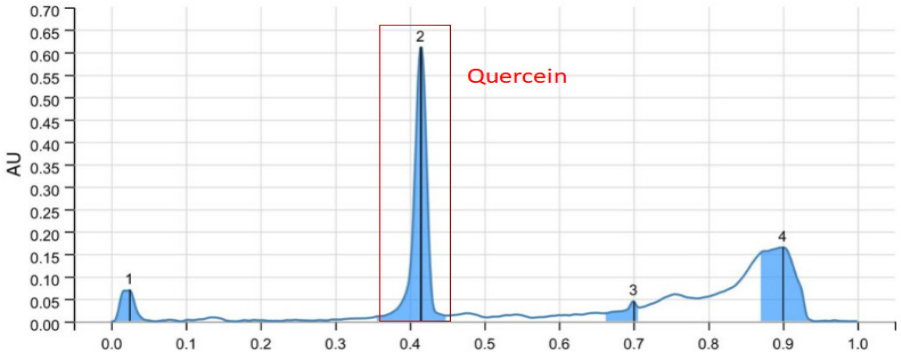

Figure 5D: HPTLC Chromatogram of methanolic extract of *Salvia hispanica* L. (Chia) Microgreens.

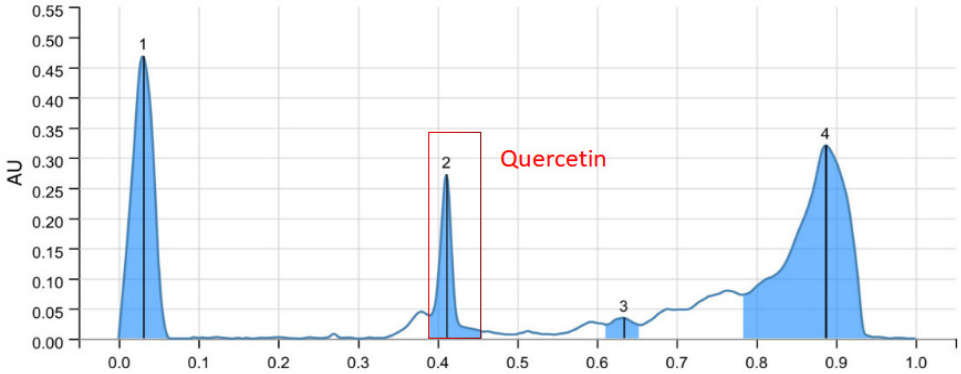

Figure 5E: HPTLC Chromatogram of methanolic extract of *Helianthus annuus* L. (Sunflower) Seeds.

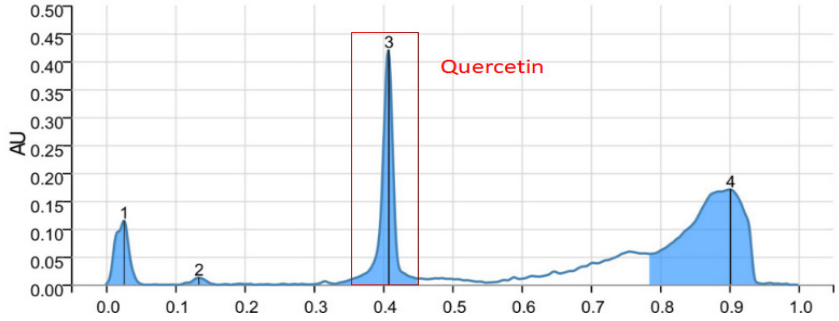

Figure 5F: HPTLC Chromatogram of methanolic extract of *Helianthus annuus* L. (Sunflower) Microgreen.

in case of microgreen the maximum total flavonoid content was 76.36±0.4 mg QE/g dry weight in *Salvia hispanica* L. followed by *Linum usitatissimum* L. (50.14±0.4 mg QE/g dry weight) and *Helianthus annuus* L. (33.7±0.3 mg QE/g dry weight) (Table 1).

The maximum amount of quercetin was found to be present in *Salvia hispanica* L. seeds (0.84±0.01 mg/100 g dry Weight) and *Salvia hispanica* L. microgreens (0.88±0.005 mg/100 g dry weight). Minimum amount were present *Helianthus annus* L. Seeds and microgreens (0.36±0.005 and 0.58±0.003 mg/100 g dry weight) (Table 4). Additionally, the proposed HPTLC method was found to be reliable, reproducible and user friendly and quality assurance of herbal formulations and raw materials. Therefore, it can be useful other microgreens content quercetin in future study.

CONCLUSION

This study evaluated the quercetin concentration of microgreens and seeds from *Helianthus annuus* L. (Sunflower), *Salvia hispanica* L. (Chia), and *Linum usitatissimum* L. (Flax) from West Bengal. The comparative quercetin content analysis among these three commonly consumed seeds and microgreens will be helpful for the nutritional choice in case of immunity boost up. The highest TFC and quercetin concentrations were found in *Salvia hispanica* L. (Chia) microgreens. However, all of the microgreens under study were found to contain more quercetin than seeds.

So, it would be advisable that consumption of the microgreen will provide higher benefits than the corresponding seeds with respect to their quercetin content. However, further research can be carried out to explore the other beneficial effect of three microgreens as well as determination of the quercetin content of the remaining microgreens can be done, which will provide the nutritional indicator among the microgreens to boost immunity of the common people in the society.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

QE: Quercetin; **HPTLC:** High performance thin layer chromatography; **TFC:** Total flavonoid content; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **AUC:** Area under curve; **RSD:** Relative standard deviation; **SD:** Standard deviation; **TLC:** Thin layer chromatography; **R_f:** Retention factor; **SE:** Standard error.

SUMMARY

For the treatment of numerous types of viral and metabolic diseases, plant materials have been employed as folk medicine since the beginning of time. The current study establishes the quantitative estimation of quercetin from commercially available seeds of *Helianthus annuus* L. (Sunflower), *Salvia hispanica* L. (Chia), and *Linum usitatissimum* L. (Flax) as well as their microgreens from Wes Bengal to support the treatment of immunological, viral, and metabolic disorders that are widely prevalent worldwide. According to the findings of this study, quercetin has been shown to be found in greater concentrations in all of the microgreens than in seeds. *Helianthus annus* L. microgreens (0.580.003 mg/100 g dry weight) had the lowest concentration while *Salvia hispanica* L. microgreens (0.880.005 mg/100 g dry weight) had the highest. Therefore, it would be reasonable to conclude that microgreen consumption will have greater health benefit than eating the corresponding seeds because of the quercetin rich content of microgreen.

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