

Targeted HPTLC Profiling and UV Spectrophotometric Estimation of Key Polyphenols Present in *Brassica* sp.: A Comparative Phytochemical Analysis

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ABSTRACT

Brassica sp., including kale, cabbage, broccoli, and cauliflower, contains bioactive compounds like flavonoids and phenolic acids, which make them nutritionally and therapeutically valuable. This study aimed to detect polyphenolic compounds in selected *Brassica* sp. (kale, cabbage, broccoli, and cauliflower) and to optimize a mobile phase to obtain better separation of these polyphenolic compounds using High-Performance Thin-Layer Chromatography (HPTLC). Standards including rutin, quercetin, chlorogenic acid, caffeic acid, and caffeine were selected based on their known antioxidant activities and occurrence in leafy vegetables. The dried powdered samples were extracted using methanol-assisted sonication. Multiple mobile phase systems were screened to achieve optimal separation, and the best-performing system, butanol: ethyl acetate:water:formic acid (9:2:1:0.5), was selected for the chromatographic profiling. The choice of mobile phase significantly influenced the resolution, migration behaviour, and detectability of phytochemical constituents. Distinct chromatographic bands were observed in each *Brassica* sample, indicating the presence of phenolic compounds. However, all standards were not consistently detected, probably due to low concentration, lack of certain compounds, or to extraction difficulties. This study highlights the critical importance of mobile phase selection in HPTLC fingerprinting and emphasizes the need to align extraction and chromatographic conditions with the expected phytochemical profile of the target plant group. Understanding the presence of flavonoids and phenolic acids in *Brassica* sp. is valuable for both nutritional evaluation and analytical standardization.

Keywords: *Brassica* sp., Caffeic acid, HPTLC, Mobile phase, Quercetin, Rutin.

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INTRODUCTION

Brassica, a genus within the mustard family (Brassicaceae), and its species are commonly referred to as mustards or cabbages. This genus was originally classified under Cruciferae due to its cross-shaped flower petals and thought to have originated in the Mediterranean-Saharan region, the genus has since adapted to colder climates, with some species developing drought and salt tolerance.^[1] The common edible species includes kale, cabbage, cauliflower, and broccoli. The genus is known for its higher nutritional value and are mainly rich in vitamins, fiber, and minerals.^[2] Besides its nutritional values, the phytochemical profile of the Brassicaceae family has been extensively characterized due to the presence of a wide array of bioactive compounds such as

glucosinolates, phenolics, and flavonoids that aids in antioxidant, anticancer, anti-inflammatory activities making *Brassica* sp. an important contributor to a balanced diet and functional food formulations.^[3,4]

Kale (*Brassica oleracea* L. var. acephala) is a nutrient dense leafy green vegetable, has gained attention for its higher concentrations of vitamins particularly vitamin C, potassium, calcium, magnesium, iron, amino acids.^[5] Kale contains high levels of polyphenols, including flavonoids kaempferol and quercetin, glucosinolates, phenolics, and carotenoids that are rich in antioxidant, anti-inflammatory capacity. Kale has been associated with decreased oxidative stress, improved cardiovascular function, detoxifying the body, and putative chemoprotection, and supports gut health and enhances immunity.^[6,7] Kale is widely used in culinary practices for its slightly bitter, sweet flavour.^[8]

Cabbage (*Brassica oleracea* L. var. capitata), a commonly edible winter crop, is low in calories (~25 kcal/100 g) but high in glucosinolates, flavonoids, and phenolic acids possessing antioxidant, antimicrobial, anticancer, and cardioprotective activities.^[9,10] Glucosinolates are hydrolyzed to isothiocyanates,



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which have been associated with decreased risk of cancer. It is also a good source of essential minerals (Fe, Ca, Mg, K, Na, P) and Vitamins C, K, and A and maintains immune, skin, and bone health.^[11]

Cauliflower (*Brassica oleracea* var. botrytis) is a low-calorie, high-nutrient vegetable that consists largely of water and dietary fiber. It yields approximately 25 kcal, 2.6 g protein, 1.2 g fiber, and negligible fat (0.28 g).^[12] Packed with Vitamins C, K, and B-complex, and minerals such as potassium, phosphorus, and calcium, it promotes general well-being. Its bioactive compounds include β -carotene, glucosinolates, sulforaphane, indole-3-carbinol, and isothiocyanates which are known for their potent anticancer, anti-inflammatory, and detoxifying properties. Phenolic acids and flavonoids help in maintaining the heart health, suppress tumor growth, and combat inflammation. The higher fiber content assists in digestion, weight control, and lowering cholesterol.^[13]

Broccoli (*Brassica oleracea* L. var. italica), indigenous to the Mediterranean region, is abundant in vitamins A, C, and K, and the minerals Fe and Ca.^[4,14] It is low in calorie content (~34 kcal/100 g) and has ~2.8 g protein, 6.6 g carbohydrates, and 2.6 g of fiber content.^[15] Its glucosinolates break down into indole-3-carbinol and isothiocyanates possessing powerful antioxidant, anti-inflammatory, and anticancer properties. Sulforaphane augments immune response, whereas flavonoids and polyphenols (quercetin) fight oxidative stress.^[2]

Despite the well-documented health benefits of *Brassica* sp. there is a need for accurate characterization and quantification of key bioactive compounds present among different *Brassica* sp. essential for understanding their nutritional value and potential therapeutic efficacy. High-Performance Thin-Layer Chromatography (HPTLC) and UV-visible spectroscopy are powerful analytical tools for profiling and quantifying these bioactive constituents.

Hence, this present research aimed to study a thorough comprehensive qualitative and quantitative evaluation of the key bioactive constituents present in the selected *Brassica* sp. (Kale, Cabbage, Cauliflower and Broccoli) through HPTLC and spectroscopic analysis which will contribute to the standardization of these species, support nutraceutical and functional food development, and enhance understanding of their pharmacological potential.

METHODOLOGY

Brassica sp.

Four varieties of *Brassica* sp. including, Kale, Cabbage, Cauliflower, Broccoli were selected for the studies. Botanical Reference Materials (BRMs) for these sp. was obtained from the American Herbal Pharmacopoeia, with lot numbers 6157, 6479, 6359 and 6480, respectively. The dried powder sample of

Kale, Cabbage and Broccoli were provided as test samples by Green Jeeva LLC, with lot numbers OKP-20240120, 2023060497, C24010204, respectively.

Chemicals and Reagents

Analytical grade solvents such as Methanol (Batch no. 3713404, 99.8%), Ethanol (Batch 2024071, 99.95%), Butanol (Batch no. 9367414) Ethyl acetate (Batch no. 5718732, 99.5%), Polyethylene glycol 400 (Batch no. 5568303, 95%) and Formic acid (Batch no. 1502438, 98%) were procured from Sisco Research Laboratories Pvt. Ltd., (SRL). Pure standards such as caffeic acid (Batch no. CO625, 98%), quercetin (Batch no. Q4951, 95%) and chlorogenic acid (Batch no. C3878, 95%) were procured from Sigma-Aldrich. Rutin (Lot no. 0001844013, 95%) was obtained from chromaDex. Caffeine (Batch no. CB106487, 98%) was obtained from Wuhan Healthream Biological technology Co., Ltd., Detection reagent Naturals products reagent (Batch no. D0281, 98%) and Sulphuric acid was procured from Tokyo Chemical Industry.

Instrumentation and Equipment

A CAMAG TLC system was used for HPTLC analysis. The setup included the Linomat V sample applicator along with a dosage syringe of 100 μ l, a twin trough glass chamber for 20 $\times$ 10 cm plates, CAMAG TLC Visualizer 3, Immersion device, Dip tank, and Wincats software (version 3.2 SP2). Pre-coated silica plates of specification 60 F₂₅₄, 20 $\times$ 10 cm size were used as the stationary phase. After sample loading, chromatographic development took place in the twin trough chamber. The derivatization took place using an immersion device and a dip tank. Sample preparation involved an ultrasonic bath (Amesys, HSN-8419) and a centrifuge (Remi, HSN 84211999(R-83B/Remi), while a TLC plate heater (CAMAG) was used to dry the derivatized silica-coated plates. A UV-vis Spectrophotometer (Shimadzu, UV-1900i) was employed for UV-vis spectroscopy of *Brassica* samples.

Physical characterization

Physical characterization, involving solubility, hygroscopicity and pH of 1% w/v aqueous solution of the *Brassica* samples were assessed. Organoleptic properties, including colour and texture, were also evaluated. These tests were carried out according to standard pharmacopeial procedures as per the WHO guidelines.

Chromatographic Method Development

HPTLC Sample Preparation: Test Samples, BRM, and Reference Standards

Reference standards such as rutin, quercetin, chlorogenic acid, caffeic acid, and caffeine were prepared in methanol at a final concentration of 1 mg/mL using an ultrasonicator, ensuring proper mixing. *Brassica* samples (test samples) and reference BRMs were finely powdered and extracted in methanol to obtain a final concentration of 100 mg/mL. The extracts were subjected to ultrasonication for 30 min, followed by centrifugation at

2000 rpm for 15 min. The supernatants were collected into 1.5 mL Eppendorf tubes for further analysis.

Chromatographic conditions and method development

Pre-coated silica gel 60 F₂₅₄ plates (20 × 10 cm, 250 µm thickness) served as the stationary phase. Various mobile phase systems with different polarities were tested to optimize band resolution and reproducibility. The optimal separation was achieved using a mobile phase consisting of butanol: ethyl acetate: water: formic acid (9:2:1:0.5, v/v/v/v). Chromatographic development was carried out in a CAMAG twin trough chamber, pre-saturated with the mobile phase vapor for 25 min using a saturation pad. The development was performed at a controlled temperature of 25±2°C with a migration distance of 70 mm. Following development, plates were air-dried and derivatized using Natural Product reagent (NP) followed by PEG reagent, and then heated at 102°C for 10 min. Visualization of bands was carried out under UV light at 254 nm and 366 nm, as well as under white light post-derivatization.

HPTLC Method optimization: Sequential Run

An optimum amount of all the *Brassica* samples and reference BRM were finely ground and dissolved in methanol having final

concentration 100 mg/mL. It was followed by ultrasonication for 30 min. Then, the mixture was centrifuged at 2000 rpm for 15 min. The supernatant from each sample was then collected into a 1.5 mL Eppendorf tube for HPTLC analysis.

Reference solutions of rutin, quercetin, caffeine, chlorogenic acid and caffeic acid were prepared by dissolving in methanol, having a final concentration of 1 mg/mL for HPTLC analysis.

In the first HPTLC run, standard solutions and BRM were applied in volumes of 2 µL, 4 µL, and 6 µL onto pre-coated silica gel 60 F₂₅₄ plates. Chromatographic development was performed using a mobile phase comprising ethyl acetate: butanol: water (18:8:4.5, v/v/v). After development, the plates were visualized under UV light at 254 nm and 366 nm. Derivatization was carried out using Natural Product (NP) and PEG reagents, and the plates were subsequently re-examined under UV and white light to observe derivatized zones.

In the second HPTLC run, reference standards (2 µL) and *Brassica* samples (4 µL and 5 µL) were loaded onto the TLC plate of 20 × 10 cm size. Development was conducted using a different solvent system consisting of ethyl acetate: water: acetic acid: formic acid (90:19:2:2, v/v/v/v). The chamber was pre-saturated for 25 min, and derivatization procedures were identical to those used in the first run. The bands were visualized under UV light at 254 nm

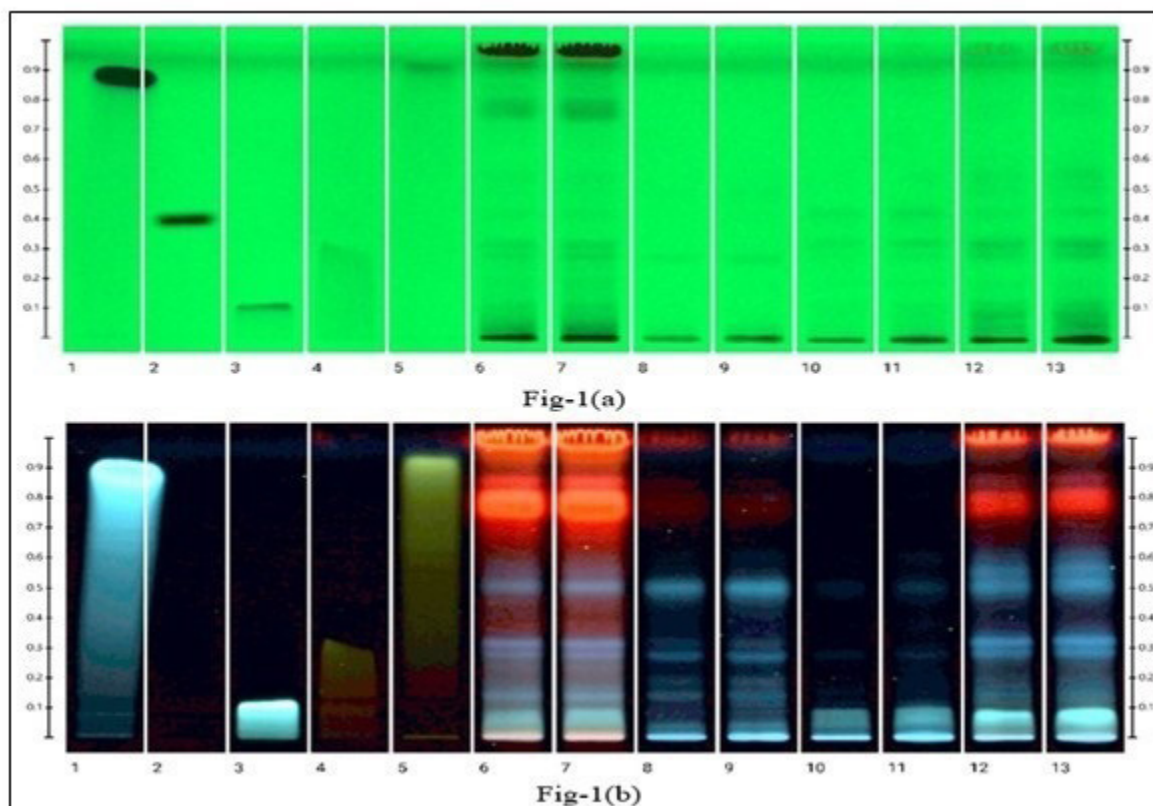


Figure 1: HPTLC profile of *Brassica* by method 1 before and after derivatisation: (a) image under 254 nm and (b) image under 366 nm. Lane numbers 1 to 5 represent the reference standards Caffeic acid, Caffeine, Chlorogenic acid, Rutin and Quercetin. Lane 6, 7 represents Kale BRM, Lane 8,9 represents Cabbage BRM, Lane 10,11 represents Cauliflower BRM, Lane 12,13 represents Broccoli BRM.

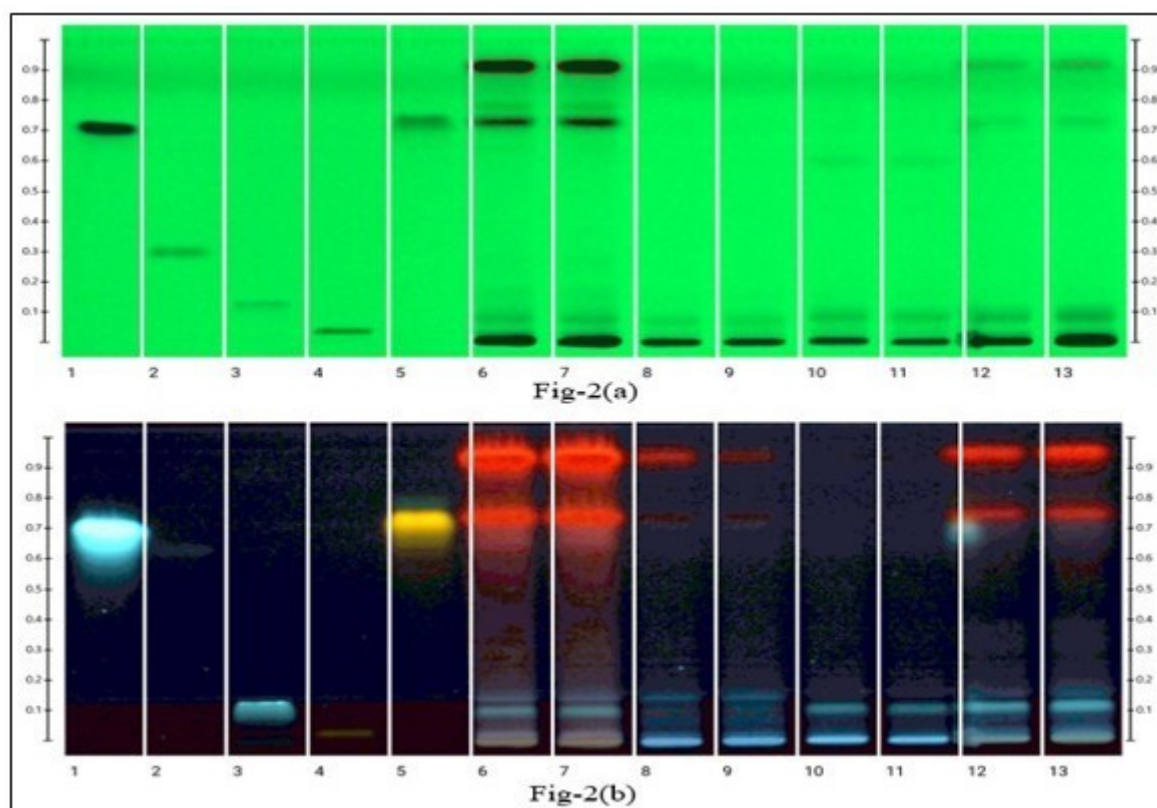


Figure 2: HPTLC profile of *Brassica* by method 2 before and after derivatisation: (a) image under 254 nm and (b) image under 366 nm. Lane numbers 1 to 5 represent the reference standards Caffeic acid, Caffeine, Chlorogenic acid, Rutin and Quercetin. Lane 6, 7 represents Kale BRM, Lane 8,9 represents Cabbage BRM, Lane 10,11 represents Cauliflower BRM, Lane 12,13 represents Broccoli BRM.

and 366 nm, as well as under white light. Derivatisation reagent Natural product and PEG reagent was used. The derivatized bands were later re-visualized under UV light at 366 nm and white light.

The third HPTLC run was carried out under the same sample loading conditions as the second run. However, the mobile phase was modified to butanol: ethyl acetate: water: formic acid (9:2:1:0.5, v/v/v/v), which provided enhanced band resolution and increased signal intensity. All other parameters, including chamber saturation, derivatization, and visualization, were consistent with the previous runs.

In the fourth and final HPTLC run, BRMs were analyzed alongside their corresponding test samples to enable comparative profiling. Cauliflower was excluded from this run due to the unavailability of the corresponding test sample. The optimized mobile phase from the third run was used again, as it had demonstrated superior separation and visualization of the target compounds in earlier runs.

Quantification of rutin, quercetin, chlorogenic acid, caffeic acid, and caffeine in *Brassica* Samples Using UV-visible Spectroscopy

The quantification of rutin, quercetin, chlorogenic acid, caffeic acid, and caffeine in *Brassica* samples was carried out using UV-visible (UV-vis) spectroscopy based on standard calibration curves. All analytical-grade standards were purchased from certified suppliers (e.g., Sigma-Aldrich) and stored according to the manufacturer's instructions. Standard stock solutions (1 mg/mL) were prepared in methanol for Rutin and quercetin and serially diluted to generate calibration curves within the linear range for each compound. Similarly, the stock solution of 1 mg/mL of Caffeic acid was prepared with ethanol: water (50:50 v/v) and for chlorogenic acid the stock solution was prepared in same concentration using methanol: water (50:50 v/v). Working standard solution of 100 µg/mL was prepared from above stock solutions and were scanned in the entire UV-vis range of 700-200 nm to determine the λ_{max} . Calibration curve with concentration ranging from 20 µg/mL to 100 µg/mL versus Absorbance were constructed effectively. The linearity equation and R^2 value were determined.

Sample Preparation for HPTLC analysis

Samples of *Brassica* plant material (e.g., Kale, Broccoli and Cabbage.) were used for the estimation of rutin, quercetin, chlorogenic acid, caffeic acid, and caffeine by spectroscopic method. Approximately 1.0 mg of powdered sample was extracted with 10 mL of selected solvent respect to the standards as mentioned above, using ultrasonic-assisted extraction for 30 min at room temperature. The mixture was centrifuged at 6000 rpm for 10 min, and the supernatant was filtered through a 0.45 µm syringe filter before analysis.

UV-vis Spectroscopic Analysis

Spectroscopic measurements were conducted using a double-beam UV-visible spectrophotometer (Shimadzu, UV-1900i). Absorbance readings were recorded at characteristic wavelengths for each analyte: rutin ($\lambda_{\max} \approx 257$ nm), quercetin ($\lambda_{\max} \approx 256$ nm), chlorogenic acid ($\lambda_{\max} \approx 325.5$ nm), caffeic acid ($\lambda_{\max} \approx 319.5$ nm), and caffeine ($\lambda_{\max} \approx 274$ nm). Quantification was performed by comparing the absorbance values of the samples at respective $\lambda_{\max}$ to those of the standard calibration curves ($R^2 > 0.995$ for all compounds). All analyses were conducted in triplicate, and results were expressed as % w/w of plant material.

RESULTS AND DISCUSSION

Physical characterization

The *Brassica* samples were analysed for various physical properties, including colour, pH, texture, and water solubility at room temperature (Table 1). The samples appeared as light green (kale) to yellowish white (cauliflower), reflecting natural pigment differences due to chlorophyll and carotenoid content. The pH values ranged between 5.1 to 5.8, demonstrating a slightly

acidic nature. The samples were non-hygroscopic in nature. The non hygroscopicity suggests its minimal moisture uptake which in turn enhances the shelf-life making it suitable for nutraceuticals-based product development.^[16] The variations in taste ranging from earthy, sweet and nutty flavours attributed to compositional differences in volatile compounds, and secondary metabolites among species. The bitter taste was mainly due to the presence of glucosinolates.^[17]

HPTLC Method optimization: Sequential Run

During the initial HPTLC run, various *Brassica* BRMs were separated by employing the mobile phase Ethyl acetate: Butanol: Water (18:8:4.5). The same phase was used to find the R_f positions, colour, and availability of standards in the BRM fingerprints. Pre-derivatisation (254 nm) and post-derivatisation (366 nm) HPTLC profiles are presented in Figures 1a and 1b show standards along with *Brassica* BRMs, wherein Lanes 1-5 are standards (caffeic acid, caffeine, chlorogenic acid, rutin, quercetin) and Lanes 6-13 are *Brassica* BRMs, 6-7: kale, 8-9: cabbage, 10-11: cauliflower, 12-13: broccoli. At 254 nm, caffeic acid (Lane 1) showed at R_f 0.89, and caffeine and chlorogenic acid (Lanes 2, 3) at R_f 0.4 and 0.12, whereas rutin (Lane 4) was not detected prior to or following derivatisation, possibly because of poor solvent extraction, instability, or low level. Quercetin (Lane 5) showed at R_f 0.9. Following derivatisation (366 nm), kale BRMs (Lanes 6-7) had distinct bands at R_f 0.15, 0.3, 0.5, 0.76, and 0.8. Cabbage BRMs (Lanes 8-9) had blue bands at R_f 0.15, 0.3, 0.5, and 0.8 but of reduced intensity. Cauliflower BRMs (Lanes 10-11) had weak bands at R_f 0.1, 0.29, and 0.3, whereas broccoli BRMs (Lanes 12-13) had blue and red bands as in kale. Blurred or absent bands indicate poor solubility or low levels of active compounds. Generally, R_f values of BRMs failed to correspond with standards,

Table 1: Physical characteristics of various *Brassica* samples.

Sl. No.	Samples	Colour	Texture	Odour	Taste	pH	Hygroscopicity	Solubility
1.	Kale leaf	Light green	Fine powder	Strong, earthy, slightly grassy	Earthy, slightly bitter, vegetal	5.8	Non-hygroscopic	Partially soluble
2.	Cabbage	Cream	Fine powder	Vegetal, neutral or slightly sweet	Mildly sweet, earthy	5.14	Non-hygroscopic	Partially soluble
3.	Broccoli	Yellowish green	Fine powder	Earthy, slightly grassy	Slightly bitter, grassy with a mild flavour	5.4	Non-hygroscopic	Partially soluble
4.	Cauliflower	Yellowish white	Fine powder	Earthy smell	Slightly nutty, crunchy	5.2	Non-hygroscopic	Partially soluble

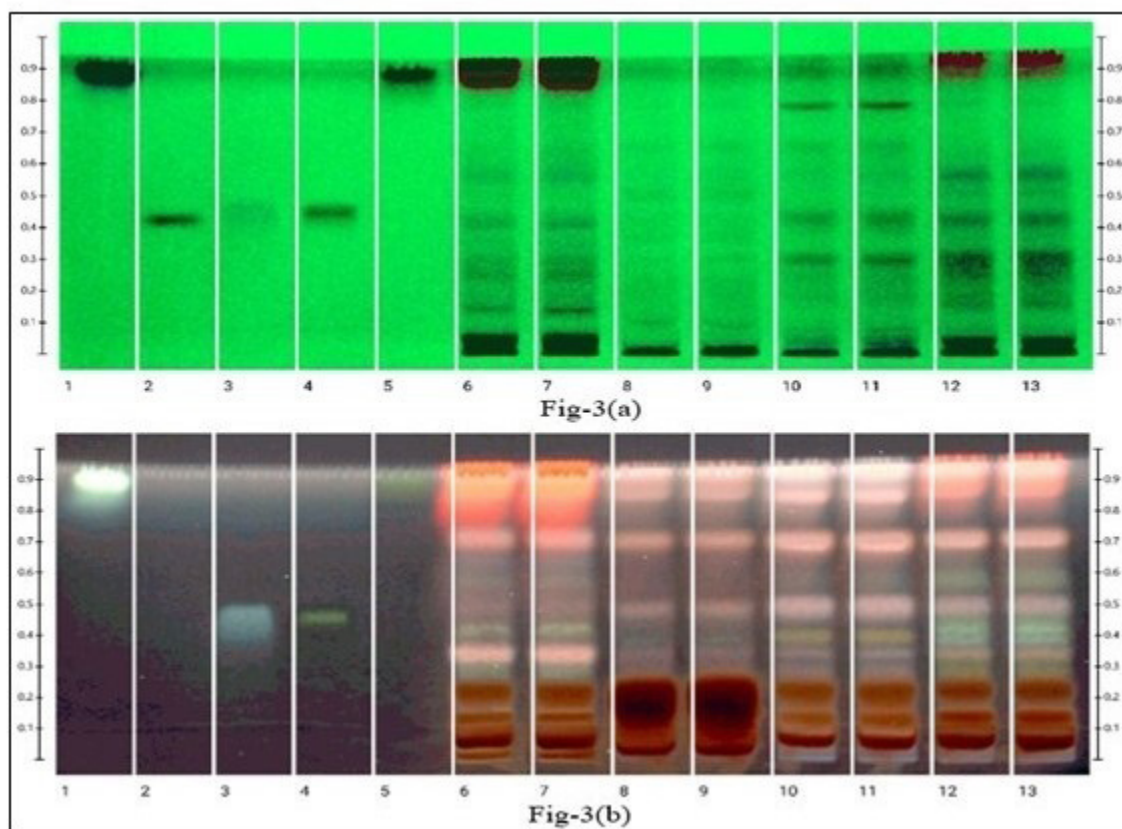


Figure 3: HPTLC profile of *Brassica* by method 3 before and after derivatisation: (a) image under 254 nm and (b) image under 366 nm. Lane numbers 1 to 5 represent the reference standards Caffeic acid, Caffeine, Chlorogenic acid, Rutin and Quercetin. Lane 6, 7 represents Kale BRM, Lane 8,9 represents Cabbage BRM, Lane 10,11 represents Cauliflower BRM, Lane 12,13 represents Broccoli BRM.

demonstrating that composition of the solvent significantly influences separation efficiency in *Brassica* species. Resolution was enhanced for a second run by adopting Ethyl acetate: Water: Acetic acid: Formic acid (90:19:2:2). HPTLC profiles (Figures 2a and 2b) had standards (Lanes 1-5) and *Brassica* BRMs (Lanes 6-13). Caffeic acid, caffeine, and chlorogenic acid at 254 nm appeared as R_f 0.7, 0.3, and 0.13 respectively; rutin was not detected; quercetin was seen at R_f 0.73. Upon derivatisation (366 nm), all BRM lanes showed a blue band at R_f 0.13 (chlorogenic acid). Kale and broccoli BRMs (Lanes 6-7, 12-13) exhibited identical banding at R_f 0.13, 0.7, and 0.9, whereas cabbage and cauliflower BRMs (Lanes 8-11) contained weak or no bands, perhaps because of incomplete solubilization or degradation of active ingredients. A third run with Butanol: Ethyl acetate: Water: Formic acid (9:2:1:0.5) provided better separation. As indicated in Figures 3a and 3b, Lanes 1-5 were standards and Lanes 6-13 *Brassica* BRMs (6-7: kale, 8-9: cabbage, 10-11: cauliflower, 12-13: broccoli). Caffeic acid was seen at R_f 0.9 at 254 nm, caffeine and chlorogenic acid at 0.41 and 0.45 (weak), rutin at 0.45, and quercetin at 0.9. After derivatisation (366 nm, 10% sulfuric acid), all BRMs showed similar brown bands (R_f 0.09-0.2) and reddish-orange bands in the upper region, indicating this solvent system was optimal for extraction and separation. Using

this optimized mobile phase, a fourth run (Figures 4a and 4b) analyzed BRMs and samples (excluding cauliflower). Lanes 1-5 contained standards; Lanes 6-7 kale BRM/sample; 8-9 cabbage BRM/sample; 10 cauliflower BRM; 11-12 broccoli BRM/sample. At 254 nm, caffeic acid (Lane 1) was observed at R_f 0.9, caffeine (Lane 2) at 0.45 (not visible at 366 nm), and chlorogenic acid (Lane 3) weakly at 0.47 (blue at 366 nm). Rutin (Lane 4) was observed at R_f 0.48 and quercetin (Lane 5) at 0.9. Following the derivatisation with NP/PEG reagent, Lanes 6-7 showed the most bands, followed by Lanes 11-12 (broccoli), while Lanes 8-10 (cabbage and cauliflower) had faint blue bands, indicating fewer extractable active components in the solvent system.

Quantification of rutin, quercetin, chlorogenic acid, caffeic acid, and caffeine in *Brassica* Samples Using UV-visible Spectroscopy

The $\lambda_{\max}$ for Rutin, quercetin, Caffeic acid, caffeine, chlorogenic acid was found to be 257 nm, 256 nm, 319.5 nm, 274 nm, and 325.5 nm (Figure 5 and Table 2). The UV spectroscopic analysis provided quantitative insights into the presence of selected phytochemical standards in *Brassica* sp. The results indicated that rutin, quercetin, chlorogenic acid, caffeic acid, and caffeine were present in very minimal amounts. Rutin, with an absorbance at

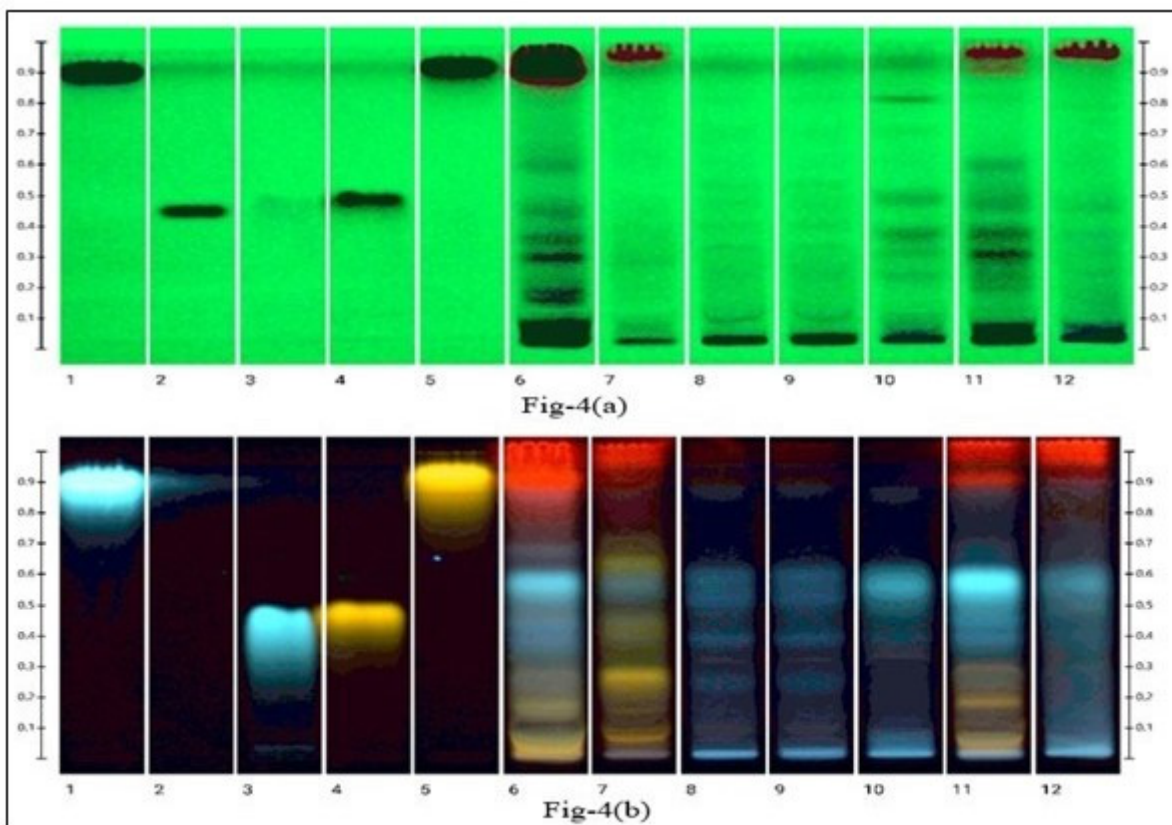


Figure 4: HPTLC profile of *Brassica* by method 4 before and after derivatisation: 4(a) image under 254 nm and 4(b) image under 366 nm. Lane numbers 1 to 5 represent the reference standards Caffeic acid, Caffeine, Chlorogenic acid, Rutin and Quercetin. Lane 6 represent Kale BRM, Lane 7 represents test sample kale powder, Lane 8 represent Cabbage BRM, Lane 9 represent test sample cabbage powder, Lane 10 represent Cauliflower BRM, Lane 11 represent Broccoli BRM and Lane 13 represents test sample broccoli powder.

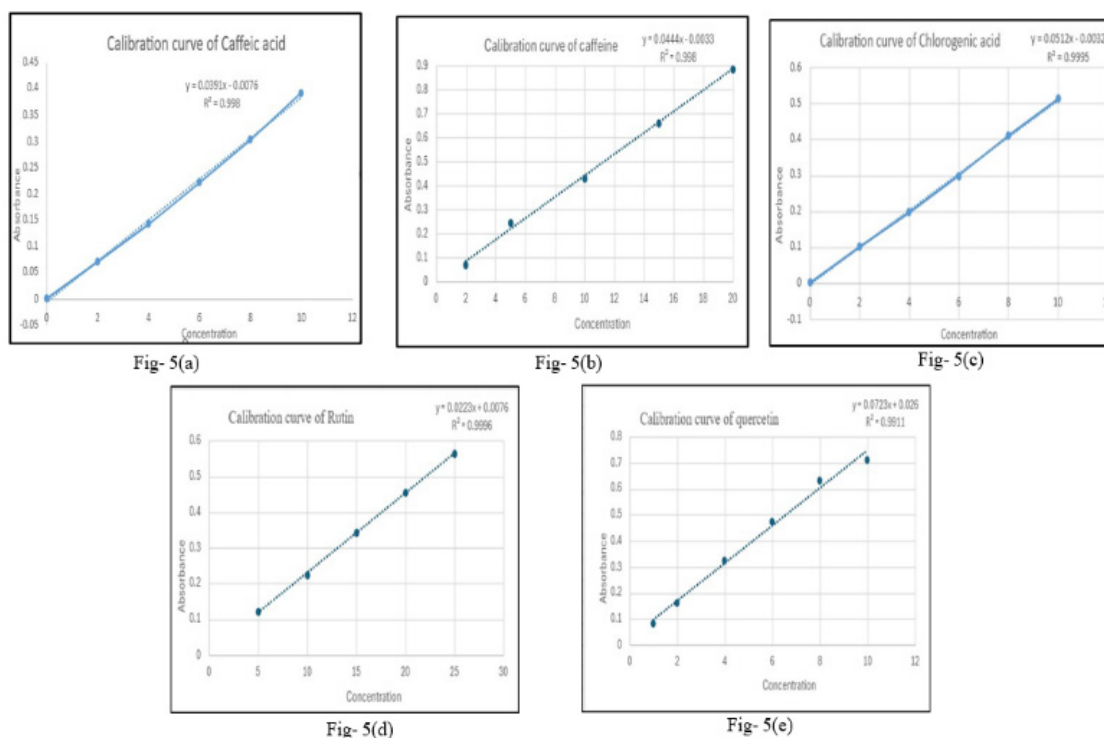


Figure 5: UV Calibration curve of standards: 5(a) Caffeic acid, 5(b) Caffeine, 5(c) Chlorogenic acid, 5(d) Rutin, 5(e) Quercetin.

Table 2: Concentration of these standards in Kale, Cabbage and Broccoli test samples.

Standards	Absorbance	Quantity (% w/w)		
		Kale	Broccoli	Cabbage
Rutin	257 nm	0.51%	2.5%	0.55%
Caffeic acid	311.5 nm	0.96%	1.52%	0.91%
Caffeine	274 nm	0.86%	1.65%	0.75%
Quercetin	256 nm	0.05%	0.35%	0.027%
Chlorogenic acid	325.5 nm	0.29%	0.49%	0.14%

257 nm, was found to be highest in broccoli (2.5%). Caffeic acid, detected at 311.5 nm, showed its highest concentration in broccoli (1.52%), with kale (0.96%) and cabbage (0.91%) containing comparatively lower amounts. Caffeine, absorbing at 274 nm, was most abundant in broccoli (1.65%). Quercetin, with an absorbance at 256 nm, was present in small quantities, peaking in broccoli (0.35%), followed by kale (0.05%) and cabbage (0.027%). Chlorogenic acid, detected at 325.5 nm, was most prevalent in broccoli (0.49%), with kale and cabbage containing 0.29% and 0.14%, respectively. UV spectroscopy is the most crucial analytical technique in drug analysis to accurately determine compound concentrations. It detects and measures drugs by analyzing the absorbance of UV light at specific wavelengths to determine purity, concentration, and stability.^[18]

CONCLUSION

This study explored the phytochemical profiling of *Brassica* vegetables using HPTLC and UV spectroscopic methods and provides complementary insights into the complex bioactive composition of *Brassica* vegetables. The HPTLC analysis demonstrated the significant impact of mobile phase selection on the separation and visualization of compounds. This underscores the importance of tailored solvent systems based on analyte polarity. While key standards such as rutin, quercetin, chlorogenic acid, caffeic acid, and caffeine were either absent or present in negligible amounts attributing to their inherently low concentrations, extraction inefficiencies, or their natural absence in the *Brassica* matrix. Quantitative determination through UV spectroscopic analysis confirmed the minimal presence of these standards, in the tested sample. The study emphasizes the need for method optimization in extraction protocols and analytical approaches to address the challenges posed by the unique phytochemical matrix of *Brassica* vegetables. Tailored analytical techniques are crucial to enhancing the detection and quantification of minor constituents while ensuring accurate representation of their bioactive potential. These findings provide a foundation for further research into the nutritional and therapeutic significance of *Brassica* bioactives, with a focus on improving analytical methodologies and understanding the roles of dominant phytochemicals.

ABBREVIATIONS

HPTLC: High-Performance Thin Layer Chromatography; **BRM:** Botanical Reference Material; **NP:** Natural Product Reagent; **PEG:** Polyethylene Glycol; **UV-vis:** Ultraviolet-visible.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

SKP: Conceptualization, Data Analysis and Interpretation, Writing - Review and Editing, NM: Conceptualization, Methodology, Data Analysis and Interpretation, Writing - Original Draft, Writing - Review and Editing, PK: Conceptualization, Methodology, Data Analysis and Interpretation, Writing - Original Draft, AR: Methodology, SSS: Writing - Review and Editing, AM: Investigation, DJ: Investigation.

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