Ind. J. Pharm. Edu. Res., 2026; 60(3s):s1170-s1179.
https://www.ijper.org

Original Article

Spectrometric and Chromatographic Profiling of Naringin from *Citrus reticulata* Blanco, Native to the Himalayan Region of Darjeeling and Sikkim

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ABSTRACT

Aim: *Citrus reticulata* Blanco (CRB), widely cultivated in the Himalayan regions of Darjeeling and Sikkim, is a remarkable source of bioactive flavonoids, particularly naringin. This study presents the methodical effort to isolate, characterize, and quantify naringin from CRB grown in the foothills of the Himalayan region of Darjeeling and Sikkim where such data have not been reported earlier to the best of our knowledge, emphasizing the impact of agro-climatic conditions and elevation on phytochemical profiles. **Materials and Methods:** Juice and Peel extracts were examined using a mixed Chromatographic (TLC, HPTLC, HPLC) and Spectroscopic (UV, IR, NMR, MS) methods on samples gathered from various altitudinal sites. The R_f and retention time values of the isolated chemicals closely matched those of the standard, verifying the effectiveness of the extraction and purification performed. **Results:** Spectral analysis (UV-vis at 282 nm, IR, ^{1}H-NMR., ^{13}C-NMR and mass spectrometry) established a strong foundation for the identity and structure of the compound. Notably, the detected concentrations of naringin in the Himalayan region CRB were significantly higher than those documented for citrus grown in the different geographical areas, indicating that elevation and agro-climatic factors enhance the phytochemical compositions. **Conclusion:** Thus, this robust analytical approach gives firm information regarding the accuracy of the techniques, which helped in the quality and quantity assessment of naringin in the Himalayan CRB. While the study discusses the pharmacological relevance of naringin, the scope does not extend to drug interaction experiments.

Keywords: *Citrus reticulata* Blanco, Darjeeling, Naringin, Plant compound evaluation, Sikkim.

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Received: 12-01-2026;
Revised: 05-03-2026;
Accepted: 22-04-2026.

INTRODUCTION

Mandarin, *Citrus reticulata Blanco* (CRB)[1] is a valuable commercial crop that accounts for 41% of the total citrus fruits produced in India. It is the most significant cash crop in Sikkim and the nearby Darjeeling hills,[2] which have similar topography and climatic conditions.[3] Sikkim occupies 5% of the total landmass of the country. It generates 2% of the citrus output nationally, with a total of 13.08 thousand hectares under cultivation and 18.99 thousand metric tons produced every year.[4] Several superior and unique ecotypes of *C. reticulata* Blanco (mandarin) are traditionally cultivated in the foot hills of the Darjeeling region and are commonly referred to as Darjeeling mandarin.[5] The subtropical, humid climate of the lower hills of the Himalayas i.e., Sikkim and Darjeeling, is mostly suitable for the cultivation of mandarins. Thus, the fruits cultivated in these areas are of the highest calibre. CRB is an origin of many bioactive substances like limonoids, flavonoids, and their glycosides. An essential flavonoid that is present in CRB is Naringin. Naringin ($C_{27}H_{32}O_{14}$ M.W.: 580.5 g mol-^{1}) is a significant derivative of a disaccharide. The (S)-naringenin is modified by a 2-O-(alpha-L-rhamnopyranosyl)-beta-D-glucopyranosyl moiety in the glycosidic bond at the 7th position.[6] The IUPAC name of naringin is (2S)-5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-3, 4-dihydro-2H-chromen-7-yl2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside, a flavanone-7-O-glycoside or 4′,5,7-trihydroxyflavanone7-rhamnoglucoside.[7] It possess anti-diabetic, anti-inflammatory and hepatoprotective properties,[8] helps in osteogenesis, metabolic syndrome, oxidative stress, genomic damage and Central Nervous System (CNS) disorders.[6,9] It has also been established in various studies that Naringin is a P-gp modulator and a CYP3A4 inhibitor .The studies have revealed that with the pretreatment of naringin up to 15mg/kg would greatly increase the bioavailability of diltiazem[10] and Verapamil.[11] It is advised that the dosage of these drugs should be adjusted when given with

DOI: 10.5530/ijper.20263469

naringin or a naringin-containing dietary supplement.[11] Thus, it is well established that Naringin influences the bioavailability of additional pharmaceuticals, including those in the category of Calcium Channel Blockers (CCB) when administered concomitantly. The production and consumption of Naringin containing CRB are very high in these regions of Sikkim and Darjeeling, though the amount of Naringin present in this variety of mandarin is not known. There are various research reports regarding the extraction and estimation of Naringin from various other citrus species such as mandarin, orange and pomelo.[4,12-15]

Even though methods for isolation, estimation, and characterization of naringin from Citrus species are well documented, no literature is available specifically for the Himalayan region, including the northeastern Indian states, where these fruits are also cultivated extensively. Hence, this work was undertaken to fill the gap by working on the isolation, estimation, and characterization of CRB of the Himalayan region of Darjeeling and Sikkim, where such data have not been reported earlier to the best of our knowledge. The interaction study of the concomitant use of Naringin with other drugs, including any Calcium channel blockers, is currently out of the scope of this manuscript.

MATERIALS AND METHODS

Chemicals and instruments/apparatus

Analytical grade chemicals and solvents were used (Merck, India) in the present study. HPLC grade solvents (Rankem, India) and water (Millipore direct Q3, USA) were used wherever necessary. Solvents were utilised as provided by commercial suppliers without further purification. Standard for naringin was purchased from Sigma Aldrich (Bangalore, India).

Collection of Plant Materials

The experimental material used in this work included specific fresh mandarins[1] collected from three different locations in Darjeeling and two locations in Sikkim. The locations to collect the samples were selected based upon their altitude, Bijanbari, 760 m (S1), North Sikkim region, 956 m (S2), Mangphu 1250 m (S3), Mirik, 1495 m (S4), and West Sikkim region, 1800 m (S5) as it was reported in previous study that the ideal elevation for Mandarin production were between 700-1300 m above sea level.[16] The plant material (CRB) ref no. RC-S/02/2025-26/Tech. 247 was taxonomically authenticated by Botanical Survey of India, Sikkim Himalayan Regional Centre, Gangtok-737103. The Fruits were collected from October to March, after which the peels were sun-dried and then coarsely powdered, whereas the juice was hand-squeezed. No lyophilization was employed in this study.

Experimental

Isolation of Naringin from the Juice

Sudto *et al.*[13] methods were further modified where, 20 mL of freshly hand-squeezed fruit juice was accurately measured and taken in a measuring cylinder and combined with an equal volume of methanol. This amalgamation was then transported to a separatory funnel and agitated/shaken for 5 min. Whatman filter paper was used to filter the solution, and the collection of the filtrate was done in a porcelain dish. It was measured using a measuring cylinder and placed in a water bath for 20 min. The remaining filtrate from the water bath was poured into a separating funnel, and 10 mL of hexane was added to it. The resulting methanolic and hexane layers were separated into different beakers. The methanolic layer was appropriately diluted with methanol for UV-visible spectrophotometric studies.

Isolation of Naringin from the Peels

Maria *et al.*[17] and Yusof *et al.*[18] methods were used as a foundation, which was also subsequently modified to develop an optimized process for the study. Firstly, the peels were initially sun-dried and thereafter coarsely pulverised into powder using a mortar and pestle. Measured amount (10 g) of the powdered sample was macerated in 500 mL of methanol for a duration 72 hr. The resulting extract was subjected to filtration, and the filtrate was concentrated on a water bath until it reached a sticky consistency. To this concentrated methanolic extract, 60 mL of water were included, and the mixture was further agitated at 70°C for 30 min. After stirring, the mixture was separated using a separating funnel. A mixture of hexane (20 mL) was then mixed in the aqueous layer and further kept for 2-3 days at 25°C to facilitate naringin crystals to crystallize out. Once the naringin crystals formed, they were diluted with isopropanol (25 mL) and further heated to reduce the volume by half. The crude naringin crystals were purified by repeated recrystallization (three times) in isopropanol until a single HPLC peak confirmed the absence of impurities. The purified crystals were collected by filtration through Whatman filter paper and air-dried at room temperature to obtain pure naringin. The appearance and purified yield of the samples were recorded.

Preparation of standard solution

A 10 µg mL^{-1} solution of standard Naringin solution was prepared by mixing it with methanol and adjusting the volume accordingly. The wavelength corresponding to maximum absorbance and absorbance values obtained were used for the identification and assessment of the isolated compounds.

Characterisation

The isolated Naringin was characterised by various spectrometric techniques such as UV, IR, NMR spectroscopy, Mass spectrometry and chromatographic techniques like TLC, HPTLC, HPLC,

along with determination of its melting point. Furthermore, the IR, UV data provided confirmatory evidence of the compounds functional group.

Determination of Melting Point

Determination of the melting point was performed using the capillary technique. Naringin powder extracts were filled into capillaries measuring 6 mm in length and 1 mm in diameter. Three readings were taken and averaged to obtain the melting point.

Thin Layer Chromatography

Thin-Layer Chromatography (TLC) and High-Performance Thin-layer Chromatography (HPTLC) analysis were conducted on Pre-coated TLC plates (10 x 10 cm) composed of 0.2 mm thickness containing silica gel 60 F254 TLC plates (Merck, Germany) supported by an aluminium sheet. The standard and the methanolic extracts were deposited onto TLC plate as circular spots using a capillary tube, maintaining a space of 7 mm between each spot and the edge of the plate, along with 10 mm from the bottom. Standard solutions (1 mg/µL) and sample solutions (1 mg/µL) were administered within a volume range of 1-2 µL. For HPTLC, methanolic solutions of isolated samples and standard (1 mg/µL) were applied (2 µL) to HPTLC pre-coated plates were utilised to create 8 mm bands (150 nL/s of dosage speed using nitrogen; positioned, 20 mm from the plate edges, 10 mm away from the lower edge along with a 12 mm inter-track spacing employing Linomat 5 automatic TLC Sampler (Linomat 5, CAMAG, Switzerland) along with 100 µL HPTLC syringe (Hamilton, Switzerland). The TLC and HPTLC plates were developed in a pre-saturated mobile phase containing methanol, water, and ethyl acetate at a ratio of 0.8:0.6:3 (v/v/v)[19] in a CAMAG twin tower chamber, up to 7 cm of migration at 25±5°C. After drying the spots and the chromatograms were observed and recorded at 254 nm and 366 nm with the TLC UV Cabinet 4 (CAMAG).[19] The evaluation of the chromatograms was carried out using a TLC scanner equipped with win CATS software (Slit width 5 x 0.45 mm; CAMAG) at 254 nm. Ultraviolet spectra of both samples and standards were recorded across 200-400 nm, which would confirm spectral purity and also assess spectral identity. The absorbance readings for standards and samples were taken at a specified wavelength using the TLC scanner with WinCATS software (Slit width 5 x 0.45 mm; CAMAG).

High-Performance Thin Layer Chromatography

Standard Naringin (1 mg/mL) and the test samples (peel isolations) were subjected to filtration using methanol as the solvent in a Whatman No.1 filter paper, which was applied as 8 mm band utilising a CAMG Linomat 5, sample applicator fitted with a 100 µL syringe. Application parameters included a band length of 8 mm, 20 mm from the left edge margin and 10 mm track spacing from the lower plate edge. The plates were then added in the CAMAG twin tower chamber for development, which was pre-saturated for 20 min using the mobile phase. The development distance was sustained at 70 nm, at 25±2°C with 45-55% relative humidity. Following development, plates were dried and scanned with a CAMG TLC scanner IV, which was operated using WinCATS software (version 1.4.9.2020). Densitometric Scanning was completed in absorption mode at 285 nm, which is the reported λ_{max} for Naringin. Slit size of 6.00 × 45 mm was applied for scanning at a speed of 20 mm/s. The technique was found to have high specificity, reproducibility, hence was found to be highly suitable for qualitative determination of naringin isolated from CRB.

UV Spectroscopy

UV-visible spectrum of isolated samples and standard naringin were documented from 200 to 800nm using a UV-1800 Shimadzu Spectrophotometer (Japan) equipped with a 10 mm matched quartz cells. All the samples were prepared at a 10.0 µg mL^{-1} concentration by diluting with methanol. Prior to measurement, each sample underwent a double dilution process of 1:100. This was carefully considered during the final concentration calculations. The wavelength corresponding to maximum absorbance was determined and used to identify the isolated samples. The absorbance values were used to estimate the amount of Naringin present in the juice.

Infrared Spectroscopy

Data acquisition was performed using Agilent Cary 630 FTIR equipped with Resolution Pro v5.2 and Microlab FTIR software v4.0 (California, USA). Cary 630 multireflection ZnSe ATR sensor was used, where 128 scans were averaged at a resolution of 2 cm^{-1} for each spectrum between 4000 and 625 cm^{-1}. This ATR module was used with a sampling press to compress the sample against the crystal. The background spectrum was recorded by scanning air prior to each measurement.

Nuclear magnetic resonance spectroscopy

For NMR studies (^{1}H and ^{13}C NMR), spectra were captured using Bruker, ascend 400 MHz, an automatic sample changer sample Xpress, along with a 5 mm BBFO PLUS probe, optimized for nuclei direct observation. The analysis was carried out in deuterated Dimethyl Sulfoxide (DMSO) and Tetramethyl Silane (TMS) as internal standard. The measured chemical changes were expressed in parts per million (ppm). The substance structures were established using spectroscopic data analysis and compared with existing data.[20]

Mass Spectrometry

The mass spectra were collected with a quadrupole ion trap mass spectrometer by the infusion of the extracted solution into the Waters, Synapt G2, ranging from 20 to 100,000 m/z Resolution mode from which the spectra were determined. The criteria for

the ion source employed were a capillary voltage of 4000V, a gas flow rate of 10 L/min, a nebuliser pressure of 30 psi, and a gas temperature of 350°C. in addition to these, other parameters were implemented according to the instrument's suggested settings. The mass detector functioned in multiple reaction monitoring mode at unit mass resolution with a dwell duration of 200 ms.

High-Performance Liquid Chromatography

The reverse-phase HPLC method of Kanaze *et al.*[21] was used to identify and assess the purity of the isolated naringin crystals. The HPLC system (Dionex, USA) used for the analysis consisted of the Ultimate 3000 UHPLC system featuring a quaternary pump, an electronically controlled injector, a vacuum degasser and an Ultimate 3000 diode array detector. Qualitative and quantitative assessment was performed with Dionex Chrome Leon software program. A 0.22 µm Miilipak Express filter in HPLC-grade water from Millipore Direct Q3 (USA) along with membrane filter of 0.45 µm pore size, was used for the procedure. The samples were filtered using Whatman's syringe filters (NYL 0.45 µm). A reversed phase C18 column (Shimadzu Shim-pack 5 µm particle size, 250 mm × 4.6 mm), operating at an ideal temperature of 25°C, was used for the chromatographic separation. A combination of tetrahydrofuran: water: acetic acid at 21:77:2 (v/v/v) ratio was taken with a flow rate of 1.0 mL/min.[13] Injection volume used for analysis was 5 µL (100 mg/L) and column eluents were monitored at 280 nm. The responses were measured as retention time for identification and peak areas vs concentration for quantitative estimation.

Statistical analysis

All the quantitative measurements were performed in triplicates. The analysis was further expressed as mean±standard deviation (n=3). No statistical tests were applied, only descriptive statistics are reported for the quantification of naringin.

RESULTS

Determination of Melting point

The separated flavonoid, Naringin was observed to be brownish white powder.[22] It was observed that the melt range of Naringin was fairly large, extending from 220.1 to 249.7°C.

Thin Layer Chromatography

The identification of isolated Naringin from juice and peel was confirmed by comparing the corresponding R_f value obtained for the standard compound.

High Performance Thin Layer Chromatography

The isolated compounds showed a maximum R_f value of 0.27-0.28, which is similar to the standards R_f (0.27) with an average peak height of 399.5 and an area of 7885.09. The total area accounted for 98.27% which indicates a high purity level and successful isolation. A presence of a minor peak ranging at 0.50-0.60 R_f of the different samples representing 1.73%-1.77% of additional flavonoid components or minimal impurities. Thus, confirming that the method developed were of highly specific, reproducible and suitable for the quantitative and qualitative estimation of naringin from CRB samples.

UV Spectroscopy

Ultraviolet scanning was performed to determine the concentration of the five different samples from the Himalayan region of Darjeeling and Sikkim. The prepared Sample solution was detected supporting the structure characteristic absorption band at a wavelength of 282 nm (Figure 1). The concentrations of Naringin in both juice and peel extracts were calculated accordingly. According to Serpil Yalim *et al.*[23] the concentration of naringin in Turkish citrus peel and juice ranging from 0.50 to 15.7 mg/L and 0.12 to 2.63 mg/L, respectively. Similarly, Pradeep Raj Rokaya *et al.*[16] reported that Naringin content varied

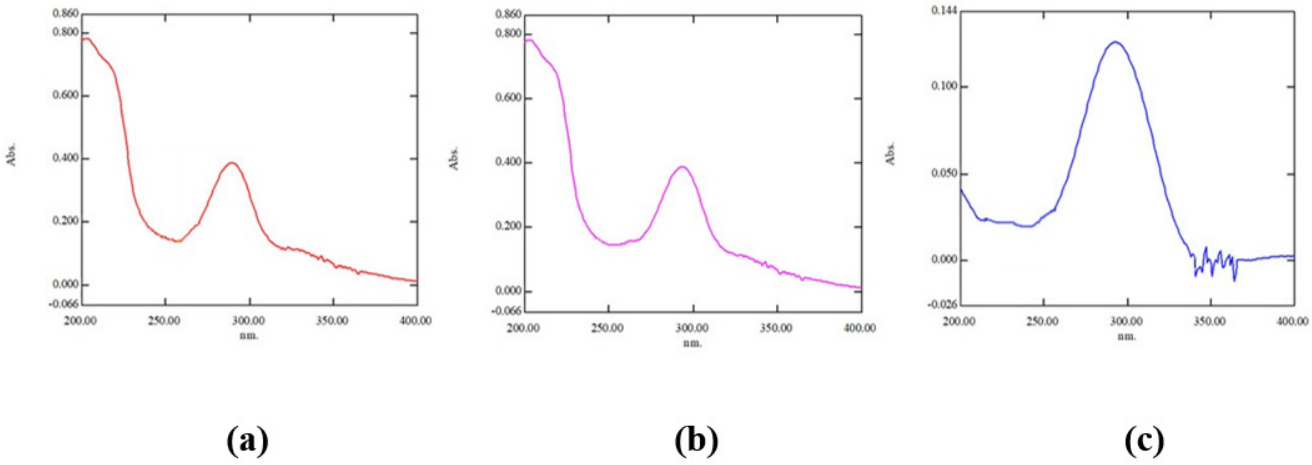


Figure 1: Figure (a) Depicts the characteristic UV spectrum of standard naringin, Figures (b) and (c) Represent the spectra from peel and juice extracts, respectively.

significantly with altitude. In comparison, the naringin levels obtained from CRB grown in the Himalayan region of Darjeeling and Sikkim were notably higher, as shown in Table 1, suggesting a potentially richer photochemical profile influenced by altitude and agro-climatic conditions.[3] The UV spectra supported the structure, showing characteristic bands

Infrared

The FTIR spectra (Figure 2) illustrate the isolated Naringin isolated from different samples and its matching standard. The spectra represent characteristic peaks at 3340 for the stretching vibration of -OH groups, at 1633 cm^{-1} for the stretching vibration of -C=O, at 1610/1525 cm^{-1} for the stretching vibration of -C=C- in the aromatic ring, at 983 cm^{-1} for the axial deformation of -C-O-C bonds.[20] The characteristic peaks from both the standard naringin and the isolated compounds are well illustrated in Table 2, further confirming that the isolated sample is Naringin itself.

Agrawal[24] asserts that the structure of an aglycosylated flavonoid may be determined by the use of ^{1}HNMR (a) and ^{13}CNMR (b) (Figure 5).

These data allow one to determine the type, location, conformation, and number of linked sugars.

^{1}H NMR(400 MHz, DMSO, TMS): δ: 1.15 (d, 3H, *J* = 6 Hz, CH3), 2.65 (dd, 1H, *J* = 2.8 Hz), 2.73 (dd, 1H, *J* = 2.8 Hz), 3.17 (d, 1H, *J* = 9.6 Hz), 3.22 (d, 1H, *J* = 9.6 Hz), 3.30 (s, 1H), 3.40 (s, 3H), 3.44 (d, 1H, *J* = 4.0 Hz), 6.75 (d, 2H, *J* = 3.2 Hz, Ar-H), 7.32-7.45 (dd, 2H, *J* = 3.2 and 2.8 Hz, Ar-H).

In ring A, two signals indicative of hydrogen atoms are present corresponding to, meta substitution at hydrogens H_6 and H_8.

Ring B exhibited a substituted aromatic *para* system, indicated by two doublets 7.32 ppm ($H_{2'}$ and $H_{6'}$) and 6.93 ppm ($H_{3'}$ and $H_{5'}$) in the molecule.[14] A hydroxyl on carbon C-4 is seen at 12.05 ppm[25] The hydrogens present in the two sugar molecules of naringin exhibit signals at 1.15 (d) ppm corresponding to C H3, between 3.0 and 4.0 ppm, and two signals related to the hydrogens attached to the anomeric carbons at 5.51 ppm.

^{13}CNMR (400 MHz, DMSO, TMS): δ ppm: 18.111, 42.550, 42.351, 60.519, 68.740, 70.050,70.981,76.411,77.317,77.580,79.092,79.190,92.634,96.511,96.541,101.102,103.640,115.01,115.020,127.470, 129.263,129.273,158.211,163.123,163.241,165.211,197.590.

The ^{13}CNMR spectrum (400 MHz, DMSO-d6, TMS) of isolated naringin shows characteristic resonance for the flavanone glycoside skeleton.C-2 and C-3 aromatic carbons at δ115.01-115.02 ppm (C-2) and δ 127.47 ppm (C-3) as per the B-ring's substitution pattern. Signals of the glycosidic moiety

Table 1: Concentrations of naringin in Peel and juice extracted from 5 different samples of *Citrus reticulata* Blanco of the Himalayan region of Sikkim and Darjeeling.

Sl. No.	Samples	Isolated compounds (mg/10 g)	% Concentration of naringin extracted from the peel	Concentration of naringin in juice (mg/L)
1.	S1	550	5.5	61
2.	S2	510	5.10	39.11
3.	S3	450	4.5	32
4.	S4	300	3.0	31.85
5.	S5	226	2.26	31

[note: All quantitative measurements were performed in triplicate and are expressed as Mean±Standard Deviation (*n* = 3)].

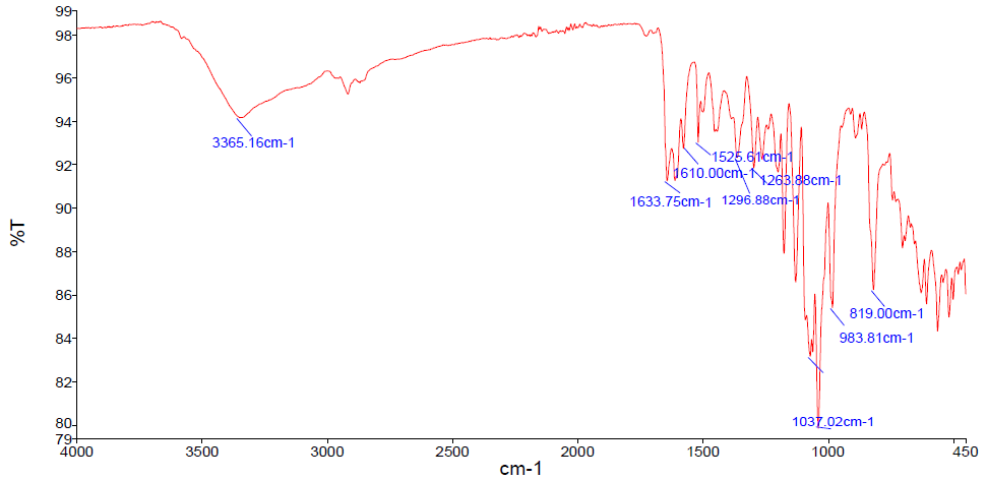


Figure 2: IR Spectra of the naringin, isolated from *Citrus reticulata* Blanco.

Table 2: IR spectra of 5 different samples isolated from *Citrus reticulata* Blanco of the Himalayan region of Sikkim and Darjeeling.

Functional group	Standard Sample 1Frequency (cm^{-1})	Sample 2	Sample 3	Sample 4	Sample 5
OH (axial deformation)	3351.76 3365 1039.32 1037	3340 1026	3325 1015	3345 1025	3360 1030
C=O	1643.75 1633.75	1622.80	1617.20	1620.80	1625.20
C=C	1519.61 1525.61 1605.51 1610.00	1520.61 1601.00	1535.50 1603.11	1524.51 1600.01	1530.40 1604.06
Aromatics	1296.88 1296.88 1263.88 1263.88	1247 1230	1257 1220	1230 1240	1230 1235
Axial deformation of C-O-C	982.81 983.81	985.21	980.31	979	983
Angular deformation C-H	819 819	815	800	820	821

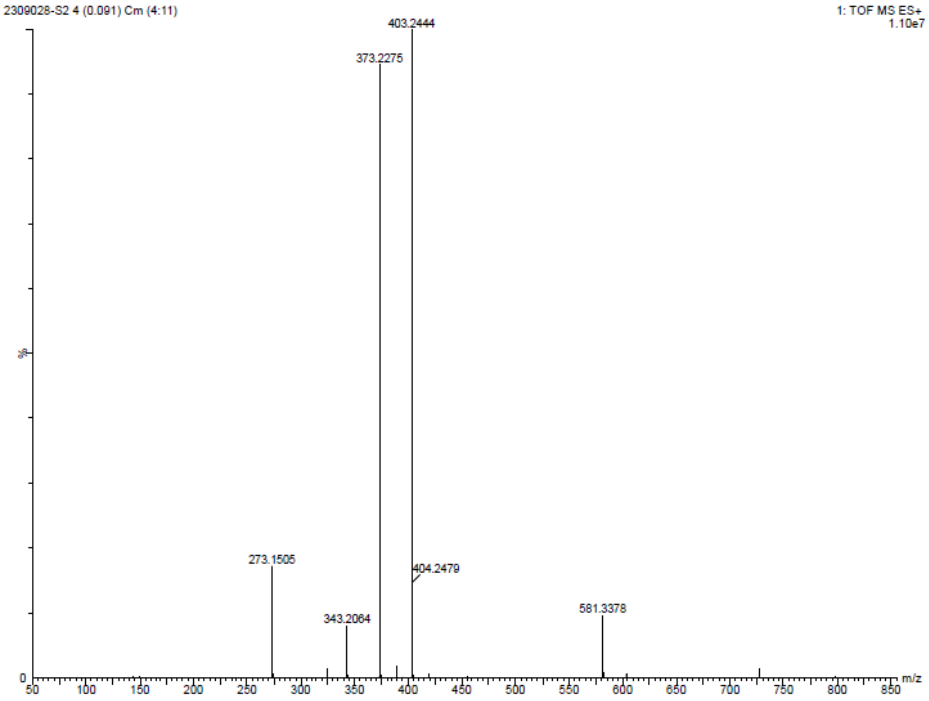


Figure 3: Mass spectrometry of *Citrus reticulata* Blanco.

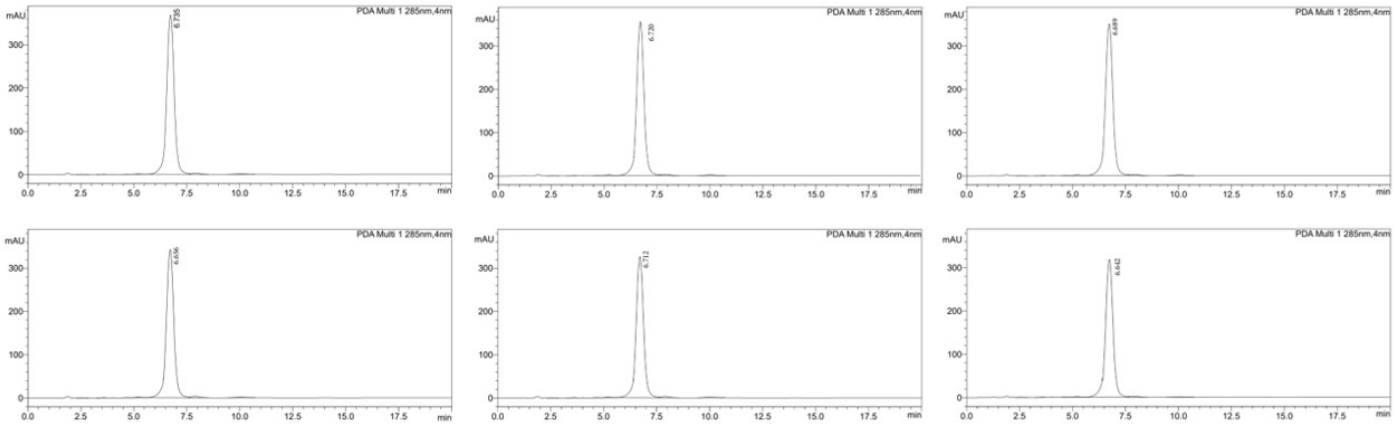
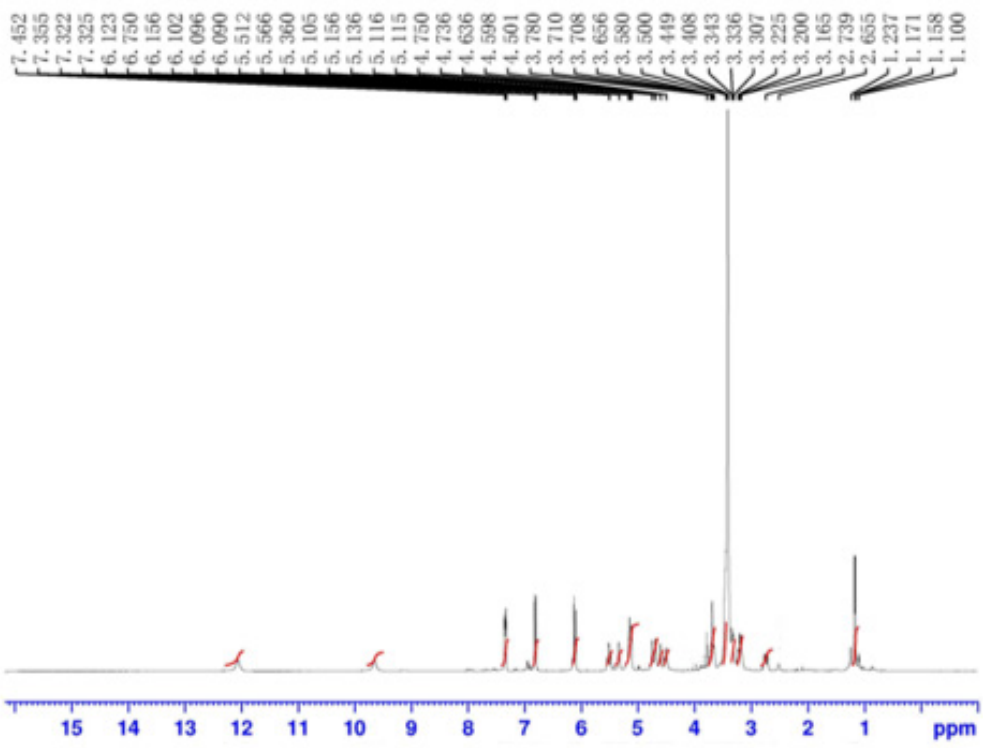
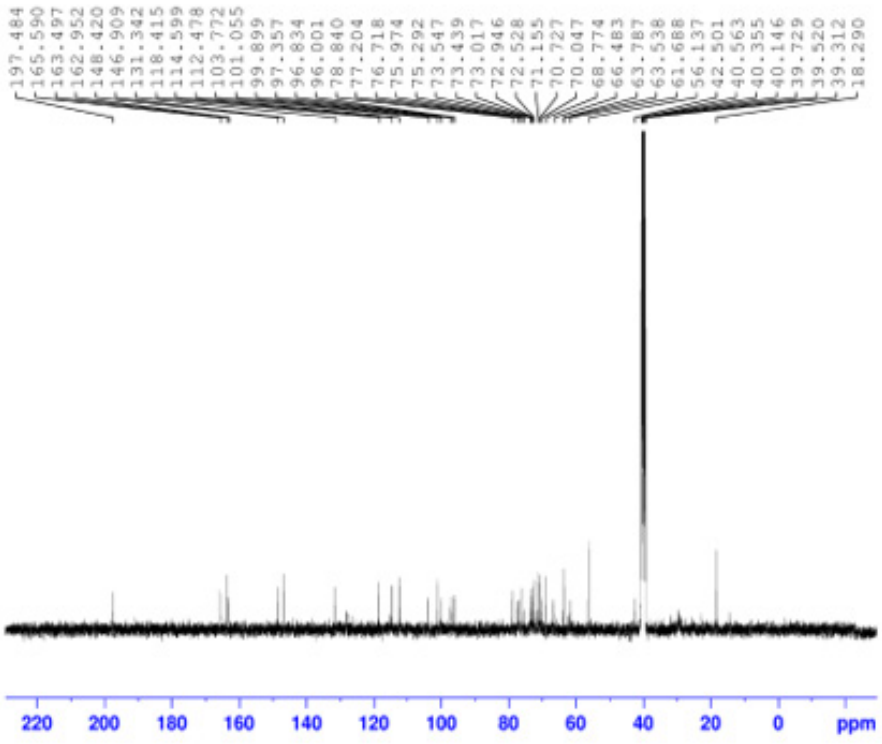


Figure 4: High-Performance Liquid Chromatography of Standard naringin (a), b-f of the isolated compound from *Citrus reticulata* Blanco.



(a)



(b)

Figure 5: ^{1}H NMR (a) and ^{13}CNMR (b) of isolated naringin from *Citrus reticulata* Blanco.

were seen at (C-6) at δ 18.11 ppm. Carbon of glucosyl (C-1) at δ101.10 ppm, establishing β- linkage. Glucosyl C-2 -C-6 between δ 60.52-79.19 ppm. Flavanone core: C-4 carbonyl at δ 197.59 ppm C-2/C-3 dihydro flavanol system at δ42.35-42.55 ppm (C-3) and δ76.41-79.19 ppm (C-2). A-ring carbons (C-6/C-8) at δ 96.51 -96.54 ppm, characteristic of 5,7-dihydroxy substitution. The ^{13}CNMR spectrum is consistent with the literature reports for naringin. The peaks and accurate chemical shifts match reflect excellent purity and structural similarity to the standard compound.

Mass Spectrometry

Mass spectra were used to better characterize the analyte. The fragmentation patterns that were identified were 273.15 for the sugar moiety, 334.20-373.22 for phenolic, and 403.24-404.24 for OH group (Figure 3). In the given conditions, the analyte generated protonated molecules at 581 m/z.

High-Performance Liquid Chromatography

The mean retention duration of Naringin was observed at 6.735± 0.0095 min when comparing standard and isolated sample chromatograms (Figure 4). This leads us to confirm that the isolated compound is Naringin only, as the retention time is similar in the standard and isolated samples.

DISCUSSION

Efficient isolation, characterization, and quantification of naringin from *Citrus reticulata* Blanco (CRB) in the Himalayan regions of Darjeeling and Sikkim addressed a key literature gap for these ecotypes.[26] Reports from comparable Himalayan altitudes have estimated naringin quantities in *C. reticulata* similar to those isolated here (peels: 2.26-5.5 mg/10g; juice: 31-61 mg/L). Spectroscopic (UV, IR, NMR, MS) and chromatographic (TLC, HPTLC, HPLC) techniques confirmed purity and identity, with melting range (220.1-249.7°C),[27] R_f (0.27-0.82),[28] λ_{max} (282 nm),[27] and RT (6.735±0.0095 min) matching standards. Peel extracts consistently showed 5-10times higher naringin than juice across all five samples (S1-S5), consistent with Turkish citrus varieties (peels: 0.50-15.7 mg/L vs. juice: 0.12-2.63 mg/L) [29] and global patterns favouring epicarp accumulation. This disparity highlights peels' potential for nutraceutical extraction, bypassing juice debittering while capitalizing on naringin's anti-inflammatory, CYP3A4-inhibitory, and bioavailability-enhancing properties-ideal for supplements targeting metabolic disorders.[30-33] The employed methanol maceration and recrystallization yielded pure crystals efficiently. The richness of the agro-climatic stressors (cooler temperatures, high UV at 760-1800 m), upregulating phenylpropanoid pathways as reported in regional studies. Mid-altitude sites (S1-S3) maximized output, suggesting targeted cultivation bioactives in GI-tagged ecotypes. Such superiority positions Himalayan CRB as a premium source, -20 times richer than sea-level equivalents. FTIR, NMR, and MS further corroborated functional groups, glycosylation, and fragmentation (581 m/z), with minimal tailing underscoring method reproducibility.[34-40] The well-defined peaks, very little tailing, and good reproducibility highlight the efficacy of the isolation and purification methods used. Collectively, these results validate a good and efficient procedure for the isolation and qualitative evaluation of naringin from CRB cultivated in the Sikkim and Darjeeling hills.

CONCLUSION

This study is to be reported, for the first time, successful isolation, characterization, and estimation of naringin from CRB grown in the Himalayan region of Darjeeling and Sikkim. Chromatographic and spectroscopic studies established the purity and identity of the isolated compound, while estimation confirmed significantly high contents of naringin, especially in peels over juices. Taken together, concordant data from TLC/HPTLC, UVvis, FTIR, ^{1}H and ^{13}C NMR, MS, and HPLC retention times, all in agreement with authentic standard naringin and published spectral data, provides strong and convergent evidence that the isolated compound is naringin.

The results further indicate that the specific agro-climatic and altitudinal conditions of these areas are responsible for a greater phytochemical content, thus Darjeeling and Sikkim mandarins are a good natural source of naringin. Given the known pharmacological effects and drug interaction potential of naringin, additional research on its bioavailability, pharmacokinetics, and clinical relevance is indicated. Future studies can also investigate the standardization of CRB extracts for nutraceutical and therapeutic uses.

ACKNOWLEDGEMENT

None.

ABBREVIATIONS

CRB: Citrus reticulata Blanco; **TLC:** Thin-Layer Chromatography; **HPTLC:** High-Performance Thin-Layer Chromatography; **HPLC:** High-Performance Liquid Chromatography; **UV:** Ultraviolet; **IR:** Infrared; **NMR:** Nuclear Magnetic Resonance; **MS:** Mass Spectrometry; **CCB:** Calcium Channel Blockers.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORSHIP

Vishala Rani Baraily (VRB), Assistant Professor, Dept. of Pharmaceutical Analysis, Himalayan Pharmacy Institute, East Sikkim, Majhitar, as the first author, contributed to the development of the technique, execution of the experimental data, writing of the original draft, and data analysis. **Dr. Jithendar**

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SUMMARY

The present study focuses on reporting the successful isolation, characterization and quantification of Naringin from *Citrus reticulata* Blanco, particularly from the Himalayan region of Darjeeling and Sikkim. A combination of Spectroscopic methods like UV-vis, FTIR, NMR, and Mass spectrometry, along with Chromatographic techniques like TLC, HPTLC, and HPLC confirmed the isolation of Naringin, confirming its purity and structural similarity with the standard. The unique agro-climatic condition helped to determine the quantity of Naringin in the peel and juice, where the quantity of Naringin was found to be higher in the prior one. These findings revealed the nature of the Himalayan mandarin and were also found to be a rich natural source of naringin and further provided a basis for further pharmacological and nutraceutical research.

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Cite this article: Baraily VR, Shrestha B, Mandhadi JR. Spectrometric and Chromatographic Profiling of Naringin from *Citrus reticulata* Blanco, Native to the Himalayan Region of Darjeeling and Sikkim. Indian J of Pharmaceutical Education and Research. 2026;60(3s):s1170-s1179.