https://doi.org/10.33472/AFJBS.6.6.2024.1937-1948

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Multi-wavelength Densitometric HPTLC Fingerprinting for Identification of BDMC in Hydro-ethanolic Extract of *Curcuma longa* L. Rhizome

Raghvendra Misra[1], Rizwan Ahmad[2], Tirath Kumar[1], Kumud Upadhyaya[1*]

[1]Department of Pharmaceutical Sciences (DOPS), Bhimtal Campus, Kumaun University, Nainital, Uttarakhand-263136, India
[2]Vivek College of Technical Education (VCTE), Bijnor, Uttar Pradesh-246701, India

*Corresponding Author: **Dr. Kumud Upadhyaya**
Department of Pharmaceutical Sciences (DOPS), Bhimtal Campus, Kumaun University, Nainital, Uttarakhand, INDIA, PIN- 263136
Email: upkuupku@gmail.com

Article Info

Volume 6, Issue 6, June 2024

Received: 07 April 2024

Accepted: 12 May 2024

Published: 04 June 2024

doi: 10.33472/AFJBS.6.6.2024.1937-1948

ABSTRACT:

HPTLC fingerprints have attained a vital role towards automated biometric authentication of primary and secondary metabolites in herbal extract. Today, worldwide researchers are in mutual agreement that the biological and medicinal property of *Curcuma longa* Linn are primarily attributed to its curcuminoids (a polyphenolic combination of secondary metabolites i.e. curcumin, demethoxy curcumin, and bisdemethoxycurcumin). This is a study aimed to validate the authenticity of bisdemethoxycurcumin (BDMC) in solvent-evaporated dried extracts of *Curcuma longa* Linn (CE) through HPTLC fingerprints. HPTLC bands were scanned and visualized at shorter (254 nm) to longer (366 nm) wavelengths of UV radiation and in white light. The chromatograms of HPTLC amply demonstrated the presence of BDMC (a bioactive detoxifier and immunomodulator) in the hydroethanolic extract of *Curcuma longa* Linn (CE). This contextual study will emphasize the significance of HPTLC to trace specific metabolites in herbal sample.

Keywords: HPTLC fingerprinting, curcumin, *Curcuma longa* Linn, herbal extract

© 2024 Raghvendra Misra *et al.* This is an open access article under the CC BY license (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

1. Introduction

Chemical identification of herbal materials requires swift, precise and reliable instruments. High performance thin layer chromatography (HPTLC) is gaining popularity among researchers due to its exceptional ability to analyze marker compounds in herbal extract[1,2]. Today, the prime objective of detecting secondary metabolites in crude drugs is to ascertain their therapeutics[3,4]. HPTLC technique uses electronic images of chromatographic fingerprints and densitogram to detect marker compounds in herbal samples[5,6]. Herbal extracts are a type of medicinal extracts prepared via extracting a part of a dried or fresh raw herbal material with specific solvent(s) viz. alcohol, water, acetone etc[7,8,9]. These extracts are frequently utilized in the formulation treating chronic (i.e. stomach ulcers, colitis, chronic constipation) and microbial mediated disorders[10,11]. Mostly, soxhlation is being used as a preeminent extraction procedure for making crude extract from the herbal sample[12, 13]. Herbal extracts obtained from the roots of natural yellow colorant turmeric (*Curcuma longa* L., Zingiberaceae) contains three polyphenolic curcuminoids namely curcumin, demethoxy curcumin (DMC), and bisdemethoxycurcumin (BDMC)[14,15]. From centuries, curcuminoids have mostly been used as vibrant colorant in food industry[16,17]. Nowadays, the world researchers have shown keen interest in curcuminoids for its several pharmacological effects viz. anti-oxidant, anti-inflammatory gastro-protective and antimutagenic activity[18,19,20]. At present, numerous analytical tools are employed for the detection of marker compounds in herbal sample, in which HPTLC has become more popular[21, 22]. The present work was carried out to develop and validate a modest, rapid and specific HPTLC method for the detection of BDMC in hydroethanolic extract of *Curcuma longa* Linn (CE). Finally, the HPTLC chromatographic fingerprints of the test (CE) vs. standard (BDMC) sample were meticulously scanned and analyzed for the qualitative assessment of the aforementioned curcuminoid.

2. Materials and Methods

2.1 Chemicals and Instruments

Dried rhizomes of *Curcuma longa* L., were procured from local market of Bijnor, U.P., India. Bisdemethoxycurcumin (BDMC, purity ≥98.0%) was used as standard drug for the analytical purpose. Methanol (purity ≥99.9%), Ethanol (95% v/v, purity ≥99.8%), Ethyl acetate (HPLC grade and purity ≥99.7%), Acetone (HPLC grade and purity ≥99.5%), Toluene (HPLC grade), Ultra-pure water (HPLC grade and 99.9% of purity) and Trifluoracetic acid (TFA) were obtained from Fischer Chemical. All other chemical utilized were AR-grade and used without further purification. Soxhlet apparatus (Singhla Scientific Industries), Hand grinder (Kalsi), HPTLC analytical instrument (CAMAG®, Muttenz, Switzerland), spotting device (Automated Linomat V), Micro syringe (Hamilton Bonaduz AG, Switzerland), (20 × 10 × 4 cm) Twin -Trough TLC chamber (CAMAG®, Muttenz, Switzerland), Ultraviolet-Visible (UV-Vis) thin layer chromatographic scanner-III (CAMAG®, Muttenz, Switzerland) with CAMAG®winCATS software.

2.2 Authentication of Herbal Material

Crude drug materials were identified and authenticated by Dr. Sunita Garg (Emeritus Scientist) and Mr. R.S. Jayasomu (Principal Scientist & Head, RHMD) CSIR-NISCAIR, India (Ref. No.-NISCAIR/RHMD/Consult/2017/3109-58-1; 13/09/2017).

2.3 Preparation of Herbal Extract

Procured herbal raw materials were aptly cleaned by rinsing separately with deionized H_2O. These materials were air dried under the shade for 7 days and finally in a hot air oven at 40°C for six hours[23]. Dried material was cut down into small pieces and milled by a hand

grinder[24] (Kalsi). The resultant powdered material was uniformly sized via a 40-mesh sieve[25]. Initially, 100 g of each air-dried powdered material was defatted with petroleum ether (500 ml, 60-80^{0}C, 4-6 hours) via Soxhlet extractor[26]. Defatting was continued until a drop of the solvent from the siphon tube evaporated without leaving a greasy spot[27,28]. After defatting the cake was removed from the extraction tube and spread evenly on a clean paper surface to air dry, allowing the petroleum ether solvent to evaporate. A 50 g of air-dried powdered defatted material was successively fractionated with different solvents in a Soxhlet apparatus[29,30]. Solvents were selected on the basis of their polarity value. During the soxhlation process, 500 ml of desired solvents were sequentially used from low polarity to high polarity. Each extraction process for specific crude extract with different solvents was run for approximately six hours. Each time before extracting with the next solvent, the powdered herbal material was dried in hot air oven below 40^{0}C. Each batch was extracted until the siphon tube of Soxhlet apparatus did not leave residue or solution in siphon tubes and showed complete clear solvent. On the complete extraction, the filtrate (solvent+ extract) was removed from boiling flask (i.e. distillation flask) and filtered with Whatman filter paper no.1 (125 mm). After that, each crude extract was concentrated by distilling off the menstruum using rotary vacuum evaporator. The extract was further dried below 40^{0}C for the complete evaporation of solvent[31,32].

Figure 1. Hydroethanolic extract of *Curcuma longa* L. (Dried and powdered)

Thereafter, the dried extracts *(**Figure 1**)* were kept separately in sterile amber colored glass container under refrigerated conditions below 8^{0}C for further use[33].

2.4 HPTLC fingerprint profiling of herbal extract

Before formulating any herbal product, it is essential to check the authenticity of the herbal components used. In this context, HPTLC fingerprinting is one of the important techniques for the qualitative analysis of herbal materials. HPTLC fingerprinting analysis for the hydroethanolic extract of *Curcuma longa* L., was performed as per Wagner and Baldt[34]. The test solution of hydroethanolic extract (CE) was prepared by dissolving 100 mg of each sample extract separately in 1 ml of HPTLC graded ethanol and centrifuged for five minutes at 3000 rpm. HPTLC development system (CAMAG®, Muttenz, Switzerland) was composed of Linomat-V automatic sample applicator as spotting device with micro syringe of Hamilton Bonaduz AG, Switzerland (100 µl), a glass pre-saturated CAMAG® automated twin trough TLC chamber (20 × 10 × 4 cm), CAMAG® Ultra violet-visible TLC Scanner-III, and CAMAG®winCATS V4.06 software. The standard and test solutions were spotted on the silica gel- coated fluorescent HPTLC plates (size: 20 × 10 cm; thickness: 0.2 mm; E. Merck, Darmstadt, Germany) in the forms of 6 mm band using a CAMAG® 100 µl micro syringe. For pre-derivatization of fluorescent HPTLC plate, the plate was exposed to iodine vapor for ten minutes. A continual application rate of 2.0 µl/s was applied and the space between two bands was 8 mm (band length). The slit dimension was fixed at 6.00 mm×0.30 mm and scanning speed was kept at 100 nm/s (spot spectrum scanning) and 20 mm/s (sample track screening). The eluent consisted of toluene: ethyl acetate: formic acid (7:3:0.5) was used for

each chromatographic run. Linear ascending development was carried out in a glass pre-saturated CAMAG® automatic twin-trough TLC chamber (20 × 10 × 4 cm) pre-saturated with the eluent (solvent phase). The optimized chamber saturation time for the above eluent was 20 minutes at controlled room temperature (25^0C±3) and relative humidity (60%±5). TLC plates were developed in the development chamber up to 8.0 cm and air dried using hot air oven. The densitometric scanning was done via CAMAG® TLC scanner-III in the reflectance absorbance mode. These plates were mainly visualized in a UV cabinet using dual wavelengths (i.e. 254 nm and 366 nm) for the chromatographic documentation. The entire process was performed under CAMAG®winCATS V4.06 software (CAMAG®, Muttenz, Switzerland).

2.5 Measurement of retention factor (Rf) value

Illumination of HPTLC plates were observed in the white light and with UV light of short and long wavelengths (λ, 254 & 366 nm) using CAMAG®TLC visualizer. The distance travelled via the analyte (b) and distance travelled via the eluent (a) were recorded (as indicated in ***Figure 2***) after every observation using HPTLC chromatogram[35,36]. After that Rf value was measured using the formula:

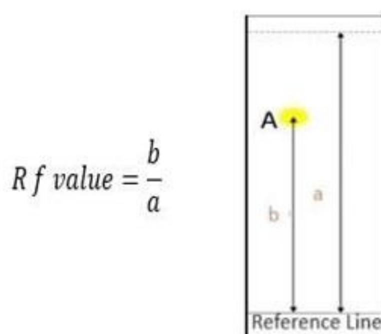

Figure 2. Migration distance of analyte (b) and eluent (a)

The HPTLC plates were scanned at 200, 250, 300, 350, and 400 nm of wavelengths for which the results were recorded (***Figures 4 to 15***).

3. Results and Discussion

A number of specific solvents and solvent systems in various configurations were tried for the chromatographic run. Eluent comprising toluene: ethyl acetate: formic acid (7:3:0.5) was found to be best for better resolution of BDMC with Rf value of 0.13 **(*Figure 16*).** 80 mm solvent front and 10 ml volume was applied to HPTLC plates that helps in recognizing individual component within the sample. Glass HPTLC Silica gel 60 F_{254} plates were placed in photo documentation chamber of CAMAG® TLC Scanner III and different chromatographic images were captured at different scanning wavelengths. The different Rf values at various fingerprint region were calculated and recorded by CAMAG®winCATS V4.06 software. Solvent front linearity was measured by least square linear regression analysis of the calibration spot. BDMC exhibited good linear response in conc. range of 50-500 ng/spot with significant correlation coefficient ≥0.997 ($p<0.05$). X-coordinate (abscissa, horizontal line) and Y-coordinate (ordinate, vertical line) in HPTLC chromatogram represents average peak area in arbitrary unit (AU) and Rf values respectively **(*Figure 3*).** The HPTLC chromatograms of hydroethanolic CE showed symmetrical shaped peaks over a stable horizontal baseline (abscissa).

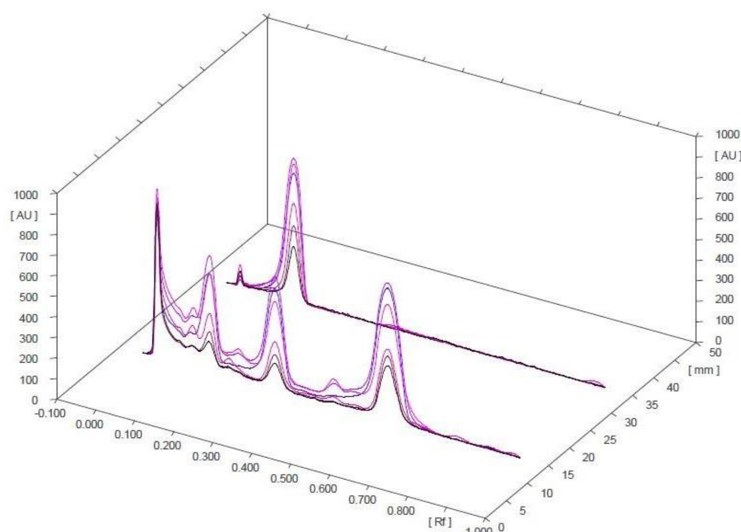

Figure 3. Abscissa and ordinate in HPTLC chromatogram of CE at different scanning wavelengths

At different scanning wavelengths, the peaks were well separated from one another, and there were no overlaps or bands reported **(*Figure 3*).** The BDMC peak in hydroethanolic extract of *Curcuma longa* L., was identified by comparing the spot with that of standard BDMC by measuring the Rf value. The retention factor (Rf value) of BDMC in hydroethanolic CE and standard BDMC were found to be similar (0.13) when HPTLC plates were visualized via the CAMAG®TLC visualizer at a shorter (254 nm) to a longer (366 nm) wavelength of UV and in white light **(*Figure 16*).** Furthermore, the graphic reflection and fine recording of the entire three-D overlay of HPTLC chromatogram of all tracks at numeral wavelengths make the process more suitable for swift and precision-based analysis of several components present in the commercial sample of *Curcuma longa* Linn.

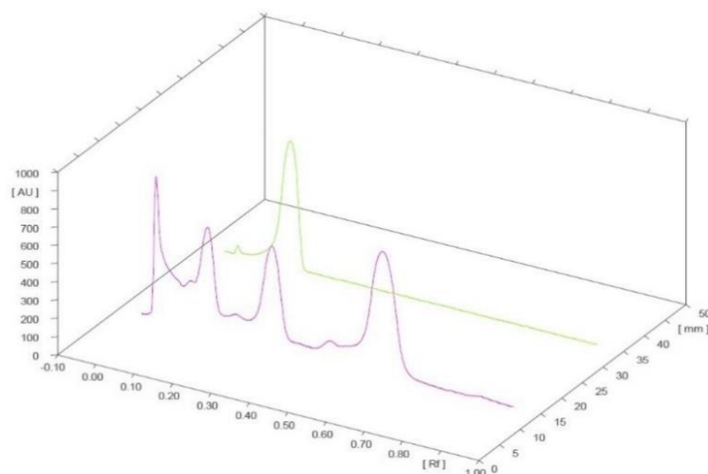

Figure 4. Three-D overlay of HPTLC chromatogram of all tracks at 200 nm of scanning wavelength [CE sample (Test) vs. BDMC (Standard)]

Track 1, ID: CE Sample [Test]

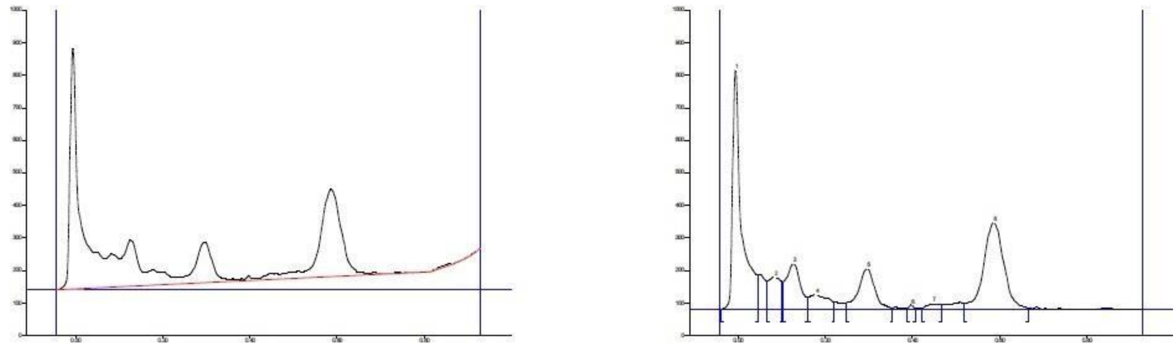

Figure 5. HPTLC chromatogram of hydroethanolic CE (Test) at 200 nm of scanning wavelength

Track 2, ID: Bisdemethoxycurcumin (BDMC) [std., curcuminoid]

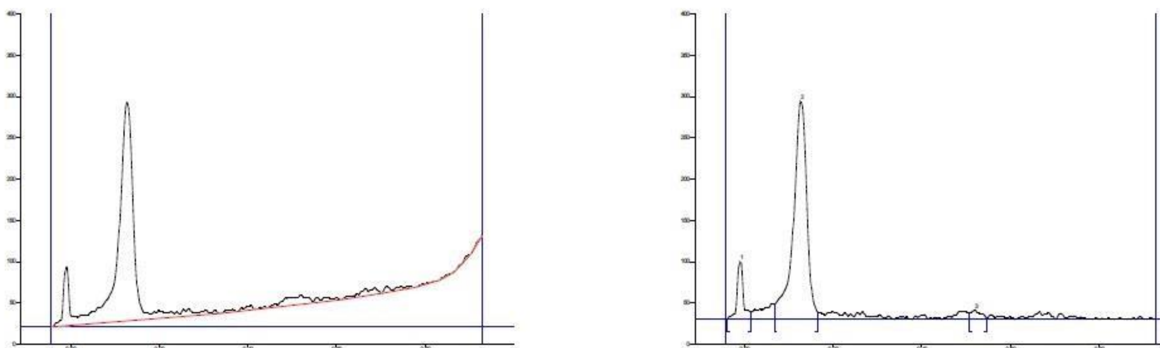

Figure 6. HPTLC chromatogram of BDMC (Standard) at 200 nm of scanning wavelength

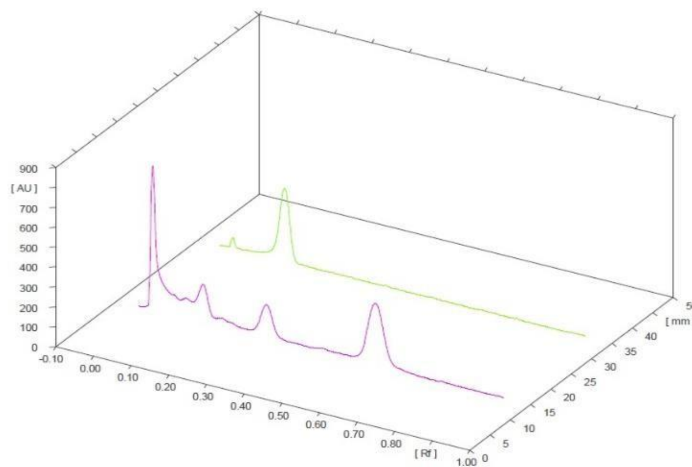

Figure 7. Three-D overlay of HPTLC chromatogram of all tracks at 250 nm of scanning wavelength [CE sample (Test) vs. BDMC (Standard)]

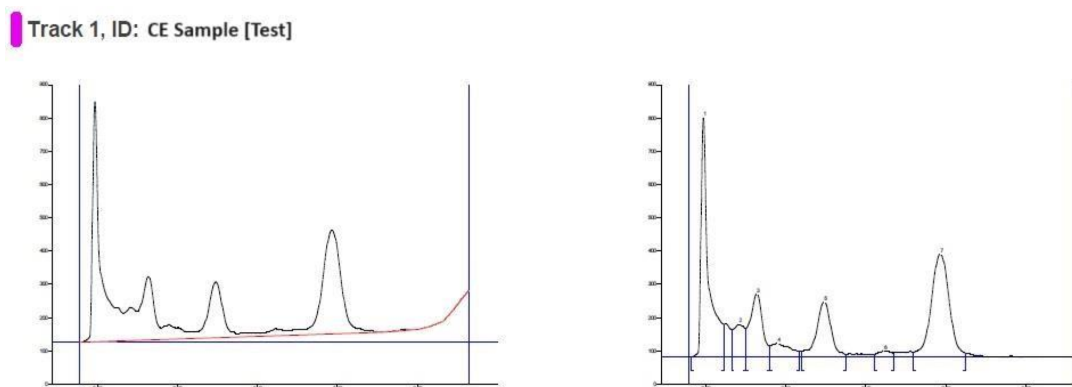

Figure 8. HPTLC chromatogram of hydroethanolic CE (Test) at 250 nm of scanning wavelength

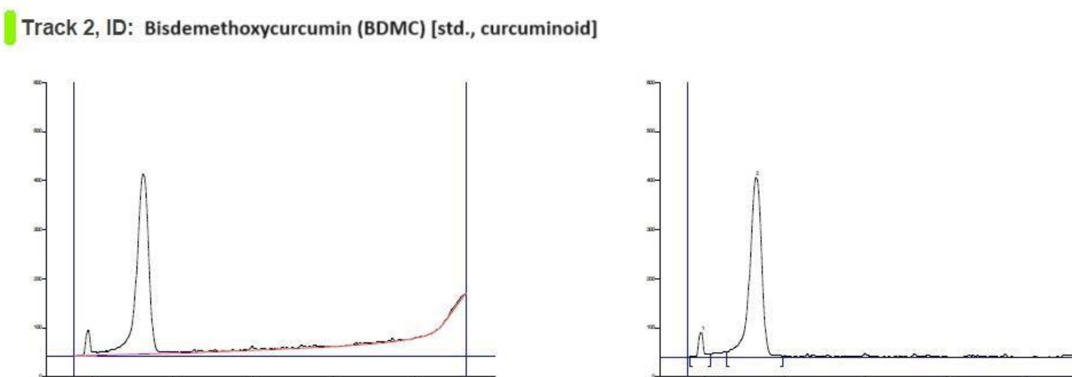

Figure 9. HPTLC chromatogram of BDMC (Standard) at 250 nm of scanning wavelength

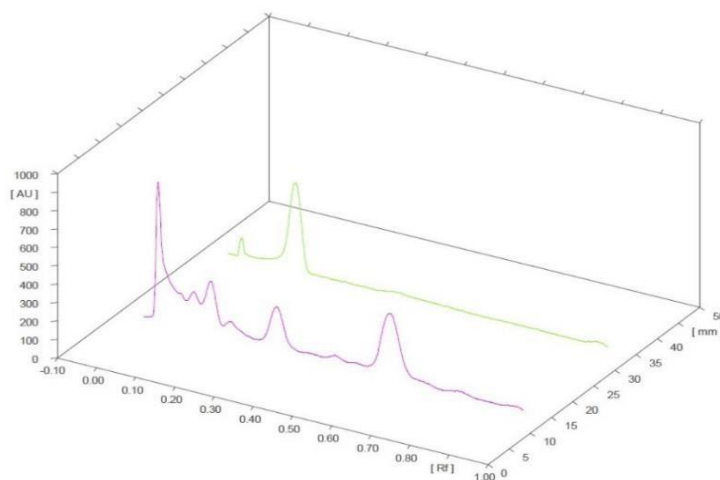

Figure 10. Three-D overlay of HPTLC chromatogram of all tracks at 300 nm of scanning wavelength [CE sample (Test) vs. BDMC (Standard)]

Track 1, ID: CE Sample [Test]

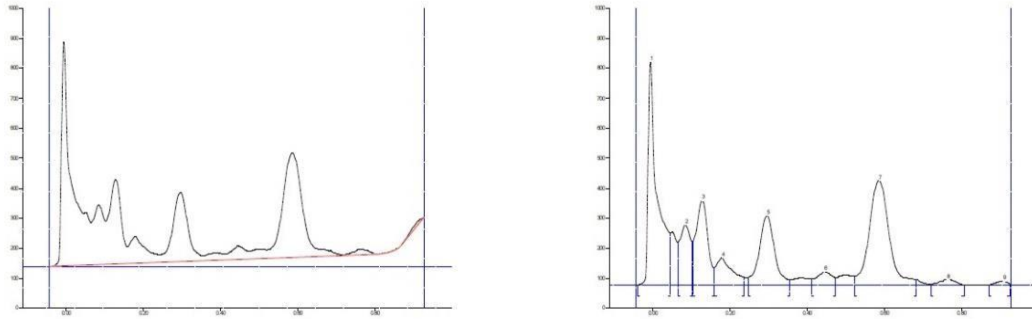

Figure 11. HPTLC chromatogram of hydroethanolic CE (Test) at 300 nm of scanning wavelength

Track 2, ID: Bisdemethoxycurcumin (BDMC) [std., curcuminoid]

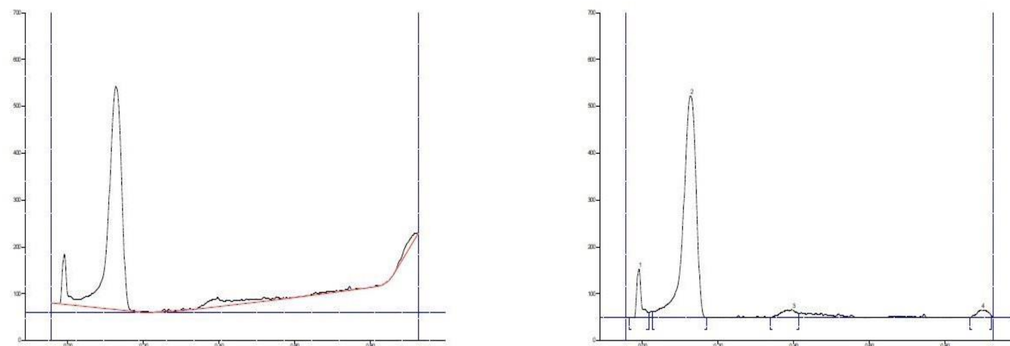

Figure 12. HPTLC chromatogram of BDMC (Standard) at 300 nm of scanning wavelength

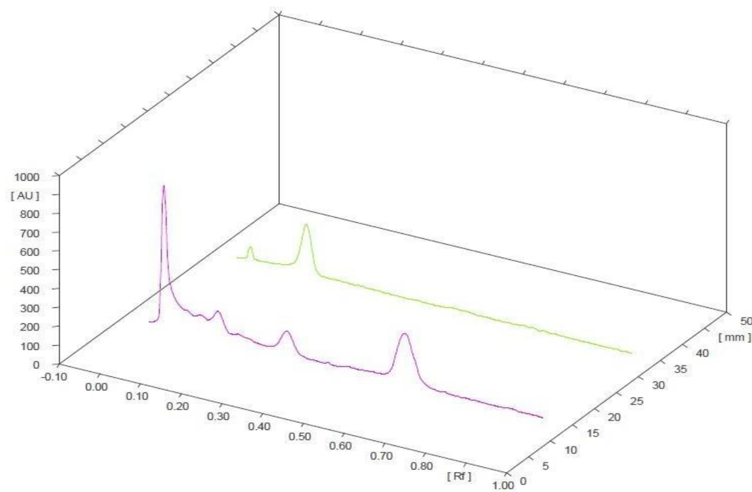

Figure 13. Three-D overlay of HPTLC chromatogram of all tracks at 400 nm of scanning wavelength [CE sample (Test) vs. BDMC (Standard)]

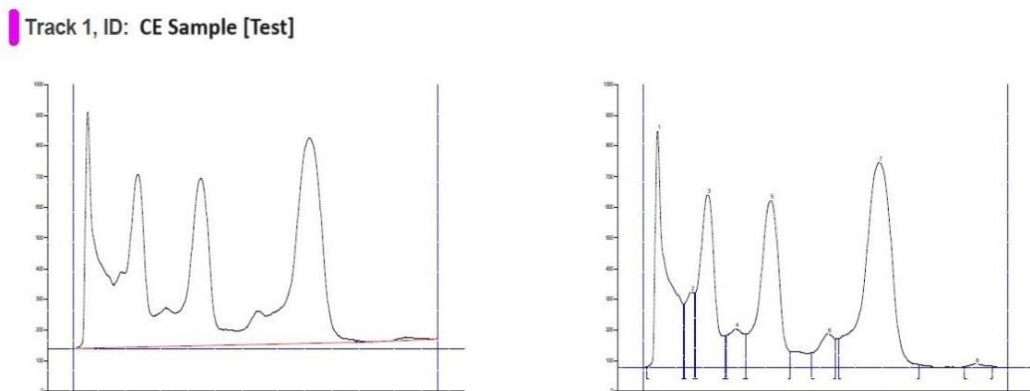

Figure 14. HPTLC chromatogram of hydroethanolic CE (Test) at 400 nm of scanning wavelength

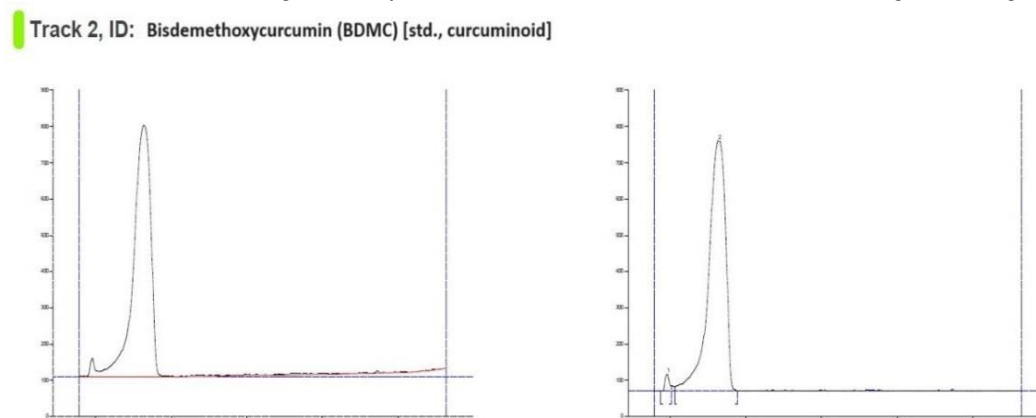

Figure 15. HPTLC chromatogram of BDMC (Standard) at 400 nm of scanning wavelength

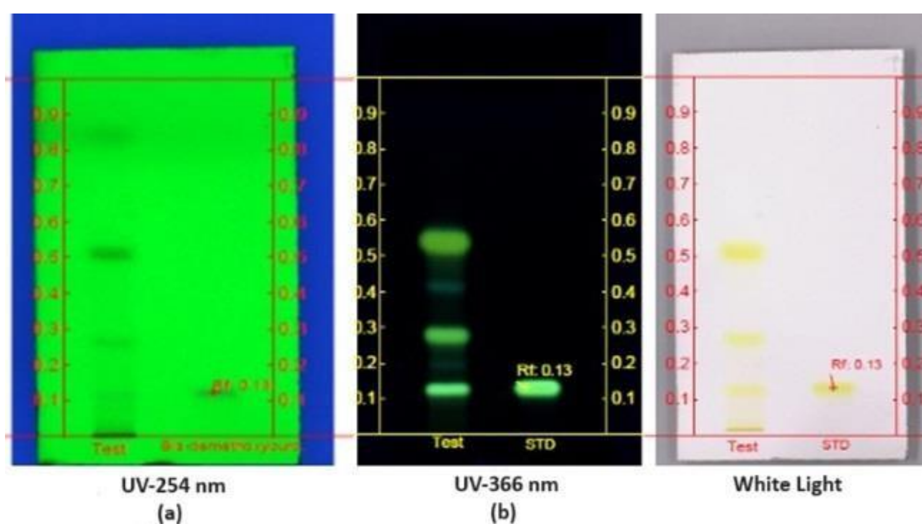

Figure 16. HPTLC Chromatograms of hydroethanolic CE (Test) vs. BDMC (Standard) as observed in UV-254 nm, UV-366 nm and white light
(Illumination Instrument- CAMAG TLC Visualizer, Digital Camera-DXA252, Computar, 16 mm, f4.0)

4. Conclusion

The above developed HPTLC method for determining BDMC in ethanolic CE is modest, economical, and highly sensitive. Statistical analysis has also concluded that the method is very precise and reproducible for the identification of BDMC in the sample. This methodology may be employed for routine evaluation of specific curcuminoid components in pharmaceutical and dietary supplement formulations.

5. Acknowledgments

The authors are very thankful to all the faculty members, staff, and researchers of the Department of Pharmaceutical Sciences (DOPS), Kumaun University, the Indian Pharmacopoeia Commission and the National Botanical Research Institute (NBRI) for their assistance and providing the requisite facilities related to our research work.

6. Conflicts of Interest

No conflict of interest.

7. References

1. Attimarad, M., Ahmed, K.K., Aldhubaib, B.E. and Harsha, S. (2011). High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. *Pharm Methods;*2(2), 71-5.
2. Twaij, B.M. and Hasan, M.N. (2022). Bioactive secondary metabolites from plant sources: Types, synthesis, and their therapeutic uses. *Int. J. of Plant Biology;*13(1), 4-14.
3. Newman, D. J. and Cragg, G.M. (2007). Natural products as sources of new drugs over the last 25 years. *J. of Natural Products;* 70 (3),461–477.
4. Hussein, R. A. and El-Anssary, A.A. (2019). Plants secondary metabolites: The key drivers of the pharmacological actions of medicinal plants. *Intech Open.* doi: 10.5772/intechopen.76139.
5. Cobzac, S.C.A., Olah, N.K. and Casoni, D. (2021). Application of HPTLC multiwavelength imaging and color scale fingerprinting approach combined with multivariate chemometric methods for medicinal plant clustering according to their species. *Molecules ;*26(23), 7225.
6. Mauji, R., Abdin M.Z., Khan M.A. and Jha P. (2011). HPTLC fingerprint analysis: A quality control of authentication of herbal phytochemicals. *Verlag Berlin Heidelberg: Springer*; 1-397.
7. Abubakar, A.R. and Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *J. Pharm. Bio allied. Sciences*; 12(1), 1-10.
8. Zhang, Q.W., Lin, L.G. and Ye, W.C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chin. Med. ;*13(20), 2-26.
9. Sasidharan, S., Chen, Y., Saravanan, D., Sundram K.M. and Yoga, Latha L. (2011).Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr. J. Tradit. Complement Altern Med.*; 8(1): 1-10.
10. Awaad, A.S., El-Meligy, R.M. and Soliman, G.A. (2013). Natural products in treatment of ulcerative colitis and peptic ulcer. *J. Saudi. Chem.* ;17, 101–124.
11. Khanna, V., Poddar, U., and Yachha, S.K. (2010). Etiology and clinical spectrum of constipation in Indian children. *Indian pediatrics;* 47, 1025-1030.

12. Gopalasatheeskumar, K. (2018). Significant role of Soxhlet extraction process in phytochemical research. *Mintage Journal of Pharmaceutical & Medical Sciences*; 7 (1), 43-47.
13. De Castro, M.L. and Garcıa-Ayuso, L.E. (1998). Soxhlet extraction of solid materials: An outdated technique with a promising innovative future. *Anal. Chim. Acta.*; *369*, 1–10.
14. Kulyal, P., Kuchibhatla, L.N., Maheshwari, K.U., Babu, K.N., Tetali, S.D., and Raghavendra, A.S. (2016). Highly sensitive HPLC method for estimation of total or individual curcuminoids in curcuma cultivars and commercial turmeric powders. *Current science;*111. 1816-1824.
15. Sanghvi, K., Chandrasekher, K.S., Pai, V. and N Aswatha, R.H. (2020). Review on *Curcuma longa*: Ethnomedicinal uses, pharmacological activity and phytochemical constituents. *Res. J. of Pharm. and Technology; 13(8), 3983-3986.*
16. Aberoumand, A. (2011). A review article on edible pigments properties and sources as natural bio colorants in foodstuff and food industry. *World. J. of Dairy and Food Sciences;* 6 (1), 71-78.
17. Abdeldaiem, M. H. (2013). Use of yellow pigment extracted from turmeric (*Curcuma longa*) rhizomes powder as natural food preservative and colorant. *Food Sci. and Quality Management*; 22, 56-68.
18. Ravindran, P.N., Babu, K.N. and Sivaraman, K. (2009). The golden spice of life: Turmeric the genus *Curcuma* India. *CRC Press*; 1-13.
19. Yadav, S.K., Sah, A.K., Jha, R.K., Sah, P. and Shah, D.K. (2013). Turmeric (Curcumin) remedies gastroprotective action. *Pharmacognosy Reviews*; 7(13), 13-46.
20. Yadav, R.P. and Tarun, G. (2016). Versatility of turmeric: A review the golden spice of life. *J. of Pharmacognosy and Phytochemistry*; 6(1), 41-46.
21. Amber, V., Jain, V., Sahu, U., Kumar, N. and Joshi, N. (2023). Development of herbal drugs and its validation: An overview. *Research J. of Pharmacy and Technology*; 16(8), 2964-2966.
22. Upton, R., David, B., Gafner, S. and Glasl S. (2020). Botanical ingredient identification and quality assessment: Strengths and limitations of analytical techniques. *Phytochem. Rev.;* 19(5) :1157-1177.
23. Krakowska-Sieprawska, A., Kiełbasa, A., Rafińska, K., Ligor, M. and Buszewski B. (2022). Modern methods of pre-treatment of plant material for the extraction of bioactive compounds. *Molecules*; 27(3) :730.
24. Balekundri, A., Mannur, V. (2020). Quality control of the traditional herbs and herbal products: A review. *Futur. J. Pharm. Sciences*; 6, 67.
25. Merkus, H.G. (2009). Sieves and Sieving. In: Particle size measurements. *Particle technology series*, vol 17, *Springer, Dordrecht*.
26. De Castro, M.L., Garcıa-Ayuso, L.E. (1998). Soxhlet extraction of solid materials: An outdated technique with a promising innovative future. *Anal. Chim. Acta*; *369*, 1–10.
27. Waqas, M., Sharma, P.K., Asif, M., Yadav, S. and Chandra kala. (2021). Physicochemical, phytochemical and toxicological study of hydroalcoholic extract of *Aerva javanica* Roots. *Asian Plant Research Journal*; 8(2), 35-40.
28. Kokate, C.K. (2005). Practical pharmacognosy. *Vallabh Prakashan;*107–111.
29. Khandelwal, KR (2003). Practical pharmacognosy techniques and experiments. *Nirali Prakashan*.
30. Harborne, J.B. (1984). Phytochemical methods: A guide to modern techniques of plant analysis. 2nd ed. London: *Chapman and Hall* ;4-16.

31. Kulkarni, S.J., Maske, K.N., Budre, M.P. and Mahajan, R.P. (2012). Extraction and purification of curcuminoids from turmeric (*Curcuma longa* L.). *Int. J. of Pharmacology and Pharmaceutical Technology*; 1(2), 81-85.
32. Trease, G. and Evans, S.M. (2002). Pharmacognosy. 15th ed. London: *Bailer Tindal*, 23-67.
33. Bhalodia, N.R. and Shukla V.J. (2011). Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant. *J. Adv. Pharm. Technol. Res.;* 2(2) :104-9.
34. Wagner, H. and Baldt, S. (1996). Plant drug analysis. A thin layer chromatography atlas, *Springer*, Berlin, Germany, 2nd ed.
35. Kumar, S., Jyotirmayee, K., and Sarangi, M. (2012). Thin layer chromatography: A tool of biotechnology for isolation of bioactive compounds from medicinal plants. *Int. J. Pharm. Sci. Rev. Res.* ;18(1), 126-132.
36. Chatwal, G.R. and Anand, S.K. (2008). Instrumental methods of chemical analysis. *Himalaya publishing house*; 5th ed., 2.599-2.616.