Indian Journal of Research in Homoeopathy

Volume 15 | Issue 3 Article 2

29-9-2021

High-performance thin-layer chromatography fingerprinting and antioxidant study of Mangifera indica mother tincture

Binit Kumar Dwivedi
Dr D P Rastogi Central Research Institute of Homoeopathy, Noida, Uttar Pradesh, India, dbinit@gmail.com

Manoj Kumar
Dr D P Rastogi Central Research Institute of Homoeopathy, Noida, Uttar Pradesh, India, manoj12370@gmail.com

Rakhi Mishra
Dr D P Rastogi Central Research Institute of Homoeopathy, Noida, Uttar Pradesh, India, rakhimishra020@gmail.com

Follow this and additional works at: https://ijrh.researchcommons.org/journal

 Part of the Alternative and Complementary Medicine Commons

Recommended Citation

Dwivedi BK, Kumar M, Mishra R. High-performance thin-layer chromatography fingerprinting and antioxidant study of Mangifera indica mother tincture. Indian J Res Homoeopathy [Internet]. 2021 Sep 29; 15(3):Article 2. Available from: https://ijrh.researchcommons.org/journal/vol15/iss3/2. Free full text article.

This Original Article is brought to you for free and open access by Indian Journal of Research in Homoeopathy. It has been accepted for inclusion in Indian Journal of Research in Homoeopathy by an authorized editor of Indian Journal of Research in Homoeopathy.

High-performance thin-layer chromatography fingerprinting and antioxidant study of Mangifera indica mother tincture

Abstract

Background Mangifera indica is widely used in the Homoeopathy systems of medicine. In Homoeopathy, Mang. ind. is one of the indicated medicines for passive haemorrhages, uterine, renal and gastric problems. Mangiferin is a xanthanoid present in leaves, stalks barks, peel and kernel of the mango plant. Mangiferin has antioxidant, antiviral, anticancer, antidiabetic, antiaging, immunomodulatory, hepatoprotective and analgesic properties. Therefore in the current study, we have tried to identify mangiferin and its antioxidant properties in Mang. ind. homoeopathic mother tincture. Objective The aim of the present study wais to identify Mangiferin, Total Phenol Content (TPC) and Total Flavonoid Content (TFC) in Mang. ind. homoeopathic mother tincture. Materials and Methods Mangiferin (A) was used as a primary reference standard and in sample In-house prepared mother tincture (B) and two different market samples (C and D) were used. An authentic sample of Mang. ind. bark was received from the Centre of Medicinal Plants Research in Homoeopathy, Emerald, Tamil Nadu, India for preparation of in house mother tincture. Results We have successfully identified Mangiferin in Mang. ind. in-house prepared mother tincture (B) and two market samples (C and D) by HPTLC. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) was determined by Ultraviolet–visible spectroscopy. Conclusion High Performance Thin Layer Chromatography study confirms the presence of Mangiferin whereas U. V spectroscopy proves to be a useful tool for in-vitro antioxidant assay study.

Acknowledgments and Source of Funding

N/A

This original article is available in Indian Journal of Research in Homoeopathy: https://ijrh.researchcommons.org/journal/vol15/iss3/2

Original Article

High-performance thin-layer chromatography fingerprinting and antioxidant study of *Mangifera indica* mother tincture

Binit Kumar Dwivedi*, Manoj Kumar, Rakhi Mishra
Dr. D P Rastogi Central Institute of Homoeopathy, Noida, Uttar Pradesh, India

Abstract

Background: In homoeopathy, *Mangifera indica* is one of the indicated medicines for passive hemorrhages, uterine, renal, and gastric problems. Mangiferin is a xanthanoid present in leaves, stalks, barks, peel, and kernel of the mango plant. Mangiferin has antioxidant, antiviral, anticancer, antidiabetic, anti-aging, immunomodulatory, hepatoprotective, and analgesic properties. Therefore, in the current study, we have tried to identify mangiferin and its antioxidant properties in *Mang. ind.* homoeopathic mother tincture. **Objective:** The aim of the present study was to identify mangiferin, total phenol content (TPC), and total flavonoid content (TFC) in *Mang. ind.* homoeopathic mother tincture. **Materials and Methods:** Mangiferin (A) was used as a primary reference standard and in sample in-house prepared mother tincture (B) and two different market samples (C and D) were used. An authentic sample of *Mang. ind.* bark was received from the Centre of Medicinal Plants Research in Homoeopathy, Emerald, Tamil Nadu, India, for the preparation of in-house mother tincture. **Results:** We have successfully identified Mangiferin in *Mang. ind.* in-house prepared mother tincture (B) and two market samples (C and D) by high-performance thin-layer chromatography (HPTLC). TPC and TFC were determined by ultraviolet–visible spectroscopy. **Conclusion:** HPTLC study confirms the presence of mangiferin, whereas U. V spectroscopy proves to be a useful tool for *in vitro* antioxidant assay study.

Keywords: High-performance thin-layer chromatography, Homoeopathy, *Mangifera indica*, Physicochemical, Phytochemical, Ultraviolet spectroscopy

Introduction

Mangifera indica L. belonging to the family Anacardiaceae[1] is one of the important tropical fruits with nutritional and medicinal value.[2,3] It is cultivated throughout the country.[4] India is exclusively famous for its exotic mango varieties. In India, about 1500 varieties of mango grown and each of the main varieties of mango have a unique taste and flavor. In the present study, we used *Mang. ind.* stem bark for the study. Mango trees are evergreen plant which withstands in dry periods very efficiently. It can be used as food supplement.[5] The different parts of Mang. ind. have been utilized for medicinal uses throughout the globe. Mangifera indica is believed to posses diverse pharmacological activities such as antioxidant,[6] antitumor, immunomodulatory, antiHIV,[7] antiviral,[8] inhibit bowel carcinogenesis,[9] antipyretic activity,[10] anticancer,[11] antdiabetic,[12] anthelmintic,[13] antimicrobial,[14] antibacterial,[15] and neuroprotective activity.[16] *Mang. ind.* is one of the indicated medicines for passive hemorrhages, uterine, renal, gastric, pulmonary, and intestinal in homoeopathic literature.[16]

Access this article online

Quick Response Code:

Available in print version only

Website: www.ijrh.org

Chemical profiling of majority of plants refers to the generation of qualitative and quantitative molecular description of the whole extract, that is, mother tincture of plant secondary metabolites to establish plant identity and product quality using chemical analytical method such as high-performance thin-layer chromatography (HPTLC) and ultraviolet (UV)–visible spectroscopy.[17] The fingerprint is a unique pattern which chemically represents a sample, based on detected compounds, and reflects the entire chemical profile of the different constituents within the plant material. Each individual plant has its own fingerprint and much evidence is found for fingerprint profile of *Mang. ind.* homoeopathic mother

***Address for correspondence:** Dr. Binit Kumar Dwivedi, Dr. D P Rastogi Central Research Institute for Homoeopathy, Noida, Uttar Pradesh, India.
E-mail: dbinit@gmail.com

Received: 26 June 2020; **Published:** 29 Sep 2021

is an open access journal, and articles are distributed under the terms of the Creative mons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to ix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

How to cite this article: Dwivedi BK, Kumar M, Mishra R. High-performance thin-layer chromatography fingerprinting and antioxidant study of *Mangifera indica* mother tincture. Indian J Res Homoeopathy 2021;15:166-172.

tincture. HPTLC fingerprint profiles of mother tincture are becoming widely accepted as an effective method to describe the complexity of components present in the plants. Hence, in the present study, we have worked on identification of mangiferin in homoeopathic mother tincture using HPTLC and UV spectroscopy.

Materials and Methods

Collection of plant materials

The stem bark of *Mang. ind.* was collected by the botanist at Centre of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Tamil Nadu, and authenticated by the botanist of CMPRH. Mangiferin ($C_{19}H_{18}O_{11}$), rutin hydrate, and ascorbic acid were purchased from Sigma-Aldrich (India), sodium hydroxide pellets sodium nitrite and aluminum chloride anhydrous powder were purchased from Merck (India). Solvents ethyl acetate, glacial acetic acid, formic acid, chloroform, and methanol were of analytical grade purity (Merck Ltd.), Folin–Ciocalteu's phenol reagent was purchased from M/s Merck Ltd., Germany, and HPLC water from ELGA Lab Water.

Physicochemical study

Stem bark was dried and coarsely powdered and subjected for the determination of loss on drying at 105°C. Total ash, acid-insoluble ash, physicochemical parameters, and UV spectroscopy study were performed by standard methods.

Preparation of mother tincture

To 100 g of coarsely powdered bark, 670 mL strong alcohol and 360 mL water were added to make 1000 mL of mother tincture using percolation method (as per the Homoeopathic Pharmacopeia of India, Volume VII).[18]

Preparation of standard solution

The stock solution was prepared by weighing accurately 10 mg in 10 mL volumetric flask followed by dilution in methanol. The concentration of standard is 1000 µg in 1000 µl.

HPTLC studies

For HPTLC analysis, HPTLC CAMAG Linomat V (Switzerland) with vision CATS software was used as analytical instrument. HPTLC analysis were performed using different solvent systems, and finally, ethyl acetate: glacial acetic acid: formic acid: water (7:1:1:1, v/v/v/v)[19,20] was used for the identification of mangiferin in in-house sample (B) (track 7–9) and market sample (C and D) (track 10–14) with reference standard mangiferin (A) (track 1–6). Twenty-five milliliters of mother tincture were taken in a 50 mL beaker. To remove the ethanol, solution was evaporated on water bath and extracted 3 times with 20 mL chloroform. Chloroform extract was combined and concentrated up to 2 mL volume. HPTLC of chloroform extract of mother tincture and reference standard mangiferin was carried out on silica gel 60 F254 pre-coated plate using mobile phase ethyl acetate: glacial acetic acid: formic acid: water (7:1:1:1,v/v/v/v). Camag Linomat V was used as sample applicator; for the development of mobile phase, a saturating chamber Camag Twin Trough glass chamber (20 × 10) was used. The concentrated chloroform extract was spotted in the form of band width 8.0 mm with CAMAG microliter syringe. Spots were made on silica gel 60 F254 pre-coated plate (Merck) 20 × 10 cm plate with an aid of sampling machine and solvent front was run up to 70 mm height. Densitometric scanning was performed at 254 nm and 366 nm by CAMAG TCL Scanner and VISION CATS software.

UV spectrophotometer

UV analysis was performed on UV Spectrophotometer SPECORD 200 Plus with win aspect software (Analytik Jena, Germany). The samples were prepared with ethanol in the ratio of volume (1:99**,** v/v) (mother tincture: ethanol). Spectrophotometer set at range 190–600 nm, samples, and standard were put in cuvettes. Before analysis, cuvettes were washed with ethanol and analysis was performed on win Aspect software for the UV analysis. Samples (in-house) used for UV analysis were prepared by mixing one part of mother tincture and 99 parts of absolute alcohol (1:99) and filtered through membrane filter before U.V. analysis. Analysis peak was observed at λ max 316 nm and 366 nm [Figure 1].

Antioxidant study

Determination of total phenolic content (TPC)

The TPC of the extracts was determined using the Folin–Ciocalteu's reagent, which is expressed in terms of ascorbic acid equivalent. Ascorbic acid was used as standard compound different concentrations (0.26615–8.517 mM) of ascorbic acid were prepared and analyzed at 742 nm and calibration curve was plotted as absorbance versus concentration. For TPC determination, ascorbic acid was used as standard approximately 50 µl of the mother tincture was mixed with 5 mL 10% Folin–Ciocalteu's (phenol reagent) and 4 mL of sodium carbonate. The mixture was allowed to stand for 1 h in dark. After 1 h, the color changed from yellow to blue. The absorbance of the solutions was measured at λmax of 742 nm using a UV–visible spectrophotometer (UV Spectrophotometer SPECORD 200 Plus Analytik Jena, Germany). Ascorbic acid (0.26615–8.517 mM) was used as standard to prepare a calibration curve from which phenolic content in terms of ascorbic acid equivalent of mother tincture was determined. The TPC was calculated from the calibration curve [Figure 2], and in final results, TPC of the in-house mother tincture and two market sample was calculated

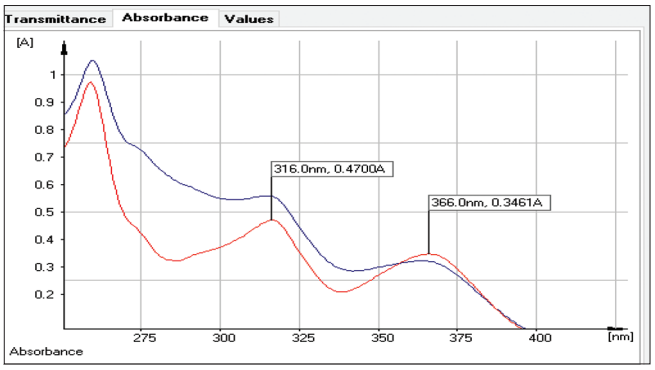

Figure 1: Ultraviolet absorption spectra (λ_{max}) of mother tincture (blue) and reference standard mangiferin (red)

as the ascorbic acid equivalents.[21]

Determination of total flavonoid content (TFC)

TFC was estimated using aluminum chloride colorimetric assay method. Rutin hydrate was used as standard compound different concentrations (15.625–250 µg/mL) of rutin were prepared and analyzed at 508 nm and calibration curve was plotted as absorbance versus concentration. For TFC determination, an aliquot of 1 mL of the mother tincture mixed with 4 mL distilled water add 300 µl (5%) sodium nitrite in it. After 5 min, add 300 µl (10%) aluminum chloride then after 5 min add 2 mL methanol and 2 mL (1M) sodium hydroxide then add 2.4 mL distilled water to make up the volume up to 10 mL. The mixture was shaken vigorously and left to stand in dark at room temperature. The resulting mixture color changes yellow to pink. The absorbance of the reaction mixture was measured at λ_{max} 508 nm with a spectrophotometer. A standard calibration curve was constructed using rutin hydrate standard solutions of 15.625–250 µg/mL of each standard which was treated in the same manner as the samples above to generate calibration curve [Figure 3]. The TFC of each mother tincture in-house and two market samples was determined from the curve and the results recalculated and expressed as the rutin hydrate equivalents.[22]

Results

Physicochemical studies

The determined data under the physicochemical study for the raw drug are summarized in Table 1 and that of mother tincture preparation and its standardization in Tables 2 and 3, respectively.

HPTLC fingerprinting

The results from HPTLC fingerprint for chloroform extract of *Mang. ind.* bark are given in Figures 4-7. The presence of mangiferin was confirmed with R_f value 0.58 in all in-house sample (B) (track 7–9) and market samples (C and D) track (10–14). The phytoconstituents in the chloroform extracts had several visible color spots in TLC plate. The composition of the mobile phase for TLC was optimized by testing different solvent mixtures of varying polarity. The colored spots were visualized under the UV light between 254 and 366 nm with mobile phase ethyl acetate: glacial acetic acid: formic acid: water (7:1:1:1, v/v/v/v) indicates the presence of mangiferin and other phytoconstituents. At U.V white light for track (1–6), one spots appear at R_f 0.58 (reference standard mangiferin A), for in-house sample (B) track (7–9), three spots appear at R_f 0.26, 0.58 (standard mangiferin), and 0.79 (all yellow), and for sample (C) track (10–12), three spots appear R_f 0.27 (light yellow), 0.57 (yellow), and 0.64 (yellow), and for sample (D) track (13–14), two spots appear at R_f 0.58 (yellow) and 0.64 (yellow) [Figure 4]. At UV 254 nm for track (1–6), one spot appears at R_f 0.58 (reference standard mangiferin A), for in-house sample B (7–9), four spots appear at R_f 0.26, 0.58, 0.72,

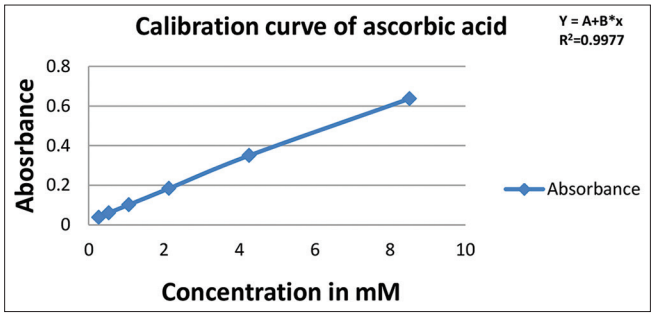

Figure 2: Ascorbic acid calibration curve at λ_{max} 742 nm

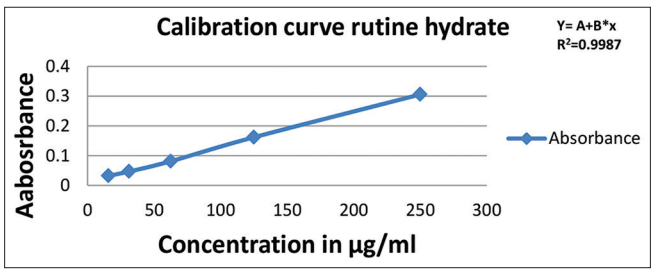

Figure 3: Rutin hydrate calibration curve at λ_{max} 508 nm

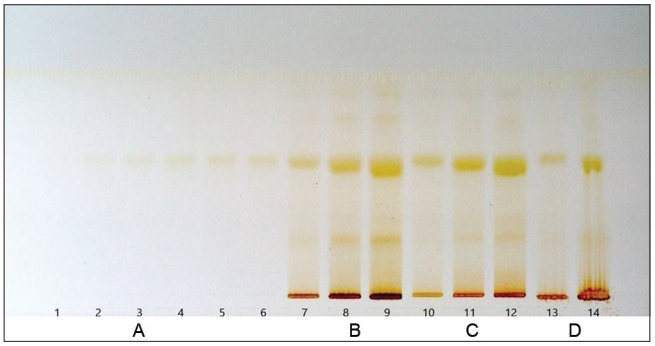

Figure 4: High-performance thin-layer chromatography fingerprints of *Mang. ind.* under white light of standard mangiferin track (1–6), track (7–9) in-house sample B, track (10–12) commercial market sample C, and track (13–14) market sample D

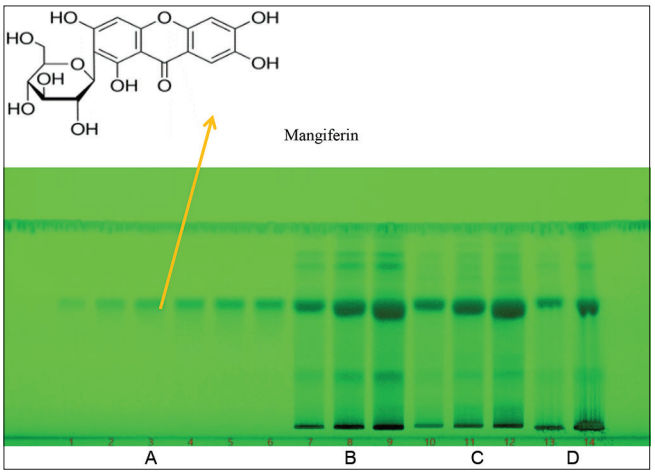

Figure 5: High-performance thin-layer chromatography fingerprints of *Mang. ind.* at UV 254 nm of standard mangiferin track (1–6), track (7–9) in-house sample B, track (10–12) commercial market sample C, and track (13–14) market sample D

Table 1: Standardization of raw drug

Parameters	Quantitative values
Loss on drying at 105°C	Not more than 22.3% w/w
Total ash value	Not more than 6.18% w/w
Acid-insoluble ash	Not more than 0.84% w/w
Alcohol-soluble extractive value	Not less than 14.15% w/w
Water-soluble extractive value	Not less than 16.3% w/w

Table 2: Formulation of mother tincture (percolation technique used)

Drug strength	1/10
Preparation	
Mang ind. coarse powder	100 g
Strong alcohol	670 mL
Purified water	360 mL

Table 3: Standardization of mother tincture

Parameters	Observations
Organoleptic properties	
Appearance	Clear liquid
Color	Brownish color
Odor	Characteristic
Physicochemical tests	
Sediments	Nil
Wt. per mL	0.9191 g
Total solid	1.09% w/v
Alcohol content	64.6% v/v
pH	6.00

and 0.79 (all brown), for sample (C) track (10–12), four spots appear at R_f 0.27, 0.57, 0.64, and 0.72 (all brown), and for sample D track (13–14), two spots appear at R_f 0.59 and 0.76 (all brown) [Figure 5]. While at UV 366 nm for track (1–6), one spot appears at R_f 0.58 (reference standard mangiferin), for in-house sample (B) track (7–9), four spots appear at R_f 0.24 (yellow), 0.58 (yellow), 0.67 (blue), and 0.77 (yellow), for sample (C) track (10–12), four spots appear at R_f 0.24 (yellow), 0.57 (yellow), 0.68 (blue), and 0.78 (yellow), and for sample (D) track (13–14), five spots appear at R_f 0.22 (light blue), 0.27 (light yellow), 0.58 (yellow), 0.67 (blue), and 0.76 (yellow) [Figure 6]. Spectral studies revealed that the peaks obtained from both the standard and for sample were identical [Figures 1 and 7]. Isometric chromatogram of *Mang. ind.* reference standard mangiferin (A), in-house sample (B), and market sample (C) and (D) [Figure 7] is vital fingerprint parameters to ensure the reliability and reproducibility of the drug and considered valuable standards in pharmacopoeia.

Antioxidant study

After optimization of the conditions for the spectrophotometric determination of phenolic using the Folin–Ciocalteu reagent, TFC using aluminum chloride colorimetric assay method, all parameters analyzed showed adequate results [Tables 4 and 5]

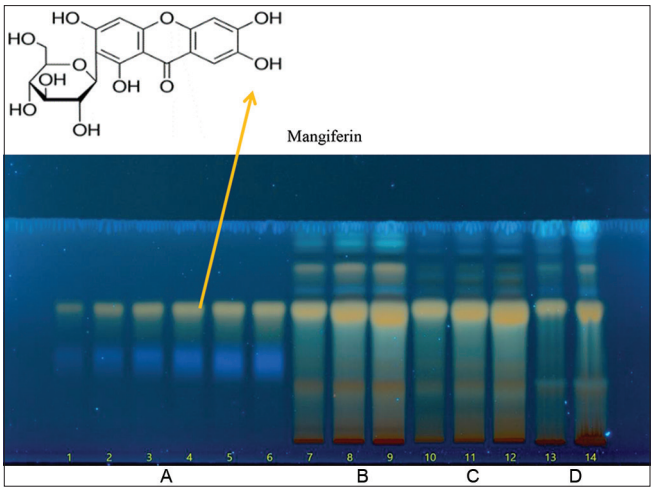

Figure 6: High-performance thin-layer chromatography fingerprints of *Mang. ind.* at UV 366 nm of standard mangiferin track (1–6), track (7–9) in-house sample B, track (10–12) commercial market sample C, and track (13–14) market sample D

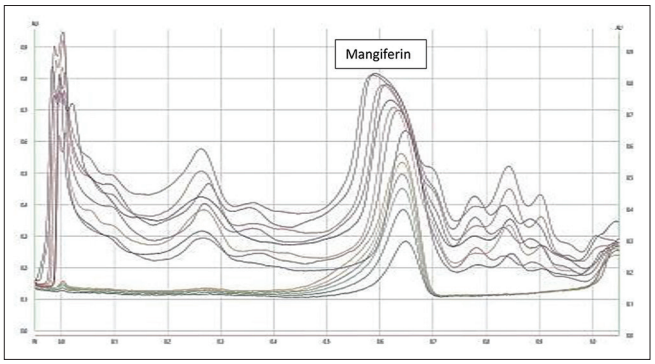

Figure 7: High-performance thin-layer chromatography isometric chromatogram of standard mangiferin (A), in-house sample (B), and commercial market samples C and D of *Mang. ind.* bark chloroform extracts

[Figures 2 and 3]. The UV–Vis spectrophotometric method described here was successfully validated as suitable for the determination of TPC and TFC of *Mang. ind.* This methodology using the Folin–Ciocalteu and aluminum chloride colorimetric assay reaction complies with the requirements for analytical use and for ensuring the reliability of the results.

Discussion

The results obtained from the study could be utilized for scientific validation and formulating standards for the quality assurance of the drug. Based on extensive literature reviews, various combinations of solvent systems were studied with an aim to have an appropriate mobile phase composition for best and efficient HPTLC chromatographic separation of mangiferin in *Mang. ind.* chloroform extract but no appropriate resolution of band observed, whereas in mobile phase ethyl acetate: glacial acetic acid: formic acid: water (7:1:1:1, v/v/v/v), efficient band resolution of mangiferin observed with improved R_f value of 0.58. Thus, it was finalized the best appropriate

Table 4: Quantity of total phenolic content (ascorbic acid equivalents) in in-house and market samples

S. No.	Sample	Concentration in mM	Absorbance
1.	In-house sample	40.47	2.9728
2.	Market sample C	32.71	2.4078
3.	Market sample D	42.52	3.1225

Table 5: Quantity of total flavonoid content (rutin hydrate equivalents) in in-house and market samples

S. No.	Sample	Concentration in μg/mL	Absorbance
1.	In-house sample	2159.6	2.5604
2.	Market sample C	1818.81	2.1580
3.	Market sample D	2498.52	2.9603

mobile phase composition for entire HPTLC qualitative study. UV spectroscopic study exhibits, prominent peaks, which serve as characteristic standards. The present study reveals that as part of pre-formulation study, the mother tincture of stem bark of *Mang. ind.* showed promising physicochemical characteristics. The study suggests the chloroform extract of *Mang. ind.* of xanthanoid (mangiferin) medicinal importance that justifies its medicinal usage in homoeopathy. This is the reason for cure and healing property of stem bark of *Mang. ind.* mother tincture.

Conclusion

HPTLC fingerprinting analysis confirms the presence of mangiferin, whereas UV spectroscopic analysis plays an important role for the determination of TPC and TFC in *Mang. ind.* in-house prepared homoeopathic mother tincture and two market samples.

Financial support and sponsorship

Nil.

Conflicts of interest

None declared.

References

1. Parvez GM. Pharmacological activities of mango (*Mangifera indica*): A review. J Pharmacogn Phytochem 2016;5:1-7.
2. Luo F, Lv Q, Zhao Y, Hu G, Huang G, Zhang J, *et al*. Quantification and purification of mangiferin from Chinese mango (*Mangifera indica* L.) cultivars and its protective effect on human umbilical vein endothelial cells under H_2O_2-induced Stress. Int J Mol Sci 2012;13:11260-74.
3. Padh H, Parmar S, Patel B. Stability indicating HPTLC method for estimation of mangiferin in bulk and dosage form. Int J Pharm Biol Sci 2017;7:71-7.
4. Government of India, Ministry of Health and Family Welfare. Department of ISM and H. The Ayurvedic Pharmacopoeia of India. Vol. 3. New Delhi: Government of India, Ministry of Health and Family Welfare; 2001. p. 9-10.
5. Laulloo SJ, Bhowon MG, Soyfoo S, Chua LS. Nutritional and biological evaluation of leaves of *Mangifera indica* from Mauritius. J Chem 2018;2018:1-9.
6. Guha S, Ghosal S, Chattopadhyay U. Antitumor, immuno modulatory and anti-HIV effect of mangiferin, a naturally occurring glucosyl xanthone. Chemotherapy 1996;42:443-51.
7. Zheng MS, Lu ZY. Antiviral effect of mangiferin and isomangiferin on herpes simplex virus. Chin Med J 1990;103:160-5.
8. Yoshimi N, Matsunaga K, Katayama M, Yamada Y, Kumo T, Qiao Z, *et al*. The inhibitory effects of mangiferin, a naturally occurring glycosylxanthone in bowel careinogenesis of male F344 rats. Cancer Lett 2001;163:163-70.
9. Singh SK, Sinha KS, Prasad SK, Kumar R, Bithu BS, Kumar SS, *et al*. Synthesis and evaluation of novel analogues of mangiferin as potent antipyretic. Asian Pac J Trop Med 2011;4:866-9.
10. Garrido G, Gonzalez D, Lemus Y, Garcia D, Loderio L, Quintero G, *et al*. *In vivo* and *in vitro* anti-inflammatory activity of *Mangifera indica* L. extract (Vimang). Pharmacol Res 2004;50:143-9.
11. Ojewole JA. Antiinflammatory, analgesic and hypoglycemic effects of *Mangifera indica* Linn. (*Anacardiaceae*) stem-bark aqueous extract. Methods Find Exp Clin Pharmacol 2005;27:547-54.
12. Singh M, Khatoon S, Singh S, Kumar V, Rawat AK, Mehrotra S. Antimicrobial screening of ethnobotanically important stem bark of medicinal plants. Pharmacognosy Res 2010;2:254-7.
13. Islam MN, Rahim MA, Farooque AM. Standardization of time and grafting techniques in Mango under Bangladesh condition. Asian J Plant Sci 2004;3:378-86.
14. Bhuvaneswari K, Periyanayaga K. Pharmacognostical and phyto-physicochemical profile of the leaves of *Mangifera indica* L. var alphonso anacardiaceae-valuable assessment of its quality. Asian J Pharm Clin Res 2013;6:173-4.
15. Shah KA, Patel MB, Patel RJ, Parmar PK, *Mangifera indica* (Mango). Pharmacogn Rev 2010;4:42-8.
16. Boericke W. Pocket Manual of Homoeopathic Materia Medica. 9th ed. New Delhi: B. Jain Publishers; 1927. p. 424.
17. Rocchetti G, Senizza B, Zengin G, Okur MA, Montesano D, Yildiztugay E, *et al*. Chemical profiling and biological properties of extracts from different parts of *Colchicum szovitsii* Subsp. *Szovitsii*. Antioxidants (Basel) 2019;8:632.
18. Government of India, Ministry of Health and Family Welfare. Homoeopathic Pharmacopoeia of India. Vol. 7. New Delhi: The Controller of Publications; 1999. p. 85.
19. Khurana RK, Rao S, Beg S, Katare OP, Singh B. Systematic development and validation of a thin-layer densitometric bioanalytical method for estimation of mangiferin employing analytical quality by design (AQbD) approach. J Chromatogr Sci 2016;54:829-41.
20. Wagner H, Bladt S. Plant Drug Analysis, a Thin Layer Chromatography Atlas. Berlin: Springer; 1984. p. 196.
21. Srinivasan S, Wankhar W, Rathinasamy S, Rajan R. Free radical scavenging potential and HPTLC analysis of *Indigofera tinctoria* Linn (Fabaceae). J Pharm Anal 2016;6:125-31.
22. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid contents in propils by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-82.