IJBPAS, June, 2023, 12(6): 2536-2545

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

CHEMICAL FINGERPRINTING OF CURCUMIN AND BERBERIN IN SELECTED AYURVEDIC FORMULATION AND HERBS RECOMMENDED FOR SKIN INFECTION

KAKAD KALYANI S[*1], NAWALE S[2], PATIL PM[3], KUTE R[4], MALODE SS[5], NEMMANIWAR AS[1], DHOLE ON[1] AND RAUT PL[1]

1: Department of Pharmacognosy, Progressive Education Society's Modern College of Pharmacy, Sector -21, Yamunanagar Nigdi, Pune-411044, Maharashtra, India

2: Department of Pharmacognosy, Gokaraju Rangaraju College of Pharmacy, Nizampet, Bachupally, Hyderabad, 500090 (Osmania University), India

3: Associate Professor, Progressive Education society's Modern College of Pharmacy, Sector - 21, Yamunanagar

4: Inducare Pharma Pvt. Ltd., 1750, Morgaon road, Jejuri, Tal. – Purandar, Dist. – Pune.

5: Department of Pharmaceutics, NGSPM'S College of Pharmacy, Brahma Valley Education Campus, Anjaneri Nashik-422213

[*]**Corresponding Author: Dr. Kakad Kalyani Shrinivas: E Mail: kk_pharma20@rediffmail.com**

Received 10th July 2022; Revised 15th Aug. 2022; Accepted 15th Sept. 2022; Available online 1st June 2023

https://doi.org/10.31032/IJBPAS/2023/12.6.7099

ABSTRACT

Natural products have great potential to treat diverse kinds of skin diseases. In India more than 75% peoples are depends on the traditional health care system and now a days different natural product-based products are used to treat skin diseases. The objective of this study is to detect the active compounds of particular Ayurvedic medicines suggested for skin treatment. The selected polyherbal liquid preparation traditionally recommended for management of various kinds of skin related infections, like acne, fungal infection, vitiligo, psoriasis, chronic itching.

Phytochemical fingerprinting was developed and optimized and it gives good resolution in herbal product and recommended herbs. High performance thin layer chromatographic analysis showed presence of active phytoconstituents like Curcumin and Berberin in ayurvedic formulation and herbs. This analytical study provided scientific information concerning active ingredients of certain medicines with established therapeutic potentials like antiviral, immunomodulatory and anti-inflammatory activities.

Keywords: *Berberis aristata* DC; Berberidaceae; Curcuma *longa* L; Zingiberaceae; Berberin; Curcumin; HPTLC

INTRODUCTION

Berberis aristata is generally known as Daruharidra, Daru haldi, Indian Barberry, tree turmeric. It is a spikey, hard, yellow herb goes to the family berberidaceae. This plant is mainly grown-up in the sub-Himalayan region, Nilgiri hills of southern India and hilly areas of Nepal **[1]**. *Berberis aristate* is considered the most significant herbal plant in the ayurveda, Siddha and Unani medicinal system due to its therapeutic importance. Traditionally the plant is used as tonic, demulcent, diaphoretic, diuretic and diuretic and alternative to treat diseases like wound healing, skin diseases, rheumatism, snake bite, menorrhagia, jaundice and eye problem **[2]**. The main alkaloid component of plant is Berberin which is either in leaves, roots, rhizome, stem bark. In present study berberin was identified in trunk extract of *Berberis aristata* DC and this plant containing polyherbal formulations. Daruharidra is principally useful for skin complications and it has anti-psoriatic and anti-inflammatory activity **[3]**. Daruharidra benefits in management of acne and reported as antibacterial and anti-inflammatory properties **[4]**.

According to Ayurveda, applying a paste of Daruharidra powder in various forms helps in quicker curing due to its Ropan (healing) property **[5]**.

Turmeric (*Curcuma longa*) is herb going to ginger family, which is mostly grown in various Asia region. Turmeric, is used as spice which has a standing place in the cuisines of Iran, Malesia, India, China, Polynesia, and Thailand. Turmeric is correspondingly known to have been used for times in India and China for the therapeutic handlings of illnesses such as dermatologic diseases, infection, anxiety, and sadness. Turmeric's properties on health are mostly due to occurrence of a lipophilic polyphenol substance called "curcumin," rhizome is a major source of curcumin from this species

[6]. Curcumin is recognised just to have antioxidant, anti-inflammatory, anticancer effects, antidiabetic and dermatologic diseases etc. **[7-8]**. In current study curcumin was identified in its polyherbal formulation and in crude form that is in rhizome of *curcuma longa* L.

Puril is a polyherbal liquid formulation (Inducare Pharma Pvt. Ltd. Jejuri Pune) contains diverse medicinal plants such as Manjistha, Hirda, Behada, Amalaki, Kharsaal, Patha, Halad, Daruhalad, Kutaki, Anantmool, Nimbsaal, Vidang, Nagarmotha, Padmakashtha, Pittapapada. It is generally used for the treatment of skin related problems. Majorly Puril syrup use for acne, fungal infections, Vitiligo, Psoriasis and chronic etching.

MATERIAL AND METHODS

2.1 Chemicals and reagents

Berberin and Curcumin was purchased from Yucca Enterprises Mumbai and other reagents, solvents and precoated silica gel 60 F_{254} HPTLC plates (20 × 20 cm) were procured from Merck (Germany).

2.2 Plant material

Daru halad (*Berberis aristata* DC; family: Berberidaceae) trunk powder, Halad (*Curcuma longa* L; Family: Zingiberaceae) was procured from Manakarnika Aushadhalaya Pimpri Chinchwad Pune Maharashtra. Ayurvedic formulation Puril syrup (Inducare Pharma Pvt. Ltd) was collected from corporate office of Inducare pharma Pvt. Ltd. Puril syrup contains 15 herbs, includes extracts of *Berberis aristata* DC (100mg each 5ml), *Curcuma longa* L (100mg each 5ml).

2.3 Preparation of standard solution

A stock solution of Berberin (1000µg/ml) and Curcumin (1000µg/ml) was prepared by dissolving 10mg of accurately weighed standard compounds in 10ml methanol. For calibration 0.5-2.5µl standard solution was applied to HPTLC plate in the range 500-2500ng per band.

2.4 Preparation of sample solution

Depending on solubility of the marker compounds, methanol solvents were used for extraction purpose. Methanolic extract of trunk powder of Daru halad and rhizome powder of Haladi were used for chemical profiling. Weighing 10mg of dried powdered of Daru halad and rhizome powder of Haladi and ultrasonicator assembly was used for extraction, methanol was selected as appropriate solvent for extraction procedure follow for 15 min in methanol.

For formulation measure 5 ml Puril syrup mixed with 20ml methanol this solution kept for sonication for 15 minutes, filtered it and further use for HPTLC analysis.

2.5 HPTLC instrumentation and experimental conditions

Method development parameters includes sample and test solution preparation, HPTLC instrumentation condition, preparation of developing chamber, derivatization reagents carried out as per guideline mention in United States Pharmacopeia (USP. Chapter, 203). According to this chromatographic analysis was done on pre-coated silica gel 60 F_{254} plates (10×10cm with 200µm thickness HPTLC). Samples of extracts, formulations and standards were applied by using microsyringe (Linomat syringe, Hamilton-Bonaduz schweiz, Camag, Switzerland) in band length 8mm wide and 8mm apart by Camag Linomat 5 sample applicator (Camag, Muttenz, Switzerland). The application rate of sample on plate was $150nl^{-1}$. The plate was developed in previously saturated 10×10 cm twin-trough glass chamber (Camag, Muttenz, Switzerland) at room temperature. Initially different mobile phases were used for chromatogram development from this best resolution was observed in the composition of n-propanol: toluene: methanol: glacial acetic acid (4:4:2:0.5 v/v) for Berberine and Curcumin. Dry thin layer chromatographic plate was observed and separation of bands it helps in analysis of Berberin and Curcumin in respective plant species and in ayurvedic formulations. For determining the linearity range of standard berberin and curcumin, a series of spots of different volumes (0.5, 1, 1.5, 2. 2.5, 3 µl) were applied so as to get 500-2500ng quantity of standard per band, respectively. Linearity was evaluated in triplicate Analysis carried out at 366nm in absorbance remission mode by TLC scanner III (Camag, Muttenz, Switzerland) and win CATS version 1.4.0 software (Camag, Muttenz, Switzerland) were used in this study. Microsoft excel was also used to treat data statistically **[9]**.

RESULTS AND DISCUSION

Phytochemical fingerprinting was developed for skin care herbs and polyherbal products. The mobile phase was developed and optimized and it gives good resolution in herbal product **(Figure 4)**. Rf value and overlay spectra of standard compounds compare with sample helps in a identification of compounds. The curcumin and berberin were recognized from the tested formulation **(Figure 4 & 5)**. A respectable linearity relationship was initiate to be with (r^2) correlation coefficient value of 0.9890 for berberin, (r2) of 0.9936 for curcumin **(Figure 3)**.

Bioactive constituents are precursor for drug development. As per traditional knowledge medicinal plants are used for

therapeutic properties including anti-inflammatory, anti-viral, antidiabetic **[10]**.

Chemical profiling of Haldi and Daruharidra showed presence of therapeutically active important phytoconstituents such as Curcumin and Berberin majorly used for dermatologic related disorders. Berberin is ($C_{20}H_{18}NO_4$), **(Figure 1)** a natural bioactive ingredient mainly exists naturally in the various usefull parts of medicinal such as roots, rhizomes, stem bark plants from Ranuculaceae, Rutaceae and berberidaceae families. Berberin also used in the inhibition and treatment of skin psoriasis and pigmentation disorders. Natural sources have large potential to treat different kinds of skin ailments **[11-13]**.

Curcumin is a ($C_{21}H_{20}O_6$), **(Figure 2)** polyphenol is identified as of particular polyherbal formulation that might have come from the ingredient plant *Curcuma longa* L. As per ethnomedicinal claims curcumin having potential role in health care system. Recently curcumin in various forms use for skin diseases and also reported as anti-inflammatory, antineoplastic etc.

Curcumin may signify an effective agent in the treatment of several skin conditions, inflammatory and neoplastic diseases **[14-16]**.

The phytochemical identified from Daru halad (*Berberis aristate*), Halad (*Curcuma longa* L) and Puril syrup are active molecule with probable biological activities such as anti-inflammatory, antiviral etc.

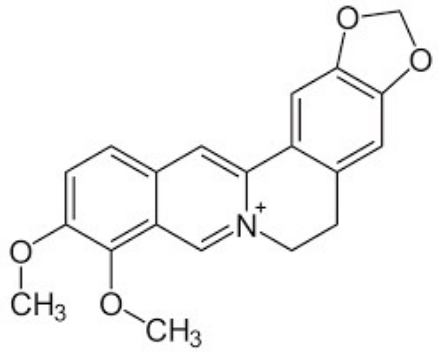


Figure 1: Chemical structure of Berberin

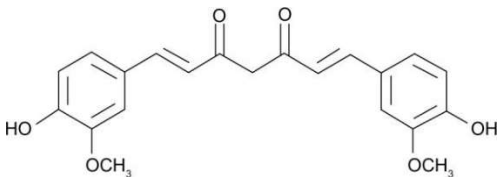


Figure 2: Chemical structure of Curcumin

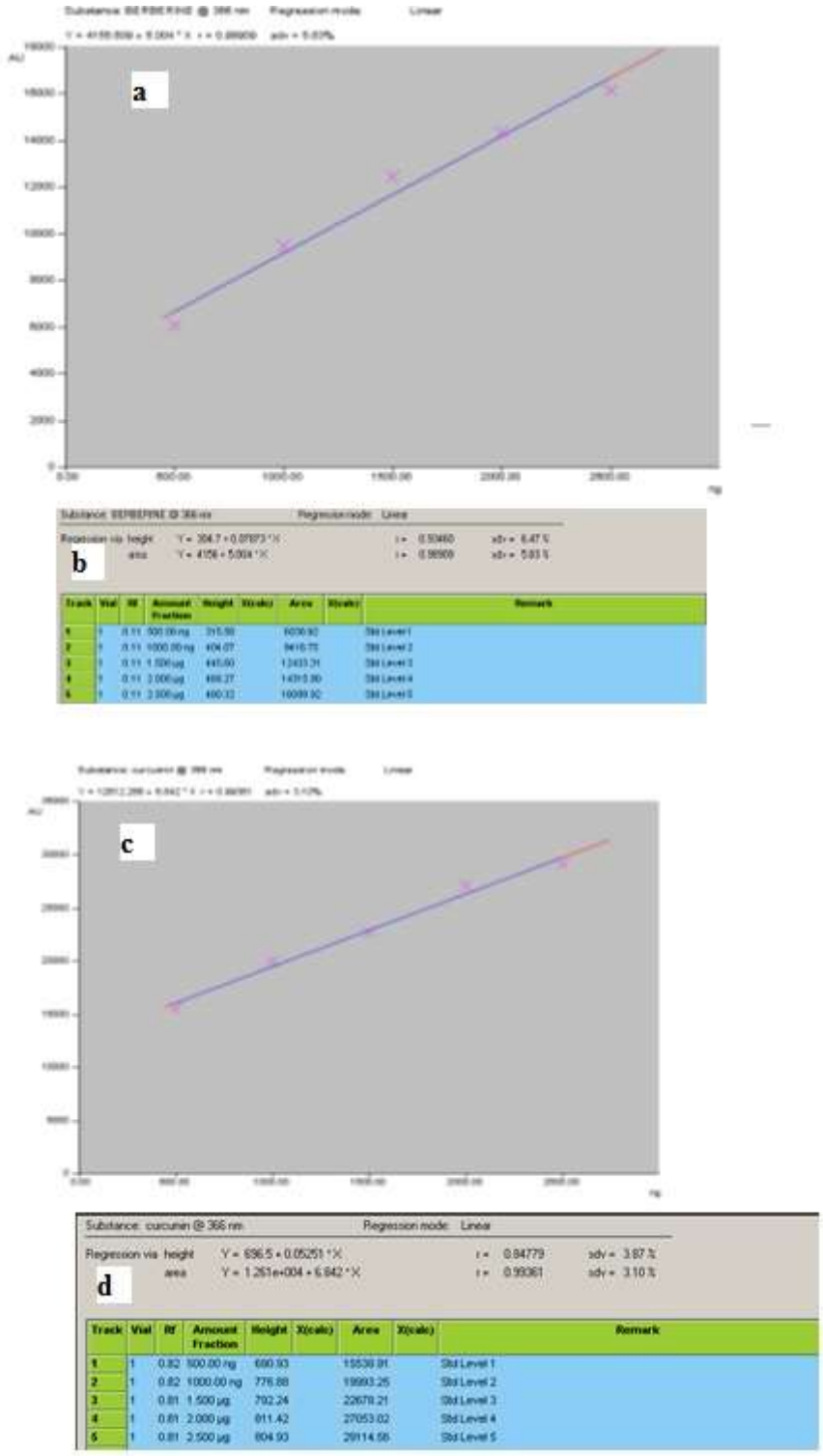
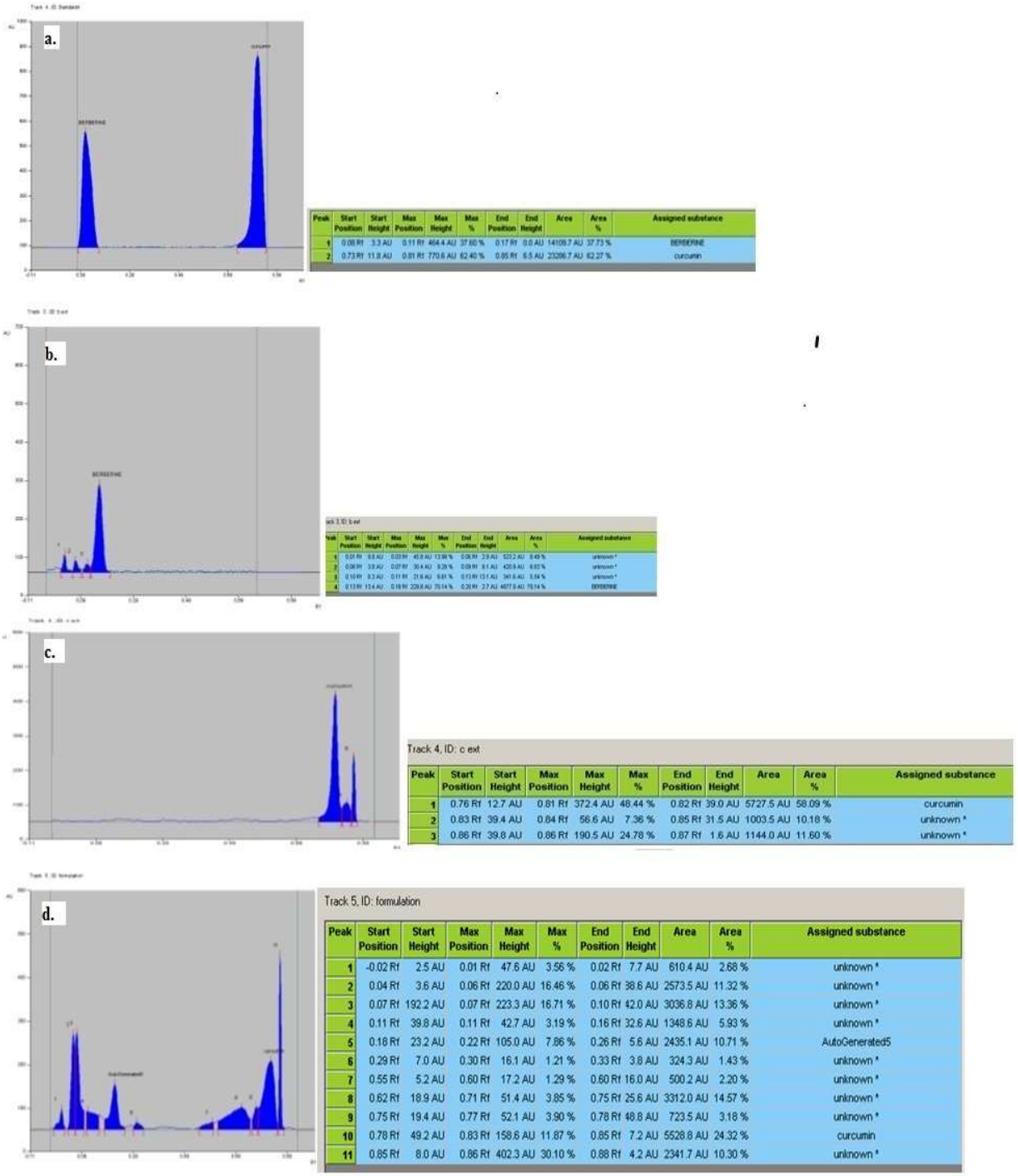


Figure 3: Calibration curve of standards a/b Calibration curve of Berberin, c/d Calibration curve of Curcumin at 366nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.08 Rf	3.3 AU	0.11 Rf	464.4 AU	37.60 %	0.17 Rf	0.0 AU	14109.7 AU	37.73 %	BERBERINE
2	0.73 Rf	11.8 AU	0.81 Rf	770.6 AU	62.40 %	0.85 Rf	6.5 AU	23286.7 AU	62.27 %	curcumin

Track 4, ID: c ext

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.76 Rf	12.7 AU	0.81 Rf	372.4 AU	48.44 %	0.82 Rf	39.0 AU	5727.5 AU	58.09 %	curcumin
2	0.83 Rf	39.4 AU	0.84 Rf	56.6 AU	7.36 %	0.85 Rf	31.5 AU	1003.5 AU	10.18 %	unknown *
3	0.86 Rf	39.8 AU	0.86 Rf	190.5 AU	24.78 %	0.87 Rf	1.6 AU	1144.0 AU	11.60 %	unknown *

Track 5, ID: formulation

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	2.5 AU	0.01 Rf	47.6 AU	3.56 %	0.02 Rf	7.7 AU	610.4 AU	2.68 %	unknown *
2	0.04 Rf	3.6 AU	0.06 Rf	220.0 AU	16.46 %	0.06 Rf	38.6 AU	2573.5 AU	11.32 %	unknown *
3	0.07 Rf	192.2 AU	0.07 Rf	223.3 AU	16.71 %	0.10 Rf	42.0 AU	3036.8 AU	13.36 %	unknown *
4	0.11 Rf	39.8 AU	0.11 Rf	42.7 AU	3.19 %	0.16 Rf	32.6 AU	1348.6 AU	5.93 %	unknown *
5	0.18 Rf	23.2 AU	0.22 Rf	105.0 AU	7.86 %	0.26 Rf	5.6 AU	2435.1 AU	10.71 %	AutoGenerated5
6	0.29 Rf	7.0 AU	0.30 Rf	16.1 AU	1.21 %	0.33 Rf	3.8 AU	324.3 AU	1.43 %	unknown *
7	0.55 Rf	5.2 AU	0.60 Rf	17.2 AU	1.29 %	0.60 Rf	16.0 AU	500.2 AU	2.20 %	unknown *
8	0.62 Rf	18.9 AU	0.71 Rf	51.4 AU	3.85 %	0.75 Rf	25.6 AU	3312.0 AU	14.57 %	unknown *
9	0.75 Rf	19.4 AU	0.77 Rf	52.1 AU	3.90 %	0.78 Rf	48.8 AU	723.5 AU	3.18 %	unknown *
10	0.78 Rf	49.2 AU	0.83 Rf	158.6 AU	11.87 %	0.85 Rf	7.2 AU	5528.8 AU	24.32 %	curcumin
11	0.85 Rf	8.0 AU	0.86 Rf	402.3 AU	30.10 %	0.88 Rf	4.2 AU	2341.7 AU	10.30 %	unknown *

Figure 4: a. HPTLC Chromatogram of standard Berberin and Curcumin (a), Extract of Daru halad (Berberis aristate), Trunk part (b), Extract of Halad (Curcuma longa L) /Rhizome part (c), sample formulation (Puril syrup) (d).

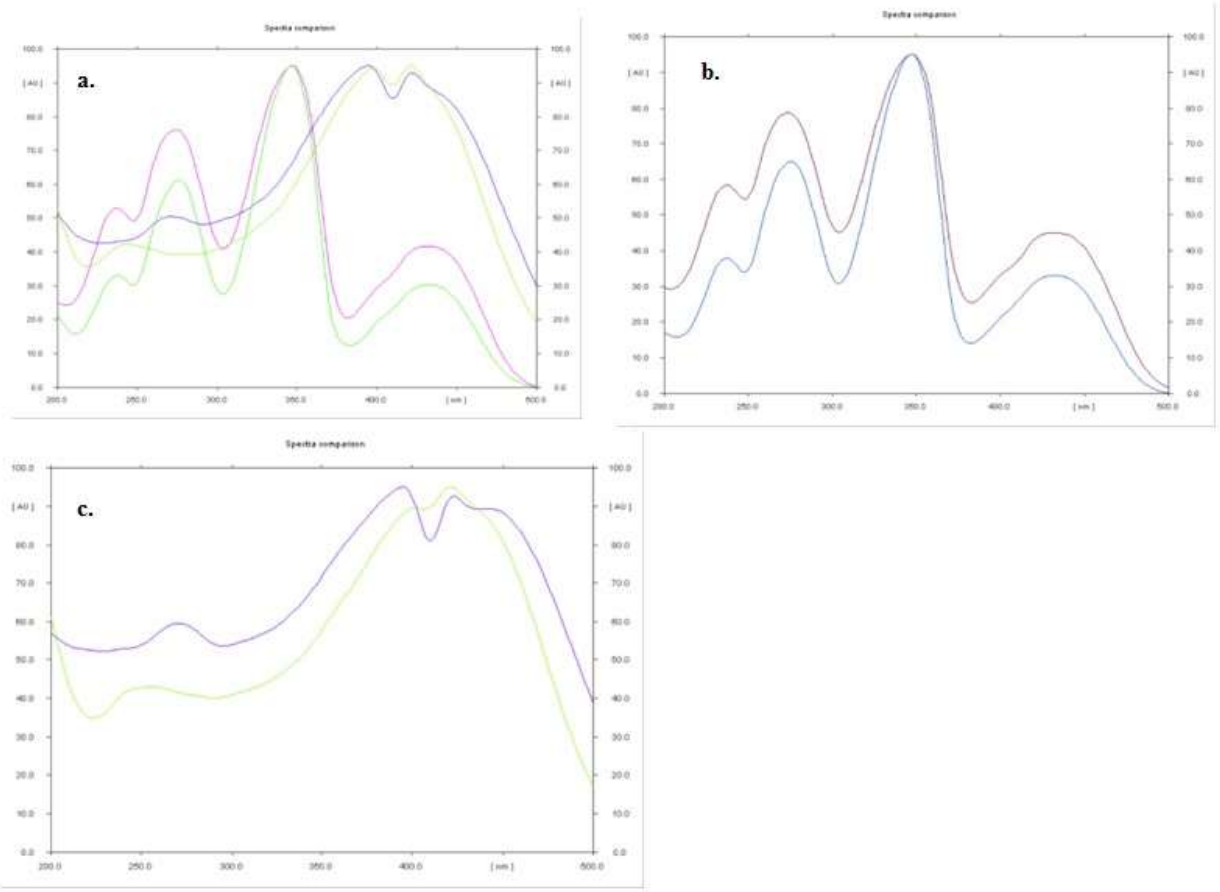


Figure 5: Overlay UV absorption spectra of standard Berberin and Curcumin in peaks of standard and formulation (a), Berberin in paeks of standard and extract (b), Curcumin in peaks of standard and extract Halad (c)

CONCLUSION

In human health and natural therapy skin diseases are comprehensive challenges among patients and physician researchers. In Europe and Asian countries herbal remedies have been used successfully for dermatological disorders. The present work done on specific data regarding the active ingredients of the drugs analyzed with authenticate pharmacological potential like antiviral, anti-inflammatory etc.

ACKNOWLEDGMENTS

Authors are thankful to Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Pimpri, Pune for providing instrumental facility.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

[1] Choudhary S, Kaurav H, Madhusudan S. and Chaudhary G. Daruharidra (Berberis aristata):

Review based upon its Ayurvedic Properties. International Journal for Research in Applied Sciences and Biotechnology 2021; 8 (2): 98-105. https://doi.org/10.31033/ijrasb.8.2.12.

[2] Neag MA, Mocan A, Echeverría J, Pop RM, Bocsan CI, Crişan G, Anca D. Buzoianu AD. Berberine: Botanical Occurrence, Traditional Uses, Extraction Methods, and Relevance in Cardiovascular, Metabolic, Hepatic, and Renal Disorders, Front Pharmacol. 2018; 9: 557.

[3] Nille GC, Chaudhary AK. Potential implications of Ayurveda in Psoriasis: A clinical case study. J Ayurveda Integr Med. 2021;12(1):172-177. doi: 10.1016/j.jaim.2020.11.009.

[4] Prasad SB, Kaur D. In Vitro Anti Acne Activity of Ethanolic Extract of Stem of Berberis aristate. International Journal of Pharmacognosy and Phytochemical Research 2017; 9(2); 190-192.

[5] Varshney S, Dhyani S. Medicinal herbs having incredible wound healing effects. Res. Ayurveda Pharm 2015; 6(5): 573-579.

[6] Kocaadam B, Şanlier N. Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. Crit Rev Food Sci Nutr 2017; 57(13): 2889-2895.

[7] Rahmani AH, Alsahli MA, Aly SM, Khan MA, and Yousef H. Aldebasi YH. Role of Curcumin in Disease Prevention and Treatment. Adv Biomed Res. 2018; 7: 38. doi: 10.4103/abr.abr_147_16.

[8] Vaughn AR, Branum A, Sivamani RK. Effects of Turmeric (Curcuma longa) on Skin Health: A Systematic Review of the Clinical Evidence. Phytother Res. 2016; 30(8):1243-64. doi: 10.1002/ptr.5640.

[9] Wagner H, Bladt B. Plant Drug Analysis. 2nd ed. Springer Publications, 1996.

[10] Ahamad J, Kaskoos RA, Ali F, Mir SR. Critical Review on the Unexplored Therapeutic Treasure of Himalayan Ayurvedic Drug Daruharidra (Berberis Aristata), Current Traditional Medicine. 2020; 1: 1-0.

[11] Neag MA, Echeverría AMJ, Pop RM, Bocsan CI, Crişan G, Anca D. Buzoianu AD. Berberine: Botanical Occurrence, Traditional Uses,

Extraction Methods, and Relevance in Cardiovascular, Metabolic, Hepatic, and Renal Disorders. Front Pharmacol. 2018; 9: 557.

[12] Zhang SSX et al. Berberine downregulates CDC6 and inhibits proliferation via targeting JAK-STAT3 signaling in keratinocytes, Cell Death Dis 2019; 10(4): 274. doi: 10.1038/s41419-019-1510-8.

[13] Huang TH, Lin CF, Alalaiwe A, Yang SC, Fang JY. Apoptotic or Antiproliferative Activity of Natural Products against Keratinocytes for the Treatment of Psoriasis. Int J Mol Sci. 2019; 20(10): 2558.

[14] Sharifi-Rad J, Rayess YE. Turmeric and Its Major Compound Curcumin on Health: Bioactive Effects and Safety Profiles for Food, Pharmaceutical, Biotechnological and Medicinal Applications, Front Pharmacol. 2020; 11: 01021.doi: 10.3389/fphar.2020.01021.

[15] Mohd Zaid NA, Sekar M, Bonam SR , Gan SH , Lum PT, Begum MY, Mat Rani NNI, Vaijanathappa J, Wu YS, Subramaniyan V, Fuloria NK, Fuloria S. Promising Natural Products in New Drug Design, Development, and Therapy for Skin Disorders: An Overview of Scientific Evidence and Understanding Their Mechanism of Action. 2022; 16 Pages 23—66DOI https://doi.org/10.2147/DDDT.S326332.

[16] Vollono L, Falconi M, Gaziano R, Iacovelli F, Emi Dika E, Terracciano C, Bianchi L, Campione E. Potential of Curcumin in Skin Disorders, Nutrients. 2019; 11(9): 2169. doi: 10.3390/nu11092169