IJPSR (2021), Volume 12, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 July 2020; received in revised form, 07 December 2020; accepted, 08 May 2021; published 01 July 2021

HPTLC SCREENING OF AMINO ACID PROFILE OF *CURCUMA* SPECIES FROM MELGHAT FORESTS DIST. AMRAVATI MAHARASHTRA

Mangesh Dagawal [*1] and Prabha Bhogaonkar [2]

Department of Botany [1], Smt. Radhabai Sarda Arts, Commerce and Science College, Anjangaon Surji Amravati - 444705, Maharashtra, India.
Government Vidarbha Institute of Science & Humanities [2], Amravati - 444604, Maharashtra, India.

Keywords:

Curcuma inodora Blatt, *Curcuma pseudomontana* J. Graham, *Curcuma longa* L, Melghat Forest, HPTLC, Amino acid Profile

Correspondence to Author:
Dr. Mangesh J. Dagawal

Head,
Department of Botany, Smt. Radhabai Sarda Arts, Commerce and Science College, Anjangaon Surji Amravati - 444705, Maharashtra, India.

E-mail: mdagawal@gmail.com

ABSTRACT: Genus *Curcuma* L. (Zingiberaceae), comprising of 120 species, is distributed throughout South and South-East Asia, with few species extending to China, Australia, and South Pacific. Four species of *Curcuma* are reported from Melghat. Of these *C. longa* L. is cultivated while *C. inodora* Blatt., *C. pseudomontana* J. Graham and *C. decipiens* are wild. *C. decipiens* being rare could not be collected. *Curcuma inodora* Blatt. Known as 'Jangali Halad' is a common herb of Melghat at higher elevations. In the Melghat area, populations of *C. inodora* are found to show many distinct variations in aerial as well as underground characters. Twelve distinct variants of *C. inodora* and *C. pseudomontana* and *C. longa* were collected. HPTLC screening of free amino acids showed the presence of four to eleven amino acids in *Curcuma inodora* accessions, while in *C. pseudomontana* and *C. longa*, ten amino acids each were found. Glycine and Proline were found in all samples studied. Amino acid composition is specific for each species and variants; hence can be used as identifying character. HPTLC screening of amino acids reflects the distinctness as well as relatedness of the species.

INTRODUCTION: Genus *Curcuma* L. (Zingiberaceae) comprising of 120 species and is distributed throughout South and South-East Asia, with a few species extending to China, Australia, and the South Pacific. Four species of *Curcuma* are reported from Melghat [1, 2]. Of these *C. longa* L. is cultivated while *C. inodora* Blatt. *C. pseudo-montana* J. Graham and *C. decipiens* are wild. *C. decipiens* being rare, could not be collected. *C. inodora* is widely distributed throughout Maharashtra and is very common and abundant in Melghat. It is commonly called 'Jangli halad' and used in traditional medicine by locals.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(7).3750-55

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(7).3750-55

Fresh rhizome paste is applied over the cut as a strong antiseptic. The smoke of dried rhizome is used to hypnotise the person; some use it in Tantrik, Vashikarana and Mayajal Kriyas [3.] Paste of root stock is applied in glandular diseases and piles [4, 5, 6], psychosomatic disorders, and constipation [7, 8]. *C. pseudomontana* is used in traditional medicine to cure jaundice and diabetes [9], body swellings, and increased lactation [10]. Fresh tubers are eaten as blood purifiers [11].

The dried rhizome of *Curcuma longa* L. has been a rich source of beneficial phenolic compounds known as curcuminoid [12, 13]. It is used in several ways in Ayurveda and traditional medicine the world over. Amino acids are primary metabolites, occurring in plants both in the Free State and as the basic unit of proteins. HPTLC method is most suitable as separation is faster, more efficient, and results are more reliable and reproducible. In

combination with digital scanning HPTLC also provides accurate and precise R_f values [14].

The present attempt has been made to analyze free amino acids in methanol extract of Curcuma species through HPTLC.

MATERIALS AND METHODS: *Curcuma pseudomontana* J. Graham, *Curcuma longa* L. and twelve variants of *Curcuma inodora* Blatt. Were collected from various locations in Melghat Forests for HPTLC screening of Amino acids.

Identification of *Curcuma* species was done by using standard floras [2, 15, 16, 17]. For HPTLC studies, leaves were washed with distilled water, air-dried, powdered, and stored at room temperature for further analysis. HPTLC screening was done following Wagner [18].

HPTLC for Amino Acids:

Sample Preparation: 500 mg of each sample was extracted with 5 ml methanol by sonication for 30 min. Then these solutions were filtered and filtrate used for chromatography.

Preparation of Standard Amino Acid Solution for HPTLC Analysis: Solutions of 15 Amino acids (Argenine, Lucine, Methionine, Proline, Threonine, Histidine, Phenylalanine, Serine, Tryptophan, Glutamic acid, Glycine, Isoleucine, Lysine, L-Valine, and Cystine) were prepared at the concentration of 1 mg/ml each.

Chromatography: 15 standard amino acids and sample solutions were used for HPTLC analysis. Standard amino acids were run to determine the R_f for reference. Then 14 sample solutions and a mixture of standard 5 amino acids were run simultaneously on single plate. Likewise, all samples were run with all standard amino acids (in a batch of five) using n–Butanol: Acetic Acid: Water (60: 1.5: 25 v/v/v) as solvent system. 2 µl of the standard solution and 5 µl of sample solution were loaded as 8 mm band length on Cellulose F TLC plates using Hamilton syringe with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through Vision CATS server-PH, version 2.5.18262.1 software. After the application of spots, chromatogram was run in TT chamber (Twin –Trough chamber) pre-saturated with the mobile phase. The air-dried plates were kept in CAMAG Visualizer, and images were captured in white light, UV 366 nm, and UV 254 nm light. The chromatograms were scanned by a CAMAG TLC Scanner after spraying with ninhydrin reagent and dried at 100°. R_f values were recorded by Vision CATS server-PH, version 2.5.18262. Best results were obtained in white light.

RESULTS AND DISCUSSION: *Curcuma pseudomontana* J. Graham (MJD-101), *Curcuma longa* L. (MJD-102) and twelve variants of *Curcuma inodora* Blatt. (MJD-103-114) were screened for Amino acid Profile.

TABLE 1: AMINO ACID PROFILE

Sample	Amino acid
Sample 1 (CI-1)	Proline, Phenylalanine, Serine, Tryptophan, Glutamic acid, Glycine, Isoleucine, Lysine, L-Valine and Cystine.
Sample 2 (CI-2)	Proline, Phenylalanine, Tryptophan, Glycine.
Sample 3 (CI-3)	Lucine, Methionine, Proline, Phenylalanine, Tryptophan, Glutamic acid, Glycine, Isoleucine, Lysine, L-Valine and Cystine
Sample 4 (CI-4)	Lucine, Methionine, Proline, Phenylalanine, Tryptophan, Glycine, Isoleucine, Lysine, L-Valine
Sample 5 (CI-5)	Lucine, Methionine, Proline , Phenylalanine, Serine, Tryptophan, Glutamic acid, Glycine, Isoleucine, Lysine, L-Valine
Sample 6 (CI-6)	Methionine, Proline, Phenylalanine, Tryptophan, Glycine, Isoleucine, Lysine, L-Valine
Sample 7 (CI-7)	Methionine, Proline, Phenylalanine, Tryptophan, Glycine, Isoleucine, Lysine, L-Valine
Sample 8 (CI-8)	Proline , Threonine, Phenylalanine, Serine, Glycine
Sample 9 (CI-9)	Proline , Phenylalanine, Tryptophan, , Glycine , Isoleucine, Lysine, L-Valine
Sample10 (CI-10)	Proline , Phenylalanine, Serine, Tryptophan, Glutamic acid, Glycine , Isoleucine, Lysine, L-Valine
Sample11 (CI-11)	Proline , Threonine, Serine, Glycine , Isoleucine, , L-Valine
Sample12 (CI-12)	Proline , Histidine, Phenylalanine, Serine, Tryptophan, Glutamic acid, Glycine , Isoleucine, Lysine, L-Valine
Sample13 (CP13)	Methionine, Proline , Phenylalanine, Serine, Tryptophan, Glutamic acid, Glycine , Isoleucine, Lysine, L-Valine
Sample14 (CL14)	Argenine, Proline , Histidine, Serine, Tryptophan, Glutamic acid, Glycine , Isoleucine, Lysine, L-Valine

Variants of *C. inodora* selected for screening were coded as CI-1, CI-2, CI-3, CI-4, CI-5, CI-6, CI-7, CI-8, CI-9, CI-10, CI-11, and CI-12 and *Curcuma pseudomontana* coded as CP-13 and *Curcuma longa* as CL-14 Photoplate -1. Extracts of all samples were run along with the mixture of standard amino acids as mentioned in methodology. Since amino acids are very sensitive minor differences also result in changing the Rf. Therefore every time, along with sample extracts mixture of five standard amino acids was run. Specific amino acids were identified with reference to the standard. Amino acid composition of all samples is presented in **Table 1** and HPTLC photoplates Photoplate no. 2 to 5.

CI-1 **CI-2**

CI-3 **CI-4** **CI-5** **CI-6**

CI-7 **CI-8** **CI-9** **CI-10**

CI-11 **CI-12** **CP-13** **CL-14**

PHOTOPLATE 1: CURCUMA ACCESSIONS PHOTOPLATE

PHOTOPLATE 2: HPTLC OF AMINO ACID

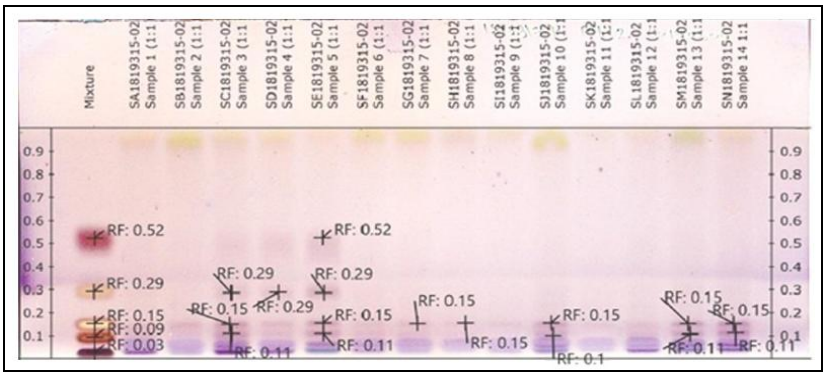

FIG. 1: HPTLC IMAGE OF 14 SAMPLE FOR AMINO ACID MIXTIRE OF STANDARD AMINO ACID MIXTURE A IN WHITE LIGHT AFTER DERIVATISING IN NINHYDRIN REAGENT

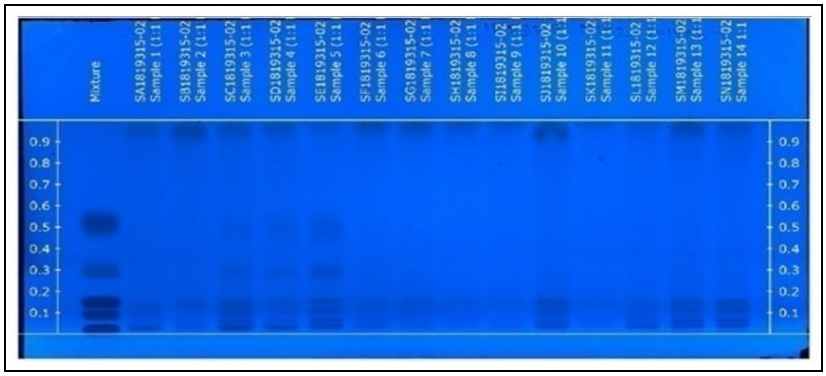

FIG. 2: HPTLC IMAGE OF 14 SAMPLE FOR AMINO ACID MIXTIRE OF STANDARD AMINO ACID MIXTURE A UNDER WAVELENGH 366 NM AFTER DERIVASING IN NINHYDRIN REAGENT STANDASRD AMINO ACID MIXTURE A- ARGENINE, LUCINE, METHIONINE, PROLINE AND THREONIN

PHOTOPLATE 3: HPTLC OF AMINO ACID

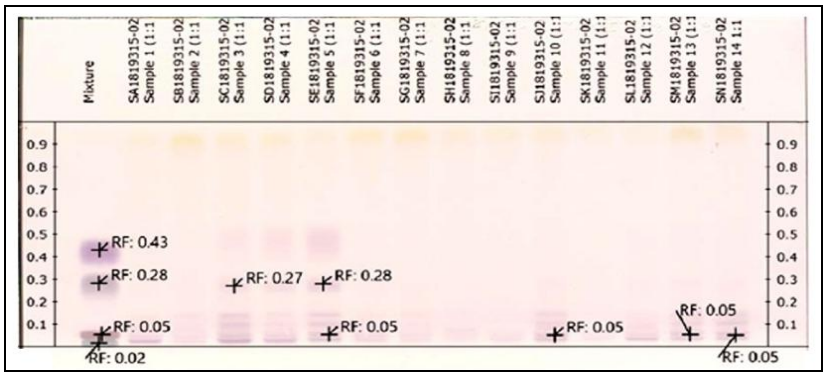

FIG. 3: HPTLC IMAGE OF 14 SAMPLES FOR AMINO ACID MIXTIRE OF STANDARD AMINO ACID MIXTURE B IN WHITE LIGHT AFTER DERIVATISING IN NINHYDRIN REAGENT

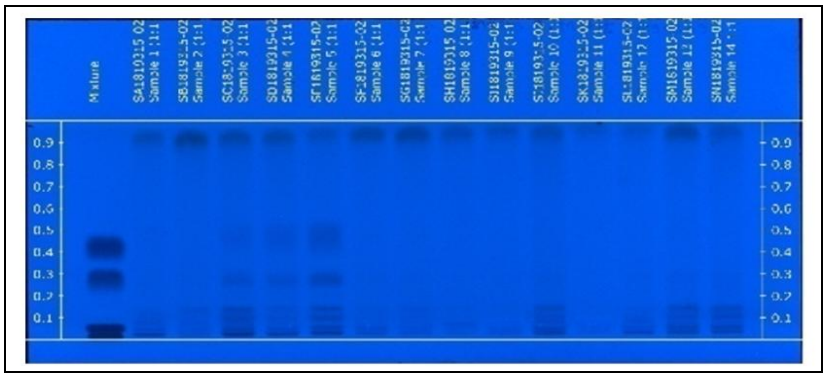

FIG. 4: HPTLC IMAGE OF 14 SAMPLE FOR AMINO ACID MIXTIRE OF STANDARD AMINO ACID MIXTURE A UNDER WAVELENGH 366 NM AFTER DERIVASING IN NINHYDRIN REAGENT STANDASRD AMINO ACID MIXTURE B- HISTIDIN, PHENYLALANINE, SERINE AND TRYPTOPHAN

PHOTOPLATE 4: HPTLC OF AMINO ACID

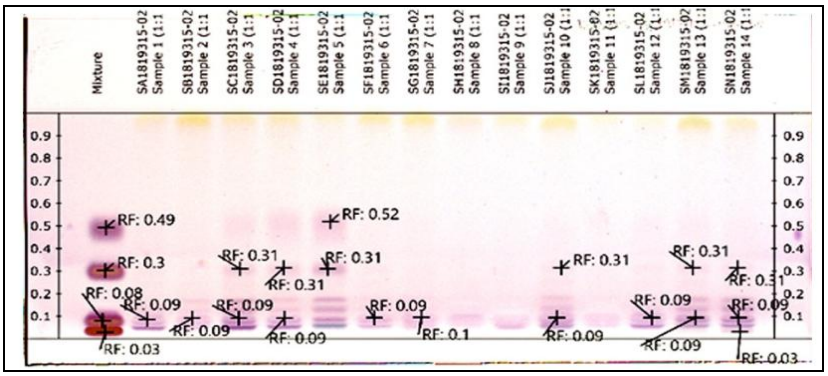

FIG. 5: HPTLC IMAGE OF 14 SAMPLES FOR AMINO ACID MIXTURE OF STANDARD AMINO ACID MIXTURE C IN WHITE LIGHT AFTER DERIVATISING IN NINHYDRIN REAGENT

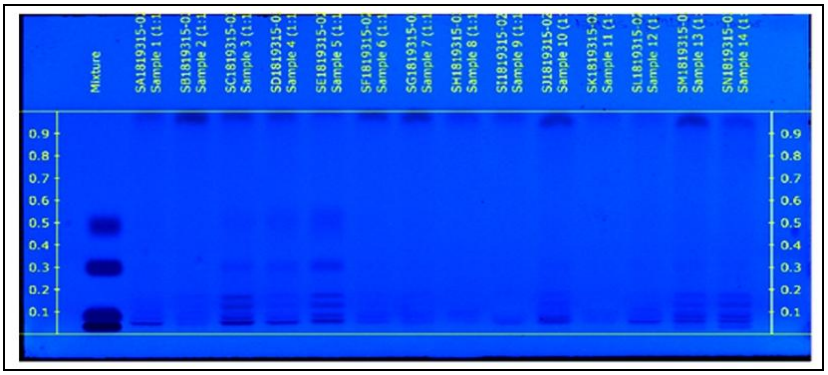

FIG. 6: HPTLC IMAGE OF 14 SAMPLE FOR AMINO ACID MIXTURE OF STANDARD AMINO ACID MIXTURE A UNDER WAVELENGTH 366 NM AFTER DERIVASING IN NINHYDRIN REAGENT STANDARD AMINO ACID MIXTURE C- GLUTAMIC ACID, GLYCINE, ISOLEUCINE, LYSINE, AND L-VALI

PHOTOPLATE 4: HPTLC OF AMINO ACID

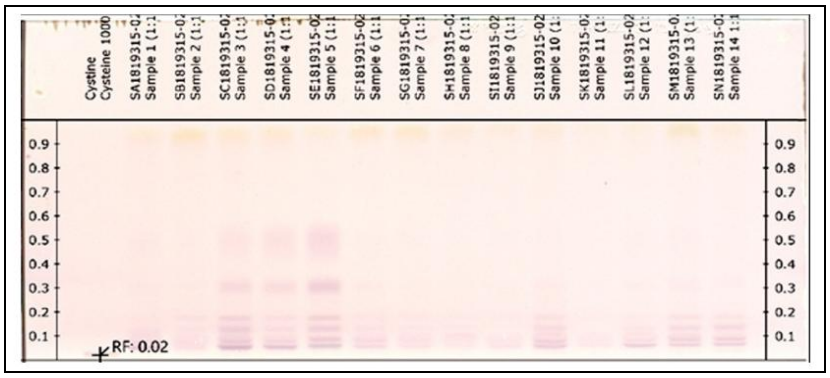

FIG. 7: HPTLC IMAGE OF 14 SAMPLES FOR AMINO ACID MIXTURE OF STANDARD AMINO ACID CYSTIN IN WHITE LIGHT AFTER DERIVATISING IN NINHYDRIN REAGENT

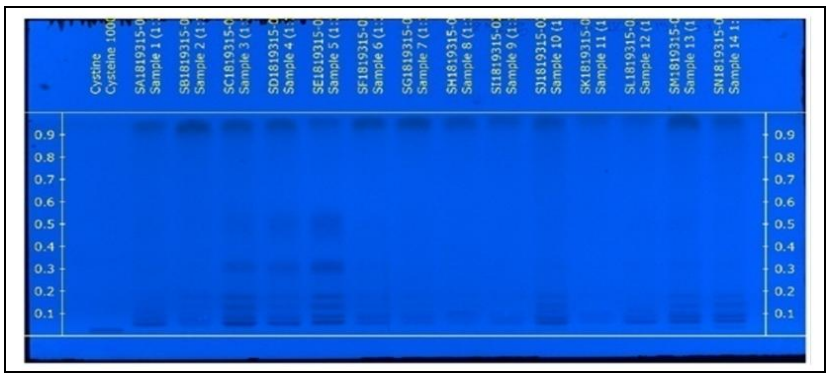

FIG. 8: HPTLC IMAGE OF 14 SAMPLES FOR AMINO ACID MIXTURE OF STANDARD AMINO ACID CYSTIN IN WHITE LIGHT AFTER DERIVATISING IN NINHYDRIN REAGENT

The amino acid profile of a species is a taxonomically important character. The profile reflects the distinctness as well as relatedness of the species. Variants CI-3 and CI-5 contain a maximum number of amino acids, *i.e*., 11 while, CI-12, CP-13, and CL-14 all contain 10 amino acids each, though there is a difference in amino acid profile composition. CI-2 shows only 4 amino acids. Glycine and Proline are found in each sample. They characterize the *Curcuma species* studied.

CONCLUSION: All the three *Curcuma* species, including variants, are taxonomically characterized by the presence of Glycine and Proline, while other amino acids present characterize particular species and variants. Variants of *C. inodora* are quite distinct as far as the amino acid profile is concerned; they possibly represent further evolutionary lines within the species.

ACKNOWLEDGMENT: Authors are thankful to the Director, Biodiversity Board, Maharashtra State, Nagpur, and Chief Conservator of Forest Melghat, Tiger Project, Amravati, for their permission to field visits and required sample collection outside the reserve area. They are also thankful to Dr. Gaganjyot Kaur, NFB, Khalsa College, Mumbai her help in HPTLC studies.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Dhore MA and Joshi PA: Flora of Melghat Tiger Reserve. Directorate, Project Tiger, Melghat, Paratwada, Dist. Amravati. Maharashtra 1988.
2. Bhogaonkar PY and Devarkar VD: Additions to the Flora of Melghat. The Directorate Project Tiger Melghat, Amravati, India (Technical Bulletin no. VII) 1999.
3. Devarkar VD: Ethnobotanical studies of Melghat Dist. Amravati with special reference of ethnomedicine. Thesis submitted for the degree of Doctor of Philosophy, Amravati University (now- Sant Gadge Baba Amravati University), Amravati 2001.
4. Shah GI and Gopal GV: An Ethnobotanical profile of the Dangies. J Econ Tax Bot 1982; 3: 355-64.
5. Mudaliar SK, Kulkarni HG and Sharma BD: Unpublished ethnobotanical information of G. M. Ryan and G. A. Gammie. J Econ Tax Bot 1987; 11(2): 311-20.
6. Bhogaonkar PY and Kadam VN: Ethanobotanical survey of Umarkhed Area (Dist. Yavatmal, M.S.) 1 - Monocotyledonous drug plants. J Bot Soc Univ Sagar 2005; 40: 36-42.
7. Rommand-Monnier F: *Curcuma inodora*. Assessment using IUCN Categories and Criteria 3.1(IUCN 2001). Royal Botanic Gardens, Kew. (SRLI conservation assessments, pending approval by IUCN) 2009.
8. Jagtap SD: Traditional ethnomedicinal knowledge confined to the Pawra tribe of Satpura hills, Maharashtra, India. Ethnobotanical Leaflets 2009; 13: 98-115.
9. Panal SB, Ramkrishna H and Devender R: Ethnomedical studies for endemic diseases by the tribes of Munchingiputtu Mandal, Visakhapatanam district, Andhra Pradesh, India. Intr J Med Arom Plants 2012; 2(3): 453-59.
10. Ramarao N, Rajendran A and Henry AN: Increasing the secretion of breast milk –indigenous practices in Andhra Pradesh. Ancient Science of Life XIX 2000; (3-4).
11. Acharya V, Sharma V, Patra PK, Naik ML and Kanungo VK: Plants used by Kamar, Gond and Halba tribes of Dhamtari district of Chattisgarh for relief of sickle cell disease. Recent Research in Science and Technology 2012; 4(3): 1-3.
12. Lechtenberg M, Quandt B and Nahrstedt A: Quantitative determination of curcuminoids in Curcuma rhizomes and rapid differentiation of *Curcuma domestica* Val and Curcuma zanthorrhiza Roxb. by capillary electrophoresis. Phytochem Anal 2004; 15: 152-58.
13. Sirinivasan KR: Chromatographic study of the curcuminoids in *Curcuma longa*. J Pharm Pharmacol 1953; 5: 448-53.
14. Mofet CA: Clarkes analysis of drugs and poisons. London Pharmaceutical Press 2004; 392.
15. Cooke T: The Flora of the Presidency of Bombay. Botanical Survey of India Culcutta 1967; 1(2-3).
16. Yadav SR and Sardesai MM: Flora of Kolhapur District. Shivaji University Vidyanagar Kolhapur 2002.
17. Dhore MA: Flora of Amravati District with Special Reference to the Distribution of Tree Species, Ph. D. Thesis. Published by Amravati University, (now- Sant Gadge Baba Amravati University) Amravati 1986.
18. Wagner H and Bladt S: Plant Drug Analysis -and A Thin Layer Chromatographic Atlas, Springer- Verlag Berlin Heidelberg 1996.

How to cite this article:
Dagawal M and Bhogaonkar P: HPTLC screening of amino acid profile of *Curcuma* species from Melghat forests dist. Amravati Maharashtra. Int J Pharm Sci & Res 2021; 12(7): 3750-55. doi: 10.13040/IJPSR.0975-8232.12(7).3750-55.