RESEARCH ARTICLE JPAR 2020

Journal of Pharmaceutical Advanced Research

(An International Multidisciplinary Peer Review Open Access monthly Journal)

Available online at: www.jparonline.com

Histochemical and chromatographic characterization of different promising varieties of *Curcuma longa* L.

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Received: 20.01.2020 Revised: 24.01.2020 Accepted: 27.01.2020 Published: 30.01.2020

ABSTRACT: Background: Histochemical and phytochemical characterization is generally resolve many of taxonomic ambiguities **Aim:** The present study was aimed to carry out the Histochemical and phytochemical characterization for correct taxonomic identification of selected taxa. **Methods:** The selected Taxa were characterized taxonomically by morphologically, histochemically and chromatographically. **Results**: The results of the present study highlights the intraspecific variability of different cultivars of *Curcuma* with respect to their morphological, anatomical and phytochemical characters. **Conclusion:** The variability noted with respect to morphological, anatomical and phytochemical parameters can be utilized for judicious selection of particular taxa and their taxonomic identification in the absence of conventional identification characters.

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Keywords: Anatomy, Phytochemistry, Intraspecific variability, *Curcuma longa* L.

INTRODUCTION:

The genus Curcuma (Family: *Zingiberaceae*) comprising over 80 species of rhizomatous herbs, is endowed with widespread adaptation from sea level to altitude as high as 2000 m in the Western Ghats and Himalayas [1]. Having originated in the Indo-Malayan region, the genus is widely distributed in the tropics of Asia to Africa and Australia. Curcuma species exhibit inter- and intra-specific variation for the biologically active principles coupled with morphological variation with respect to the above-ground vegetative and floral characters as well as the below-ground rhizome features besides for curcumin, oleoresin and essential oil. Curcuma is gaining importance world over as a potential source of

new drug(s) to combat a variety of ailments as the species contain molecules credited with anti-inflammatory, hypocholestraemic, choleratic, antimicrobial, insect repellent, anti-rheumatic, anti-fibrotic, anti-venomous, antiviral, anti-diabetic, anti-hepatotoxic as well as anti-cancerous properties [2].

In addition to *C. longa*, the other economically important species of the genus are *C. aromatica*, used in medicine and toiletry articles, *C. kwangsiensis*, *C. ochrorhiza, C. pierreana, C. zedoaria, C. caesia* etc. used in folk medicines of the South-East Asian nations; C. alismatifolia, *C. roscoeana* etc. with floricultural importance; *C. amada* used as medicine, and in a variety of culinary preparations, pickles and salads, and *C. zedoaria, C. malabarica, C. pseudomontana, C. montana, C. decipiens, C. angustifolia, C. rubescens, C. haritha, C. caulina* etc. all used in arrowroot manufacturing. Crop improvement work has been attempted mainly in *C. longa* and to a little extent in *C. amada*. At present there are about 20 improved varieties of *C. longa* in India [3].

Turmeric is known as the "golden spice" as well as the "spice of life." It has been used in India as a medicinal plant, and held sacred from time immemorial. Turmeric has strong associations with the socio-cultural life of the people of the Indian subcontinent [4]. Turmeric is cultivated most extensively in India, followed by Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia, and Philippines. On a small scale, it is also grown in most tropical regions in Africa, America, and Pacific Ocean Islands. India is the largest producer, consumer and exporter of turmeric [5]. In the search for taxonomic characters all sources of taxonomic evidence are scanned. A natural system of classification should be based on the analysis and harmonization of evidence from all organs, tissues and parts.

Morphological characters of plants have been used extensively both for producing classification and for diagnostic purposes and they are still indispensable to the taxonomist. Recent researches have shown that contributions to systematics can come from any branch of botany. Chemical characters of plants can often find as wide application in classification as do characters from gross morphology. The occurrence and distribution of the various types of chemical substances present in plants form the taxonomic evidence. However, all kinds of chemical substances do not reveal information useful to the taxonomist [6]. Distribution of secondary compounds of low molecular weight such as non-protein free Amino acids, Phenolics and Betalins, Alkaloids, Terpeniods, and steroids provide valuable clues to the systematist [7-9].

Histochemical and phytochemical investigation of plant material has a variety of goals, such as determination of the presence of various groups of chemical compounds, their tissue specificity, distribution, and quantitative analysis of active compounds, isolations of substances from the plant tissues for their further identification or physicochemical characterization and finally correct taxonomic identification of the plant.

MATERIALS AND METHODS:

Collection and drying of plants:

The plants used for the investigation that is Panjab haldi, Ranga and Subha varieties of *Curcuma longa* were collected from experimental farm of Indian Institute of Spices Research, Peruvannamuzhi, Kozhikode district.

Morphological study:

Morphological characters of rhizome of each variety were recorded and photographs were taken using Digital Camera.

Anatomical and Histochemical Study:

Anatomical and Histochemical studies were done for identifying and comparing different varieties of turmeric.

The cross sections of rhizomes of different varieties of turmeric were taken by hand sectioning. The thin sections were stained using various histochemical stains like, safranine, sudan red, iodine and boric acid. Stained materials were mounted using glycerine and observed under trinocular microscope with photomicrographic system.

Preparation of extracts:

The methonolic extracts of the rhizomes of different varieties of *Curcuma longa* were prepared for the investigation. The extraction was done using Reflex condenser. 2.85 g of shade dried rhizome material of each variety was taken in the RB flask containing methanol. The setup at boiling temperature was kept for about four hours. The extracts thus obtained is filtered and concentrated to 10 ml and 10 µl from this was used for Thin layer chromatography (TLC) and High Performance Liquid chromatography (HPLC) studies.

Reflex condenser is a distillation technique involving the condensation of vapors and the return of this condensate to the system from which it originated. It is used in industrial and laboratory distillations. It is also used in

chemistry to supply energy to reactions over a long period of time. The term reflux is very widely used in industries that utilize large scale distillation column and fractionators such as petroleum refineries, petrochemical and chemical plants and natural gas possessing plants. A liquid reaction mixture is placed in a vessel open at the top. This vessel is connected to a Leibig or Vigreux condenser, such that any vapors given off are cooled back to the liquid and fall back into the reaction vessel. The vessel is then heated vigorously for the course of the reaction. The purpose is to thermally accelerate the reaction by conducting it at an elevated temperature (*i.e.* the solvents boiling point). The advantage of this technique is that it can be left for a long period of time without the need to add more solvent or fear of the reaction vessel boiling dry as any vapor is immediately condensed in the condenser.

Thin Layer Chromatography (TLC) study:

Thin layer chromatography was developed by Stahl in 1958. It is the most widely used technique for the separation of drugs, plant pigments, pen inks and also for explosive materials. It is based on the principle that a mobile phase moves by capillary action across a thin layer of finally divided stationary phase (absorbent like silica gel) bound on to a plate. When a mixture of sample is applied to the plate and develop with the mobile phase, the sample component moves across the plate at different degrees depending on their solubility.

Rf = Dst/Dsv ……(1)

Where, Dst and Dsv are distance travelled by solute and solvent.

Experimental procedure:

The plant extract was applied to the pre-coated silica plate about 1cm away from the lower edge of the slide using capillary tube. The loaded silica plate was kept inside a coupling jar containing running solvents: Toluene: ethyl acetate: Methanol (7:2:1). Solvent carrying different component were separated into distinct bands on the plates. The distance of the each component that travelled was measured and the Rf value of each component was calculated after derivetization using Anisaldehyde sulphuric acid reagent.

High Performance Liquid Chromatography studies (HPLC):

Agilent 1200 High Pressure Liquid Chromatographic system equipped with prep pump, a Rheodyne injector, Diode Array Detector in combination with Chem32, Chemstation software was used.

Chromatographic conditions:

Mobile phase : Acetonitrile (A): water (B) (30:70)
Mode of flow : Binary
Column : C18-scalar (5 µm, 4.6 × 150 mm) Agilent.
Detector : Diode Array Detector
Flow rate : 1 ml/min
Run time : 20 min
Injection volume : 20 µl

RESULTS AND DISCUSSION:

In the present study considerable differences were observed between different varieties of *Curcuma longa* (Panjab haldi, Ranga and Subha) in their histochemical and chromatographic characters.

Anatomical and Histochemical studies:

The basic anatomical features of the rhizomes were similar in all the varieties of *Curcuma longa* (Panjab haldi, Ranga and Subha) studied. Transections of rhizomes are round in shape and bounded by the epidermis composed of almost rectangular cells (Figs 1 to 3). Unicellular and branched epidermal hairs are present all over the rhizome. Vascular bundles are arranged in wavy patches and broken vascular elements are visible after safranine staining due to the pressure suffered by the underground rhizome.

Histochemical studies between varieties (Panjab haldi, Ranga and Subha) revealed substantial variation in the number and distribution of curcumin cells and starch depositions. Maximum number of curcumin cells were found in the endodermoidal region of *C. longa* var. subha and least among the cortical cells of *C. longa* var. ranga. In all the three varieties number of curcumin cells were more in the endodermoidal region than cortical. The curcumin cells were more evident after staining with boric acid.

The iodine staining of different varieties of *C. longa* revealed substantial variation in the number and distributions starch deposition. Least starch was observed in the variety ranga and maximum in variety subha. Starch depositions were also more in the endodermoidal region.

There are no reports regarding the comparative anatomical studies between different varieties of *C. longa* but Sherlija *et al*. [5], Gogoi *et al.* [7] and Sasikumar *et al.* [10] have reported rhizome anatomical characters of different Curcuma species and reported variation in the morphology of epidermal cell walls, nature and number of epidermal cells per unit area, stomatal frequency and index besides trichome length.

Thin Layer Chromatographic (TLC) Study:

Thin Layer Chromatographic (TLC) Study conducted revealed considerable variation between different varieties of *C. longa* (Fig 4).

UV 366:

Compounds at Rf 0.57, 0.82 and 0.85 were present only in the variety Ranga. Whereas compounds at Rf 0.32, 0.42, 0.47 and 0.51 were present in all the three varieties studied (Table 1).

Table 1. Details of TLC under UV 366.

Rf	Track-1 Punjab	Track-2 Ranga	Track-3 Subha
0.23 (Blue)	1	1	1
0.26 (green)	0	1	1
0.30 (green)	1	0	1
0.32 (Light brown)	1	1	1
0.42 (yellow)	1	1	1
0.47 (yellow)	1	1	1
0.51 (yellow)	1	1	1
0.57 (light blue)	0	1	0
0.82 (Light green)	0	1	0
0.85 (blue)	0	1	0

UV254 and Visible light:

Compounds at Rf 0.47 and 0.51 were present in all the three varieties studied under UV 254 and visible light. Compound at Rf 0.78 was present only in subha. Compounds at Rf 0.17 and 0.23 were absent in Ranga but present in the other two varieties (Tables 2 and 3).

Table 2. Details of TLC under UV 254.

Rf	Track-4 Punjab	Track-5 Ranga	Track-6 Subha
0.10	0	0	1
0.17	1	0	1
0.23	1	0	1
0.41	1	1	1
0.47	1	1	1
0.51	1	1	1
0.78	0	0	1

High Performance Liquid Chromatographic study:

Comparative High Performance Liquid Chromatographic studies between selected varieties of *C. longa* revealed substantial qualitative and quantitative phytochemical variation (Fig 5 to 7). Two major compounds at retention time 2.3 and 2.5 s were present in all the three varieties studied. The compound at retention time 2.3 was maximum (29.73 %) in the variety Panjab haldi and was least (23.87 %) in variety subha. The compound at retention time 2.5 s was also more (19.68 %) in the variety Panjab haldi and was least (12.94 %) in variety subha. With regard to several other compounds at various retention times, qualitative and quantitative variations were observed between different varieties studied.

Table 3. Details of TLC under visible light.

Rf	Track-4 (Punjab)	Track-5 Ranga	Track-6 Subha
0.10	0	0	1
0.17	1	0	1
0.23	1	0	1
0.41	1	1	1
0.47	1	1	1
0.51	1	1	1
0.78	0	0	1

Phytochemical comparison between different varieties under uniform chromatographic conditions are not available but there individual reports. Genetic variability for yield, yield attributes and curcumin content in different varieties of turmeric have been reported by many workers**.**

CONCLUSION:

Turmeric is one of the most precious and powerful plant on earth and is being used as a natural wonder by the ancient people of India. Turmeric is proving beneficial in the treatment of many diseases such as Cancer, HIV/AIDS, Alzheimer's.

Consequently, agents that can modulate multiple cellular targets are now attractive objects of research. Curcumin is one such agent and has potential to treat various diseases. More extensively well controlled clinical trials are now needed to fully investigate its potential. Regardless of all these Curcumin has established as a foodstuff and also a natural medicine because of its low cost, proven chemopreventive and therapeutic potential and potent pharmacological activities of turmeric at *in-vivo* and *in-vitro* which made it a nature's precious drug. Curcumin is rapidly moving from kitchen shelf toward the clinic.

The results of the present study highlights the intraspecific variability of different cultivars of *Curcuma* with regard to morphological, Anatomical and phytochemical characters.

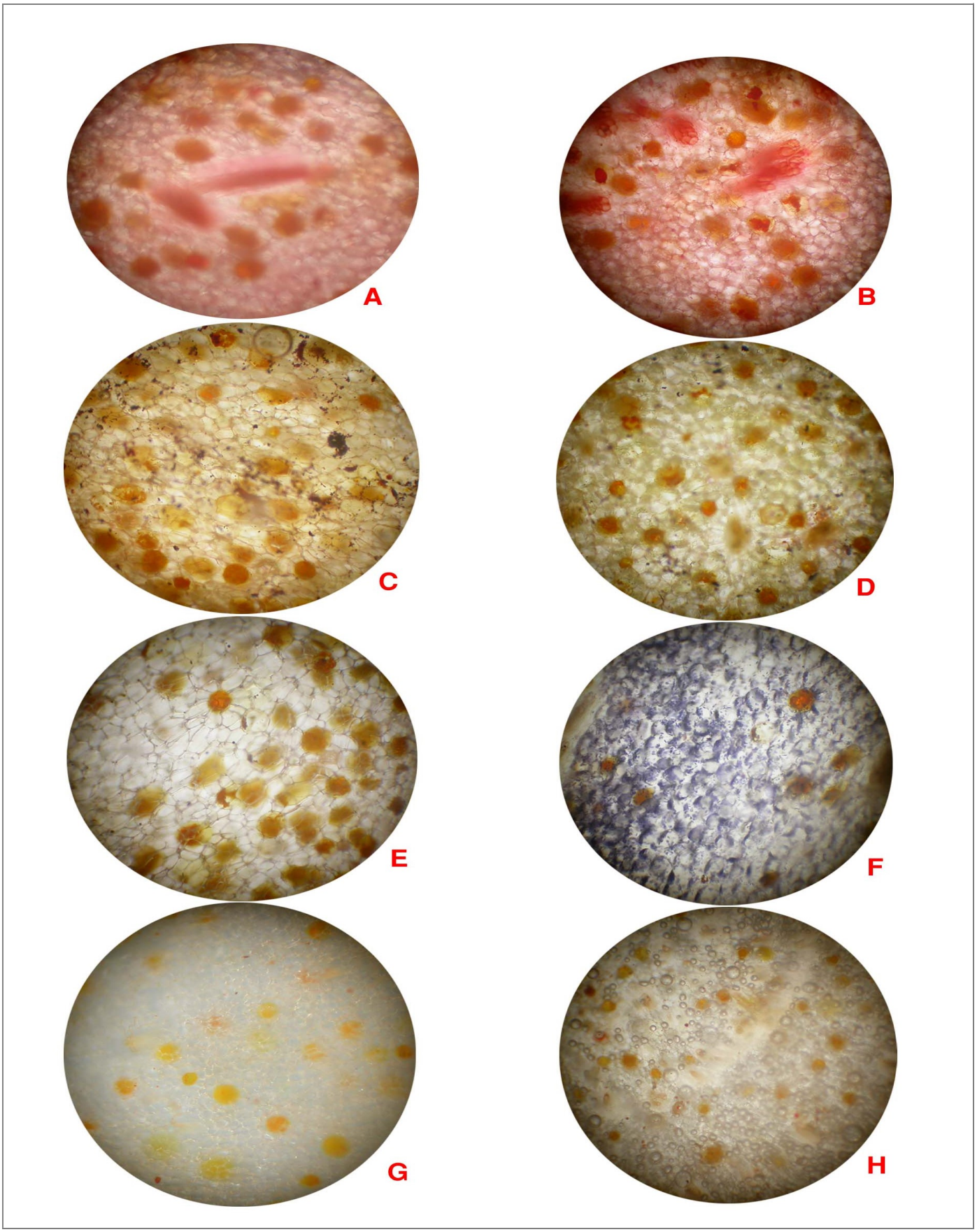

Fig 1. Histochemical studies in *C. longa* var. *Punjab* haldi. A and B are cortical and endochemcial regions after safranine staining. C and D are Cortical and endochemcial regions after sudan red staining. E and F are Cortical and endochemcial regions after iodine staining. G and H are Cortical and endochemcial regions after boric acid staining.

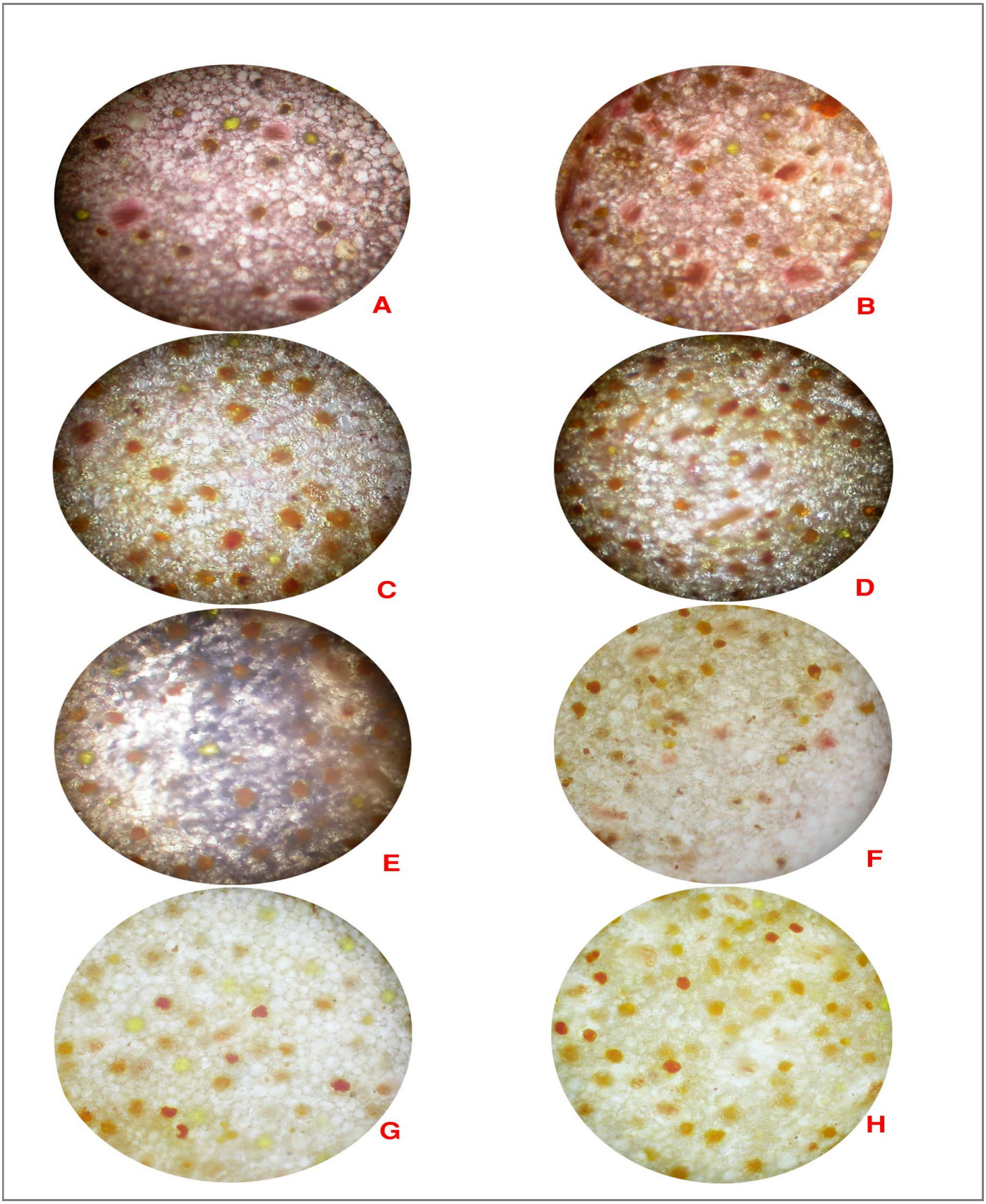

Fig 2. Histochemical studies in *C. longa* var. *ranga.* A and B are cortical and endochemcial regions after safranine staining. C and D are Cortical and endochemcial regions after sudan red staining. E and F are Cortical and endochemcial regions after iodine staining. G and H are Cortical and endochemcial regions after boric acid staining.

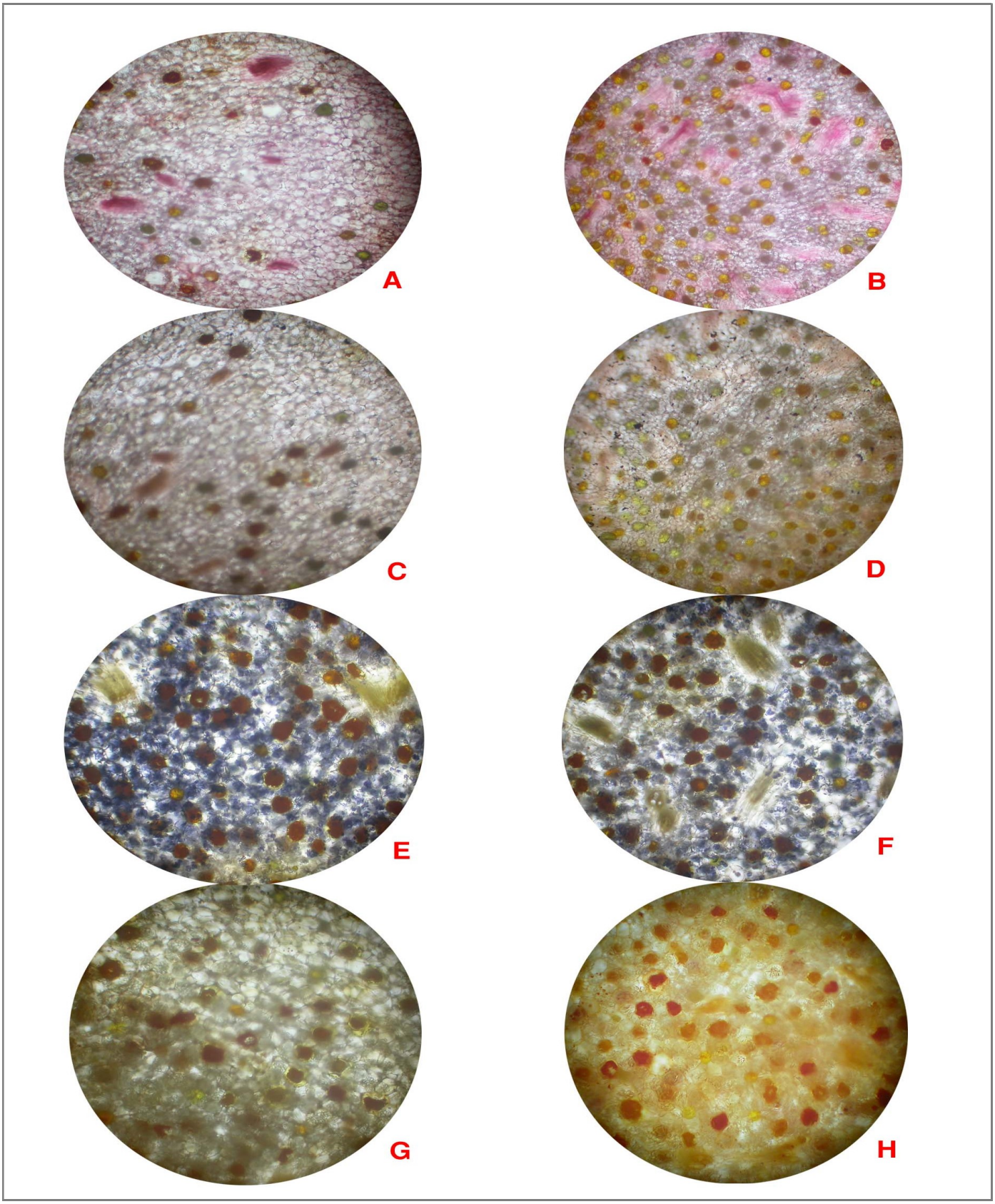

Fig 3. Histochemical studies in *C. longa* var. *subha,* A and B are cortical and endochemcial regions after safranine staining. C and D are Cortical and endochemcial regions after sudan red staining. E and F are Cortical and endochemcial regions after iodine staining. G and H are Cortical and endochemcial regions after boric acid staining.

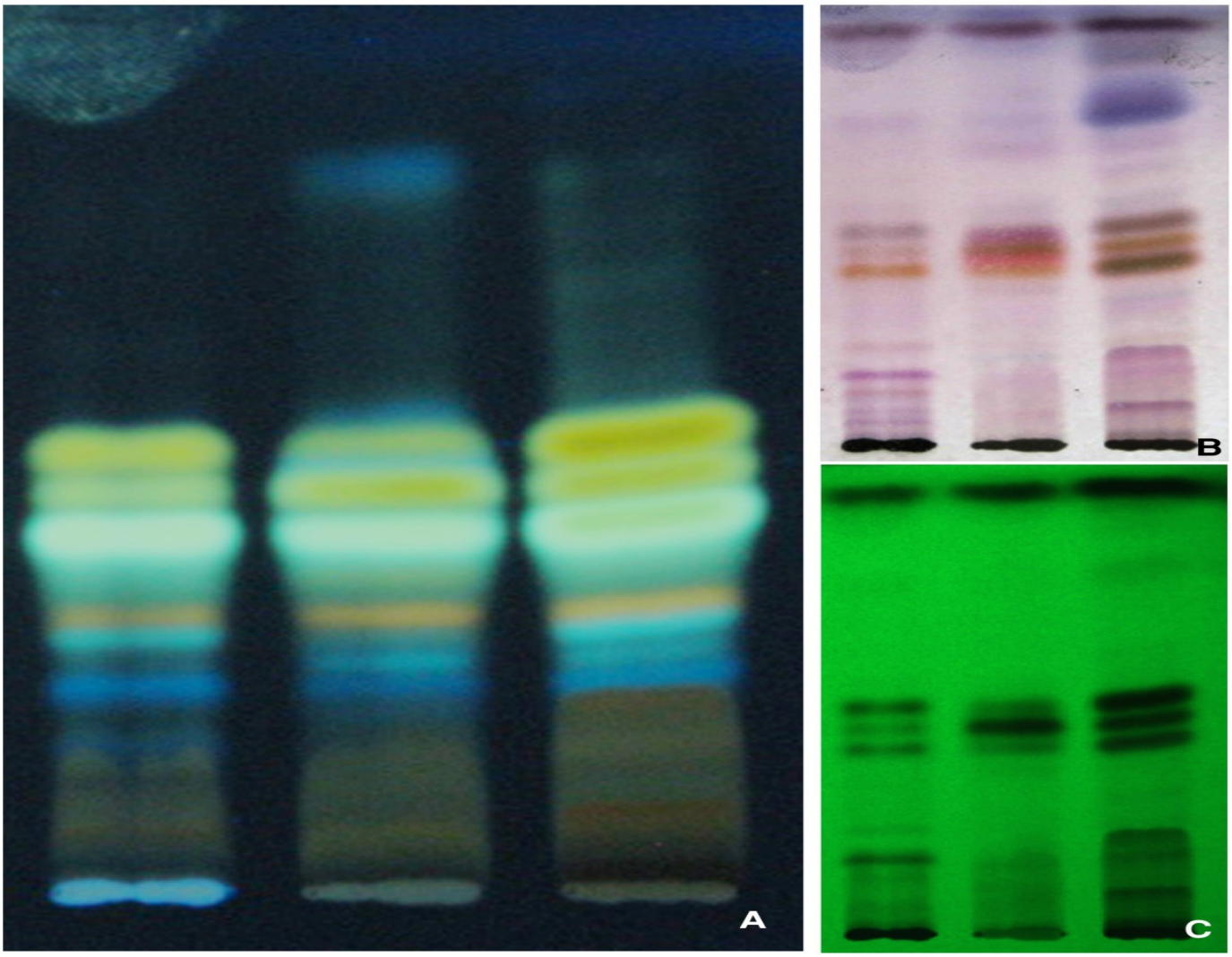

Fig 4. Thin Layer Chromatographic studies in selected varieties of *Curcuma longa.* A. Chromatogram under UV 366. B. Chromatogram under UV 254 and C. Chromatogram under visible light.

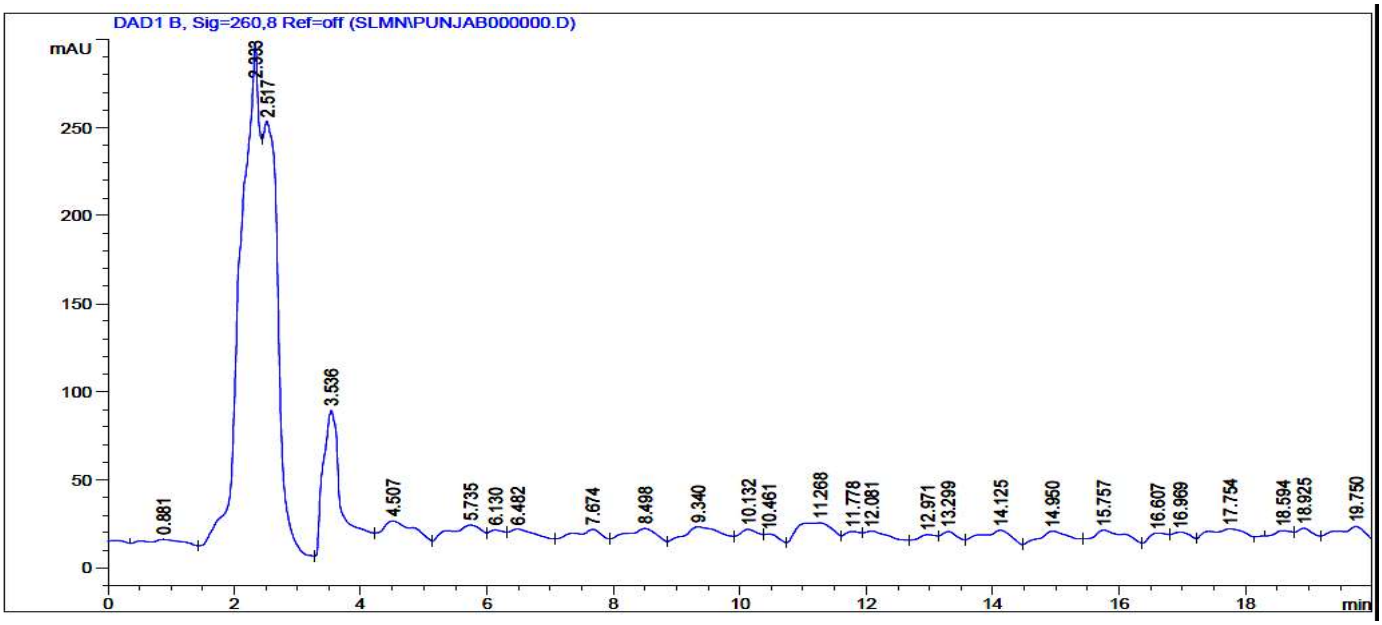

Fig 5. HPLC Chromatogram of *C. longa* var. *Punjab* haldi.

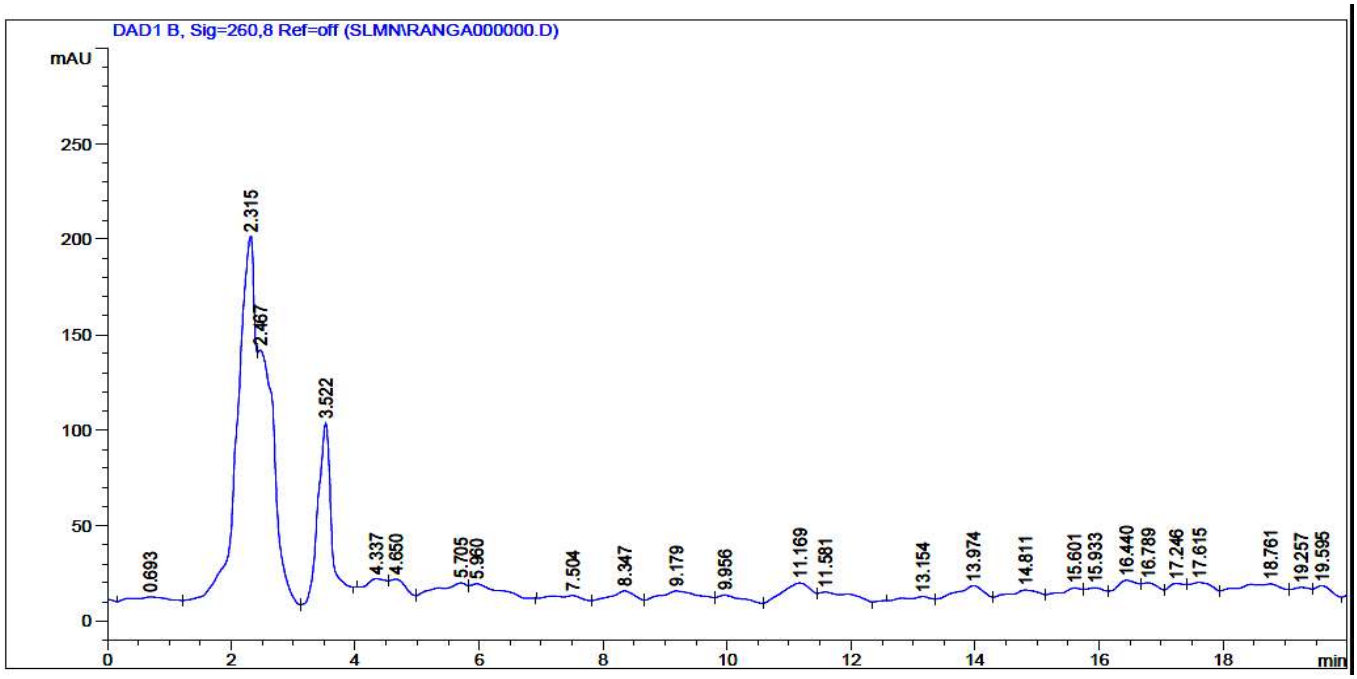

Fig 6. HPLC Chromatogram of *C. longa* var. *ranga*.

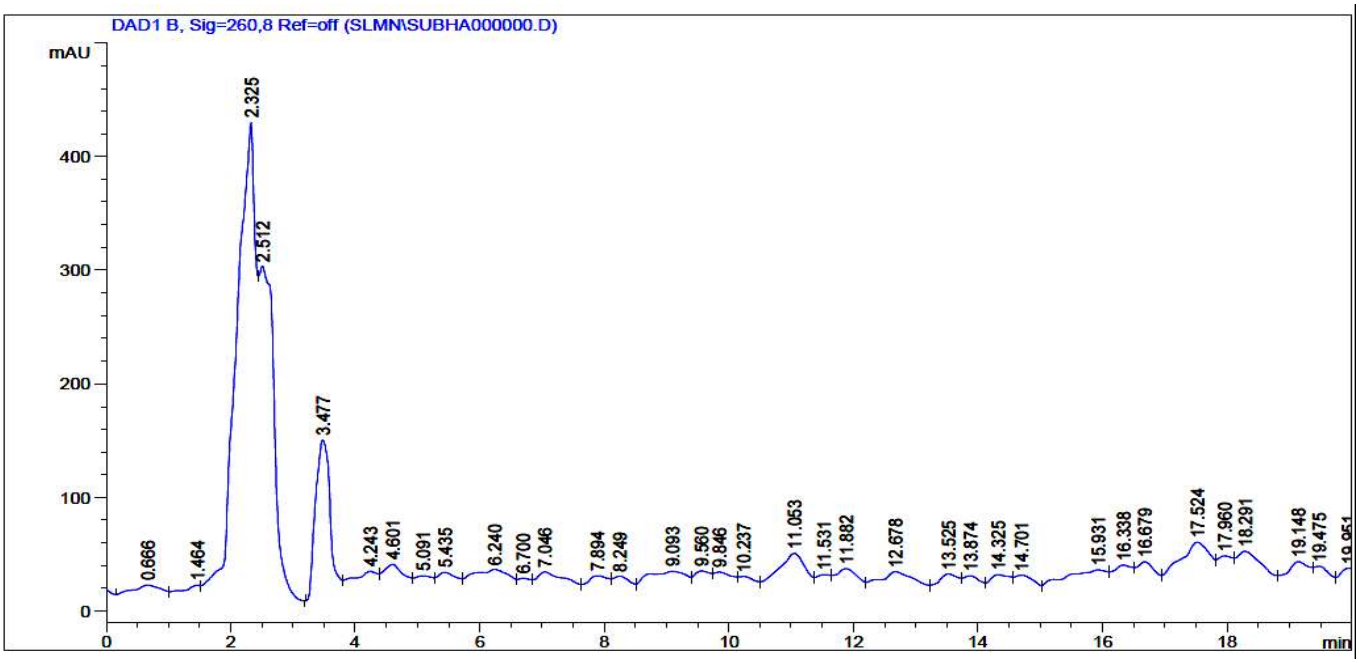

Fig 7. HPLC Chromatogram of *C. longa* var. *subha*.

The variability noted in the above mentioned parameters can be utilized for judicious selection of particular variety and also its taxonomic identification in the absence of conventional identification characters.

ACKNOWLEDGEMENTS:

Authors are grateful to the funding agency RUSA State Project Directorate (MHRD), St. Joseph's College (Autonomus), Devagiri, Calicut, Kerala for providing financial support to complete the present study.

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Conflict of Interest: None
Source of Funding: Nil
Paper Citation: George S, Sebastian DP, Thomas B*. Histochemical and chromatographic characterization of different promising varieties of *Curcuma longa* L. J Pharm Adv Res, 2020; 3(1): 757-766.