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TLC based biochemical diversity analysis among local black turmeric (*Curcuma caesia* Roxb.) germplasm

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Abstract
In present study the 24 black turmeric germplasm were collected from Madhya Pradesh and evaluated for their phytochemical characterization. The rhizome powdered samples were continuously extracted using a soxhlet apparatus with ethanol and 95% ethanol, respectively. Quantification and qualitative evaluation of curcumin was done using Thin layer Chromatography which shows the presence of different curcumin concentration in all black turmeric germplasm. The presence of different spots in the various extract is in line with qualitative analysis which indicated that cucurmin extracts of black turmeric constituted different phytochemical compounds with different Rf values The retention factor (Rf) values and heights for three curcuminoids Curcumin I (Curcumin), Curcumin II (Demethoxycurcumin), and Curcumin III (Bisdemethoxycurcumin) were assessed across 24 plant genotypes and a standard to evaluate the separation efficiency and variability of curcuminoid content among the genotypes. Curcumin I displayed the highest Rf values, indicating it possesses the least polarity of the three compounds, while Curcumin III demonstrated the lowest Rf values, signifying its greater polarity. The control standard values were within the observed range for each curcuminoid, confirming the method's reproducibility. Notable differences in the heights and Rf values among the genotypes highlight the diversity in curcuminoid content.

Keywords: Black turmeric (*curcuma caesia* Roxb.), rhizome, curcumin, thin layer chromatography

Introduction
The black turmeric (*curcuma caesia* Roxb.) is a medicinal and perennial herb that belongs to the family Zingiberaceae and sterile triploid (2n=3x=42). The species is native to northeast and central India, also sparsely found in papi hills of Godavari, root hills of the Himalayas and Northern hill forest of Sikkim (Kumar *et al.*, 2021) [6], and widely distributed in India, Bangladesh, China, Nepal, Malaysia and Thailand, also reported from Java and Myanmar as cultivated species (Liu *et al.*, 2013) [8]. In India black turmeric is found in West Bengal, Madhya Pradesh, Orissa, Chhattisgarh and Uttar Pradesh states.

Black turmeric grows in a variety of tropical climates, from sea level to 1500 meters above mean sea level. It prefers temperatures between 20 and 35 degrees Celsius and receives 1500 mm of rain on average annually (Lakshmi *et al.*, 2018) [7]. The herb's potential medical benefits have led to its significant economic value. All plant parts leaves roots, bulbs, and rhizomes are utilized in the herbal medicine systems of Ayurved Unani, and Siddha (Kumar *et al.*, 2021) [6]. Rhizomatous perennial black turmeric grows erect to a height of 0.5-1.0 m. It features broad, vertical oblong leaves, a huge tuberous rhizome, and a pale yellow bloom with a reddish border (Paliwal *et al.*, 2011) [9]. The bluish-black or buff interior portion of the rhizome is arranged in a circle that is commonly mistaken for the growth ring (Baghel *et al.*, 2013) [2]. Curcumin is a polyphenol and is lipophillic in nature, hence insoluble in water and also in ether but soluble in ethanol, dimethylsulfoxide, and other organic solvents (Aggarwal *et al.*, 2003) [1]

TLC could be used for qualitative and semiquantitative analysis, required less organic solvent, and required less time for analysis. Moreover, optimization of the TLC system could be performed in curcumin analysis for medicinal plant authentication (Kotra *et al.*, 2019) [5].

Materials and Methods

Experimental Material

24 genotypes of black turmeric obtained from Madhya Pradesh and further work done at Department of Molecular Biology and Biotechnology (MBBT), Rajasthan College of Agriculture, MPUAT, Udaipur. The genotypes were studied under G1, G2, G3, G4, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, G16, G17, G18, G19, G20, G21, G22, G23, G24.

Field Studies

Field work will be carried out at Research farm, Hi tech nursery farm, Department of "Horticulture" Rajasthan College of Agriculture, MPUAT, Udaipur, during rabi 2023-24 which is situated at South Eastern part of Rajasthan.

Evaluation of curcumin content in black turmeric genotypes

Qualitative evaluation of curcumin was done by using Thin layer Chromatography (Edward and Kessmann, 1982) [3]. In the present study, total 24 genotypes of black turmeric were used for extraction of curcumin content. Curcumin is quantitatively extracted by refluxing the material in ethanol and is estimated spectrometrically at 425nm (Sadasivam and Manickam, 1992) [11].

Extraction Process

Black turmeric rhizome powder was extracted with 95% ethanol in a soxhlet assembly until all the colouring matter is extracted. The obtained crude extract was concentrated to semisolid brown coloured mass by evaporating ethanol.

Thin layer Chromatography (TLC)

The stationary phase most commonly used is silica gel with different solvent systems including benzene, ethyl acetate, ethanol, chloroform, acetic acid, hexane, and methanol for chromatographic separations (Ravindran *et al.*, 2007 and Gupta *et al.*, 1999) [10, 4]

- TLC of the isolated curcumin was performed on precoated silica gel G plates (Stationary Phase)
- Mixture of chloroform and methanol in the ratio 95:5 will use as solvent system (Mobile Phase).
- Curcumin was used as standard.
- Detection was done by spraying the plate with vanillin-sulphuric acid reagent.

The R_f values for the separated spots were calculated and compared with R_f value of pure curcumin

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Results and Discussion

The chromatographic analysis using Thin Layer Chromatography (TLC) was conducted with various solvent systems on extracts of black turmeric Table 1. The retention factor (Rf) values and heights for three curcuminoids Curcumin I (Curcumin), Curcumin II (Demethoxycurcumin), and Curcumin III (Bisdemethoxycurcumin) were assessed across 24 plant genotypes and a standard to evaluate the separation efficiency and variability of curcuminoid content among the genotypes. Curcumin I displayed the highest Rf values, indicating it possesses the least polarity of the three compounds, while Curcumin III demonstrated the lowest Rf values, signifying its greater polarity.

The control standard values were within the observed range for each curcuminoid, confirming the method's reproducibility. Notable differences in the heights and Rf values among the genotypes highlight the diversity in curcuminoid content. All 24 genotypes consistently showed the highest values for both Curcumin I and III, suggesting a greater concentration or larger molecular presence.

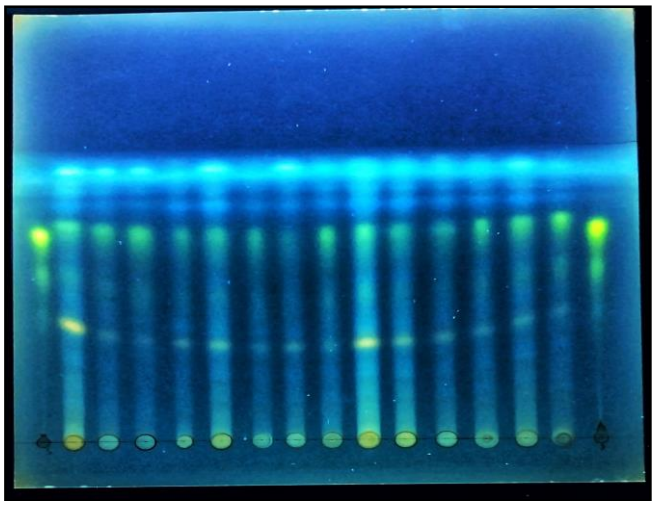

Plate 1: Thin layer chromatography plate of black turmeric genotype from G1 to G15

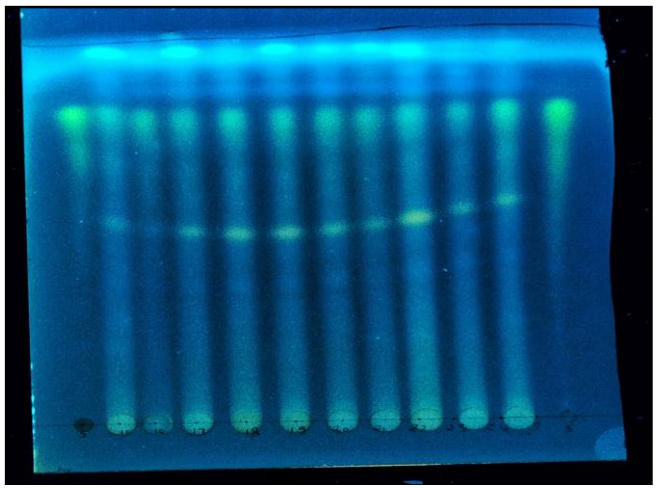

Plate 2: Thin layer chromatography plate of black turmeric genotype from G16 to G24

Table 1: Thin-Layer Chromatography (TLC) Analysis of Curcuminoid Heights and Rf Values in Plant Genotypes

Plant Genotypes	Height (cm	Rf Value (Curcumin I)	Height (cm)	Rf Value (Curcumin II)	Height (cm)	Rf Value (Curcumin III)
STANDARD	8.8	0.72	7.1	0.58	6.2	0.42
1	9	0.73	7	0.57	4.9	0.4
2	8.8	0.72	6.7	0.54	4.5	0.36
3	8.7	0.72	6.5	0.53	4.3	0.35
4	8.6	0.71	6.3	0.51	4.2	0.34
5	8.5	0.7	6.4	0.52	4.1	0.33
6	8.7	0.69	6.1	0.5	4	0.32
7	8.8	0.71	6	0.49	3.9	0.31
8	8.9	0.72	6.2	0.5	4	0.32
9	8.7	0.72	6.3	0.54	4.1	0.33
10	8.6	0.7	6.6	0.52	4	0.32
11	9	0.73	6.4	0.54	4.3	0.35
12	9.1	0.74	6.6	0.55	4.5	0.36
13	9.2	0.75	6.8	0.59	4.9	0.4
14	8.9	0.72	7.2	0.58	5	0.4
15	8.9	0.72	7.1	0.58	5.2	0.42
16	10.3	0.79	8.7	0.67	6.8	0.52
17	10.2	0.79	8.5	0.65	6.6	0.51
18	10.1	0.78	8.4	0.65	6.4	0.49
19	10.2	0.79	8.3	0.64	6.3	0.48
20	10.3	0.79	8.2	0.63	6.2	0.48
21	10.1	0.78	8.1	0.62	6.4	0.49
22	10.3	0.79	8	0.66	6.5	0.5
23	10.3	0.79	8.3	0.68	6.7	0.54
24	10.4	0.8	8.6	0.68	7	0.54

Conclusion

Study evaluation of chromatographic analysis using Thin Layer Chromatography (TLC) was conducted for 24 genotypes of black turmeric. Curcumin I displayed the highest Rf values while Curcumin III demonstrated the lowest Rf values, signifying its greater polarity. All 24 genotypes consistently showed the highest values for both Curcumin I and III, suggesting a greater concentration or larger molecular presence.

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