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Characterization of phenolic compounds of turmeric using TLC

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Abstract
In present study the 25 turmeric germplasm were evaluated for their phytochemical characterization. The total phenolics are secondary byproducts and play a significant role in defense mechanism of plant. Quantification and qualitative evaluation of phenolics was done using Thin layer Chromatography which shows the presence of different phenolic compounds in all turmeric germplasm. The results of TLC of total phenolics from the turmeric germplasm indicated the presence of caffeic acid in all the turmeric germplasm except the germplasm collected from Faizabad. Only one germplasm from Faizabad ie FZ4 show the presence of caffeic acid. The Ferulic acid was found to be present in 5 germplasm of turmeric, o-Caumaric acid also present in 5 germplasm of turmeric and presence of p-caumaric acid is found only in one germplasm of turmeric. Hence the specific turmeric germplasm is found to be suitable for the pharmacological production of these active biomolecules.

Keywords: Turmeric, Caffeic acid, Phytochemical, Thin layer Chromatography

Introduction
Turmeric synthesizes a vast range of secondary metabolites such as curcumin, oleoresin and essential oil with a significant portion consisting of phenolic compounds and flavonoid compounds (Crozier *et al*., 2006) [5]. These phytochemicals are structurally diverse, and many are distributed among a very limited number of species within the plant kingdom. This character allows them to act as bio diagnostic markers in chemotaxonomic studies. Secondary metabolites, other than providing plants with unique survival Oran adaptive strategies, are of commercial significance to humankind.
Curcumin a yellow pigment is a phenolic compound and a major phytochemical constituent of Curcuma species, has been linked with suppression of inflammation; angiogenesis; tumorigenesis; diabetes; diseases of the cardiovascular, pulmonary systems, skin and liver (Anand *et al*., 2008) [1]. It has also shown of wide spectrum of chemo preventive, antioxidant and anti-tumor properties (Kaur *et al*., 2010) [10].
The most imperative fraction of turmeric is named as curcuminoids. Among curcuminoids, Curcumin is mostly accountable for all biological activities of turmeric. TLC fingerprint is a simple and rapid identification method for authentication of *C. longa* rhizome. The study revealed different analytical parameters of the crude drug which will be useful in identification of genuine drug and control of adulterations. Thin layer chromatography (TLC) are routinely used as valuable tool for qualitative determination of small amounts of compounds. Selection of a suitable stationary phase and solvent depends on the classes of polyphenols to be examined. TLC is still in common use for preparative separations (Lee and Widmer 1996) [11] and as a rapid low-cost screening method for determining the secondary metabolites in plants (Fernández de Simón *et al*., 1992) [7].
TLC applications for quantitative analysis of phenolic acids (Azar *et al*. 1987, Agbor-Egbe and Rickard, 1990) [2] are usually carried out using normal phase chromatography on cellulose or silica layers and separating the compounds with a mixture of hydrocarbon carriers (toluene, dioxane or benzene) and polar organic modifiers (acetone, butanol, ethanol or acetic acid). Gocan and Cimpan (2004) [8] reviewed the use of TLC to analyze the medicinal plants and quantify flavanoids, coumarins, saponins, alsaloids and other classes of compounds by photodensitometery.

Materials and Methods
Extraction of Phenolic Compounds
For the present study the 25 germplasm of turmeric namely FZ 1, FZ 2, FZ 3, FZ 4, FZ 5, FZ 6, JH 1, JH 2, JH 3, JH 4, JH 5, JH 6, JH 7, JH 8, JH 9, JH 10, JH 11, JH 12, JH 13, JH

14, JH 15, JH 16, JH 17, Vallabh Sarad and Vallabhpriya were collected from Faizabad, Jhansi and Department of Horticulture, SVPUA&T, Meerut. Total phenolic acids from the leaves of turmeric germplasm were extracted by using the standard method of Bray and Thorpe (1954) [4] with some modification. 0.5 gm of leaf sample was taken and homogenized in 10 ml 75% methanol in a mortar pestle and left for overnight extraction. The samples were then centrifuged in a cooling centrifuge (CPR 24, Remi, India) at 9000 rpm using a fixed angle rotor R-242 (Remi, India) for 25 min at room temperature. The supernatant was collected and the volume was measured for further analysis. The pellet containing the cell debris was discarded.

Quantitative determination of total phenolicsin turmeric germplasm

For quantitative estimation of total phenolic compounds extracted from leaves of 25 germplasm of turmeric take 0.5 ml of the extract and mixed with 8.5 ml double distilled water and 0.5 ml follin cio-calteu's reagent (This was diluted 1:2 with double distilled water before use). The reaction mixture was incubated for 3 min and then 1 ml of Na_2CO_3 (25%W/V) was added to stop the reaction. The reaction mixture was left at room temperature for 1 hour and the absorbance was recorded at 725 nm.

Qualitative analysis of total phenolics

Separation of polyphenols was done by Thin Layer Chromatography (TLC) using TLC plate coated with silica 20 X 20(Merck, Darmstadt, Germany). 8 µl of plant methanolic extract of turmeric germplasm and standard of phenolic acids were separately applied on thick silica gel. The plate was run using different solvent mixture (Table 2). After development plates were removed and dried and spots were visualized in Ultraviolet light and sprayed with different spraying spraying agent according to the solvent used. Various spots on the plates were marked and their Rf values were calculated using the following formula:

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

Result

Quantitative evaluation of total phenolic compounds of turmeric

For the phytochemical characterization of secondary metabolite of turmeric germplasm was analysed quantitatively and qualitatively. The total phenolic content in turmeric extracts was determined by using Folin-Ciocalteu (FC) colourimetric method based on oxidation-reduction reaction (Waterhouse 2002). The reaction mixture absorbance was measured thrice at 725 nm against blank and calculated the average absorbance values. The total phenolics in terms of OD values are shown in Table 2. The highest amount of total phenolics was 1.699 OD units/gmf found to be in genotype Fz-4 whereas the minimum amount of total phenolics was 0.376OD units/gfw in Fz-5 genotype of turmeric. However the genotype Fz1, Jh2, Jh6, Jh8 and Vallabh Sharad shows good amount to total phenolics whereas Fz5, Jh12 and Jh16 shows lesser amount of total phenolic content. In terms of total phenolics the FZ-4 genotype of turmeric is seems to be good for survival under harsh environmental condition

Qualitative evaluation of total phenolic compounds of turmeric

The results of preliminary evaluation of total phenolics from 25 germplasm of turmeric were further evaluated for their quality analysis using Thin Layer chromatography (TLC). It is the most common method used for detection and separation of phenolic acid from plant extract and was performed after Edward and Kessmann, (1982) [6] as described earlier.

A total of five solvent systems and different spraying reagents were initially analyzed with available standards of phenolic acids viz. caffeic acid, p-coumaric acid, o-coumaric acid, sinapic acid and ferulic acid. The majority of phenolic compounds can be detected on chromatogram by their colour of fluorescence in UV light (Table 3). It is however usually preferable to detect them by means of a more specific reagent the best of which is $FeCl_3$. The caffeic acid, p-coumaric acid, o-coumaric acid, sinapic acid and ferulic acid gave blackish green, orange, yellow, purplish pink and redish brown colour respectively. Conversely the colours of standard phenolic acids developed by using methanolic KOH were not clearly distinguished and gave very close shades of yellow (Figure 1). Hence the $FeCl_3$ was preferred for further characterization of phenolic acids in chromatograms.

From various solvent systems tested, the standard phenolic acids were best separated in solvent system-1, which comprised of benzene, acetic acid and water (37:45:18). The rest of solvent system showed comparatively lower level of separation and were not able to separate them on the basis of their retention factor (R_f). R_f value characterized the spots of standard phenolic acid after treating them with $FeCl_3$. The R_f values for different phenolic acid standards in different solvent systems are presented in Table 4. The results showed that in solvent system I, the R_f values for caffeic acid, p-coumaric acid, o-coumaric, sinapic acid, ferulic acid were 0.36, 0.54, 0.50, 0.59 and 0.68 respectively. The same in solvent system II changed to 0.84, 0.88, 0.84, 0.88, and 0.90; in solvent system III changed to 0.97, 0.97, 1.0, 0.95, and 1.0; with solvent system IV changed to 0.87, 0.90, 0.85, 0.87 and 0.87 respectively.

The results of TLC of total phenolics from the turmeric germplasm indicated the presence of diversity in Phenolic acid in all studied turmeric germplasm. On the basis of resolution properties in different solvents, the samples of turmeric were allowed to run in solvent system I. The TLC plates were visualized under UV light (Figure 2) and treated with $Fecl_3$ (Figure 3). After treating with $FeCl_3$, a spot of green, blackish green, brown and yellowish brown color was clearly observed in the samples of turmeric. The green color spot could not be identified as it did not match with the available standards. The blackish green color spot was identified as caffeic acid which was present in all the turmeric germplasm except the germplasm collected from Faizabad. The caffeic was found to be present in only one germplasm of Faizabad ie. FZ6. The turmeric germplasm from Jh10, Jh11, Jh12, Jh13 and Jh14 shows the presence of significant amount of Ferulic acid in the form of reddish brown spot which was absent in the rest of the turmeric germplasm. The turmeric germplasm from Jh15, Jh16, Jh17, Vallabh Sharad and Vallabh Priya shows the presence of significant amount of o-Caumaric acid in the form of yellow spot which was absent in the rest of the turmeric germplasm. The turmeric germplasm from Jh15 only shows the presence of p-caumaric acid in the form of orange spot which was absent in the rest of the turmeric germplasm. This was further confirmed with the rf

value of standard phenolic acids. The Rf value was calculate using the standard formula for each intense spot obtained (Table 6). Lower polarity compounds are carried throughout the mobile phase, resulting in high RF values. Higher polarity compounds are generally retained, resulting in lower RF values. The colour and R_f value matched with that of standard and indicated the presence of caffeic acid, ferulic acid, o-caumaric acid and p-caumaric acid in turmeric germplasm.

For the phytochemical profiling the total phenolics were isolated and analysed quantitatively and qualitatively. The genotype Fz1, Jh2, Jh6, Jh8 and Vallabh Sharad shows good amount to total phenolics whereas Fz5, Jh12 and Jh16 shows lesser amount of total phenolic content. Although in methanol extract presence of phenolic content in all germplasm was observed. Similar result was observed by Devkota *et al*., (2015) and Nisar *et al.,* (2015). Ismail *et al.,* (2012) also noticed the presence of these phenolic acids in turmeric germplasm during their study. Caffeic acid is regarded as the most common of phenolic compounds distributed in the plant flora followed by chlorogenic acid known to cause allergic dermatitis among humans (Kar, 2007).

Phenolics essentially represent a host of natural antioxidants. The most important role may be in plant defence against pathogens, thus are applied in the control of human pathogenic infections (Puupponen- Pimiä *et al*., 2008) [14]. The phenolics are known to be produced in higher amounts in harsh environmental conditions with strong light intensities (Hakkinen, 2000; Roger, 2001) [9]. In any case, the variations in the amount of total phenolics are known to be influenced by various factors such as climatic conditions, geographic position and vegetational stage of plants and leaves (Maksimovic *et al*., 2005) [12]. Brandsteterova and Caniova (2002) also observed the different concentration of phenolic acid in different plants and suggested that each plant sample could be specific enough for the presence of different phenolic acids and their quantity.

Table 1: Solvent systems used for TLC separation of phenolic compounds of turmeric

S.no	Solvent system	Concentration	Sprayer
1	Benzene: acetic acid: water	37:45:18	UV, 2% $FeCl_3$in water, Methanolic KOH
2	Ethyl acetate: toluene: acetic acid	50:40:20	UV, 2% $FeCl_3$in water, Methanolic KOH
3	Ethyl acetate: formic acid: acetic acid: water	100:11:11:27	UV, 2% $FeCl_3$in water, Methanolic KOH
4	Ethyl acetate: acetic acid	80:20	UV, 2% $FeCl_3$in water, Methanolic KOH
5	Ethyl acetate: methanol	80:20	UV, 2% $FeCl_3$in water, Methanolic KOH

Table 2: Total phenolic content of 25 germplasm of turmeric

S.NO	Name of turmeric Genotype	Optical Density 725nm(OD)	S.NO	Name of turmeric Genotype	Optical Density 725nm(OD)
1.	JHANSI 1	0.691	14.	JHANSI 14	0.711
2.	JHANSI 2	1.138	15.	JHANSI 15	0.154
3.	JHANSI 3	0.952	16.	JHANSI 16	0.493
4.	JHANSI 4	0.873	17.	JHANSI 17	0.915
5.	JHANSI 5	0.953	18.	FAIZABAD 1	1.132
6.	JHANSI 6	1.171	19.	FAIZABAD 2	0.807
7.	JHANSI 7	0.665	20.	FAIZABAD 3	0.907
8.	JHANSI 8	1.453	21.	FAIZABAD 4	1.699
9.	JHANSI 9	0.782	22.	FAIZABAD 5	0.376
10.	JHANSI 10	0.772	23.	FAIZABAD 6	0.815
11.	JHANSI 11	0.643	24.	VALLABH SARAD	1.181
12.	JHANSI 12	0.558	25.	VALLABH PRIYA	0.618
13.	JHANSI 13	0.938			

Table 3: Tests for the determination of phenolic acids on chromatograms

S. no	Phenolic acid	UV	FeCl3	Methanolic KOH
1	Caffeic acid	Blue	Blackish green	Greenish yellow
2	p-coumaric acid	Blue	Orange	Light yellow
3	o-coumaric acid	Blue	Yellow	Light yellow
4	Sinapic acid	Blue	Purple	Pale yellow
5	Ferulic acid	Blue	Reddish brown	Brownish yellow

Table 4: The R_f values of different phenolic acid standards in different solvent systems

S. no.	Phenolic acids	Solvent system 1	Solvent system 2	Solvent system 3	Solvent system 4	Solvent system 5
1	Caffeic acid	0.36	0.84	0.97	0.87	0.91
2	p-coumaric acid	0.54	0.88	0.97	0.90	0.88
3	o-coumaric acid	0.50	0.84	1.0	0.85	0.86
4	Sinapic acid	0.59	0.88	0.95	0.87	0.86
5	Ferulic acid	0.68	0.90	1.0	087	0.88

Note: Solvent system 1(Benzene:acetic acid:water=37:45:18),
Solvent system 2 (Ethyl acetate:toulene:acetic acid=50:40:20)
Solvent system 3 (Ethyl acetate:formic acid:acetic acid:water acid=100:11:11:27)
Solvent system 4 (Ethyl acetate:acetic acid=80:20)
Solvent system 5 (Ethyl acetate:methanol=80:20)

Table 5: Rf value of different spots observed in TLC from 25 genotypes of turmeric

S.No	Varieties name	Rf Value	Appeared color
1	FAIZABAD1	0.310	Light Yellow
2	FAIZABAD2	0.446	Light Yellow
3	FAIZABAD3	0.425	Reddish Brown
4	FAIZABAD4	0.382	Reddish Brown
5	FAIZABAD5	0.372	Blackish Green
6	FAIZABAD6	0.140	Purple
7	JHANSI 1	0.736	Orange
8	JHANSI 2	0.719	Orange
9	JHANSI 3	0.631	Light Yellow
10	JHANSI 4	0.614	Light Yellow
11	JHANSI 5	0.758	Blackish Green
12	JHANSI 6	0.750	Blackish Green
13	JHANSI 7	0.767	Blackish Green
14	JHANSI 8	0.758	Blackish Green
15	JHANSI 9	0.750	Blackish Green
16	JHANSI 10	0.817	Reddish brown
17	JHANSI 11	0.826	Reddish brown
18	JHANSI 12	0.817	Reddish brown
19	JHANSI 13	0.808	Reddish brown
20	JHANSI 14	0.791	Reddish brown
21	JHANSI 15	0.801	Orange
22	JHANSI 16	0.793	Orange
23	JHANSI 17	0.810	Greenish yellow
24	VALLABH SARAD	0.801	Greenish yellow
25	VALLABH PRIYA	0.784	Greenish yellow

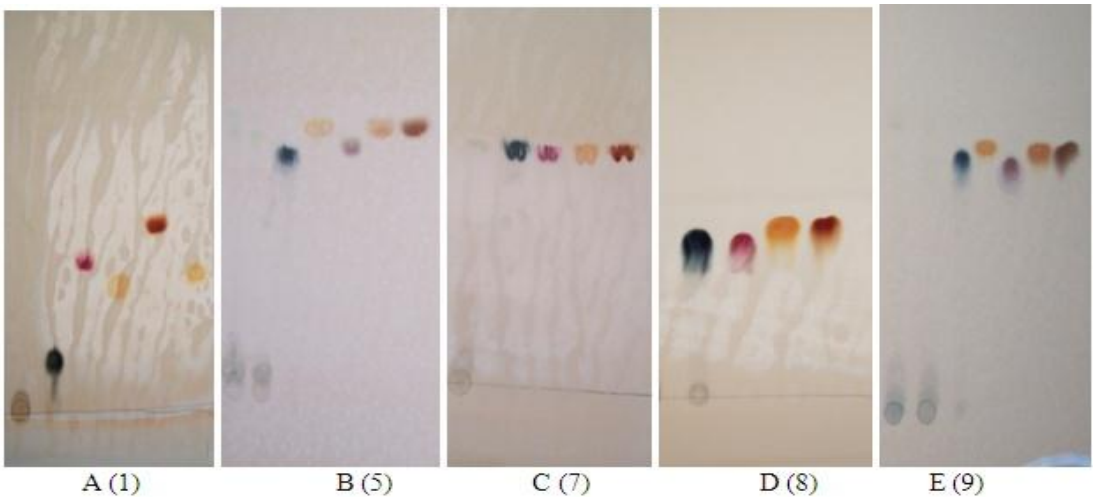

Fig 1: Separation of standard phenolic acids in different solvent systems. Spots in sequence are of caffeic acid, o-coumaric acid, sinapic acid, p-coumaric acid and ferulic acid

Fig 2: Separation of phenolic acids of 25 germplasm of turmeric using TLC and visualization under UV light

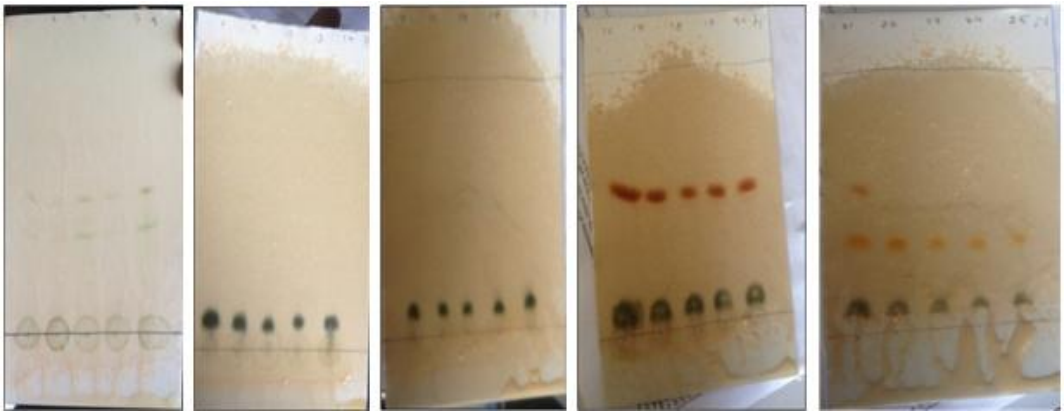

Fig 3: Separation of phenolic acids of 25 germplasm of turmeric using TLC and visualized with $FeCl_3$

Conclusions

The varietal description for identification and authentication of crop varieties and knowledge of the genetic variation in the essential for in order to conserve the genetic resources and get consistent variability, genetic studies involving morphological and molecular markers are used to detect relationship and genetic variation among germplasm. In terms of total phenolics the FZ-4 genotype of turmeric is seems to be good for survival under harsh environmental condition.

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