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Research article

STUDY OF CURCUMIN CONTENT AND ADULTERANTS PRESENT IN DIFFERENT MARKETED BRANDS OF TURMERIC POWDER

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Abstract:

Turmeric or Haldi are the rhizomes of Curcuma longa Linn., which are extensively used by traditional healer or medical practitioner for its curing properties. In Indian medicine system i.e. Siddha, Unani and Ayurveda comprises of many medicines containing turmeric. Moreover it is widely used in food preparations to give flavor and freshness. Curcumin is the main active ingredient, that is well known for its anti inflammatory, anti cancer, anti ischemic, antioxidant properties. But due to adulteration the actual therapeutic effect reduces or may become toxic to human health.

The aim of this study is to determine the curcumin content and presence of adulterants in different marketed branded or unbranded turmeric powder. The study was carried out by collecting various samples. Various chemical tests were performed and through visual inspection the adulteration and qualitative differences were determined between them. In view of the importance an extensive literature survey was carried out in journal and online databases. For the determination of curcumin in different samples of turmeric powder HPTLC, a sensitive, efficient and precise method was used. This study will reveal various analytical methods to determine adulteration and it will be a simple, accurate, sensitive and cost effective method for the quantitative estimation of curcumin.

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Furthermore, this study will bring awareness on adulteration of food and various handy methods for the detection of food adulteration.

Keywords*: Curcuma longa Linn.*, Haldi, curcumin, food adulteration, HPTLC.

Introduction:

Turmeric (*Curcuma longa* Linn.) is the ancient and sacred spice of India known as 'Indian saffron', an important commercial spice crop grown in India [1]. It is used in diversified forms as a condiment, flavoring and coloring agent and as a principal ingredient in Indian culinary as curry powder. It has anti cancer and anti viral activities and hence finds use in the drug industry and cosmetic industry. Turmeric is the dried rhizome of *Curcuma longa* L., a herbaceous perennial belonging to the family Zingiberaceae [2-6]. The main active ingredient of turmeric is curcumin (a yellow-orange dye). It is reported curcumin concentrations in turmeric ranges from 0.3% to 8.6% [7]. The varied biological properties of curcumin and lack of toxicity even when administered at higher doses makes it attractive to explore its use. According to Michael Moriarty, "The ancient Hawaiians used this herb for prevention and treatment of sinus disease, ear infection, gastrointestinal ulcers etc." [8]. Turmeric is eaten as a food both raw and cooked for flavoring in curries. The amazing health benefits include heal wounds, improve skin health, protect cognitive abilities and ease menstrual difficulties. It is known to have some medicinal properties [9-17] like anti inflammatory [18-21], anti diabetic, antimicrobial, anti cancerous [1,22,27-29], anti septic, digestive [30], improve anti oxidant properties[20,31-34], hypolipidemic [35-37] etc.

Adulterants are substances that are used or added to lower the quality of product. Simply it can be said as debasement of an article [38-41]. Adulterants can be categorized into three category and they are- Intentional (eg: sand, marbles, chips, stone, chalk, powder, harmful color etc.), Incidental (eg: pesticides, residues, etc.) and Metallic (Arsenic from pesticides, lead from water etc.) [38]. As turmeric is commonly sold by weight so the potential of using powders of toxic and cheaper agents with similar color as adulterant is maximum. Normally lead oxide and metanil yellow are used as adulterants [42]. The objective of this experiment is to

determine curcumin content and the presence of adulterant in different marketed brands of turmeric powder.

Materials and Methods:

a. Collection of sample: The samples of turmeric powder were collected from the local market of Dibrugarh, Assam. They are collected based on cost, locally produced and marketed varieties and their use in Dibrugarh University hostel mess as a student of Dibrugarh University. After visiting different market place and nearby areas, different samples are collected and performed out the laboratory test. Different samples are-
Sample 1: It is the local product of Dhemaji; manufactured and marketed by local farmer of Dhemaji District.

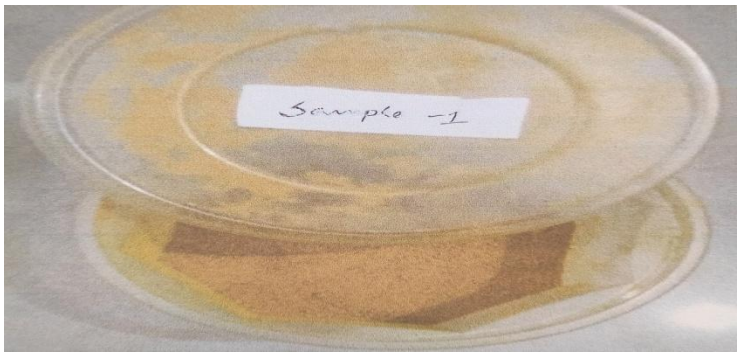

Fig 1: Sample 1

Sample 2: This is the local product of Dibrugarh, manufactured and marketed by local farmer of Dibrugarh.

Fig 2: Sample 2

Sample 3: Sigma's HALDI POWDER (Everyday Spices), a product of Spice industries Pvt. Ltd., Jail road, Jorhat- 785010 (Assam).

Fig 3: Sample 3

Sample 4: "EVEREST Turmeric" (Garden fresh, golden yellow Turmeric powder), manufactured and marketed by S. Narendra Kumar & Co. Regd. Office: krushal Centre, G.M. Road, Mumbai 400089.

Fig 4: Sample 4

Sample 5: “jmc spices Haldi Powder” manufactured and marketed by Jagdamba Masala Company.

Fig 5: Sample 5

b. Methodology: Protocol involves finding out the common adulterants and determination of curcumin content.
Chemicals and reagents:
For adulteration test: Propanolol, conc. HCl, sulphuric acid, distilled water, petroleum ether.
For the test of curcumin content: Sulphuric acid, sodium hydroxide, boric acid, acetic anhydride, alkali, conc. HCl.

Table 1: Chemical test for identification of common adulterants [38,42]:

Sl no	Test	Observation	Interference
1	Test for Metanil Yellow: 0.1g of turmeric sample was taken in a test tube and added 1ml of propanolol. Then 5-10 drops of HCl was added and color of the sample was observed.	Appearance of pink coloration indicates presence of Metanil yellow.	Dark pink color appeared in all the samples.
2	Test for Artificial color: Spice sample was extracted with petroleum ether and added 13N of sulphuric acid solution.	Red color which persists upon addition of distilled water indicates presence of artificial color. If it disappears then it is free of color adulteration.	Dark red color appeared in all the samples.
3	Test for Yellow Lead Salt: To a sample of turmeric powder conc. HCl was added.	Appearance of magenda color indicates the presence of yellow lead salt.	Magenda color appeared in sample 1,2 & 5 and sample 3 & 4 showed brownish color.
4	Test for Chalk or Yellow soap stone powder: Small quantity of	Effervescence indicates the presence of chalk or yellow soap stone.	None of the sample have shown

	turmeric powder was taken in a test tube containing small quantity of water. Then few drops of conc. HCl were added.		effervescence .

Instrumental methods for identification of Adulterants (HPTLC):

The most advanced form of instrumental TLC is commonly called HPTLC (High Performance Thin Layer Chromatography, but the term does not simply imply instrumental TLC on special high performance layers.

The main principle involved is that HPTLC have similar approach and employ the same physical principle of TLC i.e. adsorption chromatography. The mobile phase solvent flows through because of capillary action. The components move according to their affinities towards the adsorbent. Accuracy and Precision of quantification is high because samples and standards are chromatographed and measured under the identical experimental conditions on a single TLC/HPTLC plate. Its advantages are-

- Technically it is simple to learn and operate.
- Several analysts work simultaneously on the system.
- Lower analysis time and less cost per analysis.
- Low maintenance cost.

Chemical test for identification of curcumin:

Curcumin is crystalline in nature and orange yellow in color . It is soluble in alcohol, glacial acetic acid and insoluble in cold water . Melting point of curcumin is 185℃. various chemical tests for curcumin determination are described below.

Table 2: Chemical tests for the determination of curcumin

Sl n	Chemical test	Observation	Interference

o			
1	The sample powder is dissolved in sulphuric acid and then 0.1N sodium hydroxide is added.	Yields red color.	Red color appeared in all the samples.
2	Powdered sample was treated with sulphuric acid.	Gives crimson color.	Red color appeared in all the samples.
3	Aqueous solution of turmeric was mixed with boric acid and then alkali was added.	On addition of boric acid gives radish brown color which on addition of alkali changes to greenish blue.	Red color appeared in all the samples.
4	Sample was treated with acetic anhydride and conc. HCl.	It gives violet color and under uv red fluorescence is observed.	Red color appeared in all the samples.

Preparation of test solution:

200mg of each sample powder was suspended in 3mL of methanol separately. The mixtures were sonicated for 10 minutes. The resulted solutions were centrifuged and the supernatant was used as test solution.

Preparation of reference solution:

The standard curcumin solution was prepared in methanol to a concentration of 1mg/ml. The developing solvent was toluene: glacial acetic acid in the ratio of 4:1. The developing distance should be maintained as 70mm. the standard time is 20 minutes with saturation pad.

Validation procedure:

A series of spots of curcumin were applied to determine the linearity range of standard. To obtain different amount of standard curcumin per spot different volumes of standard i.e. 0.3µl-3 µl were applied. Then the plates

were scanned. Linearity curves were prepared between area and height vs amount of curcumin per spot [43].

Instrumentation and chromatographic conditions:

All the spots were used as bands and the band lengths were kept as 8 mm. 11.4 mm was kept as distance between the tracks using Camag HPTLC applicator Linomat V. Precoated silica gel 60F254 plates (20cm × 10cm) was used as stationary phase. Toluene: Glacial acetic acid in the ratio 4:1 used as mobile phase. In Camag twin the plate was developed through chamber. Ten minutes were kept as chamber saturation time for mobile phase. 8cm was taken as the length to run chromatogram. After completion of the development the TLC plates were air dried. Then on a Camag TLC scanner the densito metric analysis was carried out at 427nm [43].

Instrumental determination:

HPTLC specification:

Brand name: CAMAG world leader in planer chromatography

Software: VisionCATS

TLC plates: Silica gel 60F254, TLC alumina plate (MARC)

Sample spotter: CAMAG LINOMAT-V (semi-automatic sample spotter)

Syringe: 100µL volume, HAMILTON

Developing chamber: AMAG glass twinthrough chamber

Densitometer: CAMAG TLC scanner IV linked to VisionCATS- 2.3 (Service pack 1)

Solvent system: toluene: Glacial acetic acid= 4:1 ratio.

Procedure:

First of all the coated stationary phase plates are activated. Then samples are applied on bottom edge of the plate using an auto-sampler. The plate was developed by placing in a HPTLC development chamber made up of glass. When mobile phase moves up the layer of stationary phase, separation of compounds takes place. Normally a distance of about 5 to 15 cm is allowed and in this experiment it moved upto 7cm. the separated spots are visualized using densitometry scanners. The analyses are done qualitatively using software.

Results and discussions:

The results of chemical tests for adulterants showed that all the samples; sample 1,2,3,4,5 contained Metanil yellow and artificial color. Yellow lead is present in sample 1,2 and 5. The entire samples have shown positive result for the chemical test for identification of curcumin.

From the validation procedure carried out to determine the amount of curcumin the following results are obtained.

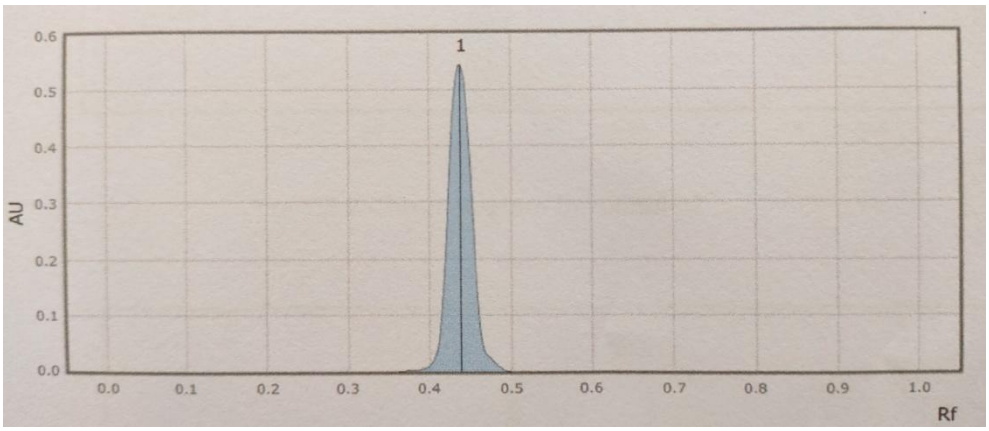

Fig 6: Chromatogram for standard curcumin

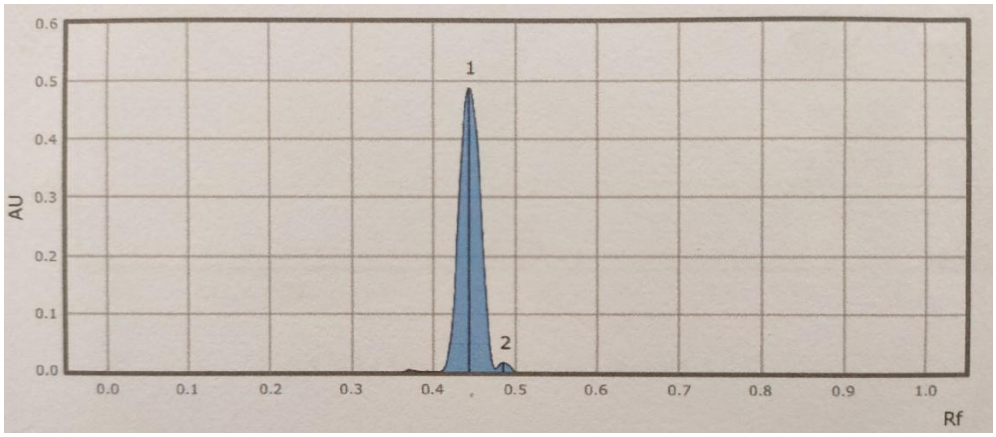

Fig 7: Chromatogram for sample 1.

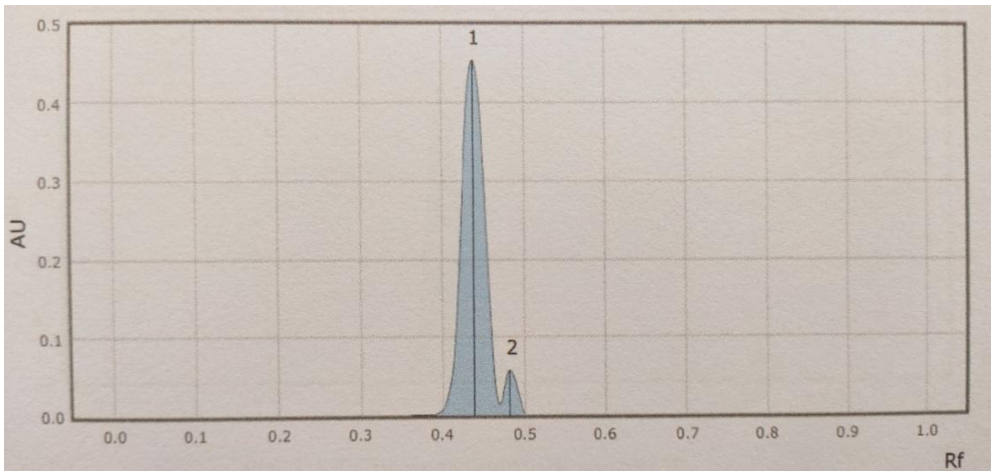

Fig 8: Chromatogram for sample 2.

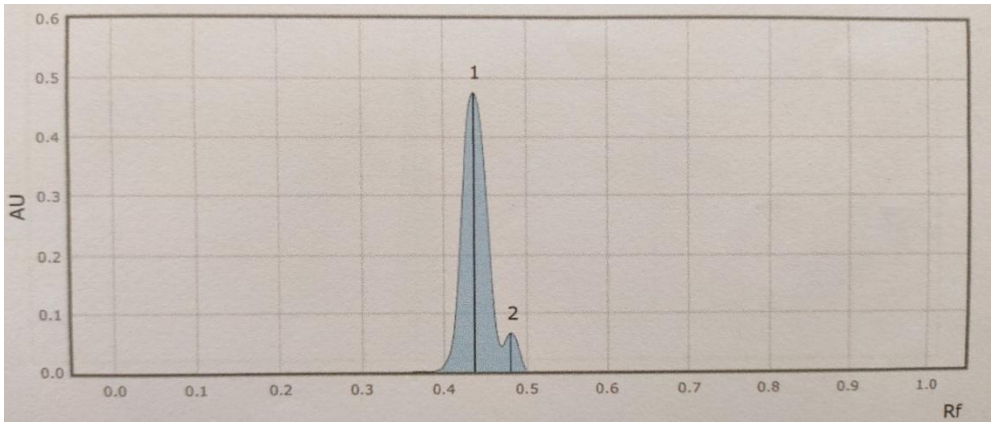

Fig 9: Chromatogram for sample 3.

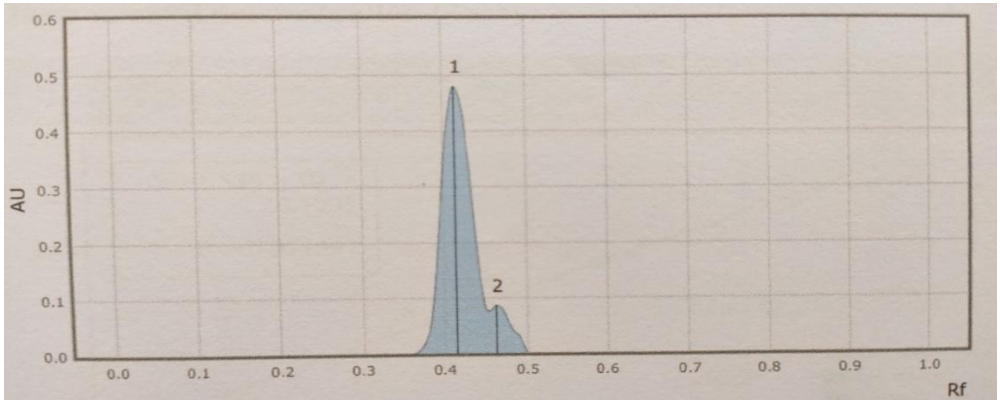

Fig 10: Chromatogram for sample 4.

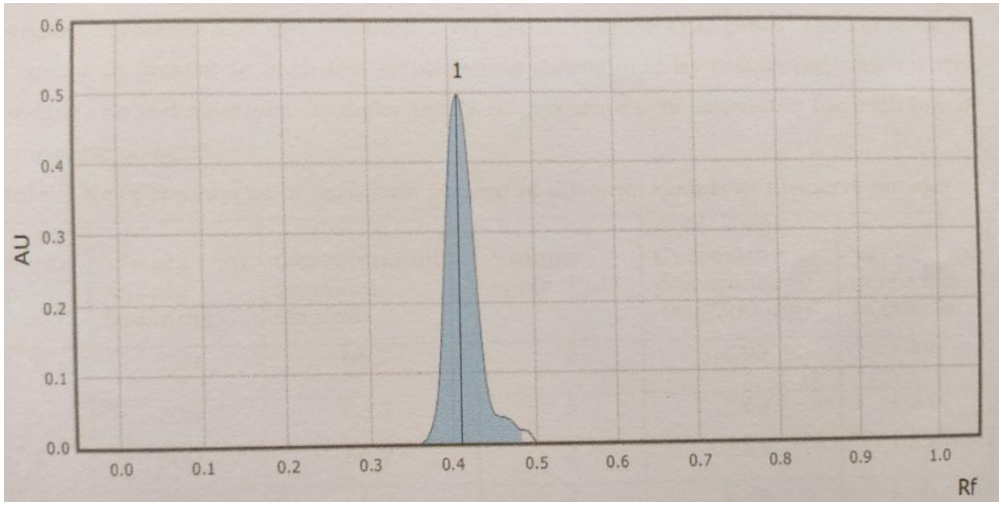

Fig 11: Chromatogram for sample 5.

Instrumental determination:

Calibration results:

Height calibration for Curcumin at 254 nm:

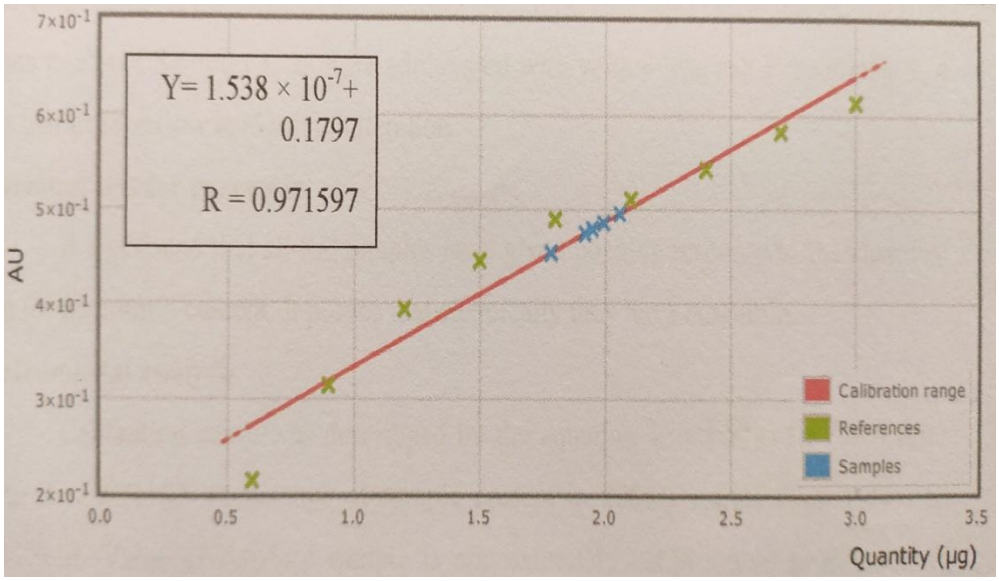

Fig 12: Calibration curve

On the HPTLC plate the standard curcumin and all the test samples were spotted. Area of the standard and all the samples were measured to determine the percentage of curcumin in each sample solution.

Table 3: Curcumin content present in different samples of turmeric powder.

Sample no	Weight taken (mg)	Prepared concentration (mg/ml)	Applied volume (µl)	Curcumin content (mg/200mg)	Curcumin percentage
1	200	66.7	2	2.93	1.49
2	200	66.7	2	2.68	1.34
3	200	66.7	2	2.88	1.44
4	200	66.7	2	2.92	1.46
5	200	66.7	2	3.01	1.54

In instrumental analysis, the equation $Y= 1.538\times10^{-7} + 0.1797$ with R= 0.971597 was used to describe the calibration curve. The curcumin content were quantified by the peak area of each chromatogram and based on a standard curve. Percentage of sample present in 200mg ranges from 1.49%

to 1.54%. R_f value of standard sample is approximately 0.434 whereas R_f value of samples 1,2,3,4 and 5 are 0.445, 0.439, 0.415 & 0.411 respectively.

Discussion:

From the chemical test carried out to check the presence of common adulterants, it was found that samples 1,2,3,4 and 5 are adulterated with metanil yellow and artificial color but none of them are adulterated with chalk and yellow soap stone powder. Samples 1,2,5 are adulterated with yellow lead salt but samples 3 and 4 are free from yellow lead salt adulteration.

From the chemical test for curcumin content, it was found that all the samples have given positive response to the test which proves they contain curcumin. The curcumin content varies in different brands or even from one batch of powder to another. Average curcumin content in percentage is reported to lie in between 1.06% to 5.70% with respect to some brands of turmeric powder [44]. From the experiment it was found that the maximum curcumin content was 1.54% and minimum curcumin content was 1.34%. All the samples contain an appropriate amount of curcumin.

Conclusion:

The adulterants upon which we conducted the tests are harmful to the human body. The health hazards caused by some of the adulterants are given below,

Metanil yellow: It is a non permitted category of food color. Continued consumption of this toxic food color leads to adverse life threatening effects in humans which can lead to cancer.

Artificial color: It may cause allergic reactions in some people and hyperactivity in sensitive children.

Yellow lead salt: lead poisoning can be easily overlooked. Exposure to high level of lead may cause anemia, weakness and kidney and brain damage. Very high exposure of lead may lead to death.

Thus, the methods developed for the quantitative estimation of curcumin and qualitative estimation of adulterants present in marketed turmeric powder can be used with good reproducibility and reliability.

From the experiment it can be concluded that if we consider the presence of adulteration then sample 4 is much safer as it has less amount of adulterant in comparison to other samples. But if we consider the curcumin content the sample 5 is better because it has highest amount of curcumin as per reported curcumin content in turmeric powder.

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