Physico-Chemical and Chromatographic Studies on Various Powdered Market Samples of Pepper, *Piper Nigrum* L. (Piperaceae)

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Abstract

A study on physico-chemical properties including powder characters, fluorescence analysis, TLC and HPTLC studies were performed on five (S1 to S5) different powdered market samples of pepper following standard procedures. The samples showed varied texture from very fine to coarse nature. All the samples revealed the presence of diagnostic characters viz., stone cells, starch grains, parenchyma cells etc. The present study revealed that the physico-chemical values were as per standards except for total ash of S2 which is little higher than prescribed. Even though there were variations in the number of spots observed in ethanol extracts among the samples and the text in the Ayurvedic Pharmacopoeia of India, the presence of piperine was confirmed by TLC and HPTLC in all the samples.

Keywords: *Piper Nigrum* L., *Piperaceae, Physico-Chemical Constants, HPTLC*

1. INTRODUCTION

Herbs have been found as a basis of traditional medicine and form an integral part of healthcare. These herbs originate from almost every part across the World and are harvested for use virtually in every continent [1]. The resurgence in the usage of traditional medicines in recent years resulted in growing demand for plant based medicines, pharmaceuticals, cosmetics and other products in India as well as in International market [2]. *Piper nigrum* L., (Piperaceae) called the King of Spices, is one of the oldest spices known has its origin from the Western Ghats of India and at present widely cultivated in various countries like Brazil, Malaysia, Sri Lanka, Vietnam including India[3]. *P. nigrum* is a perennial woody climber, grows up to a height of 4 m. The plant bears long oval shaped leaves and many small white colour flowers with a spike length up to 7-15 cm. The commonly found phytoconstituents in the plants of Piperaceae family includes volatile oil, pyrrolidone, phenolicesters, ethers etc [4]. *P. nigrum* has been reported to possess insecticidal, larvicidal, anti-apoptotic, anti-depressant, immunomodulatory, anti-convulsant, anti-microbial, anti-inflammatory, anti-mutagenic, antithyroid property etc[5,6,7]. Because of these innumerable uses *P. nigrum* finds its way as an important herb in home remedy as well as in various systems of indigenous and traditional medicine. But, raw materials of plant origin are prone to various changes due to numerous factors like seasonal, chemotypic, phenotypic variations, to name a few. Even the edaphic factors as well as harvesting, manufacturing process *viz.*, selection, drying, purification, extraction etc can cause considerable variability in material quality and in concentration of constituents. Hence, in the present work, a preliminary study on five different powdered market samples of pepper comprising of physico-chemical constants and chromatographic studies were performed and reported.

2. MATERIALS AND METHODS

Collection of Market Samples

Five different samples of pepper powder were purchased from local supermarket and were designated as S1, S2, S3, S4 and S5.

Powder Studies

A small quantity of each sample were treated with reagents like chloral hydrate, phloroglucinol and conc. HCl (1:1), iodine solution for the detection of constituents and other diagnostic characters [8]. The observed images were captured under a compound binocular microscope attached with an inbuilt analogue camera.

Physico-Chemical Constants

The following constants were determined as per standard procedures [9].

Ash Values

Total ash

About 2 g of each sample were incinerated in a silica crucible at 450 ºC until free from carbon and the percentage total ash was determined.

Acid insoluble ash

25 ml of dil. HCl was added to the above obtained total ash, boiled for 5 min and filtered through ash less filter paper. The insoluble matter was ignited in a tarred silica crucible and percentage acid insoluble ash was determined.

Extractive Values

Alcohol soluble extractive value

About 5 g of each sample were weighed and macerated for 24 h with 100 ml of 95% alcohol with frequent shaking for first 6 h and further allowed to stand for 18 h. Then it was filtered and 25 ml of the filtrate was evaporated to dryness at 105 ºC to obtain the percentage extractive value.

Water soluble extractive value

About 5 g of each sample were weighed and macerated for 24 h with 100 ml of chloroform-water with frequent shaking for first 6 h and further allowed to stand for 18 h. Then it was filtered and 25 ml of the filtrate was evaporated to dryness at 105 ºC to obtain the percentage extractive value.

Determination of Moisture Content

About 5 g of each sample were weighed in a previously tarred glass petri dish and kept in hot air oven to obtain constant weight at 105 ºC. Then, percentage moisture content was calculated.

Fluorescence Analysis

About 1 g of the samples were treated separately with reagents like dilute ethanol, methanol, 1N MeOH-NaOH, 1N EtOH-NaOH, 50% H_2SO_4, 50% HNO_3, 5% KOH, 1N HCl, acetone. Then, the treated samples were observed under 254 nm, 365 nm and visible light (10).

TLC Studies[9, 11]

Extract preparation

Ethanol extract of the samples were prepared with 95% ethanol in a soxhlet apparatus, filtered and dried at a temperature not exceeding 50 ºC. The dried extracts were stored in a desiccator until further use.

TLC development and detection

TLC plates (5 cm x 20 cm) using silica gel G was prepared, air dried and activated at 105 ºC. Mobile phase comprised of ethyl acetate:toluene (3:7) and the chamber was saturated for 2 h. The plates were spotted with samples prepared in ethanol and developed to a distance of 90%. The plates were then air dried and observed under visible light (425 nm) and long UV (366nm). The plates were also exposed to iodine vapours; dragendorff's reagent followed by 5% methanolic sulphuric acid; and vanillin sulphuric acid reagent followed by heating at 110 ºC and observed under visible light (425 nm).

TLC and HPTLC of Piperine [9,11]

Sample preparation

2 g of each sample were refluxed for 15 minutes with 10 ml of methanol, filtered and reduced to 2ml for further study.

Standard preparation

25 mg of standard piperine was dissolved in 2.5 ml of methanol

Stationary phase

Handmade TLC plates using silica gel G and TLC aluminium sheet pre coated with Silica gel GF_{254} obtained from Merck was used for TLC and HPTLC respectively.

TLC development and detection

The mobile phase comprised of ethyl acetate:toluene (3:7) and the chamber was saturated for 2 h. Samples prepared in methanol were spotted and developed to a distance of 90%.

Then the plates were air dried for 15 to 20 minutes and sprayed with vanillin sulphuric acid reagent followed by heating at 110 ^{o}C for 5 minutes.

HPTLC development and detection

HPTLC study was carried out in Camag HPTLC system with inbuilt WINCATS 4 software. 6 µl of the samples and standard were applied as 6 mm band in pre coated aluminium TLC plates using Hamilton syringe.

The plate was developed in twin trough chamber containing ethyl acetate:toluene (3:7) saturated for 2h. Then the plate was air dried and sprayed with vanillin sulphuric acid reagent followed by heating at 110 ^{o}C for 5 minutes. The derivatised plate was scanned and documented at 425 nm.

3. RESULTS AND DISCUSSION

Powder Studies

The powder texture ranged from fine to coarse and the increasing order of coarse nature was found to be of S1<S3<S4<S2<S5 (Fig. 1).

All the sample powders revealed the fragments of diagnostic characters like parenchyma, isodiametric, elongated, and beaker shaped stone cells and starch grains (Fig. 2) as mentioned in the Ayurvedic Pharmacopoeia of India (API).

S1 S2 S3 S4 S5

Fig. 1 Different powder samples of pepper

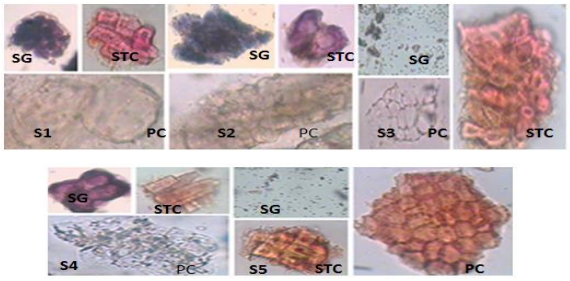


Fig. 2 Powder microscopical characters

(SG- Starch grain; STC- Stone cells; PC- Parenchyma)

Physico-Chemical Constants

Ash value is one of the important parameter that helps in ascertaining the crude drug materials' purity and quality while in powder form. Ash containing phosphates, silicates of sodium, potassium and magnesium; carbonates are obtained as the samples are incinerated.

Total ash depicts the process followed and care taken during the preparation of the crude drugs, while acid insoluble ash provides an insight in the amount of silica present, particularly sand. Extractive value provides the amount of constituents extracted by the given solvent with a suitable amount of sample. A lower extractive value may represent the exhaustion and/or adulteration in crude drug samples. Moisture content in samples should be preferably in the lower side, as it may enhance the deterioration of phytoconstituents. The various physical constants described earlier were determined for all the samples and reported in Table 1.

Fluorescence Analysis

Powder samples when treated with various reagents and exposed to different wavelengths exhibits characteristic colour that may be used to identify samples in the preliminary stage.

The colour exhibited by the samples under 366 nm upon treatment with various reagents was carbon flint alone, while the other various colour exhibited by the samples under 254 and 425 nm on exposure to above said reagents are tabulated in Table 2 and Table 3.

TLC Studies

The ethanol extract prepared by continuous hot percolation method was used for TLC studies. The percentage yield of ethanol extract by continuous hot percolation method is reported in Table 4. TLC of ethanol extract of S1 revealed 10, 3, 8, 6, 5 number of spots under 366 nm, 425 nm, on exposure to iodine vapours, treating with dragendorff's reagent followed by methanolic sulphuric acid and vanillin-sulphuric acid respectively, while S2, S3, S4 and S5 revealed 8, 4, 7, 5, 3; 11, 3, 10, 3, 4; 10, 3, 8, 4, 4 and 10, 4, 8, 6, 7 number of spots respectively upon corresponding exposure and treatments. The R_f values were indicated in the corresponding figures (Fig. 3-7).

Table 1. Physico-chemical constants of various samples (% w/w)

Sample ID/ Parameters	Ash value		Extractive value		Moisture content
	Total	Acid insoluble	Water soluble	Alcohol soluble	
S1	2.86	0.46	6.23	6.02	8.75
S2	5.80	0.48	7.38	9.89	10.30
S3	4.06	0.49	8.36	9.04	9.13
S4	4.17	0.48	8.94	8.49	9.53
S5	4.94	0.50	7.98	7.16	3.15

Table 2. Fluorescence analysis of samples under short UV (254 nm)

Reagents	S1	S2	S3	S4	S5
Powder as such	Sultry	Dark drama	Vivid green	Green gold	Dark drama
Dil. EtOH	Green gold	Mehendi N	Mehendi N	Mehendi N	Mehendi N
MeOH	Vivid green	Pine-N	Mehendi N	Mehendi N	Mehendi N
1N MeOH-NaOH	Vivid green	Mehendi N	Mehendi N	Sultry	Mehendi N
1N EtOH-NaOH	Mehendi N	Pine-N	Dark drama	Mehendi N	Dark drama
50% H_2SO_4	Carbon flint	Carbon flint	Carbon flint	Carbon flint	Carbon flint
50% HNO_3	Pine-N	Meadow path	Carbon flint	Meadow path	Meadow path
1N HCl	Green gold	Mehendi N	Vivid green	Green gold	Mehendi N
Acetone	Green gold	Pine-N	Vivid green	Mehendi N	Amazon mass
5 % KOH	Green gold	Vivid green	Dark drama	Green gold	Dark drama

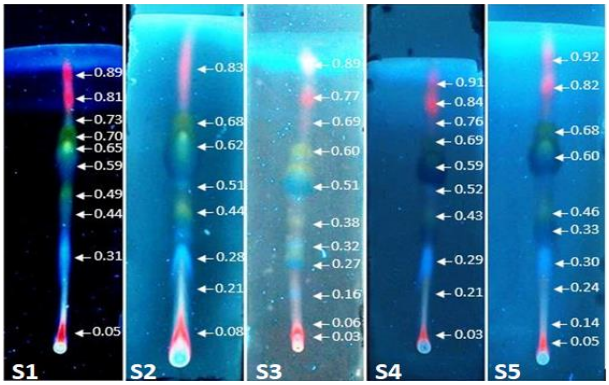


Fig. 3 TLC of various samples under 366 nm

Table 3. Fluorescence analysis of samples under visible light (425 nm)

Reagents	S1	S2	S3	S4	S5
Powder as such	Green gold	Green gold	Vivid green	Green gold	Dark drama
Dil. EtOH	Green gold	Mehendi N	Mehendi N	Green gold	Green gold
MeOH	Green gold	Mehendi N	Mehendi N	Mehendi N	Vivid green
1N MeOH-NaOH	Green gold	Mehendi N	Vivid green	Vivid green	Vivid green
1N EtOH-NaOH	Green gold	Mehendi N	Vivid green	Mehendi N	Dark drama
50% H_2SO_4	Moody maroon	Mehendi N	Moody maroon	Maroon	Maroon
50% HNO_3	Ming red	Moody maroon	Moody maroon	Teracota N	Moody maroon
1N HCl	Sandstone	Green gold	Burnt Yellowstone	Tropical tan	Green gold
Acetone	Sultry	Mehendi N	Moody maroon	Sand stone	Mehendi N
5 % KOH	Burnt Yellowstone	Vivid green	Sultry	Burnt yellow stone	Vivid green

(Note: All color comparison is based on the "Asian paints" premium gloss enamel card. Asian paints limited, Mumbai)

Table 4. Percentage yield of samples by continuous hot percolation (% w/w)

Samples	S1	S2	S3	S4	S5
% yield	6.26	9.50	10.40	10.40	11.23

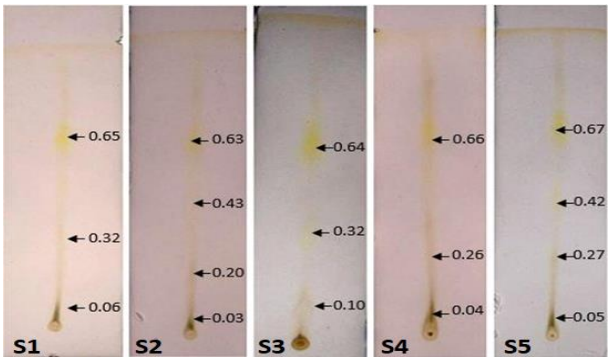


Fig. 4 TLC of various samples under 425 nm

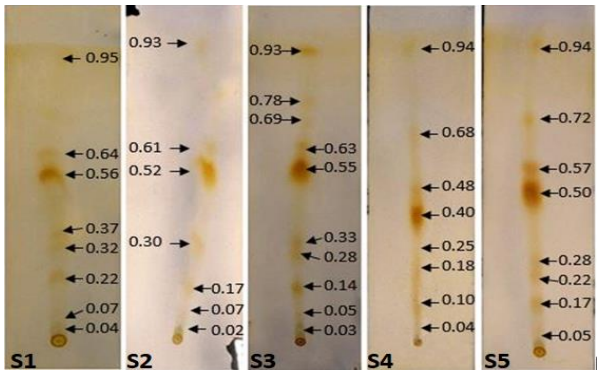


Fig. 5 TLC of various samples on exposure to I_2 vapours

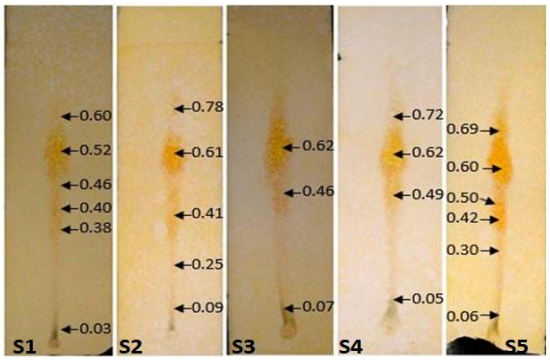


Fig. 6 TLC of various samples after treating with Dragendorrf's followed by 5% MeOH-H_2SO_4

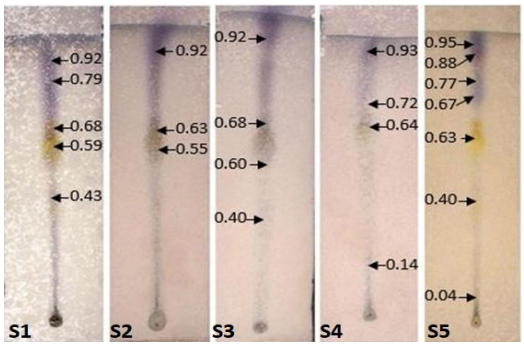


Fig. 7 TLC of various samples on exposure to vanillin-H2SO4

TLC and HPTLC of Piperine

The methanol extract of all the samples and standard piperine showed lemon yellow spot with similar R_f values on exposure to vanillin-H_2SO_4 (Fig. 8). The results suggested that all the samples contained piperine. Further, the methanol extract was also subjected to HPTLC study. Standard piperine was observed as lemon yellow spot with a R_f value of 0.39, while all the samples also showed lemon yellow spots with R_f ranging from 0.37 to 0.39 on exposure to vanillin-H_2SO_4 (Fig. 9, 9a).

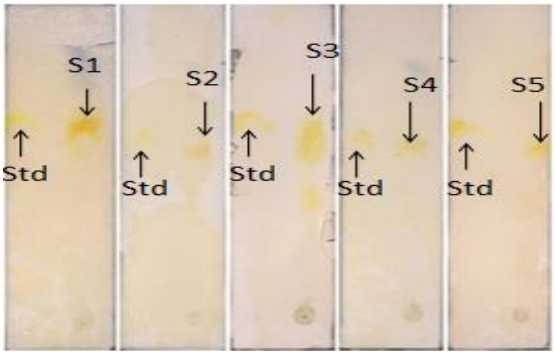


Fig. 8TLC of methanol extract of various samples for piperine

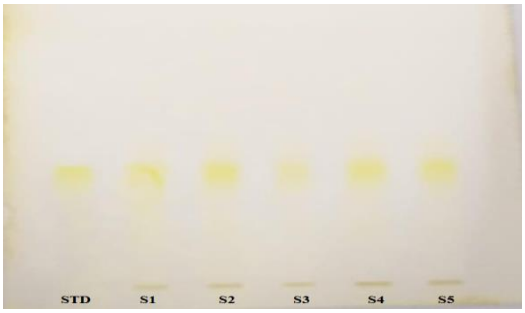
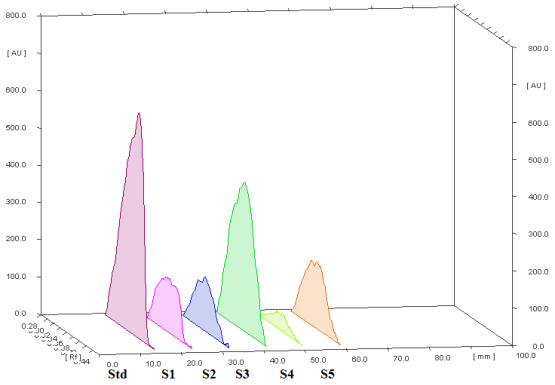


Fig. 9 HPTLC of methanol extract of various samples for piperine



Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.29 Rf	3.4 AU	0.39 Rf	605.7 AU	100.00%	0.43 Rf	0.0 AU	34784.8 AU	100.00%	unknown *
2	1	0.30 Rf	0.2 AU	0.36 Rf	145.0 AU	51.92%	0.38 Rf	133.1 AU	6396.0 AU	68.82%	unknown *
2	2	0.38 Rf	133.3 AU	0.39 Rf	134.3 AU	48.08%	0.42 Rf	2.6 AU	2897.1 AU	31.18%	unknown *
3	1	0.30 Rf	0.1 AU	0.35 Rf	125.3 AU	46.48%	0.35 Rf	123.9 AU	3077.0 AU	38.62%	unknown *
3	2	0.36 Rf	123.6 AU	0.37 Rf	144.3 AU	53.52%	0.43 Rf	0.9 AU	4889.9 AU	61.38%	unknown *
4	1	0.30 Rf	41.6 AU	0.38 Rf	400.6 AU	100.00%	0.44 Rf	1.0 AU	27443.7 AU	100.00%	unknown *
5	1	0.30 Rf	0.0 AU	0.37 Rf	44.7 AU	49.17%	0.37 Rf	43.5 AU	1412.4 AU	57.36%	unknown *
5	2	0.38 Rf	43.5 AU	0.38 Rf	46.2 AU	50.83%	0.43 Rf	3.2 AU	1049.9 AU	42.64%	unknown *
6	1	0.29 Rf	9.5 AU	0.35 Rf	173.7 AU	49.25%	0.36 Rf	164.2 AU	5708.5 AU	48.51%	unknown *

Fig. 9a: Chromatogram of methanol extract of various samples for piperine

4. CONCLUSION

Five marketed samples of pepper powder were collected and its physico-chemical properties was performed and reported. The physico-chemical constants determined were as per the standards of the API, except for total ash of the sample 2 that showed a marginally higher value of +0.8%. TLC of ethanol extract; TLC and HPTLC of methanol extract were performed. Even though ethanol extract showed variation in the number of spots obtained among the samples as well as the number of spots mentioned in the API, the TLC and HPTLC of methanol extract confirmed the presence of piperine in all the samples. The R_f values of piperine in the samples were matching with the R_f values of standard piperine. More over from the HPTLC chromatogram obtained for

methanol extract, it is derived that among the samples, S3 had maximum piperine content while S4 with minimum content. Hence, it may be concluded that the physico-chemical constants were as per standard even though there was some variation among the samples. Piperine is considered as the marker compound in pepper and all the samples (S1, S2, S3, S4 & S5) were found to contain piperine by chromatographic studies.

ACKNOWLEDGMENT

Authors thank management of Ramaiah University of Applied Sciences, Bangalore, Karnataka, India for providing facilities and encouragement for the work.

CONFLICTS OF INTEREST

There are no conflicts of interest among the authors.

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