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Phytochemical and Pharmacognostic studies on *Piper nigrum* L.

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Abstract
Piper nigrum is an evergreen climber, origin in Sri Lanka and India. Pepper is known as the master spice because it can be stored for many years without losing its irresistible aroma. Black or white pepper is a product of the same tropical Vine Piper Nigrum. Pepper berries grow in racemes like grapes. They turn from green to yellow and then to red as they ripen. Black pepper is made from under ripe berries. As they dry, the color changes to the familiar deep mahogany shade. White pepper is made from the completely ripe Piper Nigrum berry. Pepper is one the most widely used spices in the world, no exception in India. Many people prefer pepper instead of chillies to make the preparation hotter and spicier. Traditionally Pepper is used as energizer, for increasing circulation, to warm and relieve muscle aches and stiffness, for fighting colds, flu and infections. East Africans are believed that body odor produced after eating substantial amounts of pepper repels mosquitoes. Pepper powder is used to flavour varieties of food in all parts of world. The oils extracted from peppercorns goes into perfumes and flavorings. It is also an effective insecticide against houseflies. Gardeners use pepper sprays against several kinds of pests.

Keywords: *Piper nigrum,* Black Pepper, TLC Physicochemical, Anatomical, Microbial limits

Introduction
Piper nigrum Linn. (Fam.Piperaceae) commonly known as pepper, 'king of spices', are cultivated from Konkan Southwards, especially in North Konkan Kerala, and in Assam. Pepper powder are extensively known for their culinary application as they add their distinctive flavor to the dish as well as amplify the taste of other ingredients. Various reports suggest ethno pharmacological and medicinal application of pepper targeting several disease including; asthma, boils, cholera, colic diseases, cough etc. The phytochemical profile reveals the presences of several alkaloids namely; Piperine, Chavicine, Piperidine, Piperetine in the essential oils. Piperine and Piperedine is known to possess anti-inflammatory properties that is mentioned in the below studies. Pepper are Climbers woody. Nodes clearly enlarged and rooting, glabrous. Petiole 1-2 cm, glabrous; leaf blade ovate to ovate-oblong, rarely suborbicular, 10-15 × 5-9 cm, thick, ± leathery, glabrous, base rounded, usually slightly oblique, apex acute; veins 5-7(-9), apical pair arising 1.5-3.5 cm above base, alternate, others basal; reticulate veins prominent. Flowers polygamous, usually monoecious. Spikes leaf-opposed, to as long as leaves; peduncle nearly as long as petioles, glabrous; bracts spatulate-oblong, 3-3.5 × ca. 0.8 mm, adaxially adnate to rachis, only margin and broad, rounded apex free, shallowly cupular. Stamens 2, 1 on each side of ovary; filaments thick, short; anthers reniform. Ovary globose; stigmas 3 or 4, rarely 5, one third of the fruit is covered by a thick pericarp which comprises of single layered epicarp, with lignified stone, wide mesocarp, with tangentially elongated parenchymatous and oil cells and a endocarp composed of a row of beakershaped stone cells. Fruits colour vary from black when unripe to drupe red when ripe.globose, 3-4 mm in diam. fruits are harvested from December to April [1, 2].

One of the report explain that inflammation plays a key role in obesity-related pathologies such as cardiovascular disease, type II diabetes, and several types of cancer. Some spice-derived components, which are naturally occurring phytochemicals, elicit antiobesity and anti-inflammatory properties [3]. Piperine (1-peperoyl piperidine) was isolated from Piper nigrum Linn for the evaluation of anti-inflammatory activity in rats [4]. Different acute and chronic experimental models like carrageenin-induced rat paw edema, cotton pellet granuloma, and croton oil-induced granuloma pouch, were employed and simultaneously, biochemical estimations were made to elucidate the underlying mechanism of the action. Piperine acted significantly on early acute changes in inflammatory processes and chronic granulative changes. EtOH extract of Piper nigrum L was subjected to check effects on nitric oxide, three test compounds, amide alkaloids, pipernigramides showed inhibitory effect further, in the carrageenan-induced paw edema test, demonstrated anti-inflammatory effects [5]. The oral administration of *P. nigrum* ethanol extracts on ovalbumin (OVA)-induced allergic asthma rat model exhibit reduced accumulation of inflammatory cells as well as suppressed the levels of total IgE, anti-OVA IgE, anti-OVA IgG_1 and histamine release in serum thus suggesting it as treating allergic asthma [6]. In another report ten *Piper nigrum* alkaloids were examine for their anti-inflammatory effect, chabamide emerged as a promising candidate for the treatment of inflammatory diseases [7].

Considering the above studies, it can be suggested that pepper is a good candidate for anti-inflammatory activities. Piperine in most studies indicates its importance as an anti-inflammatory agent. Besides, the plant also possesses its own bundle of bio active phytochemical and biological properties including;, beneficial effects on cardiovascular health, diabetes, cancer etc. Hence, it can be put forth for further research to exploit them as nutraceutical and pharmaceutical agent. Keeping all these points in a view in the present study standardization of *Piper nigrum* is done by pharmacognosy approach.

Materials and Methods

Voucher specimen: The plant materials were collected and Identity was confirmed with the voucher specimen number Pip nig/FP/VVPL/10/02/20 [8]. Physico-chemical values such as the percentage of total ash, acid-insoluble ash, water-soluble ash, and water and alcohol-soluble extractives were calculated as per the Indian Pharmacopoeia [9]. Preliminary phytochemical tests was performed according to [10]. TLC fingerprinting profile carried as per [11]. For the Anatomical studies transverse sections (TS) and powder microscopy studies, were prepared and stained [12, 13]. A standard, Limit for total microbial count provided by WHO Guidelines [14] was followed and also Indian herbal pharmacopoeia.

Result and Discussion

Pharmacognosy

Table 1: Pharmacognosy features

Physicochemical Constants			Organoleptic Characters	
Parametrs	**Values**	**Limit**	**Parametrs**	**Values**
TA	4.45%	NMT 5%	Taste	Hot
AIA	0.25%	NMT 0.5%	Color	Blackish
ASE	7.3%	NLT 6%	Odour	Strong
WSE	7.95%	NLT 6%	Texture	Smooth

TA - Total Ash; **AIA** - Acid Insoluble Ash; **ASE** - Alcohol Soluble Extractive; **WSE** - Water Soluble Extractive; **NMT**-Not More Than; **NLT**-Not Less Than

All of the parameters of these studies for the sample was found to be under the range of limits as prescribed by Ayurvedic Pharmacopeia of India. The above-mentioned Organoleptic properties are unique for the particular plant (table 1)

Preliminary Phytochemical Analysis

Table 2: Preliminary Phytochemical Analysis

Sl.no	Secondary Metabolite	Result	Inference
1	Alkaloid : Mayer's Test	+	Creamy white precipitate
2	Phenol: Ferric chloride Test	+	Intense coloration
3	Tannins: Lead Acetate test	+	White Precipitate
4	Steroids: Salkowski test	-	No red coloration at the junction
5	Flavonoid: Flavanoid Test	+	Colour change
6	Saponins: Foam test	-	No Foam Formation

The methanoic extract of the plant showed the presence of secondary metabolites such as; alkaloids, flavonoids, tannins and phenols but showed negative result for the presence of saponins steroids (table-2).

Table 3: TLC Profile

TLC Finger Printing Profile								
Under Visible Light								
Rf Values	0.22	-	-	-	-	-	-	-
Sprayed with 10% H_2SO_4								
Rf Values	0.06	0.2	0.29	0.36	0.45	0.98	-	-
Sprayed with Anisaldehyde								
Rf Values	0.21	0.29	-	-	-	-	-	-
Under Short UV (254 nm)								
Rf Values	0.07	0.18	0.27	0.33	0.41	-	-	-
Under Long UV (366 nm)								
Rf Values	0.06	0.17	0.24	-	-	-	-	-

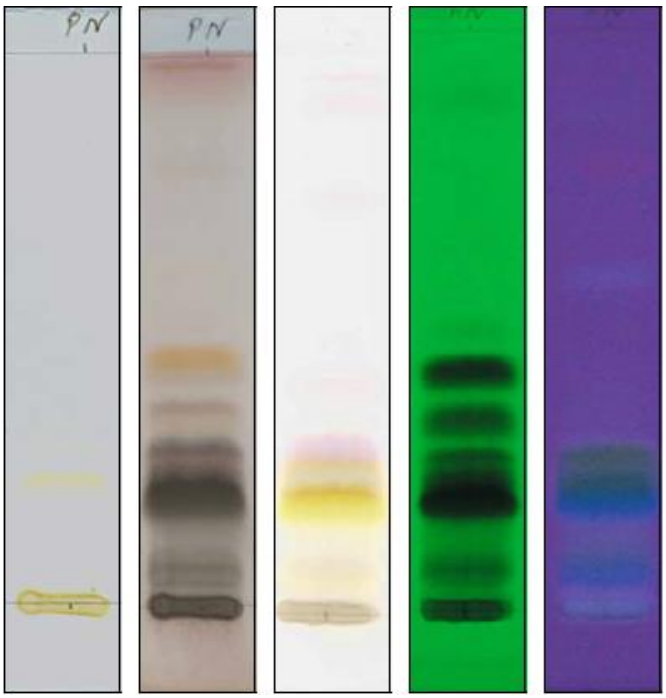

Fig 1: TLC Chromatograms

Piper nigrum showed one band under visible light, 6 bands when sprayed with 10% H_2SO_4 and 2 bands when sprayed with Anisaldehyde. Further, showed 5 and 3 bands under short and long UV light respectively. The results are qualitative TLC finger print profile of plant under study (table-3,fig-1).

Anatomical Characters

T. S of fruit shows thick pericarp which consists of epicarp, mesocarp, and endocarp, Epicarp composed of single layered, slightly sinuous, tubular cells forming epidermis, Bellow that lignified stone cells, which are radially, arranged which are adjacent to parenchyma cells,Mesocarp is wide composed of band of tangentially elongated parenchyma cells and tangentially elongated oil cells are presenting outer region and few fibro vascular bundles, Single row of cells in the inner region of mesocarp. Endocarp composed of a row of beaker

shaped stone cells, testa single layer, yellow coloured thick walled sclerenchyma cells, Perisperm contains parenchymatous cells having a few oil globules and filled with abundant oval to round; simple and compound starch grains and few aleurone grains (fig-2).

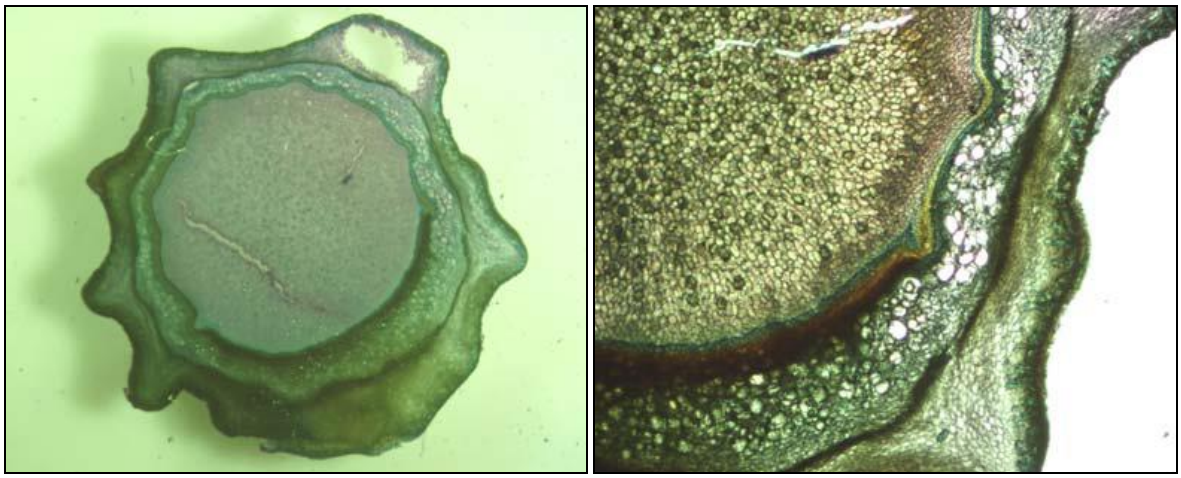

Fig 2: Anatomical Characters of *Piper nigrum*

Powder Characters

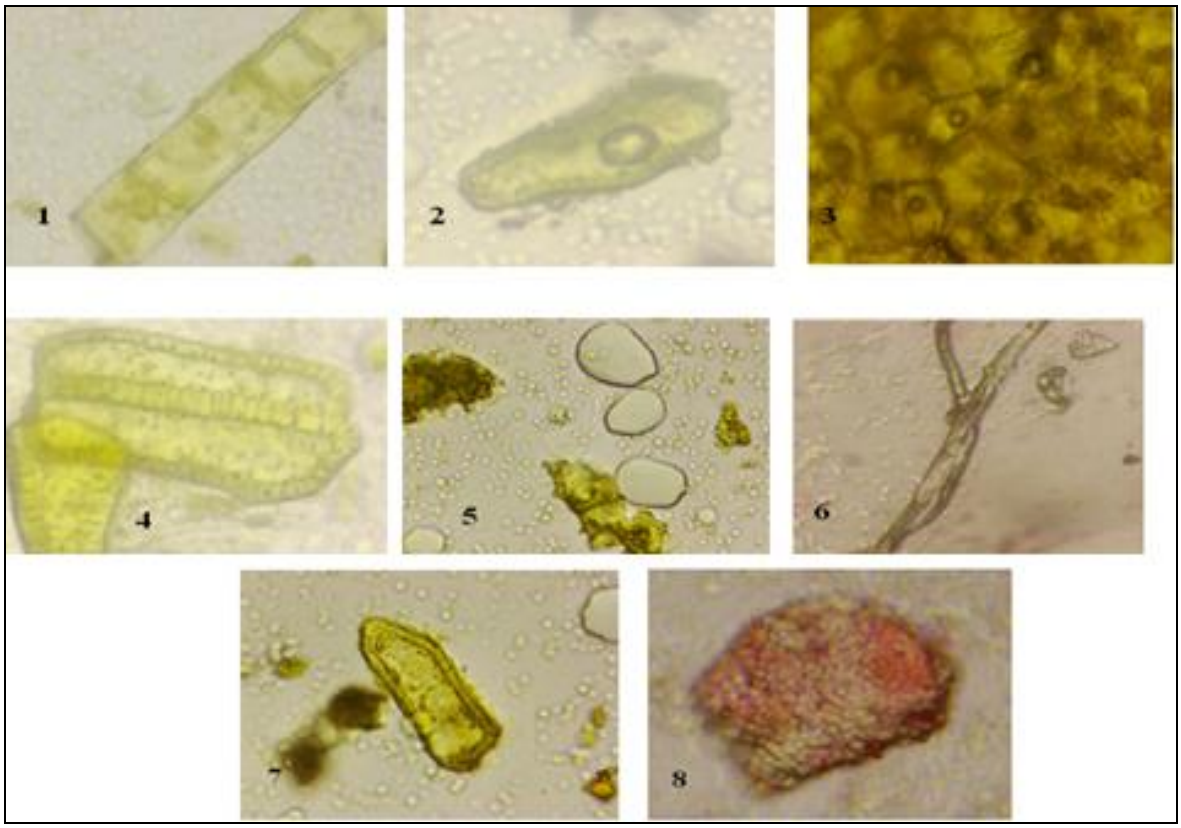

Fig 3: Powder characteristics of *Piper nigrum (*1) Fibrous sclereids (2) Cells of perisperm containing starch and oil cells (3) Mesocarp cells (4) Isolated sclereids from the outer mesocarp (5) Strach granules (6) Fibrous sclereids from the stalk (7) Isolated sclereids (8) Cells from perisperm containing starch from the outer mesicarp

Powder analyses are unique and play a significant role in authentication of crude sample (fig-3).

Microbial Limit Test

Total Aerobic Bacterial Count (TABC): 2 x 10^3

Total Yeast and Mould Count (TYMC): 0.5 x 10^3

(Microbial contamination limit for raw herbs - TABC: $<10^7$, TYMC: $<10^5$)

The values were well within the limits as prescribed by Indian herbal pharmacopoeia

Conclusion

The present study was aimed at authentication and identification of *Piper nigrum* by pharmacognostic approach which included physicochemical, microscopic and phytochemical method. The Physico-chemical characteristics and microbial limit test was performed and it was found that all the parameters of the study was found to be well within the range as prescribed by Ayurvedic Pharmacopeia of India and WHO Guidelines Preliminary Phytochemicals of the sample revealed the presence of secondary metabolites. TLC studies were performed. Both Anatomical and powder Characters are unique to the plant.

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