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Quality Specifications on *Piper nigrum* L. - A Spice and Herbal Drug of Indian Commerce

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Abstract *Piper nigrum* L. is used as drug in Ayurvedic, Unani and Siddha system of medicine. Dried fruit is employed commercially as a condiment; it is warming, drying and stimulating to the circulatory, digestive and respiratory system. The approved modern therapeutic application for Piper is support based on its long history of use in well established systems of medicines viz. Ayurveda, Unani and Siddha. The present communication deals with detailed pharmacognostic studies and review related to quality aspects of pepper.
Keywords *Piper nigrum* L.*, Drug Standardization, Quality Specifications*

1. Introduction

Piper nigrum L. is commonly known as Pepper or Kalimirch; it is widely used as a pungent condiment. The fruit of the piper plant is a common ingredient in many recipes. *Piper nigrum* plant is a woody, perennial climber, indigenous to Cochin, China, and India and also widely cultivated. One another name of piper is 'sun marcia', because it has the same qualities as the macrocosmic source of heat and light. It is warming, drying and stimulating to the circulatory, digestive and respiratory systems.

Piper was introduced into Europe about the year 1000 it was known by Theophrastus and other ancient writers. The medicinal values of Piper as a counterirritant depend on its more pungent and delicately aromatic. This spice is also used as a homeopathic treatment for a variety of conditions. Piper is credited with a number of medicinal properties in different systems of medicine viz., Ayurveda, Siddha and Unani. Piper is two type black piper and white piper , whiter piper is also consist of the fruits of *Piper nigrum* which have ripened and formed after the separation of pericarp of fruit , the fruit have soaked in salt water or lime water (1).

2. Methodology

Drug samples were collected from different places with a view to find out any significant difference present within the same species. For studying powder, Jackson and Snowdon (2) were followed. To

determine physico-chemical constants, Indian Pharmacopoeia (3) was consulted and for fluorescence study schedules mentioned by Trease and Evans (4) was followed. Colours were named by consulting Rayner (5). Standard prescribed procedures for histochemical studies (6), (7), (8), (9); organic group detection (10); U.V. Spectrophotometry (11) and Chromatography (12), (13), (14) were adopted from relevant literature resource. The informatics is complied by reviewing the available literature.

3. Result

A) Family: Piperaceae

B) Genus: *Piper*

Piper nigrum L., Sp. Pl., 29, 1753; Hook. f. in Fl. Brit. Ind., 5: 83, 1985.

Perennial, branching, climbing, stem glabrous and rooting at the nodes. Leaves simple, alternate, cordate, broadly ovate, apex pointed, 5-9 nerved and dark green. Flowers spikes usually deciduous. Fruiting spikes variable in length and robustness, rachies glabrous, fruit drupe, globular, red when ripe, turning black after drying (Figure 1 A).

C) Distribution

It is mostly cultivated in the hot and moist climate, in evergreen forests up to 1,500 m in India, Srilanka and other tropical countries and also widely cultivated. The species has probably originated in hills of South Western India, of North Kanara to Kanyakumari and wild in Travancore and Malabar (15).

D) Drug Specification

Fruits rounded, hard, wrinkled, 0.4 to 0.5 cm in diameter and greyish-black to black. The pericarp is thin and encloses a single seed with a hollow centre.

E) Nomenclature

The plant is known by different vernacular names e.g. Golmorich, Kalamorich and Morich (Bengali), Kalimori (Gujarati), Kalimirach (Hindi), Kurumulaku (Malayalam), Kalamiri (Marathi), Milagu (Tamil), Miriyalu and Marichamu (Telugu), Filfil siyah and Kalimirach (Urdu).

F) Cultivation, Collection and Storage Practices

In India pepper is cultivated mostly as a mixed crop in homestead garden. It grows in various parts of India mainly Kerala, Tamilnadu and Karnatak.The veins require trained on to existing trees like jack, mango, coconut bamboo, orange etc. Peppers require warm and humid climate with annual rainfall over 200 cm. Pepper vein can be propagated either vegetatively or by seeds. Vegetative propagation is more adopted because of slow regeneration from seed; although the seeds raised plant remain productive for a long period and yield more in later years. The plant is cropped twice during August-September. and other in March-April. The pepper vein start to fruit 2-3 years later, the fruit is ready for harvest from middle of December to middle March. After the harvesting, the spikes are removed from the vines and dried in the sun. Store protected from moisture and against attack by insect and rodents, polyethylene shall be used for packing.

G) Chemical Constituents

Pepper fruit contain 2% - 4% volatile oil (sabinene, areyophyllene, beta-pinene, alph-pinene camphene, limonene, myrcene, and piperonal), 5% - 9% alkaloids (piperine, piperidine, piperanine, pieretine and chavicine), fixed oil and chromium. The sharp taste is due to Piperiene, a nitrogenous substance (1).

H) Medicinal Properties

The fruits are acrid, bitter, anthelmintic, carminative, alerant, aphrodisiac, alexeteric, antiperiodic, deobstruant, diuretic, digestive, emmenagogue, rubefacient, stimulant and stomachic. Medicinally the pepper fruits are used in arthritis, throat troubles, hoarseness, colic, gastric ailments, vertigo ,unconsciousness, paraplegia, scorpion sting, convulsions pimples, eczema, scabies, alopecia, other skin diseases, pharyangodynia, asthma, fever, cough, catarrh, dysentery, dyspepsia, vomiting, diarrhoea flatulence, hiccough, hemorrhoids, urethrorrhea and dermatopathy, eye diseases, oedema and obesity (16).

I) Pharmacological Activities

Pepper plays a great role in digestions, useful for low appetite, sluggish digestion, abdominal pain, toxins and borborygmus. Its anthelmentic qualities help to remove worms. The drug stimulant the thermal receptors and increases secretion of salvia and gastric mucous. It has an antimicrobial effect. It influences liver and metabolic function, and has been insecticide effect (17). It has also another pharmacological activities viz. antioxidant, anticonvulsant, sedative, insecticidal, pesticidal, muscle relaxant, antipyretic, anti-inflammatory, antifungal, tendincidal, hepatoprotective, antimicrobial, antiulcer, antibacterial, lipolytic etc (15).

J) Commercial Potentialities

The commercial supplies come from Indonesia, Singapore and India. The main markets for Indian pepper have been U.S.A., U.S.S.R., Germany, France, Italy, Netherland, Belgium, U.K. and Canada. It is available throughout India and is available in the market in varying rates.

K) Important Formulations

Karpoorasava, Ayaskriti, Shrikhandasava, Eladi ghrita, Kasisdai ghrita, Taptaraja taila, Marchadi gutika, Marchadi yaila, Marchadi churna, Marchadya ghrita, Marchadya Taila Ashtangalvana churna, Laghulayi churna, Brihnmarichadya taila, Taramandura guda, Trikatu Churna Jawarankusha rasa, Guduchi lauha, Trikatu churna, Apratisaraanjana, Kshanmakshika yoga, Mrityunjaya rasa, Attatic Curanam, Civanar Amirtam, Cuvacakkutori,Elatic Curanam, Nilvakaic Curanam, Panca Tipakkinic Curanam, Talicati Vatakam, Tirikatuku Curanam (18), (19).

L) Adulterations and Substitute

Aframomum melegueta (Rosc.) Schum., Lantana camera L. *, Polygonum amphibium* L.*, Seshnus molle* L.*, Vitex agnuscastus* L.*, Vitex altisimma* L. *and Carica papaya* L. is the adulteration of Piper nigrum L.

M) Other Uses

Peepers are used as a major condiment employed for seasoning freshly prepared foods. Oil of pepper is used in flavouring sansage, canned meats, soups, table sauces, beverages and liquors; oil

is also used in soaps and medicine. Green fresh peppers used in preparing pickles. They can also use as an insecticide against clothes moths.

N) Regulatory Status

An official drug under Drugs & Cosmetic Act 1940 and Rules and food commodity (spice) in Food Safety and Standards Act, 2006, Rules, 2011 and Regulations 2011.

Ayurvedic Pharmacopoeia of India, Part I, Vol. III (18)
Ayurvedic Formulary of India, Part I (20)
Unani Pharmacopoeia of India Part I & Vol. IV (21)
National Formulary of Unani Medicine Part I (22)
Siddha Pharmacopoeia of India, Part I, Vol. I (19)
Siddha Formulary of India, Part I (23)
Indian Pharmacopoeia, 2010 (3)
Food Safety and Standards Regulations 2011 (24) (for food commodity)

O) Organoleptic Characteristics

Fruits rounded, hard, wrinkled, 0.4 - 0.5 cm in diameter and greyish-black to black. The pericarp is thin and encloses a single seed with a hollow centre (Figure 1 B, C). The powder colour is blackish-grey; odour, aromatic; taste, pungent (Figure 1 D).

P) Micro-Morphological Characteristics

The epicarp adherent to the outermost sclerenchymatous layer of the mesocarp; containing pigment and calcium oxalate crystals. The sclereids are varying in shape and size, usually polygonal to rectangular. Mesocarp of the parenchyma thick-walled, irregular shaped cells and scatter oleoresin cells. Endocarps compose of a single layer of lignified cells associated pigment layer of testa in surface view. Perisperm thin-walled, polygonal or ovoid cells; containing starch granules and oil cells (Figure 2).

Q) Histochemistry

Micro-chemical tests and behaviour of specific reagents towards plant/drug tissues: Observations and results of micro-chemical tests and behaviour of specific reagent towards plant tissues are presented in Table 1.

Table 1: *Micro-Chemical Tests and Behaviour of Specific Reagents towards Plant Tissues and Cells Contents*

Reagent	Test for	Inference	Histological Zone/Cell Contents Responded.
Dragendorff's reagent	Alkaloid	+	Perisperm cells
Marme's reagent	Alkaloid	+	Same as above
Wagner's reagent	Alkaloid	+	Same as above
Potassium hydroxide solution (5% w/v)	Anthocynin	-	Not Responded
Sulphuric acid (66% v/v)	Anthocynin	-	Not Responded
Acetic acid	Calcium oxalate	+	Calcium oxalate crystals in epicarp cells
Potassium hydroxide solution (5% v/v) + Hydrochloric acid	Calcium oxalate	+	Same as above

Sulphuric acid	Calcium oxalate	+	Same as above
Kedde reagent	Cardiac glycoside	-	Not Responded
Iodine Solution followed by Sulphuric acid	Cellulose	-	All cellular region
Sudan III	Fixed oil and fats	+	Perisperm cells
Chlor-zinc-Iodine Solution	Latex	-	Not Responded
Aniline sulphate Solution followed by Sulphuric acid	Lignin	+	Stone cells
Phloroglucinol HCl	Lignin	+	Same as above
Lugol's solution	Protein	+	Endosperm cells
Millon's reagent	Protein	+	Same as above
Picric acid	Protein	+	Same as above
Heating with KOH (5% w/v) + H_zSO_4	Suberin	-	Not Responded
Sudan III	Suberin	-	Not Responded
Weak Iodine solution	Starch	+	Starch grains in perisperm cells
Potassium hydroxide solution (5% w/v)	Starch	+	Same as above
Sulphuric acid	Starch	+	Same as above

R) Organic Groups of Chemical Constituents

The extracts of the drug were tested for presence of different organic groups and results are presented in Table 2.

Table 2: *Major Group of Organic Chemical Constituents of Drug*

Organic Groups of Chemical Constituents	Reagents / Tests	Inference
Alkaloid	Dragendorff's and Mayer's reagents	+
Anthraquinone	Borntrager reaction	+
Coumarin	Alcoholic potassium hydroxide	+
Flavonoid	Shinoda reaction	-
Glycoside	Mollisch's test	-
Protein	Xanthoprotein test	+
Resin	Ferric chloride regent	+
Saponin	Libermann-Burchard reaction	-
Steroid	Salkowski reaction	-
Tannin	Gelation test	+

S) Identity, Purity and Strength

Physico-Chemical Constants: The analytical values in respect of physico-chemical constant of drug were established and results are reported in Table 3.

Table 3: *Analytical Values of Physico-Chemical Constants*

Physico-Chemical Constants	Analytical values
Moisture content, % w/w, Not more than	6.0
pH	6.7
Total Ash, % w/w	5.5
Acid insoluble ash, % w/w, Not less than	0.5
Alcohol soluble extractive % w/w, Not less than	6.0
Water soluble extractive % w/w, Not less than	6.0
Essential Oil , % v/w, Not less than	_

T) Fluorescence and Spectroscopy

Fluorescence Characteristic of Powdered drug under Ultra-Violet Light*:* Powdered drug was screened for fluorescence characteristic with or without chemical treatment. The observations pertaining to their colour in daylight and under ultra-violet light were noticed and are presented in Table 4.

Table 4: *Fluorescence Characteristic of Powdered Drug under Ultra-Violet Light*

Treatments	Sample	
	Colour In Day Light	Nature of Colour in Fluorescence
Powder as such	Dark khaki	Greenish brown
Powder with		
Carbon tetra chloride	Greenish brown	Dark brown
Ethyl acetate	Greyish brown	Dark brown
Hydrochloric acid	Deep green	Yellowish brown
Nitric acid + water	Yellowish brown	Greyish yellow
Sodium hydroxide + methanol	Brown	Brown
Sodium hydroxide + water	Greyish brown	Brown
Sulphuric acid + water	Yellowish brown	Brown
Buffer- pH 5	Brownish	Brown
Buffer- pH 7	Brownish	Greyish brown
Buffer- pH 9	Brownish	Dark grey

Ultra-Violet Spectroscopy: The data related to Ultra-Violet Spectrophotometric characteristics as computed in Table 5.

Table 5: *Ultra-Violet Spectrophotometer Characteristic of Drugs*

Specifications	Data
Tincture dilution ml/ml	1
Maximum absorption peak	1.053 0.755
λ Maxima at, nm	342.05 260.45

Chromatographic Profile**:** Thin-Layer Chromatography: Best separation for TLC fingerprinting was obtained by using different layers and solvent systems. Inferences are shown in Table 6.

Table 6: TLC Fingerprinting Data

Drug	Mobile Phase/Solvent System	Derivatizing Reagents	Visualizations	No. of Spots	R_f Values of Bands
Piper nigrum L.	Toluene: Ethyl acetate: Formic (5:4.5:0.5) v/v	Anisaldehyde-Sulphuric Acid	Under 254 nm	5	0.51 (grey), 0.56, 0.61, 0.70 and 0.78 (all dark grey)
			Under 366 nm	4	0.51, 0.56 (both sky blue), 0.61 (greenish yellow) and 0.83 (sky blue)
			After Derivatization	5	0.51, 0.56, 0.61, 0.78 and 0.83 (all brown)

U) Regulatory Quality Specifications

Table 7 and 8 is showing the regulatory specifications for fruits of *Piper nigrum* L. in different regulatory compendium.

4. Discussion

The Pepper fruit is used in a number of classical and patent and propertiery formulations of Ayurveda, Siddha and Unani preparation. It is also most commonly used as a spice. Present communication will be very helpful for the quality control and to check the adulteration of Pepper.

Table 7: *Regulatory Specifications for fruits of P. nigrum Linn. in different regulatory compendium*

Quality Specification	Ayurvedica Pharmacopoeia of India (API) Pt.-I, Vol.-III	Unani Pharmacopoeia of India (UPI) Pt.-I, Vol.-IV	Siddha Pharmacopoeia of India (SPI) Pt.-I, Vol.-I	India Pharmacopoeia 2010	Food Safety and Standards Regulations 2011
Official Title	Marica	Filfil Siyah	Milaku	Maricha	Peeper Black(Kalimirch)
Botanical Species	*P. nigrum* L. (Fam. Piperaceae)	*P. nigrum* L. (Fam. Piperaceae)	*P. nigrum* L. (Fam. Piperaceae)	*P. nigrum* L. (Fam. Piperaceae)	*P. nigrum* L. (Fam. Piperaceae)
Morphological part/Official part	Fully mature dried fruits	Fully mature dried fruits	Fully mature dried fruits	Unripe fruits contains not less than 2.5 % w/w of piperine	Dried mature fruits
Description	I. Macroscopic II. Microscopic III. Powder	I. Macroscopic II. Microscopic III. Powder	I. Macroscopic II. Microscopic III. Powder	I. Macroscopical II. Microscopical	_
Identity, Purity & Strength					
Foreign Matter	2.0 %, not more than	2.0 %, not more than	2.0 %, not more than	2.0 %, not more than	1.0 %, not more than
Total Ash	5.0%, not more	5.0%, not more	5.0%, not more	7.0%, not more	6.0 %, not more

	than	than	than	than	than
Acid insoluble ash	0.5%, not more than	0.5%, not more than	0.5%, not more than	2.0%, not more than	-
Alcohol soluble extractive	6.0%, not less than	6.0%, not less than	6.0%, not less than	6.0%, not less than	-
Water soluble Extractive	6.0%, not less than	6.0%, not less than	6.0%, not less than	6.0%, not less than	-
Volatile Oil (Assay)	-	-	-	-	2.0 %, not less than
Thin layer chromatography	TLC profile and TLC of piperine	TLC profile and TLC of piperine	TLC profile	TLC profile	_
Extraneous Matter including dust dirt, stones, lumps of earth, chaff, stalk, stem or straw ,edible seeds of fruit other than coriander and insect damaged seeds	_	_	_	_	5.0 %, w/w, maximum
Insect Damaged Matter (partially or wholly bored by insects)	_	_	_	_	1.0, w/w %, not more than
Powder's Specification	_	_	_	_	Rough or fine powder obtained by grinding clean, dried piper fruits.
Moisture content in powder	_	_		12.0 %, not more than	12.5%, w/w, not more than
Total ash in powder	_	_	_	_	6.0 %, w/w, not more than
Acid insoluble ash in powder	_	_	_	_	1.2%, w/w, not more than
Added colouring matter in powder	_	_	_	_	Free from added colouring matter

Figure 1: *Organoleptic Characteristics*

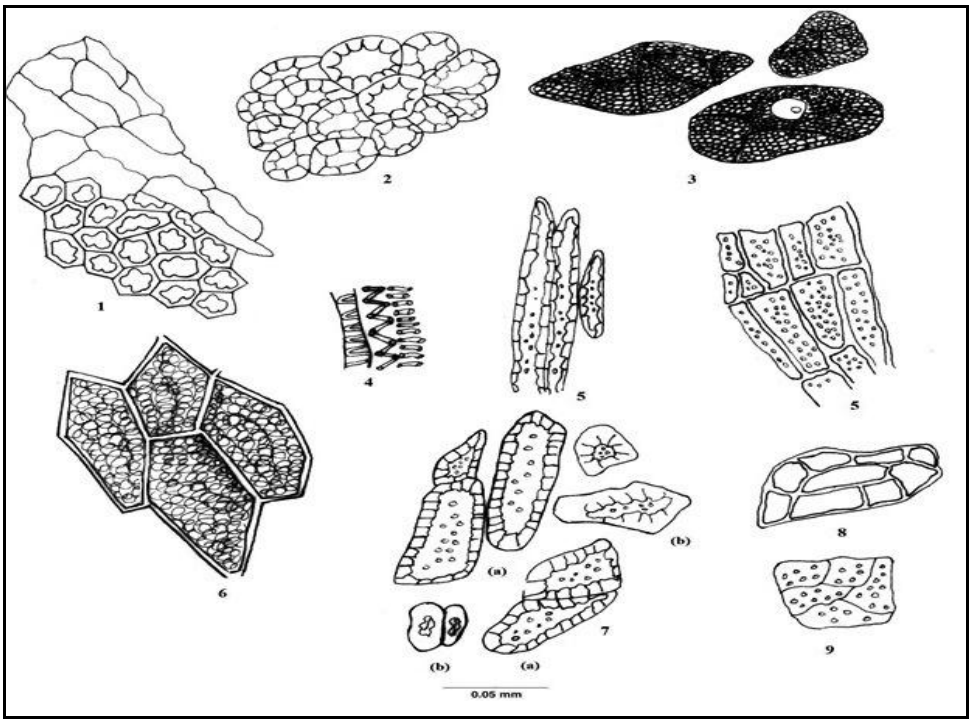

Figure 2: *Micro-morphological Characteristics*

1.Endocarp cells with mesocarp cells; 2. Sclerenchymatous cells; 3. Cells of perisperm contaning starch and oil cells, 4. Vessels from vascular strands, 5. Fibrous sclereids, 6. Inner epidermis of seed-coat, 7. (a) Isolated sclereids from the outer mesicarp, (b) Strongly thickened sclereids, 8. Mesocarp cells, 9. Outer layer of perisperm contaning aleurone.

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