PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHYSICOCHEMICAL STUDIES OF *PIPER NIGRUM* LINN. FRUIT (PIPERACEAE)

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ABSTRACT

Piper species are reported to have great medicinal value in Indian medicine. Fruits of *Piper nigrum* Linn is commonly known as “Kalimiri” belongs to the family of Piperaceae and widely used as a pungent condiment. Traditionally it is used as antipyretic, diuretic, aphrodisiac, immune-stimulant, antioxidant, hepatoprotective, digestive, rubefacient, counter irritant, antiseptic, antispasmodic agent. Present work is related to standardization of *Piper nigrum* by using Pharmacognostic (Macroscopy, Cytomorphology, Physical constants) and Photochemical study of fruit. All parameters were studied according to the WHO and Pharmacopoeial guidelines to standardize the *Piper nigrum*.

Keywords: Cytomorphology, Pharmacognostic study, *Piper nigrum* Linn, Standardization.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine system that has been in existence for thousands of years¹. Many plants derived molecules have shown a promising effect in therapeutics. Among the plants investigated to date, one showing enormous potential is the pepper family otherwise known as piperaceae.² The herb bears characteristic and large oval shaped leaves, it also has spikes of many small and white colored flowers, the commercial pepper itself is made from the many clusters of small round fruits borne on the plant, these small fruits are the pepper of common use, and these fruits ripen from a green to red coloration as they mature on the vine.³ *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.⁴ 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.^{4,5} The members of piperaceae family show the presence of phenolic esters and ethers, pyrrolidone, volatile oil & ligands.⁶

MATERIAL AND METHODS

Collection and authentication of plant

The dried ripe fruits of *Piper nigrum* Linn were collected in the month of December from the local market of Pune. Their identity and authentication was done by Department of Pharmacognosy, Marathwada Mitra Mandal's College of Pharmacy, Pune by correlating their morphological and microscopical characters with those given in literatures.

Macro morphology

The fruit of *Piper nigrum* was subjected to macroscopic studies which comprised of organoleptic characteristics viz. colour, odour, appearance, taste, shape, texture etc. of the drug. These parameters are considered as quit useful in quality control of the drug and were evaluated as per standard WHO guidelines.

Cytomorphology

Dried ripe fruits of *Piper nigrum* were hand-sectioned transversely and paradermally with a sharp diamond edge blade. Photomicrographs of different magnification were taken with Moticam 2300 camera and were analyzed by Motic Image- plus2.0 software. Histology and histochemistry of the drug was performed according to the methods described by Brain & Turner.^{7,8,9}

Powder Characteristics

Preliminary examination and behavior of the powder with different chemical reagents was carried out and microscopical examination was carried out as per reported methods.^{9,10}

Micrometry

Quantitative microscopy of the transverse sections and fruit powder were performed to determine the size and dimensions of tissues, cells and cell contents.^{11,12}

Physicochemical Evaluation

Physicochemical parameters such as foreign organic matter, moisture content, ash values, Extractive values were determine according to the official method of the WHO guidelines on quality control methods for medicinal plant material.¹³

Fluorescence Analysis

Dried fruits were powdered and observed under day light, short ultra violet light and long ultra violet light after treatment with different reagents like chloroform, ethyl acetate, methanol, petroleum ether (BP.60-80°C), 50% Sulphuric acid, 50% Nitric acid, 50% Hydrochloric acid, 10% Sodium hydroxide, etc.^{14,15,16}

Preliminary Phytochemical Screening

The qualitative chemical tests carried out for the identification of the nature of different phytoconstituents present in the powdered crude drug. The tests were carried out by using standard conventional protocols.^{8,13}

Table 1: Macro morphological Description

Characters	Observation
Colour	Blackish grey
Odour	Aromatic
Taste	Pungent
Size	0.4-0.5 cm in diameter
Shape	Globular or oblong
Type	Simple

Table 2: Histochemical Properties of Transverse Section of *Piper Nigrum* Fruits

Reagent	Inference	Histological zone
Dragendroff's reagent	Orange red colour	Alkaloids
Wagner reagent	Reddish brown	Alkaloids
Sudan red III	Red	Oil globules in endosperm
Weak iodine solution	Blue	Starch grain in perisperm
Phlorogucinol + HCL	Pink	Lignified sclereid

Table 3: Micrometry of Some Cells

Type of Cells	Length in micrometer (μm)
Epicarp	0.9
Sclereid	6.2
Mesocarp	5.0
Testa	2.1
Perisperm	71.5

Table 4: physicochemical parameters

Parameters (w/w %)	Observation (w/w %)
Foreign organic matter	1.40 $\pm$ 0.025
Moisture content	3.43 $\pm$ 0.015
Total ash	4.29 $\pm$ 0.06
Acid insoluble ash	0.44 $\pm$ 0.050
Water soluble extractive value	10.03 $\pm$ 0.061
Alcohol soluble extractive value	12.19 $\pm$ 0.055
pH	06.60 $\pm$ 0.00

*Values are expressed as mean $\pm$ standard deviationTable 5: Flourescence analysis of *piper nigrum* linn. Fruit powder

Reagents	Visible light	Short UV (254 nm)	Long UV (366 nm)
Distill water	Off white	Slight green	Brown
Pet. Ether (60 ⁰ -80 ⁰)	Light brown	Greenish brown	Brown
Chloroform	Light brown	Green	Yellowish green
Methanol	Light yellow	Green	Dark green
Conc. HCL	Deep green	Yellowish brown	Brown
Conc. HNO ₃	Yellowish brown	Grayish yellow	Brown
Conc. H ₂ SO ₄	Yellowish brown	Brown	Dark brown
Picric acid	Greenish brown	Greenish yellow	Dark green
10% NAOH	Grayish brown	Brown	Brown

Conc. – Concentrated, HCL- Hydrochloric acid, HN0₃- Nitric acid, H₂SO₄ – Sulphuric acid, NAOH- Sodium hydroxide

Table 6: Preliminary Photochemical Screening

Parameters	Observation		
	Aqueous extract	Methanolic extract	Ethanolic extract
Carbohydrates	+	+	+
Proteins	+	+	+
Tannins	+	+	+
Phenols	+	+	+
Coumarin	+	+	+
Alkaloids	+	+	+
Antraquinones	+	+	+

*+ indicates presence

Figure 1: Macro morphology of *Piper nigrum* fruit

Figure 2: T.S of *Piper nigrum* fruit

Figure 3: Micrometry of *Piper nigrum* fruit

Figure 4: Powder microscopy of *Piper nigrum* fruit
*a- starch, b- sclereid, c- mesocarp, d- testa, e- stone cell, f- epicarp

RESULT AND DISCUSSION

Macromorphological description

The *Piper nigrum* fruits are globular in shape, 3-6mm in diameter. The external surface is dark brown or grayish black and strongly reticulated wrinkled with remains of stigma at apex. The organoleptic evaluation of the fruit and fruit powder revealed that both were grayish black or dark brown in colour, with aromatic odour and pungent taste. The results of macromorphology were depicted in Table 1 and Figure 1 shows the morphology of *Piper nigrum* fruits.

Cytomorphology

Transverse section of fruit shows a well-differentiated thick pericarp, testa and inner mass of perisperm and enclosing a small embryo. Pericarp consists of an external epicarp, a large parenchymatous mesocarp and a single layer of endocarp containing stone cells. The sclereids are varying in shape and size, usually polygonal to rectangular. Mesocarp consists of tangentially elongated parenchymatous cells. Elongated oil cells are also seen in outer region of mesocarp and fibrovascular bundle and a layer of oil cells are present in the inner region of mesocarp. Endocarp is made of beaker shape stone cells and testa is represented as a single layer of yellow colour cells, thick walled sclerenchymatous cells. Perisperm contains few oil globules, starch and a few aleurone grains.¹⁷ Figure 2 and reveal the detail microscopy of fruit and Table 2 reveal the histochemistry of drug.

Powder Characteristics

The Powder of *Piper nigrum* fruit is Blackish grey, with aromatic odour and pungent in taste. The microscopic examination of the powder shows epicarp, mesocarp, sclereid, yellow colour testa, beaker shape stone cells, starch and isolated oil cells. (Figure 4)

Micrometry

The results of micrometric characters of tissues, cells and cell contents were depicted in Table 3. Measurements of different cells are frequently necessary for the quantitative identification of closely allied substances. In most cases, these allied substances are mixed with the original drugs as adulterants and substituent.^{12,18} Thus the adulterants and/or substituent's present in crude drugs can be distinguished by this way with the aid of optical microscopy (Figure 3).

Physicochemical Evaluation

Evaluation of crude drug ensures the identity of drug and determines the quality and purity of drugs. The main reason behind the need for the evaluation of crude drug is biochemical variation in the drug, effect of treatment, storage of drug, adulteration and substitutions.^{19, 20} The results of the physicochemical parameters of fruit powder lie within the limit which is mentioned in table 3. The results of foreign organic matter denote presence of any organism, part or product of an organism, other than that named in the specification and description of the herbal material concerned^{12,18} which was found to be 1.40 ± 0.025 . Insufficient drying favors spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles. Not only the ultimate dryness of the drug is important equally important is the rate at which the moisture is removed and the condition under which it is removed thus the determination of moisture content also provide the method of preparation of drug.^{11,12} and it is observed that the moisture content of the drug was found to be $3.43 \pm 0.015\%$ w/w which signify that the

drug is properly dried and properly stored. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign matter such as metallic salts or silica.^{13,18} An analytical result for total ash was found to be $4.24 \pm 0.04\%$ w/w. The amount of acid-insoluble siliceous matter present was $0.44 \pm 0.050\%$ w/w; As the ash values of the crude drugs lies within the fair limit which signify its quality and purity and gives idea about the total inorganic content. The water soluble extractive value indicated the presence of sugar, acids and inorganic compounds;^{12,13} the water soluble extractive value found to be $10.03 \pm 0.061\%$ w/w and alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids.^{13,18} The alcohol soluble extractive value was found to be $12.19 \pm 0.055\%$ w/w which signify the nature of the phytoconstituents present in plant. As the pH was determined near to 6.6 which were near to neutral because of the alkaloid nature.

Fluorescence analysis

The results of fluorescence analysis were shown in Table 5. Fluorescence is an essential parameter for first line standardization of crude drug. in fluorescent light is always greater wavelength than the exciting light. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in day light.^{21,22}

Preliminary Photochemical screening

The preliminary phytochemical investigation of powdered fruit, ethanolic extract were performed which shows the presence of Alkaloids, essential oil, coumarin glycosides, phenol and tannins derivatives, carbohydrates, proteins type of major secondary metabolites which revealed their potent therapeutic activity.²³ The results of the screening were expressed in Table 6.

CONCLUSION

Standardization is essential measure for quality, purity and sample identification. Macromorphology and microscopy along with the Quantitative analytical microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. Physicochemical and Chemical analysis of fruit confirm the quality and purity of plant and its identification.

The present study was useful for further pharmacological and therapeutic evaluation along with the standardization of plant material.

REFERENCES

1. Madhvi Bindu B *et al.* Extraction, Identification, Formulation and Evaluation of Piperine in Alginate beads. International journal of pharmacy and pharmaceutical science. 2009; 1(2): 156-168.
2. Reshmi SK, Sathya E and Devi SP. Isolation of Piperidine from *Piper nigrum* and its antiproliferative activity. African Journal of Pharmacy and Pharmacology. 2010; 4(8): 562-573.
3. Kolhe SR, Borole P, Patel U. Extraction and evaluation of Piperine from *Piper nigrum* Linn. International journal of Applied Biology and Pharmaceutical Technology 2011; 2: 144-149.
4. Rai Nitin *et al.* Quality specification on *Piper nigrum* L. - A Spice and Herbal drug of Indian Commerce. International Journal of Advanced food Science and Technology. 2012; 1(1): 1-11.
5. Leug Albert Y. *et al.* Encyclopedia of Common Natural Ingredients (used in food drugs and cosmetic). A Willey Interscience Publication, Johan Wiley & Sons; 1996.
6. Qadry JS. Pharmacognosy. 16th ed. Delhi: Published by Professor J. S. Qadry; 2010. p. 404-405.
7. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Bristol: Wright-Scientechnica; 1975. p. 4-9

8. Kokate CK. Practical Pharmacognosy. 4th ed. Delhi: Vallabh Prakashan; 1997. p. 107-111
9. Iyengar MA. Study of crude drugs. 14th ed. Manipal: Manipal Press; 2009. p. 67-68
10. Gokhale SB, Gokhale A, Kulkarni Y, Yele S. Experimental Pharmacognosy. 1st ed., Pune: Nirali Prakashan; 2011. p. 2.1-2.8
11. Evans WC. Pharmacognosy. 16th ed. London: W.B Saunders company Ltd.; 2009. p. 541-570
12. Mukharjee PK. Quality Control of herbal drugs. 1st ed. Business horizon publication; 2002. p.186
13. World Health Organization. Quality control methods for medicinal plant materials. WHO/PHARM/92.559; 1998. p. 4-46
14. Pimple BP, Patel AN, Kadam PV, Patil MJ. Microscopic evaluation and physicochemical analysis of *Origanum majorana* Linn leaves. Asian Pacific Journal of Tropical Disease; 2012. p. S897-S903
15. Gupta PC, Sharma N, Rao CV. Pharmacognostic studies of the leaves and stem of *Careya arborea* Roxb. Asian Pacific Journal of Tropical Biomedicine; 2012. p.404-408
16. Kumar D, Gupta J, Kumar S, Arya R, Kumar T, Gupta A. Pharmacognostic evaluation of *Cayratia trifolia* (Linn.) leaf. Asian Pacific Journal of Tropical Biomedicine. 2012; 2: 6-10. [http://dx.doi.org/10.1016/S2221-1691\(11\)60180-9](http://dx.doi.org/10.1016/S2221-1691(11)60180-9)
17. Indian Herbal Pharmacopoeia. Part 2. Mumbai and Jammu-Tavi: A Joint Publication of Regional Research Library and Indian Drug Manufacture Association; 1998. p. 317-326
18. Anonymous. Indian Pharmacopoeia. Vol-II, Ministry of Health and Family welfare, New Delhi: Govt of India, Controller of publication; 1996. A-53-54, A-97, A-109.
19. Jarald EE, Jarald SE. Textbook of Pharmacognosy and Phytochemistry. 1st ed. New Delhi: CBS Publication; 2007. p. 96-101
20. Kadam PV, Yadav KN, Patel AN, Navsare VS, Bhilwade SK. Phytopharmacopoeial specification of *Garcinia Indica* fruit rinds. PHCOG J. 2012; 4: 23-28. <http://dx.doi.org/10.5530/pj.2012.31.4>
21. Arya V, Gupta R, Gupta VK. Pharmacognostic and phytochemical investigations on *Pyrus pashia* Buch.-ham. Ex D. Don stem bark. Journal of Chemical and Pharmaceutical Research. 2011; 3(3): 447-456
22. Kumar D, Kumar K, Kumar S, Kumar T, Kumar A, Om Prakash. Pharmacognostic evaluation of leaf and root bark of *Holoptelea integrifolia* Roxb. Asian Pacific Journal of tropical Biomedicine. 2012; 2(3): 169-175. [http://dx.doi.org/10.1016/S2221-1691\(12\)60036-7](http://dx.doi.org/10.1016/S2221-1691(12)60036-7)
23. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 15th ed. Pune: Nirali Prakashan; 2006. p. 15-163

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