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PHARMACOGNOSTIC EVALUATION AND INSTRUMENTAL ANALYSIS (SEM) FOR THE STANDARDIZATION OF *PIPER NIGRUM* L., (BLACK PEPPER) FRUIT

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Abstract

Piper nigrum, commonly known as "black pepper" has been used in folk medicine in the treatment of various ailments i.e., cold, flu, fever, headaches, nasal congestion, improves digestive health, and enhances brain health. Piperine is an active constituent having various pharmacological properties such as anti-fungal, anti-inflammatory, antiseptic, analgesic, and diuretic etc. The present study was aimed to assess the pharmacognostic characters and standardize the *Piper nigrum* fruit for identification, and authentication. Macro-microscopic characterization and heavy metal profile (trace elements) were determined using standard analytical methods. The macroscopic study revealed the blackish to brown color seed, aromatic odor, pungent taste and almost globular shaped with wrinkled surface, while powder microscopic analysis indicated the presence of tissue such as perisperm, epicarp, stone cells, and sclereids cells etc. The scanning electron microscopy (SEM) of the sample showed the presence of micro particles. While energy dispersive x-ray spectrometer (EDAX) showed the C, O, K, Mg, Ca, and Cl elements. These specific identities will be useful in authentication and standardization of the crude drug as well as to control the adulterations and differentiate the different varieties of black pepper drug.

Key words: *Piper nigrum* L., Spice, Trace elements, SEM-EDAX, Analgesic.

Introduction

Spices are different parts of plants, most of them are found in dried form. Due to the aroma and strong tastes, spices are usually used worldwide to develop the taste of food as well as phytopharmaceuticals (Stankovic & Comic, 2006). The use of spices as medicine is one of the old forms of known healthcare regimen and used by all civilizations and cultures since prehistoric times. Around 3500 BC the ancient Egyptians used different spices as a flavor in foods, as medicine, to prepare cosmetics, as well as embalming of dead bodies (Rosengarten, 1969*;* Barnes *et al*., 2007*).*

Black pepper (*Piper nigrum* L., family Piperaceae) regarded as the "king of spice" is a very famous spice since prehistoric times. It is a smooth woody flowering plant, widely disseminated to Bengal, Malaysia, Indonesia, Brazil, Assam, Vietnam, China, Thailand, Sri Lanka, and India (Reshmi *et al*., 2010 Trivedi *et al*., 2011; Ahmad *et al*., 2012). It has been extensively used in Pakistani cuisines as a condiment and in herbal medicines. Black pepper has been used in several systems of medicine such as Siddha, Ayurveda, Unani and Homeopathic. It contains volatile oil (Piperine, Isopiperine, Chavicine and Isochavicine, sabinene, pinene, terpenene, limonene), Phenolic compounds, flavonoids, lignins, alkaloids, amides, and steroids. Piperine is an active constituent of black pepper. The reported pharmacological studies revealed that black pepper possessed antimicrobial, antipyretic, anti-inflammatory, analgesic, anti-fungal, anti-depressant, anti-spasmodic, diaphoretic, antiseptic, anti-emetic anti-toxic, aphrodisiac, diuretic, febrifuge, hepatoprotective, sialagogue, anti-oxidant, and immune-boosting properties (Nahak & Sahu, 2011). Black pepper oil is used to relieve rheumatic pain, flu, cold, fever, emotional and physical coldness, muscular aches (Aziz *et al., 2012*; Rai *et al*., 2012). The present study was aimed to evaluate the Pharmacognostical parameters including macro and microscopic characters and detection the trace elements.

Materials and Methods

Plant material: The fruit of *Piper nigrum* L**.** was purchased from local market of Karachi - Pakistan. It was then authenticated by Professor Dr. Usman Ghani Khan, Faculty of Pharmacy, Jinnah University for Women, Karachi. The reference sample was assigned specimen number BP001 and deposited in the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Science, University of Karachi - Pakistan.

Preparation of plant sample: The fruits were cleaned, washed, and dried under the shade at room temperature. It was then grounded to a fine powder and stored into a well-closed container for further studies.

Pharmacognostic analysis

Macroscopic study: Macroscopic or organoleptic characterization such a color, odor, taste, and texture of powder was performed using standard methods (Arora *et al*., 2013; Fofie *et al*., 2014).

Microscopic study: The powder microscopy was performed according to the standard method. A pinch of powder was mounted with few drops of iodine solution (5%) on a microscopic slide and then observed under a microscope. The same procedure was adopted with chloral hydrate and glycerin solution (5%) (Iyener, 1980).

Scanning electron microscope (SEM): SEM was done at Central Science Laboratory, University of Karachi, Pakistan to evaluate the powder surface morphology and particle size using JSM 6380A Scanning electron microscope. The powder sample was placed on specimen stub with two-sided adhesive tape and coated with a thin layer of gold using quick auto coater model number JFC 1500. The process was done under a high vacuum, using 20 keV electron beam energy. The sample was placed inside the microscope's vacuum column evaporator, a ray of an electron was then passed through an electron gun and traveled from a series of magnetic lenses. The detector counted the electron and the amplifier received the signals, from each spot of the sample, number of an electron was dispersed and formed images and provided information of the sample. The detection and concentration of trace elements in the tested sample EDAX detector (EX-54175 JMU) was used which attached to the electron microscope. The SEM image under 2,000 X magnification and the examining area of 1280 X 960 surface was taken for the sample (Hameed *et al*., 2008; Sathya *et al*., 2014). Where the SEM was done?

Results

Macroscopic study: The macroscopic study revealed that fruit of *Piper nigrum* was dark brown to black in color, having an aromatic odor, and strong pungent taste. Appearance noted as almost globular shaped with rough, corrugated and wrinkled texture with4-6 mm in diameter (average = 5.4 mm diameter) and the fracture was fibrous (Table 1).

Microscopic study: The powder was brownish to black in color, an aromatic odor with pungent taste. The powder microscopic study indicated the presence of lignified endocarp cells, oil cell, hypodermal parenchyma with stone cells, pigmented epicarp, perisperm with starch granules, testa with reddish brown pigments, stone cell, mesocarp cells, cluster of calcium oxalates, pitted fiber, fibrous sclereids, annular vessel, sclereids, rectangular vessels, parenchyma layer with oil cells, and starch granules, covering trichome, stomata and pigmented pericarp with crystal calcium oxalates shown in Fig. 1.

Table 1. Macroscopic evaluation of *Piper nigrum* fruit.

Color	Blackish brown
Odor	Aromatic
Taste	Pungent
Size	4-6 mm in diameter (Average = 5.4 mm diameter)
Shape	Almost globular
Texture	Rough, corrugated and wrinkled

SEM analysis: The surface of the sample was observed in cluster, medium ellipse, and globule arrangement. The SEM image (Fig. 2) showed microparticles ranging from 2.30µm to 4.25µm. These microparticles were 2.30µm, 3.13µm, 3.24µm, 3.30µm, and 4.25µm. The scanning electron microscope-energy dispersive x-ray spectrometer (SEM-EDAX) showed the spectrum of elements (Fig. 3) and reported the percentage of trace elements such as carbon 58.73%, oxygen 37.92%, magnesium 0.24%, chloride 0.44%, potassium 2.08% and calcium 0.08% (Table 2).

Table 2. Percentage of trace elements in powder of *Piper nigrum* fruit.

Element	(keV)	Mass %	Error %	At %
C	0.277	58.73	0.3	66.47
O	0.525	37.92	1.78	32.22
Mg	1.253	0.24	0.58	0.14
Al	1.486	0.5	0.52	0.25
Cl	2.621	0.44	0.45	0.17
K	3.312	2.08	0.62	0.72
Ca	3.69	0.08	0.73	0.03
Total		100		100

Discussion

Plants or plant part serves as raw material for synthesis of many drugs. Owing to the medicinal properties attributed to a crude drug and considerably fewer side effects, it is necessary to maintain its quality and purity for the commercial market. It is noted that the crude drugs in commerce are often adulterated and do not comply with the standards prescribed for an authentic drug. Hence, it is necessary to make efforts for the standardization of plants materials. The standardization procedure can be attained by stepwise pharmacognostic studies. Pharmacognostic evaluation helps to screen the commercial varieties, substitutes, adulterants, and quality of the drugs. Thus, it is necessary to know the histological characters of genuine drug materials for the detection of adulterants. Even though many sophisticated modern techniques are available nowadays for the standardization of crude drug but still pharmacognostic studies are one of the simplest, inexpensive and it is considered as a first step towards the establishment of identity and purity of the sample (Singh *et al*., 2010). In this study, macro and microscopic studies, and elemental analysis were attempted. Even though various microscopic studies of black pepper has been reported (Kadam *et al*., 2013, Das Mansoi *et al*., 2016) but some microscopic features identified in the present study were not reported in previous studies. Results of the present study revealed some diagnostic features in terms of macro and microscopic study that could provide a better referential information for identification, standardization as well as to prevent the adulteration of *Piper nigrum.*

Consumption of spices has been markedly increased all over the world due to their medicinal value but it may be contaminated with trace and heavy metals and the results could lead to several health problems (Prabhangshu *et al*., 2015). Heavy metal contents in medicinal plants show a great variation depending on a variety of factors such as habitat, climatic condition, cultivation, pre and post harvesting and storage of plant material. Thus, the medicinal plant should be tested for contaminant load before processing it further for medicinal use. SEM–EDAX, a non-destructive analytical instrument was used to visualize the various microstructures within the sample and to assess the element concentration in the test sample (Yashvanth *et al*., 2013). Medicinal plants contain several trace elements, having an important role in biological systems. Many researchers (Krejpcio *et al*., 2007, Behnaz *et al*., 2017) have identified different elements in black pepper. The present study also identified some elements that were not reported in previous studies may be due to the differences in geographic location, weather condition, fertilizer, cultivation method and soil composition in which the spice grow.

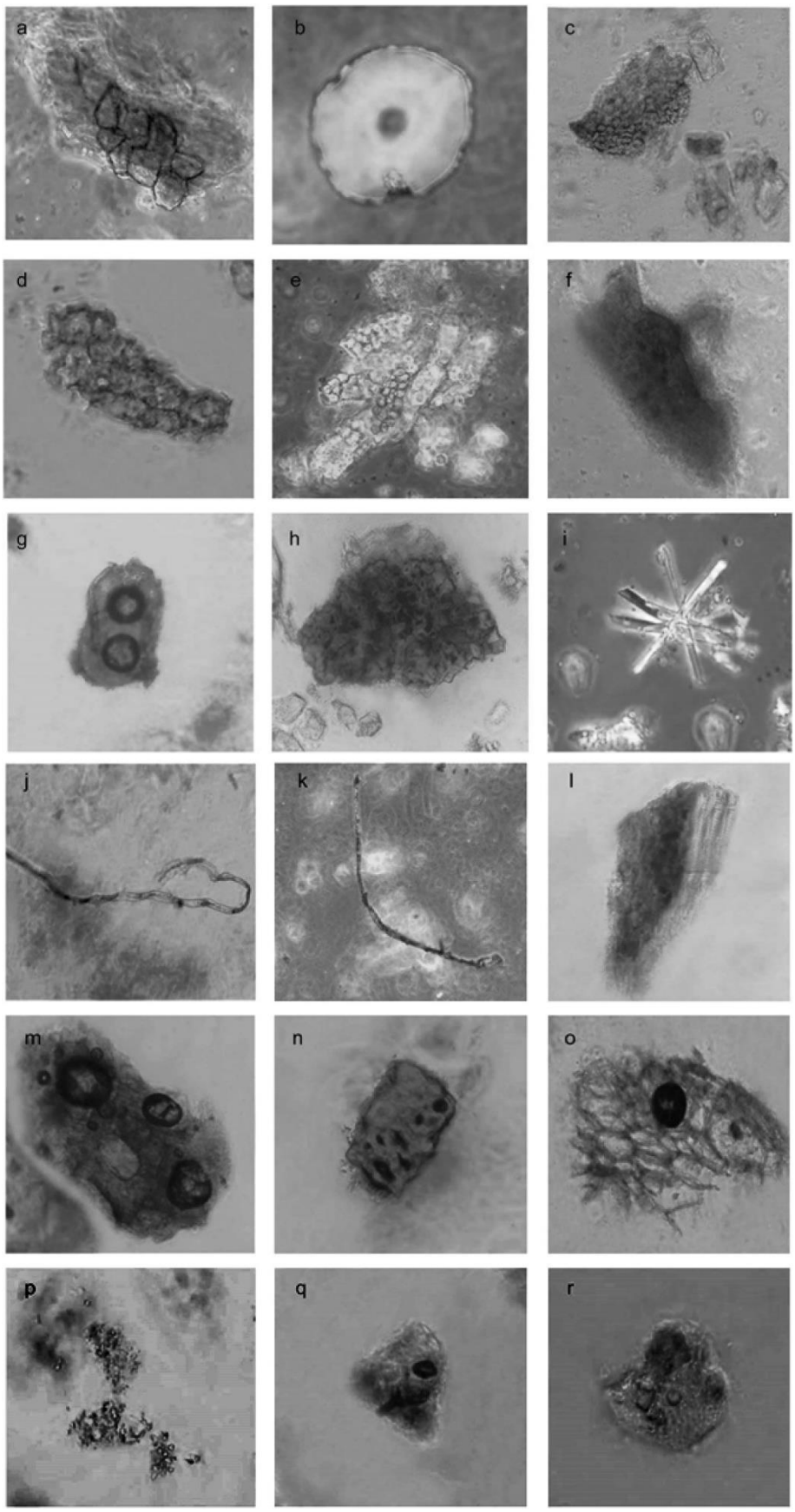

Fig. 1. Microscopic evaluation of powder *Piper nigrum* fruit (a) Lignified endocarp cells, (b) Oil cell, (c) Hypodermal parenchyma with stone cells (d) Pigmented epicarp, (e) Perisperm with starch granules, (f) Testa with reddish brown pigments, (g) Stone cell (h) Mesocarpcells, (i) Cluster calcium oxalates, *Piper nigrum* fruit (j) Pitted fiber, (k) Fibrous sclereids, (l) Annular vessel, (m) Sclereids, (n) Rectangular vessels, (o) Parenchyma layer with oil cells and calcium oxalates (p), Starch granules, (q) Covering trichome with stomata and (r) Pigmented pericarp with crystal calcium oxalates.

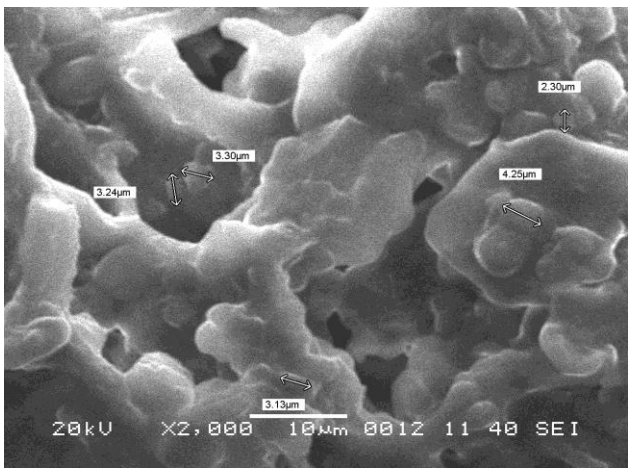

Fig. 2. SEM image of powder *Piper nigrum* fruit showing micro particle.

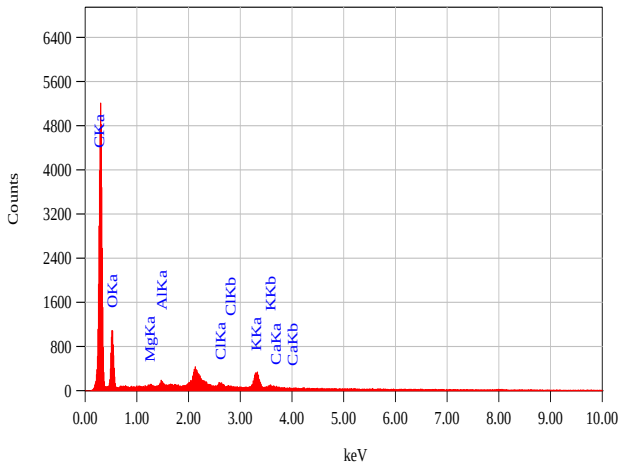

Fig. 3. The SEM-EDAX of trace elements in *Piper nigrum* fruit.

The result of trace elements showed that black pepper was an excellent source of minerals like carbon, oxygen, calcium, potassium, magnesium, and chloride. Potassium was found in maximum concentration than other elements. Potassium is an important component of cell and body fluids that help regulate heart rate, blood pressure and in smooth muscles contraction (Starlin *et al*., 2012). Calcium is essential for bones and teeth development. Magnesium is a co-factor in many enzymes that regulate growth and development. It plays a vital role in various bio-chemical reactions. (Sathya *et al*., 2014). Whereas chloride is required for the digestion of food and regulate the pH in the body and maintain the electrolyte balance in the body (Zemberlin *et al*., 2012; Starlin *et al*., 2012). Deficiency of these trace elements may cause various ailments. For instance, cardiac arrhythmias, muscular weakness, paralysis, mental confusion, osteoporosis, hypochromic anemia, leucopenia, nausea and insomnia (Dilek *et al*., 2013; Soetan *et al*., 2010).

In the present study trace elements were found in sufficient quantity, suggested that black pepper could be considered as an important source of mineral nutrients while, heavy metals such as mercury, arsenic, cadmium, and lead were not detected in the sample. Thus, *Piper nigrum* fruit appeared to have high nutritional value for human consumption.

Conclusion

The pharmacognostic aspects will contribute to the botanical identification, authentication, purity, and standardization of plant material or plant part. The findings of the present study could be helpful in setting standards of the medicinal plant as well as to distinguish the different varieties and to control adulterations of *Piper nigrum***.** These parameters can be useful in further isolation and purification of the medicinally important compound.

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