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Research Article

PHARMACOGNOSTICAL, PHYSICOCHEMICAL AND PHYTOCHEMICAL STUDIES OF PIPER LONGUM LINN. FRUITS

Sharma P*[1], Kaushik R[2], Khan AD[2], Malik MT[3], Fagna B[2]

[1]Department of Pharmacy, IEC Group of Institutions, Greater Noida, Uttar Pradesh, India
[2]Ram-Eesh Institute of Vocational and Technical Education, Greater Noida, Uttar Pradesh, India
[3]Shaqra University, Riyadh, Kingdom of Saudi Arabia, UAE

ABSTRACT

Introduction: *Piper longum* Linn, belonging to the family Piperaceae, is a climber, perennial shrub which is commonly found in India. The dried fruits of Piper longum are widely used in Ayurvedic System of medicines since time unknown. It posses significant pharmacological properties due to presence of variety of chemical constituents in it. **Objective:** The present study is aimed to evaluate the pharmacognostical, physiochemical and phytochemical parameters for *Piper longum* fruits, as per the WHO guidelines for herbal drug standardization. **Materials and Method**s: WHO emphasized the use of standardized herbs and formulations for safety and best therapeutic results. WHO guidelines were employed to determine various standardization parameters. **Results and Discussion:** Pharmacognostical studies shows that the fruits of *Piper longum* are found as green when fresh which turns grayish black upon drying. It posses pungent, bitter and acrid taste. The fruits are cylindrical with small petiole. The powder microscopy shows the presence of brown content, oleo resins, stone cells and calcium oxalate crystals. Physiochemical analysis shows variable extractive values in different solvents with maximum extractives of 20.6±0.021% in water and minimum extractive value of 6.6±0.036% in chloroform. Total Ash value of 8.3±0.015%, Acid Insoluble Ash- 1.16±0.025% and Water Soluble Ash- 5.5±0.01%, Foreign Matter- 1.62±0.12%, Moisture content of 15.70±0.051%, Bitterness value-1.96, Swelling Index-1.5±0.01 and Foaming Index- 102.33. Phytochemical analysis of the fruits of *Piper longum* shows the presence of alkaloids, volatile oil, tannins, fixed oils, and phenolics. **Conclusion:** The botanical, physical and chemical parameters obtained in this study can be used for establishing the identity and purity of the drug that will lead to safety and efficacy of the herb.

Keywords: Paracetamol, tablets, Evaluation, Market brands.

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Correspondence:
Priyanka Sharma
Department of Pharmacy, IEC Group of Institutions, Greater Noida, Uttar Pradesh, India.
Phone: +91-9999427794
Email: rahulkcsji@gmail.com

INTRODUCTION

Pepper is an English word with its root in the Sanskrit word 'Pippali'. Long pepper *(Piper longum)*, also called as Indian or Indonesian long pepper. It's a flowering vine of Piperaceae family which is cultivated for its fruit which is also used as a spice. It is found in Assam, Tamil Naidu, Andra Pradesh in India, Malaysia, Indonesia, Singapore, Srilanka and in other South East Asian regions. [1]

Piper longum is a close relative of *P. nigrum*, from which black, green, and white pepper are obtained and has a similar but generally hotter flavor. Piperine is the main alkaloid which contributes to their pungency. Ayurvedic system of medicine, describes it as a good rejuvenator. *P. longum* is a good appetizer and reduces flatulence. Piper longum acts as blood cleanser, improves digestion, helps in management of cough and epilepsy, increases blood circulation, reduces tumor growth, arthritis treatment, antipyretic and helps in treatment of various skin disorders. [1] It's an important ingredient of famous Ayurvedic formulation 'Trikatu churna'.[2]

Piperine is the major and active constituent of long pepper. The piperine content is 3-5% (on dry weight basis) in *P. longum.* The fruit of *P. longum* contains a large number of alkaloids and related compounds, the most abundant of which is piperine, methyl piperine, iperonaline, piperettine, pellitorine, piperlongumine, piperlonguminine, asarinine, piperundecalidine, refractomide A, pipercide, piperderidine, longamide and tetrahydropiperine, terahydro piperlongumine, dehydropipernonaline piperidine, pregumidiene, brachystamide, brachystamide-A, brachystine, terahydropiperlongumine and trimethoxy cinnamoyl-piperidine. Lignans Sesamin, pulvuatilol, fargesin and others have also been isolated from the fruit of *Piper longum.* Volatile oil of the fruit *Piper longum* is a complex mixture. Major components of essential oil are caryophyllene and pentadecane (both about 17.8%) and bisaboline (11%) along with volatile piperine. Other components

include thujine, terpinoline, p-cymene, p-methoxy acetophenone and dihydrocarveol. [3] Plant materials are used throughout the world both in developed and developing countries as home remedies, food supplements, over-the-counter drug products and raw materials for the food and pharmaceutical industry and represent a substantial share in the global drug market. The majority of adverse events reported related to the herbal products are attributed to poor quality of the product. World Health Organization (WHO) also appreciated and stressed the use of standardized and quality herbs and formulations and issued guidelines for establishing identity, purity, quality and efficacy for herbal materials. [4]

The present study is aimed to evaluate the organoleptic, morphological and microscopical features. Physicochemical parameters like extractive values in different solvents, ash values, foaming and swelling index, bitterness values, pH of 1 and 10% solution in water, foreign matter and moisture content. Fluorescence and phytochemical screening were also studied as per the guidelines of World Health Organization (WHO) for quality control of herbal drug materials.

MATERIALS AND METHODS

Chemicals/reagents used

All chemicals/reagents and solvents used were of analytical grade and procured from CDH, Rankem(RFCL Ltd.), Qualigens, Loba Chem and Merck.

Plant material

The plant material was purchased from local market of Greater Noida and self authenticated. The Voucher specimens of the drug samples are also preserved in the museum of Ram-Eesh Institute of Vocational & Technical Education, Greater Noida with identification number- RIT/Museum/RMIN/2017/03.

Macroscopic characters

The fresh and dried powdered formulation was observed for colour, odour, taste, size, shape, touch and fracture. The observations were recorded in Figure 1 and Table 1.

Microscopic characters

This method is used for identification of drugs on cellular level. It is used to determine structure of organised drugs by their histological characters. It includes of whole, certain parts of powdered crude drugs. The observations were presented in Figure 2.

Physicochemical evaluations

The determination of various physicochemical parameters such extractive values in different solvents, as total ash, acid insoluble ash, water soluble ash, foreign matter, moisture content, bitterness value, foaming index, swelling index and pH value of 1% and 10% solutions were carried out by the methods given in the WHO guidelines for standardization of herbal drugs. [4] [5] [6]

Extractive value

Powdered material of the drug (4g) was packed in a Soxhlet apparatus separately for each solvent like petroleum ether (60^0-80^0 C), chloroform, acetone, methanol, hydro alcohol (50:50) and water and extraction was carried out for 6 hr. Each extract was evaporated to dryness at their respective boiling points and constant extractive values were determined and recorded in Table 2.

Ash value

Total ash

The ground drug (2g) is incinerated in a silica crucible at a temperature not exceeding 450°C until free from carbon. It is then cooled and weighed to get total ash content which is recorded in Table 3.

Acid insoluble ash

Ash is boiled with 25ml dilute HCl (6N) for five minutes. The insoluble matter collected on an ash less filter paper, washed with hot water and ignited at a temperature not exceeding 450°C to a constant weight and the data was recorded in Table 3.

Water-soluble ash

Ash is dissolved in distilled water and the insoluble part collected on an ash less filter paper and ignited at 450°C to constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of soluble part of ash was obtained and recorded in Table 3.

Moisture Content

Moisture content was determined by loss on drying (LOD) method. 3 gm of the weighed quantity of the drug was taken and kept in ov enat 105^0C till a constant weight was obtained. Amount of moistur e present in the sample was calculated as reference to the air dried drug and reported in Table 3.

Bitterness Value

Bitterness value of *Piper longum* was calculated using standard procedure as mentioned in WHO guidelines for quality control of herbal medicines. Bitterness value was calculated with reference to standard bitter Quine HCl and reported in Table 3.

Swelling Index

Carry out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the herbal material concerned, previously reduced to the required fineness and accurately weighed, into a 25-ml glass-stoppered measuring cylinder. The internal diameter of the cylinder should be about 16 mm, the length of the graduated portion about 125 mm, marked in 0.2- ml divisions from 0 to 25 ml in an upwards direction. Unless otherwise indicated in the test procedure, add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature, or as specified. Measure the volume in ml occupied by the herbal material, including any sticky mucilage. The mean value of the individual determinations, related to 1 g of herbal material was calculated and reported in Table 3.

Foaming Index

Reduce about 1 g of the herbal material to a coarse powder (sieve size no. 1250), weigh accurately and transfer to a 500-ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100-ml volumetric flask and add sufficient water through the filter to dilute to volume. Pour the decoction into 10 stoppered test-tubes (height 16 cm, diameter 16 mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and adjust the volume of the liquid in each tube with water to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 minutes and measure the height of the foam. The results are assessed as follows:

- If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.
- If a height of foam of 1 cm is measured in any tube, the volume of the herbal material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.
- If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result.

The foaming index was calculated using the following formula: 1000/a and reported in Table 3.

Where,

a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

pH value (1%/10%)

The pH of 1 and 10% solutions of the Anthocephalus cadamba was measured using pH meter SYSTRONICS DIGITAL pH METER, MK VI and and reported in Table 3. [4]

Fluorescence evaluations

1mg powder of fruits of Piper longum was taken on a glass slide and treated with various reagents, acids and alkali solutions and color of the powder before and after treatment was observed under ultraviolet light at visible light, short and long wavelengths. [4] [5] The observations were reported in Table 4.

Phytochemical evaluations

The various solvent extracts of *Piper longum* fruits were subjected to phytochemical screening for alkaloids, carbohydrates, glycosides, tannins, flavonoids, phenolic compounds, volatile oil and other phytochemicals as per the standard methods [5] [6] and the results were tabulated in Table 5.

Statistical analysis

All the physicochemical evaluations were made in triplets except foaming index and pH determinations so the result was presented as Mean ± S.D.

RESULT & DISCUSSIONS

Figure 1: Powder of *Piper longum*

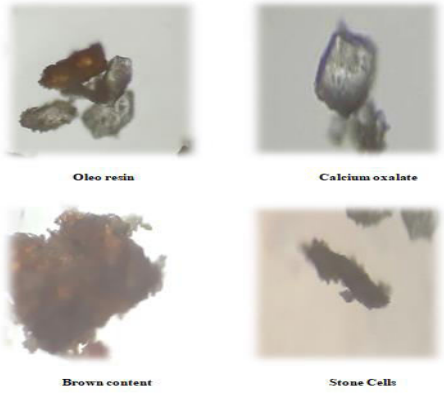

Figure 2: Microscopy of *Piper longum*

Table 1: Observations for Organoleptic characteristics of *Piper longum*

S. No.	Parameters	Inference
1	**Color**	Greyish Brown
2	**Odor**	Characteristic
3	**Taste**	Pungent
4	**Touch and Texture**	Rough
5	**Shape**	Cylindrical
6	**Size**	0.2-0.4 cm diameter; 2-3 cm in length

Table 2: Extractive values of *Piper longum* in various solvents

S.No.	Drug	Extractive values in Different solvents (%w/w)					
		PE	CF	EA	ME	A	HA
1.	*Piper longum* (fruits)	13.3 ± 0.10	6.6 ± 0.11	11.3 ± 0.11	12.6 ± 0.24	20.60 ± 0.08	9.31 ± 0.32

PE: Petroleum ether; **CF:** Chloroform; **EA:** Ethylacetate; **ME:** Methanol; **A:** Water; **HA**: Hydroalcoholic

Table 3: Physicochemical parameters

S.No.	Drug	Physicochemical parameters								
		TA (%w/w)	**AIA (%w/w)**	**WSA (%w/w)**	**FM (%w/w)**	**LOD (%w/w)**	**SI**	**FI**	**pH (1/10%)**	**BV**
1.	*Piper longum* (fruits)	8.3±0.015	1.16 ± 0.06	5.5 ± 0.08	1.62±0.12	15.70±0.051	1.5±0.01	Less than 100	6.2/5.9	1.92

TA: Total Ash; **AIA:** Acid Insoluble Ash; **WSA:** Water Soluble Ash; **FM:** Foreign matter; **LOD:** Loss on Drying; **FI:** Foaming Index; **SI:** Swelling Index; **BV:** Bitterness value

Table 4: Fluorescence analysis of *Piper longum*

S. No.	Reagent	Color Observed		
		Day Light	**Short Wavelength**	**Long Wavelength**
1.	None	Greyish black	Greenish brown	Black
2.	Distilled Water	Brown	Greenish	Greenish brown
3.	1 N NaOH in Water	Light brown	Dark brown	Black
4.	1 N NaOH in Methanol	Yellowish brown	Greenish brown	Blackish brown
5.	50% Nitric Acid	Yellowish green	Dark green	Black
6.	50% HCl	Light brown	Light Brown	Black
7.	conc. H_2SO_4	Yellowish Brown	Greenish Brown	Dark Brown
8.	Acetone	Light brown	Dark green	Black
9.	conc. HCl	Light brown	Greenish brown	Dark brown
10.	Chloroform	Light brown	Light brown	Brown

Table 5: Preliminary phytochemical screening

S.No.	Chemical Test	PE	C	EA	M	W	HA
1.	**Reducing sugars**						
a	Fehling's test	-ve	-ve	-ve	-ve	-ve	-ve
2.	**Monosaccharide**						
a	Barfoed's test	-ve	-ve	-ve	-ve	-ve	-ve
3.	**Hexose sugars**						
a	Cobalt chloride test	+ve	+ve	-ve	-ve	-ve	+ve
4.	**Fats and oils**						
a	Solubility test	+ve	+ve	+ve	-ve	-ve	-ve
b	Filter paper stain test	+ve	+ve	+ve	-ve	-ve	-ve
5.	**Amino Acid**						
a	Ninhydrin test	-ve	-ve	-ve	-ve	-ve	-ve
6.	**Proteins**						
a	Biuret test	+ve	+ve	-ve	-ve	-ve	+ve
b	5% Copper sulphate test	-ve	-ve	-ve	+ve	+ve	-ve
7.	**Alkaloids**						
a	Mayer's test	-ve	+ve	-ve	+ve	+ve	+ve
b	Hager's test	+ve	+ve	+ve	+ve	-ve	+ve
c	Wagner's test	+ve	+ve	+ve	+ve	+ve	+ve
d	Dragendroff's test	+ve	+ve	+ve	+ve	+ve	+ve
8.	**Sterols**						
a	Salkowaski test	-ve	-ve	+ve	-ve	-ve	-ve
b	Liebermann-Burchard's test	-ve	-ve	-ve	-ve	-ve	-ve
9.	**Cardiac Glycosides**						
a	Keller killani test	-ve	-ve	-ve	-ve	-ve	-ve
b	Legal test	-ve	-ve	+ve	+ve	-ve	-ve
10.	**Anthraquinone Glycosides**						
a	Borntrager's test	+ve	-ve	-ve	-ve	-ve	-ve
b	Modified Borntrager test	+ve	-ve	-ve	-ve	-ve	-ve
11.	**Saponin Glycosides**						
a	Foam test	-ve	-ve	-ve	-ve	-ve	-ve

PE: Petroleum ether; **CF:** Chloroform; **EA:** Ethylacetate; **ME:** Methanol; **A:** Water; **HA:** Hydroalcoholic

RESULTS AND DISCUSSION

Pharmacognostical studies shows that the fruits of *Piper longum* are found as green, cylindrical fruits when fresh which turns grayish black upon drying. It possessed bitter and acrid taste with a sensation of warmth. The fruits are cylindrical with small petiole. The powder microscopy shows the presence of brown content, oleo resins, stone cells and calcium oxalate crystals. Physiochemical analysis shows variable extractive values in different solvents with maximum extractives of 20.6±0.08% in water and minimum extractive value of 6.6±0.11% in chloroform, indicating a large number of phytoconstituents in the aqueous extract. Total Ash value of 8.3±0.015%, Acid Insoluble Ash- 1.16±0.006% and Water Soluble Ash- 5.5±0.08%, Foreign Matter- 1.62±0.12%, Moisture content of 15.70±0.051%, Bitterness value-1.96, Swelling Index-1.5±0.01 and Foaming Index was found to be less than 100. Florescence and phytochemical analysis of various extracts of the fruits of *Piper longum* shows the presence of alkaloids, volatile oil, tannins, fixed oils and traces of hexose sugars. Being a bitter drug, Peepli shows a bitterness value of 1.92 and a pH of 6.2(1%) and 5.9(10%).

CONCLUSION

The various pharmacognostical, physicochemical, and phytochemical standards thus obtained from this study will help in establishing the identity, purity, quality, safety, and efficacy of *Piper longum.*

These standards can be used by various industries and laboratories engaged in research and production of herbal formulations to

control the quality of their products and help in maintaining batch to batch consistency so that maximum therapeutic efficacy can be achieved.

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CONFLICT OF INTEREST

The author declares that he has no competing interests.

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