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EXTRACTION AND EVALUATION OF PIPERINE FROM *PIPER NIGRUM* LINN.

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ABSTRACT: Piperine is the naturally occurring alkaloid that gives the spice, black pepper its characteristic biting taste. Piperine is extracted from *Piper nigrum*, Linn. It is medicinally used as Anti-inflammatory, Anti-malarial, Cure for Stomach Ache, Weight Loss, Anti-leukemia, Fever prevention/reducer, Treatment for Snake Venom Poisoning and Anti-epileptic. Recent medical studies have shown piperine to be very helpful in increasing the absorption of certain vitamins such as Selenium, Vitamin B and Beta-Carotene. Piperine apparently has the ability to increase thermogenesis.Hence, Extraction and Standardization of piperine is performed. In Standardization, Solubility, Total ash content, Acid insoluble ash thin layer chromatography, Ultra-violet Spectroscopy, High Performance Liquid Chromatography were performed.Piperine was extracted successfully, it was found to be soluble in ethanol and chloroform. Total ash content and acid insoluble content was found to be 1%w/w and 0.5%w/w respectively. The R_f value was found to 0.55 and 0.33 for TLC .For UV the λ_{max} was found to be 360nm and the absorbance observed was 2.65. HPLC results showed that the λ_{max} was found to be 343nm. The melting point of piperine was found to be 125^0C.

Keywords: Piperine, Black Pepper, Extraction, Standardization

INTRODUCTION

The common herbal product known as the black pepper is a well known spice around the world .The plant itself is a perennial woody climbing vine which can reach a height of about 15 ft or 5 meters climbing on trees and along man made wooden trellises. 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. Black pepper has played a major role in shaping historical events in the world. Piperine a major constituent is a member of the Lipids family. Lipids are a group defined as consisting of fats or fat like substances. As an aromatic spice, the black pepper is characterized by a slight and perceptible musty aroma; this smell is because of the presence of many volatile oils mainly in the flesh and skin of the fruit. The very noticeable and pungent "bite" of the black pepper is due to the presence of two primary alkaloids in the pepper, these are the compounds piperine and the compound piperidine, the tangy bite also comes partly because of the many specific plant resins which are found in the seeds of the herb. Aside from its normal and common use as a spice, these pepper oils are also extracted to be used in the manufacture of many types of perfumes and commercial food seasoning materials around the world. Pepper based irritants and searing chemicals are utilized in a variety of processes. Some of these chemicals are used in a variety of liniments and mouth gargles. The pepper based compounds have also seen commercial use as chemical carminatives; they have also been used for their ability to reduce excess gas in the intestines and the stomach. Aside from these, these compounds have also been used to stimulate the activity in the human heart and kidneys in medicine. The specific compound in pepper known as piperine is commercially utilized to prepare different insecticides to be used against houseflies and other insect pests.

MATERIALS AND METHOD:

Materials

Pepper (black) was procured from local market while remaining all chemicals were obtained from college laboratory.

Method

I. **EXTRACTION OF PIPERINE FROM BLACK PEPPER**

Extract powered drug with ethanol (95%) in a Soxhlet apparatus for 3 hours. Filter the solution and concentrate under vacuum on water bath at 60ºC. Add 10 % alcoholic potassium hydroxide with constant stirring and filter. Allow the alcoholic solution to stand overnight whereupon needles of piperine separate out.

2. EVALUATION OF PIPERINE:

I. Chemical Characteristics:

1. Organoleptic properties of dried Black pepper and Piperine like colour, odour, taste were determined.
2. The extract content of the piperine was determined by taking %w/w
3. The Solubility of piperine in various solvents like petroleum ether, chloroform, ethanol, methanol and water was carried out.
4. Moreover, the determination of melting point of piperine was also carried out.

II. Physicochemical Analysis:

A. Total ash content

B. Acid insoluble ash contents are analyzed and compare with standards

III. Thin Layer Chromatography (TLC) Analysis:

Sample Details: Piperine
Adsorbent : Precoated Silica gel
Solvent System: Toluene: Ethyl acetate
70 : 30

Sample Preparation: Piperine (1 mg) was dissolved in methanol (1 ml) and this solution was applied on the TLC plate with the aid of capillary tube.

Detection: Saturated Iodine Chamber

IV. High Performance Liquid Chromatography (HPLC) Analysis

Mobile phase: Methanol: Water
69 : 31
Detection : 343 nm
Flow rate : 1.5 ml/min

V. **Ultra Violet- Spectrophotometer Analysis**

The 0.01% w/v solution of piperine in ethanol was prepared and λ_{max} was determined.

RESULTS

I. Chemical Characteristics

1. Oraganoleptic Characteristics:

Table 1: Organoleptic characteristics:

Color	Black
Odour	Aromatic
Taste	Aromatic and Pungent

2. Extract Content: The extract content of oleoresin is found to be 1.06%w/w

3. Solubility: The solubility of oleoresin in various solvents was found to be as follows:

Table 2: Solubility of piperine in various solvents.

Petroleum ether	Soluble
Chloroform	Soluble
Ethanol	Soluble
Methanol	Soluble
Water	Insoluble

4. Melting Point: Melting point was found to be 129^0 C

II. **Physicochemical Analysis**:

The total ash value and the acid insoluble ash value of the oleoresin were found to be as in table 3.

Table 3: Physicochemical analyses:

Properties	Standard value	Practical value
Total ash Content	< 6.0%w/w	1% w/w
Acid Insoluble ash Content	< 1.0%w/w	0.5% w/w

III. Thin Layer Chromatography (TLC) Analysis:

The R_f value was found to be 0.55, 0.33, as two spots were produced in Toluene: Ethyl acetate system.

Figure 1: TLC Analysis of extract

III. High Performance Liquid Chromatography (HPLC) Analysis:

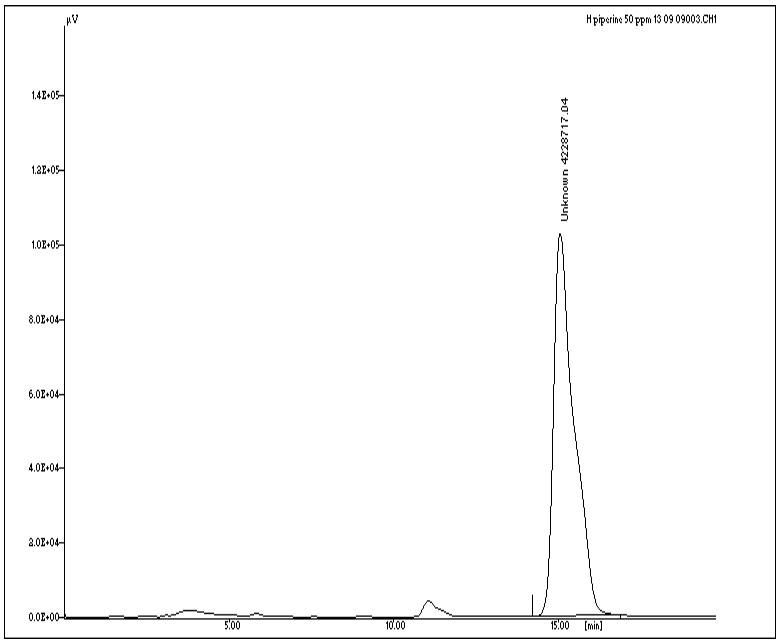

Figure 2: HPLC Analysis of extract

File name: H piperine 50 ppm
MP: water: MeOH [30:70]
Column: HiQ Sil C18W
Flow rate: 1 ml/min

Table 4: Results of HPLC

λmax	RT	Area[µV.Sec]	Plates	Resolution	Height[µV]	Asymmetry	% Area
343nm	15.043	4228717.044	3433.969	0.00	102206	1.73	100.00

IV. Ultra Violet- Spectrophotometer Analysis

Table 5: Results of UV

Absorbance	λ_{max}
2.65358	360nm

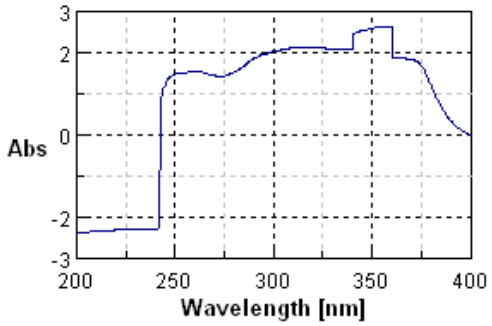

Figure 3: UV Spectroscopic analysis

DISCUSSION

There are numerous types of Indian black pepper ,that is why pepper as well as piperine is characterized by variations in colour,odour and taste which showed characteristic Black and yellow colour,aromatic odour,aromatic and pungent taste for pepper and piperine respectively.

Piperine was found to be soluble in various nonpolar solvents but insoluble in polar solvent like water.

Piperine was also characterized for its melting point which showed that it undergoes melting at 129ºC which is within the standard range (129-130ºC)

Total ash method is designed to measure the total amount of material remaining after ignition while the acid insoluble ash is the residue obtained after boiling the total ash which measures the amount of silica present .Amount of total ash was found to be 1% w/w (<6%w/w) and acid insoluble ash was found to be 0.5%w/w (1%w/w).

Thin Layer Chromatography a particularly valuable qualitative method for determination of small amount of impurities and frequently used for evaluating medicinal plant materials. Different samples have different Rf value which was determined for piperine and was found to be 0.5538 and 0.3384 (0.25 and 0.6) which showed good separation of piperine.

Piperine in *Piper nigrum* was separated from other compounds by HPLC. The separated compounds were compared with the standard Piperine peak.

CONCLUSION

The proposed method for extraction and characterization lead to rapid, accurate and sensitive method requiring less amount of solvent. Large number of samples can be suitably employed for routine quality control analysis.

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