ISOLATION AND EVALUATION OF PIPERINE FROM BLACK PEPPER AND WHITE PEPPER

Zeashan Hussain, *Vikash Kumar Chaudhri, Anurag Pandey, Raziuddin Khan, Anand Kumar Srivastava and Rishabh Maurya

Department of Pharmacy, Mahatma Gandhi Institute of Pharmacy, Lucknow-227101, Uttar Pradesh, India.

ABSTRACT
Piperine is the naturally occurring alkaloid that gives the spice, isolated from the black pepper (Piper nigrum L.) (Family: Piperaceae) and white pepper (Piper nigrum). 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. White pepper contains components rich in aromatic oils, oleoresins and alkaloids. Piperine (1-piperoyl piperidine), an active ingredient in white pepper, has numerous biologicaleffects such as anti-inflammatory, antioxidant, antimutagenic and antitumor activities.

KEYWORDS: Black pepper, white pepper and piperine.

INTRODUCTION
There is great demand for herbal medicine in the developed as well as developing countries like India, because of their wide biological activities, higher safety of margin than the synthetic drugs and lesser costs. Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans as well as valuable components of seasonings, beverages, cosmetics, dyes and medicines. Consumption of fruit and vegetables, as well as grains, has been strongly associated with reduced risk of diseases.

Natural products are organic compound that are formed by living system. The elucidation of their structure, chemistry, synthesis and biosynthesis are major areas of chemistry. Phytochemistry or the chemistry of natural products may be strategically placed somewhere
in between natural product, organic chemistry and plant biochemistry. In fact it is intimately related to the above two discipline.\textsuperscript{[3]} However, in a border sense phytochemistry essentially deals with the enormous different type of organic substances that not only elaborated but also accumulated by plant. It is also solely concerned with the following various aspects namely Natural distribution, Chemical structure, Biosynthetic structure, Biosynthesis (biogenesis), Metabolism and Biochemical function.\textsuperscript{[4]}

Piperine is the main alkaloid in such fruits of black pepper containing about 90\%. It is responsible for the hot taste and his name remains from the heterocyclic compound piperidine. Black and white peppercorns are both the fruit of the pepper plant but they are processed differently. Black peppercorns are picked when almost ripe and sun-dried, turning the outer layer black. To produce white peppercorns this outer layer is removed before or after drying, leaving only the inner seed. White pepper taste hotter than black but is less common.
Chemical structure of Piperine

![Piperine Chemical Structure](image)

**MATERIAL AND METHOD**

**Material**
Pepper (black & white), Chloroform, ethanolic potassium hydroxide, Toluene, ethyl acetate, cyclohexane.

**Method**

**Extraction of Piperine from Black or White Pepper**
Powdered black pepper or white pepper was placed in the extraction thimble of a soxhlet apparatus and extracted with chloroform for two hours to obtain the piperine. Afterwards the solvent was removed in beaker and 20 ml ethanolic potassium hydroxide solution was added with stirring. After filtering the mixture, the filtrate was allowed to stand overnight in the refrigerator. In consequence of no crystallization 4-5 ml water was added (addition of a small portion of water is a very important hint!) and the solution was allowed to stand in the refrigerator once again. Later the risen crystals were separated from solution by filtration. Then, the crystals (500 mg) were recrystallized from 10 ml cyclohexane/ Toluol (4:1 v/v). with an eluent of toluene/ethyl acetate (2:1 v/v). After working up the fractions the piperine was obtained in form of shiny yellow crystals.

![Black pepper](image)  ![White pepper](image)
Evaluation of piperine

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Property</th>
<th>Black pepper</th>
<th>White pepper</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organoleptic property</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Colour</td>
<td>Yellowish</td>
<td>Creamy</td>
</tr>
<tr>
<td>b.</td>
<td>Odour</td>
<td>Aromatic</td>
<td>Characteristic</td>
</tr>
<tr>
<td>c.</td>
<td>Taste</td>
<td>Bitter</td>
<td>Characteristic</td>
</tr>
<tr>
<td>2</td>
<td>Solubility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Water</td>
<td>Slightly soluble</td>
<td>Slightly soluble</td>
</tr>
<tr>
<td>b.</td>
<td>Petroleum ether</td>
<td>Slightly soluble</td>
<td>Slightly soluble</td>
</tr>
<tr>
<td>c.</td>
<td>Ethanol</td>
<td>Soluble</td>
<td>Soluble</td>
</tr>
<tr>
<td>d.</td>
<td>Methanol</td>
<td>Soluble</td>
<td>Soluble</td>
</tr>
<tr>
<td>e.</td>
<td>Chloroform</td>
<td>Soluble</td>
<td>Soluble</td>
</tr>
<tr>
<td>3</td>
<td>Melting range</td>
<td>90-92</td>
<td>60-62</td>
</tr>
<tr>
<td>4</td>
<td>pH</td>
<td>2.3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Rf value</td>
<td>0.77</td>
<td>0.44</td>
</tr>
<tr>
<td>6</td>
<td>Percentage yield</td>
<td>3%</td>
<td>2.25%</td>
</tr>
</tbody>
</table>

Physicochemical analysis

Thin layer Chromatography (TLC) Analysis

Sample Detail - Piperine
Adsorbent - Pre coated silica gel
Solvent System - Toluene : Ethylene (7:3)

Piperine (1 mg) was dissolved in methanol (1 ml) and this solution was applied on the TLC plate with the aid of capillary tube. The TLC plate was then placed in the chamber do not touch the surface of the eluent in the chamber and the lid was closed. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate. The plate should be removed from the chamber before the solvent front reaches the top of the stationary phase and dried. The plate was visualised in iodine chamber.

R_f value of black pepper = 0.77
R_f value of white pepper = 0.44
Ultra violet Spectroscopy

UV spectrum was recorded on uv spectrophotometer The concentration of drug in ethanol is 5 µg/ml. The $\lambda_{\text{max}}$ of black pepper was 366nm and the $\lambda_{\text{max}}$ of white pepper was 243.6nm.
RESULT
The piperinewas isolated from the black pepper and white pepper and through this we have calculated the percentage purity of the product through ultraviolet spectrophotometry analysis. The percentage yield of black pepper was 3% and of white pepper was 2.25%. The R_f value of the black pepper and white pepper 0.77 and 0.4 respectively. The melting range of black pepper and white pepper was 90°C to 92°C and 60°C to 62°C. The pH of black pepper and white pepper was  and 2 respectively.

CONCLUSION
Method for isolation of piperine with Ethanol solvent to be effective in isolating piperine with higher yield and purity. Isolated piperine was identified by M.P, TLC, UV which resulted into better pure crystals of piperine.

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