ANTIBACTERIAL ACTIVITY OF PIPERINE EXTRACTED FROM PIPER NIGRUM AGAINST E.COLI AND BACILUS SUBTILIS

Jaya Maitra* and Shilpi

School of Vocational Studies and Applied Sciences, Department of Applied Chemistry Gautam Buddha University, Greater Noida, U.P. 201310.

* Corresponding Author: Dr. Jaya Maitra
School of Vocational Studies and Applied Sciences, Department of Applied Chemistry Gautam Buddha University, Greater Noida, U.P. 201310.

ABSTRACT

Piperine is a naturally occurring alkaloid and is extracted from black pepper(Piper nigrum). As a spice it is added to many foods. It is also considered as a medicinal plant. It is medicinally used as anti-inflammatory, anti-malarial, analgesic, antidepressant etc. In the present study piperine was extracted successfully from black pepper using dichloromethane and ethanol by refluxing as well as by soxhlet extraction. It was found to be soluble in ethanol and chloroform. It was identified and evaluated by TLC and UV-visible spectrophotometry. The $R_f$ value was found to be 0.61 for TLC. For UV the $\lambda_{max}$ was found to be 343nm and the absorbance observed was 2.275. The melting point of piperine was found to be 128° C. Antibacterial property of piperine extracted from piper nigrum was studied against E.coli and Bacillus subtilis.

KEYWORDS: Piperine, Black Pepper, Extraction, Antibacterial activity.

INTRODUCTION

Piperine is 1-[5-(1, 3-Benzodioxol-5-yl)-1-oxo- 2, 4-pentadieny1] (Figure 1).

(Figure 1.Piperine)

It is an active constituent of the black pepper (Piper nigrum). The common herbal product known as the black pepper is a well known spice around the world. 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.[1] Piperine apparently has the ability to increase thermogenesis.[1]

Pepper consist of piperine alkaloid (3- 9%), pungent resin (6.0%), volatile oil (1-2.5%), piperidine and starch (about 30%).[2, 3, 7] The volatile oil of piperine has shown to have antimicrobial property.[4] Piperine has anti-inflammatory.[5, 6] analgesic[7], antiarthritic[8], CNS depressant[9], anticonvulsant[8] etc. 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. 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 compound 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.

Extraction by using various solvents is another method in which piperine is extracted by using solvents like ethanol,[9] dichloromethane[10] and glacial acetic acid.[11]

MATERIAL AND METHOD

Piperine was isolated from Piper nigrum by refluxing using dichloromethane as well as by soxhlet using
ethanol. Isolated piperine was evaluated by UV absorbance and TLC.

Black paper was taken from local market. All reagents are of analytical grade (CDH).

1. Isolation of piperine from pepper powder
   a. Isolation of piperine from pepper powder by refluxing
      10 g of ground pepper powder and 20ml of dichloromethane were placed in a 100ml round bottom flask with a magnetic stirrer. A water condenser was attached to the top of the flask and water was allowed to run through it to condense the dichloromethane vapors. Refluxing of the solution was done for 20mins. After cooling the flask, vacuum filtration was done with a Buchner funnel and pepper grounds were filtered. The grounds were washed with 10ml dichloromethane.

Purification
   The filtrate was transferred to a 50 ml round bottom flask and using a sand bath the dichloromethane was removed until dark brown oil was left. The oil was cooled in an ice bath and 6ml of cold ether was added. The flask was kept in an ice bath for 15minutes with occasional stirring to precipitate out piperine. The crystals were washed with cold ether (2 - 4ml) and 5mg of crystals were saved for TLC analysis. To re-crystallize, the piperine was placed in a test tube and dissolved it in similarly 5ml of hot 3:2 acetone:hexane solution. It was allowed to sit for 15 min at room temperature and then 30 min in an ice bath. The crystals were filtered and washed with 4ml of cold ether.

b. Isolation of piperine from pepper powder by soxhlet extraction:
   The powdered black pepper(10gm) with 95% ethanol (150ml) was extracted in a soxhlet apparatus for 2 hrs, the solution was filtered and concentrated in vacuum on a water bath at 60°C. 10ml of 10% alcoholic KOH was added to the solution and extract was decanted and after a while the insoluble was obtained.

2. Evaluation and Characterization of Piperine
   I. Physical and Chemical Characteristics:
      1. Physical properties of dried Black pepper and Piperine like colour, odour, taste, were determined.

   Table 1: Physical property

<table>
<thead>
<tr>
<th>Colour</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odor</td>
<td>Aromatic</td>
</tr>
<tr>
<td>Taste</td>
<td>Pungent</td>
</tr>
</tbody>
</table>

   2. The Solubility of piperine in various solvents like petroleum ether, chloroform, ethanol, methanol and water was carried out.

   Table 2: Solubility of piperine in various solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Soluble</td>
</tr>
<tr>
<td>Methanol</td>
<td>Soluble</td>
</tr>
<tr>
<td>Water</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>Soluble</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Soluble</td>
</tr>
</tbody>
</table>

   3. Melting point of piperine was also carried out.

   Table 3: Melting point of piperine

<table>
<thead>
<tr>
<th>Melting point</th>
</tr>
</thead>
<tbody>
<tr>
<td>128 °C</td>
</tr>
</tbody>
</table>

II. Identification of Piperine
   a. Thin Layer Chromatography (TLC) Analysis
      Sample Details: Piperine
      Adsorbent: Precoated Silica gel
      Solvent System: Acetone: n-Hexane (35: 15)

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

   Detection
      Saturated Iodine Chamber.

   Procedure
      The piperine was subjected on to the precoated and activated Silica gel TLC plates. (Plates were kept in oven for 1hr at 70°C) The mobile phase is Acetone: n-Hexane in 35:15 ratios. After the TLC run and spraying the detecting agent yellow spots of piperine were dentified visually. Rf value was calculated. Rf value = 4.5/7.4= 0.61.

   b. Ultra Violet- Spectrophotometer Analysis
      The 0.01% w/v solution of piperine in methanol was prepared and λ_max was determined.

<table>
<thead>
<tr>
<th>Amax</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>343nm</td>
<td>2.2275</td>
</tr>
</tbody>
</table>
Antibacterial activity was studied against two Bacterial cultures Gram positive bacteria (*Bacillus subtilis*) and gram negative bacteria (*Escherichia coli*-DH5α).

**Procedure**

The in vitro antibacterial activity of the ethanolic and dichloromethane (DCM) extracts of piperine was carried out by Disc diffusion method. A stock solution of 10mg/L of piperine was made from which different concentrations of piperine solution viz., 25 µl, 50 µl & 100 µl was taken for antibacterial analysis. 5 µl of extract were loaded on the disc from each concentration. The discs were then placed on the LB agar medium containing the bacterial cultures and incubated for 24 at 37°C. The diameter of zone of growth inhibition was recorded.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Name of the bacteria</th>
<th>Conc.of piperine (µl)</th>
<th>Zone of inhibition(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Bacillus subtilis</em></td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td><em>E.Coli</em></td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>4</td>
</tr>
</tbody>
</table>

**RESULT**

In the present study the antibacterial effect of piperine is shown in table 4. It indicates that zone of inhibition increases as the concentration of piperine increased. Piperine has shown an antibacterial activity against gram positive and gram negative bacteria with zone of inhibition ranged from 2mm-4mm. The maximum zone of inhibition was against gram positive bacteria *Bacillus subtilis* (3mm-7mm) than gram negative bacteria *E.coli* (5mm) for 100µl of for both the bacterial cultures.

**DISCUSSION/CONCLUSION**

The present study show that both the extracts of black paper have good antibacterial activity. Antimicrobial activity of piperine increases as the concentration increases against both the bacteria. But when compared to gram negative bacteria, gram positive bacteria are more susceptible to the extracts. The variation in the inhibition among the gram positive and gram negative bacteria is due to the cell wall and cell membrane compositions. The mechanism of antibacterial action
appears to be loss of control over cell membrane permeability.

ACKNOWLEDGEMENT
The authors are thankful to the Department of Applied Chemistry, Gautam Buddha University, for providing necessary facilities for the project work.

REFERENCES