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Research Article

**DETERMINATION OF BIOACTIVE COMPOUNDS FROM *Piper nigrum*. L BY USING HPLC ANALYSIS****S. Manjusha<sup>1\*</sup>, N.K.Parameswaran<sup>2</sup>, R.Senthil Malar<sup>3</sup>**<sup>1</sup>Department of Botany and Research Centre, Scott Christian College (Autonomous,) Nagercoil-629003, Kanyakumari district, Tamil Nadu, India.<sup>2</sup>Department of Biotechnology, Manonmaniam sundaranar University, Tirunelveli<sup>3</sup>Department of Zoology, Sivanthi Adithanar College, Nagercoil**Abstract:**

*Black pepper is important for its medical value (Parganiha et al., 2011). Medicinally black pepper can be used for digestive disorders like large intestine toxins, different gastric problems, diarrhea, and indigestion and also can be used against respiratory disorders including cold, fever and asthma. Black pepper is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, fever intermittent fever, cold extremities, colic, gastric ailments and diarrhea. In this Present Study the bioactive compounds from the plant Piper nigrum L. were determined by using HPLC Analysis.*

**Key words:** *Black pepper, Piper nigrum L., HPLC***Corresponding author:**

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**INTRODUCTION:**

*Piper nigrum* L. finds extensive use in Ayurvedic system of medicine. A number of piperidine and pyrrolidine alkaloids are known to occur in *Piper nigrum* L. the most important being piperine, known to possess a variety of biological properties like CNS stimulant, analgesic, antipyretic and antifeedant activities. Pepper is a natural spice. *Piper nigrum* L. finds an extensive application in antibacterial preparations. Fractionation of the petroleum ether extract of the berries of *Piper nigrum* L. afforded 2E, 4E, 8Z-N-isobutyleicosatrienamide, pellitorine, trachyone, pergumidiene and isopiperolein B [1]. Pergumidiene and trachyone have also been isolated from *Piper nigrum* L. All the isolated compounds have shown activity against *Bacillus subtilis*, *Bacillus sphaericus* and *Staphylococcus aureus* amongst Gram +ve bacteria, and *Klebsiella aerogenes* and *Chromobacterium violaceum* among Gram -ve bacterial strains [2].

**MATERIALS AND METHODS:****Selection of Plant material**

In this present study, the plant *Piper betle* L. leaves and seeds were collected in Pechiparai Hill Region Kanyakumari District, Tamilnadu. An adult, fresh leaves were picked out from the plant and also the matured seed were collected from the plants and transported to the laboratory for work.

The collected leaves were subjected to surface cleaning by rinsing the samples with sterile water, in order to remove dust particles present on the plant materials. The samples such as leaf and seeds were allowed to shade dry to remove moisture content. The dried samples were used for further studies.

**Preparation of Plant Extracts**

The leaves were cut into small pieces and seeds were made powdered using electric mixer grinder. All the samples were subjected to soxhlet extraction using five solvents such as Acetone, Chloroform, Dimethyl sulfoxide, Ethanol and Distilled water. Each 5grams of plant material was filled separately in the thimble and extracted successively with 60ml of solvents using a soxhlet extractor for three hours. After solvent evaporation, each of these solvent extract was weighed and preserved in room temperature until further use.

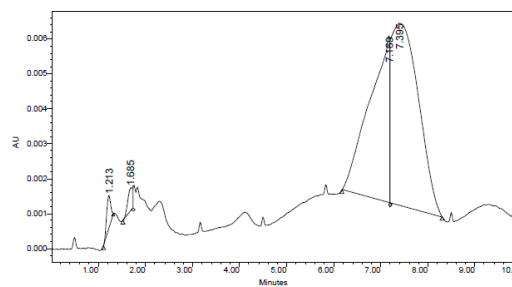
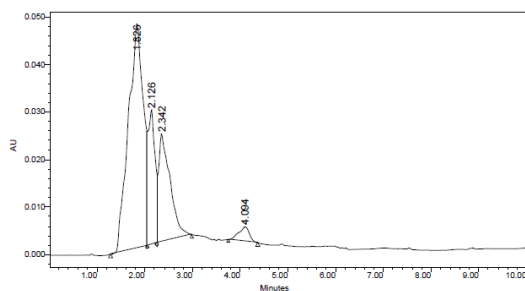
**High Performance Liquid chromatography (HPLC) analysis**

The leaf and seed samples were further analysed in high performance liquid chromatography (WATER, Germany) with the software BREEZE (ver.2.1). 10 µl of the sample extract was filled in capillary column of the instrument, run time was 10 minutes. Retention time (min), area (V\* sec), % area, height (V\* sec), % height, starting time (min) and end time (min) of the peaks were noted.

**RESULTS:****High Performance Liquid chromatography (HPLC) Analysis**

In HPLC analysis, the ethanol extracts of the samples were used. In this present study, the *Piper nigrum* L. leaf showed five peaks between the retention time of 1 to 10 minutes were as 1.551, 1.826, 2.126, 2.324 and 4.094. Among these, three peaks were found as high and larger volume, 1<sup>st</sup> one RT (min) = 1.826, area (V\* sec) = 1048235, % area = 59.81, height (V\* sec) = 47185, % height = 46.55, starting time (min) = 1.283 and end time (min) = 2.050; 2<sup>nd</sup> one RT (min) = 2.126, area (V\* sec) = 272315, % area = 15.54, height (V\* sec) = 28343, % height = 27.96, starting time (min) = 2.050 and end time (min) = 2.250 and 3<sup>rd</sup> one RT (min) = 2.342, area (V\* sec) = 384438, % area = 21.94, height (V\* sec) = 22759, % height = 22.45, starting time (min) = 2.250 and end time (min) = 2.983 (Plate 22).

*Piper nigrum* L. fruit showed five peaks between the retention time of 1 to 10 minutes were as 1.213, 1.551, 1.685, 7.169 and 7.395. Among these, two peaks were found as high and larger volume, 1<sup>st</sup> one RT (min) = 7.169 area (V\* sec) = 147457, % area = 40.52, height (V\* sec) = 4776, % height = 41.08, starting time (min) = 6.167 and end time (min) = 7.200; and 2<sup>nd</sup> one RT (min) = 7.395, area (V\* sec) = 205221, % area = 56.39, height (V\* sec) = 5228, % height = 44.97, starting time (min) = 7.200 and end time (min) = 8.300 (Plate 23). In HPLC analysis, *Piper nigrum* L. leaf showed five peaks and the major peaks were RT (min) = 1.826, area (V\* sec) = 1048235; RT (min) = 2.126, area (V\* sec) = 272315; and RT (min) = 2.342, area (V\* sec) = 384438. *Piper nigrum* L. seed showed five peaks and the major peaks were RT (min) = 7.169 area (V\* sec) = 147457; and RT (min) = 7.395, area (V\* sec) = 205221.



**HPLC Chromatogram of Leaf Extract of *Piper nigrum* L. PLC Chromatogram of Fruit Extract of *Piper nigrum* L.**

### CONCLUSION:

Almost all the members of Piperaceae are used in the traditional medicinal system. Species like *Piper nigrum* L., *Piper betle* L. and *Piper longum* L. ranks first in the siddha medicinal use. For most of the siddha medicinal preparation any one of the Piperaceae member being an ingredient. In modern days, medicinal plants are becoming probable sources of important drugs and pharmaceutical industries. Nowadays, they have come to consider traditional medicine as a source of bioactive agents which can be used in the preparation of synthetic medicine.

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