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DETERMINATION OF BIOACTIVE COMPOUNDS FROM *PIPER LONGUM* L. BY USING HP-LC ANALYSIS

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Abstract:

Plants are more important in human life and fulfill his every day needs. In recent developments free radicals are involved in many diseases. The World Health Organization [WHO] has adopted a major policy change wherein most developing nations have to make use of more traditional medical practices for primary health care. The Indian traditional system of medicine has identified the leaves with digestive and pancreatic lipase stimulant activities Piper longum L. leaves are used in eye drops for eye injury or infection as a baby lotion for the new born, for coughs, asthma, and constipation and to arrest milk secretion. Essential oil from leaves of this plant has been used for the treatment of respiratory catarrhs and antiseptic. Piper betle L. showed hypotensive, Cardio tonic, smooth and skeletal muscles relaxant actions. This present study raveled to determine the bioactive compounds from the Piper longum L.

Key words: *Bioactive compounds, Piper longum L, HPLC analysis.*

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INTRODUCTION:

Indian medicinal plants are regularly used in various system of medicine because of minimal side effect and cost effectiveness which provide scientific support to the therapeutic use of the plants in tribal medicine [1]. Black pepper [*Piper nigrum* L.] has been used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, fever [2]. *Piper longum* L. has been used as a therapeutic agent in the treatment of various pathological conditions [3]. Plants were used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants accumulated in areas where the use of plants is still of great importance [4]. *Piper longum* L. contain Piperine, Piper longamine, volatile oil, resin, gums and fatty oil. The fruits of *Piper longum* L. are useful in spleen disorders, bronchitis, tuberculosis and jaundices [5].

Thippali consists of dried fruits of *Piper longum* L. [Piperaceae] a slender, aromatic, creeping and perennial herb [7]. It is commonly used to treat stomach ache, bronchitis, cough and tumour. It is also applied externally to sooth and relieves muscular pains, rheumatism, paralysis and inflamed skin. Pippali contains an alkaloid piperine as chief constituent [8]. It is applied locally for muscular pain, inflammation and internally used as a carminative in conditions such as loss of appetite and sleeplessness [9]. In the Western part of India aqueous extract of the roots of *Piper longum* L. is used as food material [10] In addition to this, there is a major role for *Piper longum* L. is preventing the cancer development in the experimental glioma model [11,12]. The extract of the root of *Piper longum* L. and its major compound, piperine exert anti-oxidant activity and are protective in the myocardial ischemic condition [13]. The alcoholic extract of the fruits of the plant *Piper longum* L. and its component piperine showed significant immunomodulatory and antitumor activity [14]. Piper nonaline, a piperidine alkaloid derived from long pepper, possess a mosquito larvicidal activity [15]. Piperine was the first amide isolated from *Piper* species and was reported to display central nervous system depression, antipyretic, and anti-inflammatory activity [16]. The *Piper longum* L. dried fruit's oil showed significant anti-inflammatory activity on carrageen an-induced rat paw edema. Isolates from *Piper longum* L. fruit extracts showed antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria. The dried fruit of one such plant *Piper longum* L. commonly known as Indian Long pepper, used as a spice and seasoning, is known to possess multitude of pharmacological activities. The fruits and roots are attributed with numerous medicinal uses, and may be used for diseases of respiratory tract, viz. cough, bronchitis, asthma also as anti- irritant and analgesic. The fruits have been used as liver tonic, stomachic, emmenagogue, abortifacient, aphrodisiac and digestive [17] The *Piper longum* L. fruits have positive tests for the presence of volatile oil, starch, protein and alkaloids, saponins, carbohydrates, and amygdalin but no tannins [20]. Major chemical constituents are alkaloids Piperine, Piper longumine, Piper longuminine and also methyl-3, 4, 5-trimehoxycinnamate. Spices are used as the substances that increase the taste and variation of food [18] *Piper longum* L. known as long pepper is a native of northeast India and an important traditional medicinal plant. It is found in various parts of India including evergreen forests from Konkanto Travancore regions of Western Ghats. The fruits of this plant are source of famous traditional drug pippali besides being used as spice and in the manufacturing of pickle [19] The plant has tremendous medicinal values and a known curing agent against cough, leprosy, diabetes, piles, cardiac diseases, chronic fever and to improve appetite to name a few various pharmacological activities including, anti-allergy, antibacterial, anti-hepatitis and anti-tubercular have been reported from long pepper.

MATERIALS AND METHODS:

Sample collection

In this present study, the plant *Piper longum* L. leaves and seeds were collected in Edaikode, Kanyakumari District, Tamilnadu India. 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.

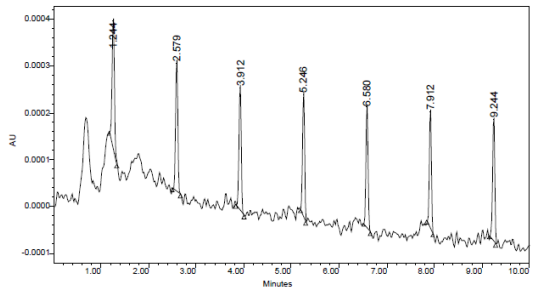
High Performance Liquid Chromatography [HPLC] Analysis

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

Piper longum L. leaf was showed seven peaks between the retention time of 1 to 10 minutes were as 1.244, 2.579, 3.912, 5.246, 6.58, 7.912 and 9.244. All peaks were found as high and larger volume, 1st one RT [min] = 1.244 area [V* sec] = 931, % area = 15.61, height [V* sec] = 268, % height = 14.94, starting time [min] = 1.167 and end time [min] = 1.333; 2nd one RT [min] = 2.579, area [V* sec] = 915, % area = 15.34, height [V* sec] = 271, %height = 15.11, starting time [min] = 2.500 and end time [min] = 2.667; 3rd one RT [min] = 3.912, area [V* sec] = 878, % area = 14.72, height [V* sec] = 257, % height = 14.33, starting time [min] = 3.833 and end time [min] = 4.000; 4th one RT [min] = 5.246, area [V* sec] = 817, % area = 13.70, height [V* sec] = 254, % height = 14.16, starting time [min] = 5.183 and end time [min] = 5.300; 5th one RT [min] = 6.580, area [V* sec] = 810, % area = 13.58, height [V* sec] = 250, % height = 13.94, starting time [min] = 6.517 and end time [min] = 6.650; 6th one RT [min] = 7.912, area [V* sec] = 761, % area = 12.76, height [V* sec] =241, % height = 13.43, starting time [min] = 7.850 and end time [min] = 7.967; and 7th one RT [min] = 9.244, area [V* sec] = 853, % area = 14.30, height [V* sec] = 253, % height = 14.10, starting time [min] = 9.167 and end time [min] = 9.300 [Fig 1]. *Piper longum* L. fruit was showed two peaks between the retention time of 1 to 10 minutes were as 1.143 and 1.987. One peak was found as high and larger volume, RT [min] = 1.987 area [V* sec] = 240708, % area = 94.71, height [V* sec] = 17768, % height = 96.22, starting time [min] = 1.450 and end time [min] = 2.283 [Fig 2].



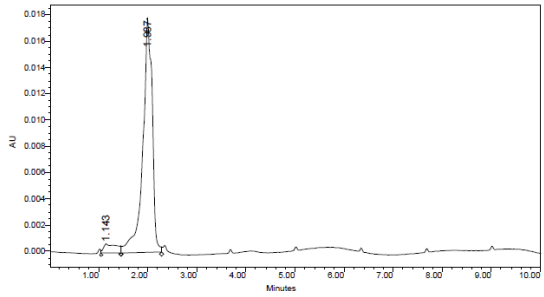


Fig : 1 HPLC Chromatogram of Leaf Extract of *Piper longum* L. Fig : 2 HPLC Chromatogram of Fruit Extract of *Piper longum* L.

DISCUSSION:

Piper longum L. leaf showed seven peaks and the major peaks were RT [min] = 1.244 area [V* sec] = 931; RT [min] = 2.579, area [V* sec] = 915; RT [min] = 3.912, area [V* sec] = 878, RT [min] = 5.246, area [V* sec] = 817; RT [min] = 6.580, area [V* sec] = 810; RT [min] = 7.912, area [V* sec] = 761; and RT [min] = 9.244, area [V* sec] = 853. *Piper longum* L. seed showed two peaks, the major peaks were RT [min] = 1.987 area [V* sec] = 240708. *Piper betle* L. leaf showed nine peaks and the major peaks were RT [min] = 1.820 area [V* sec]

= 875785; RT [min] = 1.925 area [V* sec] = 1322677.

CONCLUSION:

.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. In this study, *Piper longum* L. leaf shows seven peaks; *Piper longum* seed shows two peaks; *Piper* These results indicate that the plant samples are contained high quantities of chemical metabolites.

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