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**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.1229608>Available online at: <http://www.iajps.com>**Research Article****DETERMINATION OF BIOACTIVE COMPOUNDS FROM
PIPER BETLE L. BY USING HP-LC ANALYSIS****S. Manjusha^{1*}, N.K.Parameswaran², R.Senthil Malar³**¹Department of Botany and Research Centre, Scott Christian College [Autonomous,] Nagercoil-629003, Kanyakumari district, Tamil Nadu, India.²Department of Biotechnology, Manonmaniam sundaranar University, Tirunelveli³Department of Zoology, Sivanthi Adithanar College, Nagercoil**Abstract:**

Medicinal plants are being used since ancient time without knowing about its active ingredients. The healing power of certain plant was understood and accepted before mankind discovered the existence of microorganisms. Herbal drugs constitute a major part in all traditional systems of medicines and are a triumph of popular therapeutic diversity. In India, thousands of plant species are known to have medicinal properties and the use of different parts of several medicinal plants to cure specific ailments have been in vogue since ancient time. In this Present Study the bioactive compounds from the plant Piper betle L. were determined by using HPLC Analysis.

Key words: *Bioactive compounds, Piper betle L., HPLC***Corresponding author:**

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INTRODUCTION:

Piper betle L. is one of the popular plants which are integrated with cultural and traditional values in India. The plant is widely cultivated in different parts of India, China, Malaysia, Srilanka, Thailand and the other Pacific Asian Nations. The plant belongs to the family Piperaceae. The leaves are used traditionally in treating hysteria, headache, swelling of gum etc., [1]. The leaves are widely used as a post meal mouth freshener and the crop is extensively grown in India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries. Due to strong pungent aromatic flavour betle leaves are used as masticatory by the Asian people. Grown abundantly in many parts of India, betle is an evergreen dioecious herb that needs warm and moist growth conditions for its growth. Leaves of betle vine are used with various condiments such as areca nut [kattha], cloves, cardamom, are canut, candied rose and fennel for chewing purposes [2]. The parts of *Piper betle* L. utilized are leaves, root, stem, stalks and fruits. *Piper betle* L. has light yellow aromatic essential oil, with sharp burning taste. The chemical constituents and their pharmacological activities of the principle ingredients in the betle quid have been studied by many works [3]. Betle leaves were reported to contain volatile oil such as betel phenol and chavicol [isomeric with eugenol], tannin, sugar, vitamin-C, starch and diastase [4]. Betle phenols possess the property of reducing the central nervous stimulation, sialogogue and local anesthesia. *Piper betle* L. leaves extract contains large number of bioactive molecule like polyphenol, alkaloids, steroids, saponin and tannin [5]. Betle leaves possess activities like antidiabetic, antiulcer, antiplatelet aggregation, antifertility, cardiogenic, antitumour, antimutagenic, respiratory depressant, antihelminthic [6-8] and wound healing property. *Piper betle* L. is used to treat alcoholism, bronchitis, asthma, [9,-2] leprosy and dyspepsia, antihistaminic, antioxidant property [13,14] antimicrobial activity, anti-inflammatory [15] radio protective and immune modulatory property [16-20]. Betle leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, rheumatism, cuts and injuries [21] The betle leaves were chewed by singers to improve their voice[22]

Piper betle L. has got large number of biomolecules which show diverse pharmacological activity along with carminative, stomachic, antihelminthic, tonic, aphrodisiac, laxative activities. The leaves are used for treating cough, foul smelling in mouth, bronchitis, clears throat and styptic [23]. The plant has been used

in traditional medicine as well as in the Ayurvedic system against various disorders and infections. Unripe, dried fruits are used as an alternative to tonic. Various plant preparations like decoction of young fruits and roots are used for treating chronic bronchitis, cough, and cold also used as antidote in snake biting and scorpion sting[24-26]. The use of combination of fruits of *Piper longum*, seeds of *Embllica ribes* and borax powder has already been cited in Ayurveda as contraceptive [27].

MATERIALS AND METHODS:

Sample collection

In this present study, three plants *Piper betle* L. were selected. The plant materials such as leaves and seeds were collected. 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 μ l 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:

Piper betle L. leaf showed there are nine peaks between the retention time of 1 to 10 minutes were as 1.820, 1.925, 2.384, 4.143, 5.052, 5.237, 5.750, 5.957 and 8.594. Among these, three peak were found as high and larger volume, 1st one RT [min] = 1.820 area [V* sec] = 875785, % area = 27.02, height [V*

sec] = 70493, % height = 36.01, starting time [min] = 1.333 and end time [min] = 1.867; and 2nd one RT [min] = 1.925 area [V* sec] = 1322677, % area =

40.80, height [V* sec] = 66734, % height = 34.09, starting time [min] = 1.867 and end time [min] = 2.300 [Plate 26].

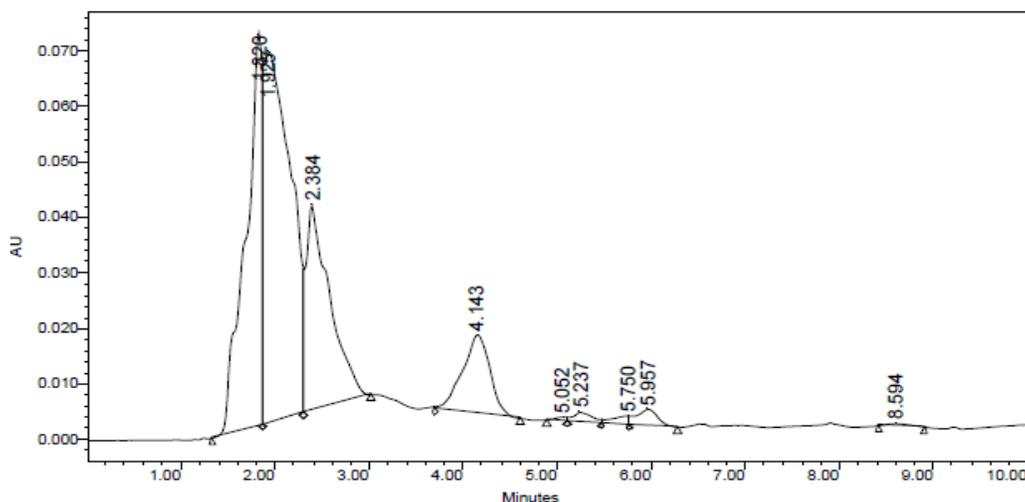


Plate 26. HPLC Chromatogram of Leaf Extract of *Piper betle* L.

Piper betle L. fruit showed six peaks between the retention time of 1 to 10 minutes were as 1.298, 1.383, 1.551, 1.669, 1.899 and 2.231. Among these six, five peak were found as high and larger volume, 1st one RT [min] = 1.298, area [V* sec] = 25190, % area = 12.96, height [V* sec] = 3317, % height = 20.03, starting time [min] = 1.117 and end time [min] = 1.367; 2nd one RT [min] = 1.383, area [V* sec] = 16494, % area = 8.49, height [V* sec] = 2499, % height = 15.09, starting time [min] = 1.367 and end time [min] = 1.500; 3rd one RT [min] = 1.669, area

[V* sec] = 55671, % area = 28.65, height [V* sec] = 4763, % height = 28.76, starting time [min] = 1.500 and end time [min] = 1.783; 4th one RT [min] = 1.899, area [V* sec] = 54236, % area = 27.91, height [V* sec] = 3439, % height = 20.76, starting time [min] = 1.783 and end time [min] = 2.100; and 5th one RT [min] = 2.231, area [V* sec] = 42727, % area = 21.99, height [V* sec] = 2546, % height = 15.37, starting time [min] = 2.100 and end time [min] = 2.567 [Plate 27].

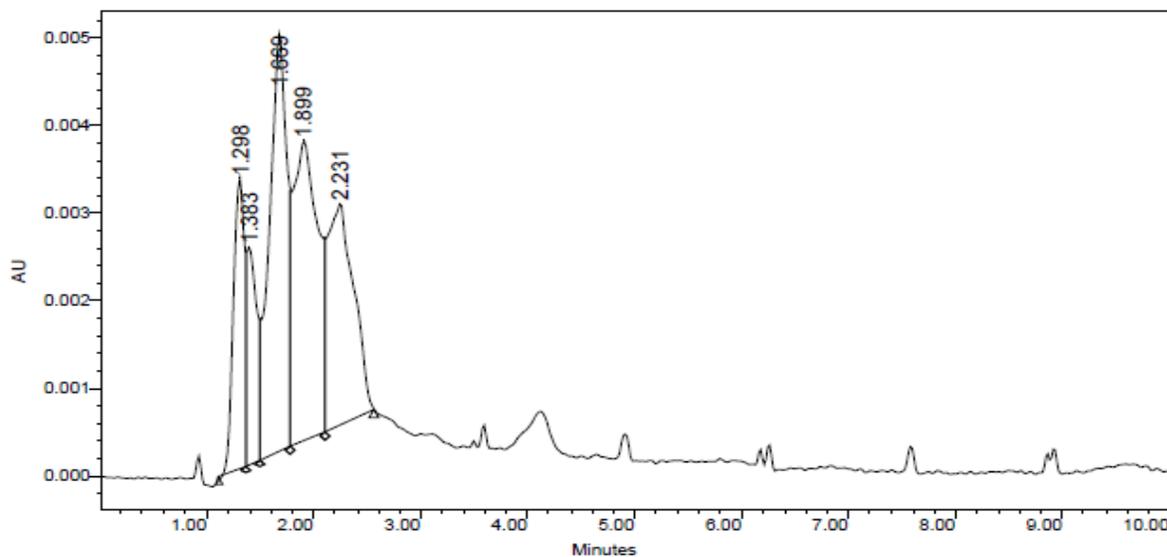


Plate 27. HPLC Chromatogram of Fruit Extract of *Piper betle* L.

DISCUSSION:

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. *Piper betle* L. seed showed six peaks and the major peaks were RT [min] = 1.298, area [V* sec] = 25190; RT [min] = 1.383, area [V* sec] = 16494; RT [min] = 1.669, area [V* sec] = 55671; RT [min] = 1.899, area [V* sec] = 54236; RT [min] = 2.231, area [V* sec] = 42727, % area = 21.99. These above results indicated that the plant samples might contain high quantities of chemical metabolites. This was supported by Upadhyay *et al.* [2013], Chauhan *et al.* [2008].

CONCLUSION:

In this study, *Piper betle* L. leaf shows nine peaks; and *Piper betle* L. seed shows six peaks. These results indicate that the plant samples are contained high quantities of chemical metabolites. Pharmacological industries have produced a number of new antibiotics in the last three decades, however, developing resistance in bacterial pathogens against commonly and currently used antibiotics has necessitated a search for structurally novel antibacterial substances from plant sources other than the traditional microorganisms [Trias and Gordon, 1997]. *Piper betle* L. most of the studies are carried out to find the antibacterial property of the plant.

REFERENCES:

- Khanra, S. and Silpakendra P.V. 1997. "Betle Leaf Based Industry". *Nabanna Bharati*, 30[2]: 169.
- Majumdar, B., Chaudari, S.R. and Roy, A. 2002. Potentiulcerogenic activity of ethanol extract of leaf *Piper betel* L. by anti-oxidative mechanism. *Indian Journal of Clinical Biological Chemistry*, 17: 49-57.
- Majumdar, B., Roy Chadhury, S., Roy, A. and Bandyopadhyay, S.K. 2003. Effect of ethanol extract of *Piper betel* L. leaf on healing of NSAID induced experimental ulcer-anovel role of free radical scavenging action. *Indian Journal of Experimental Biology*, 41: 311-315.
- Santhakumari, P., Prakash, A. and Puglendi, K.V. 2003. Modulation of oxidative stress parameter treatment with *Piper betel* leaf oxidative in Strepto zotocin induced diabetic Rats. *Indian Journal of Pharmacology*, 35: 373-378.
- Verma, A., Kumar, N. and Ranade, S.A. 2004. Genetic diversity amongst landraces of a dioecious vegetative propagated plant, betel vine [*Piper betle* L.]. *Journal of Biosciences*, 29: 319-328.
- Andhikari, P., Banerji, J., Choundhuri, D., Das, A.K., Deb, C.C., Mukherjee, P. and Chatterjee, S.R. 1990. Effect of oral administration of stalk of leaves of *Piper betle* L. on oestrous cycle and its antifertility activity in rats. *Indian Journal of Physiology and Allied Science*, 44: 116-123.
- Andhikari, P., Chowdhury, D., Banerji, J. and Chatterjee, A. 1998. Antifertility effect of crude alcoholic extract of *Piper betle* L. stalk. *Indian Journal of Physiology and Allied Science*, 52: 22-27.
- Bhide, S.V., Zariwala, M.B., Amonkar, A.J. and Ajuine, M.A. 1991. Chemo preventive efficacy of *Piper betle* L. leaf extract against benzo [a] pyrene induced forest omachtumour in mice. *Journal of Ethno pharmacology*, 34: 207-213.
- Amonkar, A.J., Padma, P.R. and Bhide, S. 1989. Protective effect of hydroxychavicol, a phenolic component of *Piper betle* L. leaf against the tobacco specific carcinogen, *Mutation research fundamental and molecular mechanism of mutagenesis*, 210: 249-253.
- Varier, P.S. 1997. *Piper betel* L. In: Varier, P.S. [ed] *Indian medicinal plants*. Kottakkal: Orient Longman, 8: 279-283.
- Koff, R.S., Gordan, G. and Sabesin, S.M. 1971. D-galactos amine hepatitis hepato cellular injury and fatty liver following a single dose. *Proceedings in Society of Experimental Biology and Medicine*, 137: 696-701.
- Shun, C.Y., Chau, J.W., Jing, J.L., Pie, L.P., Jui, L.H. and Fen, P.C. 2007. Protection effect of *Piper betle* L. leaf extract against carbon tetrachloride induced fever in rats. *Archives of Toxicology*, 81: 45-55.
- Awang, K., Pais, M., Sevenet, T., Schaller, H., Nasir, A.M. and Hadi, A.H. 1982. Eburnamiol and larutensine, alkaloids from *Kopsialarutensis*. *Phytochemistry*, 30[9]: 3164-3167.
- Schlebusch, H. and Kern, D. 1972. Stabilization of collagen by polyphenol, *Angiologica*, 9: 248-256.
- Ramji, N., Iyer, R. and Chandrasekaran, S. 2002. Phenolic antibacterial from *Piper betle* L. in the prevention of halitosis. *Journal of Ethnopharmacology*, 83: 149-152.
- Ambarta, S.P. 1986. *The Useful Plant of India*, New Delhi, CSIR Publication.
- Chaudhry, N.M.A. and Tariq, P. 2003. Bactericidal activity of black pepper, bay leaf, aniseed and coriander against oral isolates. *Pakistan Journal of Pharmaceutical Sciences*, 19: 214-218.
- Bhattacharya, S., Subramanian, M., Bauri, A., Kamat, J.P., Bandyopadhyay, S.K. and

- Chattopadhyay, S. 2005. Radio protecting property of the ethanolic extract of *Piper betle* L. leaf. *Journal of Radiation Research*, 46: 165-171.
19. Bhattacharya, S., Subramanian, M., Roychowdhury, S., Bauri, S., Kamat, J.P., Chattopadhyay, S. and Bandyopadhyay, S.K. 2006. Radio protective property of the ethanolic extract of *Piper betle* L. leaf. *Journal of Radiation Research*, 46: 165-171.
20. Agarwal, T., Singh, R., Shukla, A.D., Waris, I. and Gujrati, A. 2012. "Comparative analysis of antibacterial activity of four *Piper betle* varieties". *Advances in Applied Science Research*, 3[2]: 698-705.
21. Aggarwal, A.K., Tripathi, D.M., Sahai, R., Gupta, N., Saxena R.P., Singh, M., Mishra, R.N., Dubey., C.B. and Saxen, K.C. 1999. Management of Giardiasis by a herbal drug 'Pippali Rasayana': a clinical study. *Journal of Ethnopharmacology*, 56[3]: 233-236.
22. Usmanghani, K., Saeed, A. and Alam, M.T. 1997. "*Piper betle* L. In, Indusynic Medicine, [University of Karachi Press], Karachi". pp. 340-341.
23. Tripathi, D.M., Gupta, N., Laxmi, V., Saxena, K.C. and Aggarwal, A.K. 1999. Antigiardial and immunostimulatory effect of *Piper longum* on giardiasis due to *Giardia lamblia*, *Phytotherapy Research*, 13[7]: 561-563.
24. Chakrabarty, D. and Shah, B. 2011. Antimicrobial, antioxodative and antihemolytic activity of *Piper betle* L. leaf extract. *International Journal of Pharma and Pharmaceutical Sciences*, 3[3]: 192-199.
25. Chakraborty, D. and Shah, B. 2011. Antimicrobial, Antioxidative and Anti Hemolytic Activity of *Piper betle* L. leaf Extracts. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3: 3-7.
26. Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. *Glossary of Indian Medicinal Plants*, CSIR, New Delhi, p. 194.
27. Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. *Glossary of Indian Medicinal Plants*. Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi, pp. 256-257.
28. Chauhan, R., Dwivedi, J. and Siddiqui, A.A. 2008. Chemical standardization and quantification of *Piper* in from methanolic extract of *Piper nigrum* by HPLC method on the basis of isolated markers. *International Journal of Chemical Sciences*, 6[4]: 1726-1733.
29. Upadhyay, V., Sharm, N., Himanshu, M., Malik, M., Mishra, M., Singh B.P. and Tripathi, S. 2013. Development and Validation of Rapid RPHPLC Method for Estimation of Piperine in *Piper nigrum* L. *International Journal of Herbal Medicine*, 1[4]: 6-9.