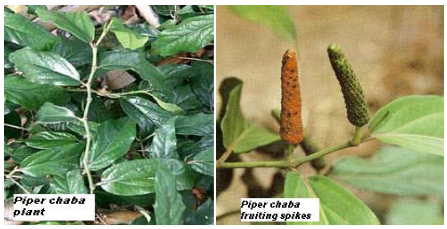
Quantification of Piperine In *P. Chaba* By HPLC And Its Bio-Potentials

GJSFR Classification - G (FOR) 270899p

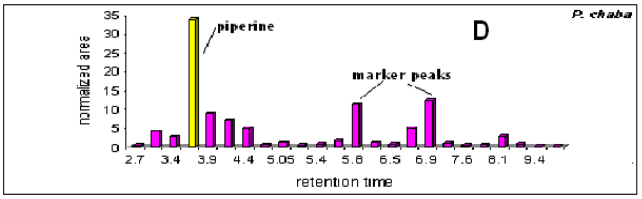
Ekta Menghani[1], Naveen Hemrajanian[2],Shalini Rajawat[3]

***Abstract*-Plants are by far the most important source of natural therapeutics, and their role in enhancing the longevity and quality of life is gaining prominence throughout the world and still the plant is often the most neglected part of plant-based medicines. Although, millions of consumers purchase medicinal plant preparations on the basis of anecdotal and scientific evidence of efficacy but very little is known about the factors that make medicinal plants different from other species. It is, therefore, necessary to standardize the medicinal plants widely used throughout the world. In view of the current importance of and interest in herbal drugs, it is necessary to prepare an International Codex containing the details of such plants so that their sale and utilization could be controlled judiciously. Therefore, in present investigations attempts have been made to isolate piperine from *P. chaba* and its quantification to evaluate the percentage of piperine for herbal validation and standardization. Further, antimicrobial, antioxidant and anti-HIV efficacy of piperine were also screened to prove its bio-potentials as bioavailability enhancer. HPLC analysis of pet.ether extract of *P. chaba*, exhibited a prominent peak of piperine at rt 3.642 min which was further ascertained by varying the concentration (1, 2, 5 and 10 mg/ml) of the extract, In the assessment of linearity, two calibration curves were plotted in the ranges 1.0 –5.0 and 5.0-10.0 mg/ml. Three replicates of each range were analyzed. The assay value of piperine was found to be 3.18%. The correlation coefficients for standard curves were 0.9933 and 0.9997. Standard deviation 8.38% and the coefficient of variation (cv) among the two curves was 5.77%. Validation of analytical method exhibited the cv of analysis less than 6%. The composite linear equations obtained from the regression analysis were y = 25210.62 x + 884438 and y = 83410 x - 2042764, where y is the area of I and x is the amount of the extract injected. Piperine possess appreciable efficacy as antimicrobial, antioxidant and anti-HIV agents but due to least toxicity it can be used as additive to toxic potent principles as bio-potent agents. Conclusively, piperine can be safely used for identification and herbal validation of *P. chaba* and as a vehicle for various biopotents.**

ABSTRACT OUTLAY



About[1]-Department of Biotechnology, Mahatma Gandhi Institute of Applied Sciences, JECRC Campus,
Jaipur -302022. INDIA;
About[2]-Department of Computer Science and Engineering, Suresh Gyan Vihar University, Jaipur



Key words: *Piper chaba*, piperine, hplc quantification, biopotentials

I. INTRODUCTION

A medicinal plant is any plant in which one or more of its part contains substances that can be used for therapeutic purposes or which are precursors for chemopharmaceutical semisynthesis. Plants have been used in traditional medicine for several thousand years (Abu-Rabia, 2005). The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine (Pei, 2001). During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world (Lev, 2006; Gazzaneo 2005; Al-Qura'n, 2005; Hanazaki *et al.*, 2000; Rossato *et al.*, 1999). Documenting the indigenous knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources.Today according to the World Health Organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary healthcare needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases (Azaizeh *et al.*, 2003). Due to less communication means, poverty, ignorance and unavailability of modern health facilities, most people especially rural people are still forced to practice traditional medicines for their common day ailments. Most of these people form the poorest link in the trade of medicinal plants (Khan, 2002). A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance (Diallo *et al.*, 1999).In the developed countries, 25 per cent of the medical drugs are based on plants and their derivatives (Principe, 1991). A group of World Health Organization (WHO) experts, who met in Congo Brazzaville in 1976, sought to define traditional African medicine as 'the sum total of practices, measures, ingredients and procedures of all kinds whether material or not, which from time immemorial has enabled

the African to guard against diseases, to alleviate his/her suffering and to cure him/herself'(Busia, 2005). Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future (Pei, 2001).Plants are by far the most important source of natural therapeutics, and their role in enhancing the longevity and quality of life is gaining prominence throughout the world and still the plant is often the most neglected part of plant-based medicines. Although, millions of consumers purchase medicinal plant preparations on the basis of anecdotal and scientific evidence of efficacy but very little is known about the factors that make medicinal plants different from other species.Current problems with medicinal plant products that compromise the quality and safety of medicinal plant products have included contamination with biological and environmental pollutants, adulteration with misidentified species, and the unsustainable harvest resulting in quantitative and qualitative variations in bioactive compounds.It is, therefore, necessary to standardize the medicinal plants widely used throughout the world. In view of the current importance of and interest in herbal drugs, it is necessary to prepare an International Codex containing the details of such plants so that their sale and utilization could be controlled judiciously.*Piper*species are known to be a rich source of *Piper* amides and their derivatives, as a result of which the plant species carry potent pharmaceutical properties like: diuretic, carminative, stimulant, etc. (Charaka Samhita, 1949; Chopra *et al*., 1956, 1969; Nadkarni and Nadkarni, 1954).Significant attention has been paid by the workers on the study of these compounds in *Piper* species (Miyakado *et al.*, 1979; Sengupta and Ray, 1987; Parmar *et al.*, 1998; Siddiqui *et al.*, 2005 a, b), but very little work on their adulteration has been carried out (Madan *et al*., 1996; Paradkar *et al*., 2001).The prime objective of this work is to study and set up certain fundamental diagnostic standards for the identification and authentication of a few important drugs such as *Piper chaba* used in the Ayurvedic System of Medicine. Efforts have been made to detect all the major and minor market adulterants with special reference to their analytical, chemical and biological screening.

II. Materials and Methods

a) *Plant material*

Authenticated samples of (Badi pippali)*Piper chaba*(from Suttind Seeds Pvt. Ltd., Delhi) and their market samples were collected and used in the presentstudy.

b) *HPLC Analysis*

The HPLC analysis was performed using a Shimadzu Model-VP 135P2 equipped with a UV spectrophotometric detector set at 254nm, column:Luna 5µC_{18}(2) 100Å (250 x 4.6 mm; 5 particle diameter), flow rate: 1ml/min, injection volume 20µl in methanol (HPLC grade).

c) *Extraction and isolation*

The fruits of *Piper chaba*and its adulterant *Piper longum* were individually extracted with ethanol for 36 hr, filtered and concentrated to dryness. Later from each, 10 mg extractof *P. chaba* and its adulterant was dissolved in 5 ml MeOH separately and used for HPLC analysis.

d) *Quantification of piperine in P. chaba by HPLC*

Pet. ether extract (piperine-rich fraction) of *P. chaba* was weighed (10, 20, 50 and 100 mg) and dissolved in 10 ml methanol (hplc grade) to prepare a concentration of 1, 2, 5 and 10 mg/ml. 200 µl of each concentrations of *P. chaba* was injected onto HPLC and the peak which appeared at the same retention time as that of standard piperine **(I)** was recorded. This value was used to calculate the amount of **I** in the extract by using the linear equation obtained from the composite standard curve. The reproducibility of quantitative analysis was verified by carrying out three replicate injections of each extract and coefficient of variation for each determination was calculated. In the present work, various calculations were achieved by Pearson's correlation formula, which is otherwise used in many forms for correlation co-efficient (*r*) and co-efficient of variation (cv):

$$r = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{N}}{\sqrt{\left(\Sigma X^2 - \frac{(\Sigma X)^2}{N}\right)\left(\Sigma Y^2 - \frac{(\Sigma Y)^2}{N}\right)}}$$

$$cv = \frac{\sigma}{x} \times 100$$

e) *Composite standard curve*

The area of corresponding piperine peak and concentration in*P. chaba* were plotted as composite standard curve.

III. Results

The quantitative evaluation of adjoining elution curves was done by calculating the resolution ($R = 2\Delta t/w_a+w_b$), where Δt is the difference between peak of interest and preceding peak and w_a and w_b are the width of peaks respectively. An easier interpretation of the HPLC tracing, as obtained in this study, was achieved when the peak area was divided by the area of reference peak and the retention time (rt) was plotted against the respective peak area gave histograms as "normalized fingerprints".In the presentinvestigations, attempts have been made to evaluate various extracts and generate some "fingerprints as markers". In *P. chaba*and its adulterants, HPLC chromatograms showed different retention time and peak area, which are characterized as "fingerprints" of *P. chaba* and its adulterants. Similarly, overlay view clearly exhibited different peaks in market samples, and thus, indicative of adulteration. The piperine concentration was also low in market samples as compared to genuine samples, and thus an efficient marker in

identification in quality control of a drug.HPLC chromatograms of extract of *P. longum* and *P. chaba* exhibited piperine at rt 3.642 and others. In *P. chaba*, two peaks at rt 5.85 and 6.98 can safely be used as marker because these peaks are absent in *P. longum* and can easily identify when adulterated with *P. chaba*. So these peaks can safely be referred as "marker peaks" (Fig. 1B and D). Further normalized fingerprints can be used as a tool for identification of the drugs.

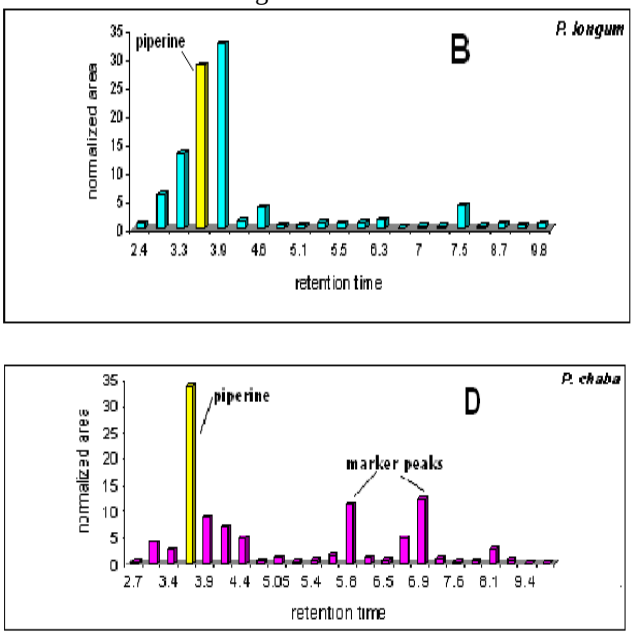


Fig. 1: HPLC Chromatograms and normalized fingerprints of alcoholic extract *P. longum* (B) and *P. chaba* (D) .

HPLC analysis of pet.ether extract of *P. chaba*, exhibited a prominent peak of piperine at rt 3.642 min which was further ascertained by varying the concentration (1, 2, 5 and 10 mg/ml) of the extract, In the assessment of linearity, two calibration curves were plotted in the ranges 1.0 –5.0 and 5.0-10.0 mg/ml (Fig. 2). Three replicates of each range were analyzed. The assay value of piperine was found to be 3.18%. The correlation coefficients for standard curves were 0.9933 and 0.9997. Standard deviation 8.38% and the coefficient of variation (cv) among the two curves was 5.77%. Validation of analytical method exhibited the cv of analysis less than 6%. The composite linear equations obtained from the regression analysis were y = 25210.62 x + 884438 and y = 83410 x - 2042764, where y is the area of **I** and x is the amount of the extract injected.

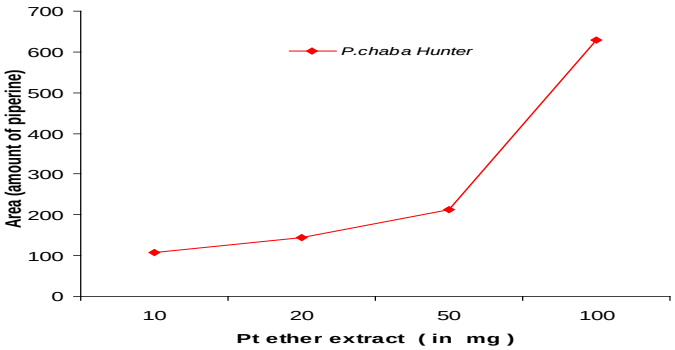


Fig. 2: The Composite standard calibration curve for quantification of piperine in *P. chaba* by HPLC

IV. Discussions

Indian herbal medicines have undoubted efficacy but still their market value is comparatively low due to unstable quality and unsuitable approaches for quality assessment. The fingerprinting is referred to as "chemical prints" established by chromatographic and spectroscopic methods for herbal drugs as markers for standardization which is easy to monitor and judge the changes within the constituents. Such chemical fingerprinting can also be used to ensure the efficacy and safety of ISM by controlling to its constituents pattern.Similarly, *Piper chaba* was studied for "HPLC chromatograms for markers in the form of peaks at different retention time (rt). An overlay view of HPLC of *P. chaba* and *P. longum* showed the peaks at rt. 5.85 and 6.98 present in *P. chaba* but were absent in *P. longum* and its market samples, thus, indicatives of adulteration in the market samples.Simultaneously, quantification of piperine in *P. chaba* was also performed for the first time and found to be 3.18% where correlation coefficient for standard curves were 0.9933 and 0.9997 with a standard deviation 8.38% and the coefficient of variation (CV) among the two curves was 5.77%.Earlier, the use of HPLC as a tool for standardization of herbals was performed by few workers (Philipp and Isengard, 1995) but no such HPLC standardization in *Piper* species was carried out so far, and thus, it is the first report of this nature to generate HPLC chromatograms of genuine v/s adulterants.

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