ABSTRACT
Variation in metabolites of herbs, due to ontogenetic, ecotypic, genotypic and chemotypic factors, leads to inconsistency in herbal medicinal products, which is one of the main challenges faced by the natural product industry. Metabolomics detect, quantify and catalogue the time-related metabolic processes of an integrated biological system. Therefore, this study aimed to use metabolomics to investigate time-related metabolic changes in fruit of *Piper sarmentosum* in order to find the suitable time of harvesting the fruit to maintain consistency in efficacy and batch-batch reproducibility. The same size of the fruit was collected from the same location from April 2006 to August 2007 at an interval of two months. Each collection was regarded as a single independent batch. The dried fruit powder of each batch was analyzed in triplicate by FTIR spectrophotometer in potassium bromide pellets. The FTIR spectra of different batches were analyzed by chemometrics, principal component analysis (PCA), to evaluate identification, classification and differentiation (ICD) using PerkinElmer application software. All the samples exhibited correlation (86.24%) with reference to PCA 1 and 9.38% on PCA2. The samples collected in November-December 2006 and July-August 2007 were grouped far from others on positive and negative side of the upper quadrants. These results indicated that the metabolic constituents in the samples varied in all the batches. However, correlation in the samples might be helpful to predicted consistency in pharmacological activity. The results of this study indicate that FTIR fingerprint profiles of the samples in combination with chemometrics is an effective tool of ICD which may be helpful for the natural product industry to indemnify the quality and batch-batch reproducibility.

Keywords: *Piper sarmentosum*, Metabolomics, FTIR spectrophotometry, Chemometrics.

INTRODUCTION
Metabolic content of the plants are influenced by ontogenetic, ecotypic, genotypic and chemotypic factors. In addition to these, growth conditions, age of the plant, collection time, drying and storage conditions contribute to inconsistency of natural products. These products are complex mixtures of various types of chemical constituents; therefore, it is always challenging to standardize herbal products. These products are usually mixed with other pharmaceuticals to make these remedies evidence-based medicines. The World health organization (WHO) in a number of resolutions has emphasized the need to ensure quality control of herbal products using modern analytical techniques because standardized extracts have several advantages as compared to crude extracts, because it ensures the identification that “the herb is what it is claimed to be”.

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Different strategies can be used to insure quality and consistency such as using markers of different categories and metabolomics. Recently, Li *et al.* (2008) have classified markers into eight categories such as therapeutic components, bioactive components, synergistic components, characteristic components, main components, correlative components, toxic components and general components. The use of markers helps in positive identification or standardisation of extracts. In markers based standardisation only markers are considered ignoring other constituents of the extracts which may have synergistic or buffering action. Recently, Hussain *et al.* (2009) have suggested the estimation of primary and secondary metabolic contents in addition to markers for complete standardisation. Many international authorities and agencies including the WHO, the European Agency for Evaluation of Medicinal Products and the European Scientific Cooperation of Phytomedicine, the US Agency for Healthcare Policy and Research and the European Pharmacopoeia Commission have started creating a new mechanism for quality control and standardisation of botanical medicines. As a result of...
these efforts, a botanical formulation is now regarded as active substance in its entity whether or not the constituents with therapeutic activity are known. This is a major step in the development of new generation of standardised herbal medicines. Several spectroscopic and chromatographic techniques like ultraviolet (UV), FTIR, Thin layer chromatography (TLC), high performance liquid chromatography (HPLC), liquid chromatography/gas chromatography-mass spectrometry (LC/GC-MS) and nuclear magnetic resonance (NMR) are used to obtain fingerprints which in combination with chemometrics provide effective tool of identification, classification and discrimination. The characterisation of metabolic profiling, metabolomics, not only helps in identification of active constituents but also improve the knowledge about efficacy, safety and complexity of a given therapeutic combination. Metabolomics, a system of cell biology, comprise of all the compounds other than proteins, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Metabolomics, detect, quantify and catalogue the time related metabolic processes of an integrated biological system under specified conditions. Metabolic fingerprints are unique patterns, which indicate the presence of particular molecules based on specialized analytical techniques and are extremely useful to identify active components, contaminants and other chemicals present in herbal products. In present study, we have used metabolomics to investigate the time-related metabolic changes in fruit of a medicinal plant, Piper sarmentosum.

Piper sarmentosum Roxb. (Piperaceae) is cultivated or found wild under shady trees in South East Asian region and is well recognized due to culinary and medicinal properties. As a wild under shady trees in South East Asian region and is well

RESULTS AND DISCUSSION

FTIR profiles are important in quality assessment of herbal materials because often it is not necessary to know identity of individual constituents that make up fingerprint. Fingerprints, characteristic to each material, give quick check of identification, classification and discrimination. IR spectrum of fruit powder indicated bands at 3650 cm

-1 (amide), 2900-2850 cm

-1 (C-H), 1634-1500 cm

-1 (aromatic domain bands), 1725-1705 cm

-1 (carbonyl) and 1200-1100 cm

-1 (alkenes). Principal component analysis (PCA) uses a mathematical procedure which transforms a number of possibly correlated variables into smaller number of uncorrelated variables called principal components. PCA1 indicate maximum possible variability and each succeeding components indicate reaming possible variability. It is theoretically the optimum transform for a data in least square terms. PCA chooses the first PCA axis as that line which goes through the centre and minimizes the square of the distance of each point to PCA line. The line traverses through the maximum variation in the data. The second PCA axis also passes through the maximum variation in the data but it must be right angle to PCA 1-axis, completely uncorrelated.

The FTIR spectra of different batches of fruit of the plant were encoded as: March-April 2006 (a, b, c), May-June 2006 (d, e, f), July-August 2006 (g, h, i), September-October 2006 (j, k, l), November-December 2006 (m, n, o), January-February 2007 (p, q, r), March-April 2007 (s, t, u), May-June 2007 (v, w, x) and July-August 2007 (y, z, z2). These spectra were then analysed in combination with chemometrics, PCA (Fig. 1). The results indicated that all the samples except the two which were collected in November-December 2006 (m, n, o) and July-August 2007 (y, z, z2) were grouped in lower two quadrants. The results of the figure indicated that samples of different batches were 86.24 % correlated with reference to PC 1, while 9.38 % uncorrelated with respect to PC 2. From looking at the figure, we can easily identify a gradient from the lower left front to the lower right indicating gradient along which PCA 1 and PCA 2 increase. From these results, we can predict similar activity in the samples which are correlated and grouped together. FTIR spectra showing differences and similarities are shown in Fig. 2.

The variations in metabolites of the plant may be minimized by the application of strict quality control protocols right
Fig. 1: Report of principal component analysis of FTIR fingerprints of Piper sarmentosum fruit from different batches collected from April 2006 to August 2007.

Fig. 2: Comparison of FTIR spectra of Piper sarmentosum fruit from different batches, A (spectra of samples showing difference); B (spectra of samples showing similarity).
from the selection of seeds, sowing at suitable time, control of cultivation conditions, collection at suitable age, drying and storage. This can goal can be achieved by the application good agricultural and collection practices and good manufacturing practices. A number of guidelines for good agricultural and collection practices have been suggested by the World Health Organization. [22] These variations may further be minimised by the application of pharmacognostic methods to know which plant, identified by botanical nomenclature and which part of the plant to which percentage is used. In present study, we have collected the fruit from the same location to minimize variable such as growth conditions, fruit size, collection time, drying and storage. The climatic conditions were the only variability. It is concluded from the results of this study that metabolomic fingerprint profiling in combination with chemometrics is an effective tool of fast and easy assessment of similarity of differentiation in samples and may be used as an analytical tool in quality assurance.

REFERENCES