CHAPTER 6

DISCUSSION
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Knowledge of the medicinal value of plants is recognized by almost every society on earth. Once a specific plant was discovered to be therapeutic, this knowledge was passed on to other as folk medicine. In India, a society with a strong cultural heritage, this knowledge attained a well-organized form. The knowledge was systematically recorded and employed as a traditional health care system called Ayurveda. Ayurveda collectively encompasses medical, psychological, cultural, religious and philosophical concepts. It is a holistic approach that has as its goal a long, healthy and happy life.

Many Ayurvedic drugs are of herbal origin. Ayurvedic literature reveals that frequent and consistent repetition of certain herbals individually / as a group was noticed in large number of prescriptions recommended for a variety of diseases. One of the groups of herbals which is documented is ‘Trikatu’ - the three acrids viz., long pepper, black pepper and ginger in equal proportions by weight. Some modern Ayurvedic practitioners have tried to explain the scientific basis underlying the increase in efficacy of these herbals. Black pepper (Piper nigrum Linn.) belongs to the family piperaceae. This perennial climber shrub is cultivated in the hot and damp parts of India. It is acrid, pungent hot, carminative and is also used also used as antiperiodic. Externally, it is rubefacials and stimulant to the skin and also a resolvent. The Extracts and essential oils of black pepper are reported to have antibacterial and antifungal activities. Experimental evidences show that the use of black pepper or piperine, the main alkaloid, enhances the bioavailability of a number of drugs like vasicine, spartain, sulphadiazine and tetracycline.

Good quality assurance is necessary for plant products, which are intended to be used as medicinal agents directly or as extracts in basic pharmacological experiments. Since the synthetic drugs are subjected to severe quality control tests, the plant products must also comply with quality standards. Thus quality assurance is necessary if plant products which are used as medicines are to fill the need of alternative and reliable medicines and if natural products are to be used as templates for new drug molecules.

The present investigation was an attempt to standardize the most common spice of the piperaceae family namely Piper nigrum Linn. commonly known as black pepper, for its bioavailability enhancing property. The investigation was carried out with an aim...
• To develop a qualitative fingerprinting method, which could help in identifying black pepper from its raw materials (powders/extracts etc.)
• To develop a quantitative HPTLC method for the determination of piperine, the main alkaloid of black pepper and apply this method for geographical and age-wise variation, to monitor accelerated stability study and to determine the black pepper content in formulations available in the market
• To check the bioavailability enhancing property of black pepper.

FINGERPRINTING

Fingerprint in essence is chemoprofiling, which means establishing a characteristic chemical pattern for the plant material or its extract. It is important to understand that plant extract consists of established classes of chemical compounds. These include the primary metabolites, secondary metabolite and inorganic salts and metals. Primary metabolites are compounds like carbohydrates, proteins, lipids that are essential for the plant physiology. Secondary metabolites are compounds which are not essential for the plant physiology as much but are formed as byproduct in the biochemical pathways. These include very interesting and useful classes of compounds like alkaloids, flavonoids, coumarines, terpenoids, anthocyanins, etc.

Our knowledge of chemistry has advanced and we have access to sophisticated analytical techniques. The secondary metabolites of plants can therefore be used for their qualitative detection and quantitative determination. It is then possible to use suitable phytochemical markers as preliminary identification methods. Preparation of fingerprints of all the species of chosen herb or plant with reference to seasonal, regional and age wise variations of phytochemicals is the first and the most important step in the standardisation of herbal/folk medicines a huge data base can be prepared on important plant materials which will be serve as a quick and reliable method of identification through the use of computerised scanning and search facilities.

It is a well-established fact that the concept of an active ingredient is not acceptable in the systems of traditional medicine. It is believed that the final effect of a herbal medicinal preparation is as a result of the synergetic effect shown by its constituent phytochemicals. Development of reliable and reproducible methods for quality control
of herbal medicines therefore is a challenging job. Availability of HPTLC with scanning facilities in the UV-Visible and fluorescence modes has made this job relatively easy.

**CHROMATOGRAPHIC METHODS**

Chromatography involves separation of components of a mixture by virtue of difference in the equilibrium distribution of the components, between two phases, the mobile phase and the stationary phase. Chromatography is used as an effective tool in the separation and estimation of individual components from a complex matrix.

High performance thin layer chromatographic method was developed for the determination of piperine from black pepper and its formulation. A normal phase chromatographic mode of separation was used. Silica gel 60 F254 plate was used as the stationary phase. The mobile phase comprising ethyl acetate: n-hexane : triethylamine in the volume ratio of (4 : 6 : 0.1) on a silica gel 60 F254 plate gave good resolution between piperine and the internal standard drotaverine. The plate was scanned at 340 nm, the wavelength of maximum absorbance for piperine.

**COMPARISON OF HPLC AND HPTLC**

The capital cost of a fully automated HPTLC system is much higher than that of a gradient HPLC system. HPTLC is often advantageous, because of its simplicity, high sample throughput and versatility. The separation mechanism for HPTLC and HPLC is fundamentally the same. Physically, however, the separation process differs in several ways. In HPLC, the stationary phase and the mobile phase are usually equilibrated prior to the introduction of the sample, while in HPTLC the mobile phase usually encounters a dry layer in the beginning of separation. In column technique such as HPLC, the flow of mobile phase results from pressure difference between the column entrance and column exit. Furthermore, the mobile phase velocity is electronically controllable up to a limit established by the maximum pressure gradient that can be maintained across the system. In HPTLC, a flow of mobile phase is a result of capillary forces and cannot be controlled as in case of column method.
One of the most important distinctions between HPLC and HPTLC is the nature of separation. Column techniques are time limited whereas there is no such limitation in HPTLC. Then in HPLC, all the components migrate the same distance and get separated in time while in HPTLC all the components have the same separation time but are separated in space.

HPTLC has an important role to play in analytical chemistry, which is complementary to other chromatographic techniques. Among the unique advantages of HPTLC is the ability to chromatograph several samples simultaneously, thereby reducing the time and cost for analysis and the chromatogram provides a permanent record. HPTLC also gains certain advantages because it is an open bed system, which offers a visual display of the entire sample, while the column techniques are closed bed systems. In the later case, the chromatographer may be unaware of the sample components that are strongly retained. The process of separation and detection are usually physically separated in HPTLC. In HPLC, the selection of the mobile phase limits the choice of a detector. For example, UV absorbing solvents cannot be used with a UV visible detector in HPLC whereas this is not a problem in HPTLC because the plate is dried prior to detection, the detection process can be optimized independently in HPTLC. In terms of sensitivity however, HPLC is far superior to HPTLC.

ACCELERATED STABILITY STUDY

Many herbal / ayurvedic formulations are available in the market with no expiry date mentioned on their labels. But it is not true that the use of these medicines is safe till the time it exists. Substances used as medicines are subjected to decomposition resulting in loss of potency. The purpose of stability testing is to provide evidences on how the quantity of medicinal substances or drugs varies with the time under the influence of a variety of factors such as temperature, humidity etc. Stability testing enables us to determine recommended storage conditions and the shelf life of the drug.

Stability studies are important due to many reasons such as the decomposition products may have a collective effect with the action of the drug and may cause toxic effects.

Black pepper samples were kept at room temperature (25°C) and portions of the same samples were stored at 40°C and 75 % RH (relative humidity) in polyethylene bags.
enclosed in a cardboard carton. These samples were analyzed for piperine content for six months, at an interval of 15 days.

**BIOAVAILABILITY ENHANCEMENT**

The literature search reveals that some herbal preparations are co-administered with synthetic drugs to enhance the bioavailability of the later. The herbals are called potantising agent. In the present work effect of piperine is studied in the bioavailability of trovafloxacin.

A randomized, two-way, two treatment, two-period, single dose, crossover bioavailability study of trovafloxacin 200 mg tablets (preparation 1) comparing with trovafloxacin 200 mg tablets along with black pepper powder 1.0 g (preparation 2), on twelve healthy adult male human volunteers under fasting conditions was carried out. The results of the bioavailability study show that the bioavailability of trovafloxacin increases significantly when it is co-administered with black pepper.