1. Introduction

1.1 History of Ayurveda

Ayurveda, ‘the science of life’ is a very comprehensive system of healing which is the most ancient system of medicine with history of over 3000 years old. The word Ayurveda is composed of two Sanskrit terms, viz, ‘ayus’ meaning life and ‘veda’ meaning knowledge. Ayurveda is an eternal science of holistic healing and healthy living. It defines life as the intelligent coordination of body, senses, mind and soul, with the totality of life and the concept of health/illness is recognized as the state of harmony/disharmony between them. Caraka has defined Ayurveda as the “science through the help of which one can obtain knowledge about the useful and harmful types of life (hita and ahita ayus), happy and miserable types of life, things which are useful and harmful for such types of life, the span of life as well as the very nature of life.” From this definition Ayurveda emphasizes upon not only leading a life which is full of happiness and which implies a personal attitude but also leading a life which will be useful to society as a whole. Ayurveda forms an important component of health care in India which is based upon centuries old observation, rich in traditional wisdom and with its own strong basic principles and philosophy as its skeleton and body. As per concepts of Ayurveda, every material of earth is made up of five basic elements, which are prithvi (earth), jal (water), tej (fire), vayu (air), aakash (space) which is true for both plants as well as human beings providing their interface [1-4].

1.1.1 Concept of Ayurveda

According to Ayurveda human body is made up of three fundamental elements called dosas, dhatus and malas. The dosas govern the physio-chemical and physiological activities of the body, while dhatus enter into the formation of the basic structure of a body cell and perform some specific actions. Malas are substances partly utilized in the body and partly excreted in a modified form after performing their physiological functions. These three elements are said to be in a dynamic equilibrium with each other for the maintenance of health and any imbalance of their relative preponderance in the body results in disease and decay. Dosas are mainly three known as vata (air), pita (fire), and kapha (water). When these three are in a state of equilibrium along with properly
functioning datus, malas, sense organs with a pleasant state of mind and spirit is considered as positive health and imbalance in the above factors results in disease [5-6].

1.1.2 Global Status of Ayurveda

Ayurveda, the traditional Indian medicine remains the most ancient yet living traditions. It is a great tradition with sound philosophical, experiential and experimental basis. Increased side effects of chemical drugs, failure of the allopathic system of medicine in various chronic ailments, high cost of new drugs, microbial resistance and emerging diseases are some reasons for public interest in complementary and alternative medicines. Global acceptance of Ayurveda is gearing up and there has been a steep rise in the demand for medicinal plants from India. The Western population is looking for natural remedies which are safe and effective. It is documented that 80% of the world’s population has faith in traditional medicine, particularly herbal drugs for their primary healthcare [7, 8].

1.1.3 Problems in Standardization of Ayurvedic Formulation [9]

Standardization of Ayurvedic / herbal drugs involves many obstacles because synthetic drugs have well defined structure, established assays, standard analytical parameters and reference standard for comparison. Therefore, quality control is not a problem for synthetic drug. There are several obstacles in the standardization of Ayurvedic / herbal product. The obstacles like identity of various plants, deliberate adulteration of plant material, problems in storage and transport. There are many problems in developing quality control methods as mentioned below:

- Herbal drugs are usually mixtures of many constituents.
- Selective analytical methods or reference compounds may not be available commercially.
- Plant materials are chemically and naturally variable.
- The sources and quality of raw materials are variable.
- The methods of harvesting, drying, storage, transportation.
- The methods of processing (mode of extraction and polarity of extracting solvent, instability of constituents, etc.).
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1.2 Diabetes mellitus
Diabetes mellitus comprises a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates and proteins; and an increased risk of complications from vascular disease. Diabetes mellitus (DM), a global health problem, is now emerging as an epidemic world over. The prevalence of diabetes in all age-groups worldwide was estimated to be 2.8% in 2000 and projected to be 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.

Many factors contribute to DM, including the aging of population, increasing obesity, due to large caloric over-consumption and decreased physical activity. Diabetes mellitus is one of the most rapidly growing diseases worldwide and is a prime cause of excess cardiovascular morbidity and mortality in Western populations. Other serious morbidities and mortalities are related to development of nephropathy, neuropathy and retinopathy due to diabetes. Despite tremendous advances in medicine during the past century, there is still no cure, which means that effective prevention and treatment is of paramount importance to prevent future increase in disease burden. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential [10-12].

The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated. Thus, plants are a potential source of anti-diabetic drugs (and others too) but this fact has not gained enough momentum in the scientific community. The reasons may be many including lack of belief among the practitioners of conventional medicine over alternative medicine, non availability of well defined alternative forms of medicine, possibility of quacks practicing such medicine providing alluring and magical cures, and natural drugs may vary tremendously in content, quality and safety. As the knowledge of heterogeneity of this disorder increases, there is a need to look for more efficacious agents with lesser side effects. Though development of modern medicine resulted in the advent of modern pharmacotherapeutics
including insulin, biguanides, sulfonylureas and thiazolidinediones, there is still a need to look for new drugs as no drug (except obligate glycemic control with insulin) has been shown to modify the course of diabetic complications. India has a rich history of using various potent herbs and herbal components for treating diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reported in numerous scientific journals [13-15].

1.3 Standardization of raw material

1.3.1 Identification of plant material [16]

Authenticity, purity and assay are important aspects of the standardization and quality control of plant materials. As the name implies authenticity means reliable and trustworthy. Quality control of botanicals starts right from identification of plant. Well-known example is of *Sankhapatupi*, an important *Medhya* drug used for improvement of memory power and intellect. *Sankhapatupi* is equated with either of the following plants depending on the region in India: *Canscora decussata*, *Evolvulus alsinoides* and *Clitoria ternata* and sometimes *Convulvularis pluricalis*.

Problems in procurement of authentic plant materials are due to:

- Collection of wildly growing plants from forests and wastelands.
- Limited knowledge of traders or suppliers of medicinal plants
- Lack of awareness of folk populace and laborers in charge of collections of the identity of the drugs.
- Non homogeneity of plant material due to collection from wild sources and different geographical locations.

1.3.2 Phytochemical variation

Consistency in composition and biological activity are essential requirements for the safety and efficacy of therapeutic agents. However, botanical preparations rarely meet this standard because of above mentioned issues. Environmental conditions such as sunlight, rainfall, altitude, temperature, soil, storage conditions as well as different harvesting procedures, time and method of collection, manufacturing processes such as
selecting, drying, purifying, extracting and genetic variability can create substantial variability in product quality and in the concentration of plant chemicals within different products. Ecological conditions like insect feeding, microbial infections may affect secondary metabolites and in turn chemical composition of the plant. Different parts of same plant (example roots, stem and leaves) may contain different concentration of chemical constituents. As botanicals are prone to contamination due to fungus, moisture etc. leading to deterioration and causing batch to batch variation in composition [17].

1.3.3 Adulteration and substitution

Adulteration of plant by substitution with inferior commercial varieties artificially manufactured substances, intentional use of pharmaceutical adulterant, exhausted drugs or cheaper plant or by another vegetative part of the same plant can affect the quality of herbal drug.

1.3.4 Heavy metal contamination

Several reports suggest that many herbal products contain undisclosed pharmaceuticals and heavy metals because of which the herbal drug may get contaminated during processing. Because of the above mentioned factors, there is a need for standardization. The manufacturer of herbal product needs to establish safety and efficacy via appropriately designed clinical trials, characterization and validation of the formulation. The key to success is using the available information strategically, identifying the gaps in the information and creating plans to fill those gaps. Other efforts such as stringent cGMP, strict analysis of raw materials for colour, aroma and content of specified actives should be made. Additional tests like thin layer chromatography fingerprinting, detection of microbial levels, extraneous matter, pesticides / herbicides residue and heavy metals should be carried out. The finished formulation should be tested for other physical tests such as dissolution, melting temperature, stability, storage conditions.
1.3.5 Parameters for standardization of crude drug/herbal medicines (As per WHO)
The following parameters are used for crude drugs/herbal medicines.

1. Authentication for
   • Botanical identity like phytomorphology, microscopical and taxonomical identity.
   • Stage of collection.
   • Parts of the plant collected.

2. Foreign matter

3. Organoleptic evaluation

4. Tissues of diagnostic importance present in the drug powder.

5. Ash values and extractive values.

6. Volatile matter

7. Moisture content determination

8. Chromatographic and spectroscopic evaluation. TLC, HPTLC, HPLC methods will provide qualitative and semi quantitative information about the main active constituents present in the crude drug as chemical markers in the TLC fingerprint evaluation of herbals (FEH).

9. Pesticide residue – WHO and FAO set limits of pesticides, which are usually present in the herbs. Mainly pesticides like DDT, BHC, Toxaphene, Aldrin cause serious side-effects in human beings if the crude drugs are mixed with these agents.

10. Microbial contamination – usually medicinal plants containing bacteria and molds are coming from soil and atmosphere. Analysis of the limits of *E. coli* and molds clearly throws light towards the harvesting and production practices. The substance known as aflatoxins will produce serious side-effects if consumed along with the crude drugs.

11. Radioactive contamination – Microbial growth in herbals are usually avoided by irradiation. Shukla and Saraf process may sterilize the plant material but the radioactivity hazard should be taken into account. The radioactivity of the plant samples should be checked according to the guidelines of International Atomic Energy (IAE) in Vienna and that of WHO.
1.4 Chromatographic fingerprint

By definition, a chromatographic fingerprint of herbal medicine is, in practice, a chromatographic pattern of pharmacologically active and/or chemically characteristic constituents present in the herbal product. Chromatographic fingerprint can successfully demonstrate both similarities and differences between various samples and the authentication and identification of herbal medicines can be accurately conducted even if the number and/or concentration of chemically characteristic constituents are not very similar in different samples of herbal medicine. Chemical fingerprints obtained by chromatographic techniques such as HPLC-UV (DAD), HPLC-ELSD, HPLC-MS, GC-MS, HPTLC-densitometry are strongly recommended for the purpose of quality control of complex herbal medicines. It can serve as a tool for identification, authentication and quality control of herbal drugs all over the world due to its simplicity and reliability.

Traditionally only a few markers of pharmacologically active constituents were employed to assess the quality and authenticity of complex herbal medicines. However, the therapeutic effects of herbal medicines are based on the complex interaction of numerous ingredients in combination, which are totally different from those of chemical drugs. Chromatographic fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of herbal drugs and their related products [18, 19].

1.5 HPLC an important analytical tool

HPLC is a popular method for the analysis of herbal medicines due to many advantages such as

- Very powerful separation ability, to separate the complex chemical components in herbal medicine.
- Application of HPLC technique to volatile and stable compounds.
- Ease of usage to learn and learning.
- Availability of many variables, such as the different compositions of the mobile phases, their pH adjustment, pump pressures, etc, to arrive at a precise method.
- Capability to couple with many detectors like PDA, UV, MS and RI detectors.
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In general, HPLC can be used to analyze almost all the compounds in the herbal medicines. Thus, over the past decades, HPLC was applied extensively in the analysis of herbal medicines. Reversed-phase (RP) columns are the most popular columns used in the analytical separation of herbal medicines. It is necessary to notice that optimal separation condition is required to obtain better separation; some new techniques have been recently developed in research field of liquid chromatography. These are micellar electrokinetic capillary chromatography (MECC), high-speed counter-current chromatography (HSCCC), low-pressure size-exclusion chromatography (SEC), reversed-phase ion-pairing HPLC (RP-IPC-HPLC) and strong anion-exchange HPLC (SAX-HPLC). They provide new opportunities for good separation for some specific extracts of some herbal medicines. Furthermore, the recent approaches of applying hyphenated chromatography and spectroscopy such as High-Performance liquid chromatography- diode array detection (HPLC-DAD), capillary electrophoresis-diode array detection (CE-DAD), Liquid chromatography and mass spectroscopy (LC-MS) and HPLC-NMR could provide the additional spectral information, which will be very helpful for the qualitative analysis and even for the on-line structural elucidation. A chemical fingerprint obtained by hyphenated chromatography, will become the primary tool for quality control of herbal medicines [19-24].

1.6 Hyphenation procedures

In the past two decades, combining a chromatographic separation system on-line with a spectroscopic detector in order to obtain structural information on the analytes present in a sample has become the most important approach for identification and/or confirmation of the identity of target and unknown chemical compounds. For most (trace-level) analytical problems in the research field of herbal medicines, the combination of column liquid chromatography or capillary gas chromatography with a UV–vis or a mass spectrometer (HPLC–DAD, CE-DAD, GC–MS and LC–MS, respectively) becomes the preferred approach for the analysis of herbal medicines. It is also true that additional and/or complementary information is urgently required in quite a number of cases. This can be provided by few examples: Atomic emission, Fourier-transform infrared (FTIR), fluorescence emission (FE), or nuclear magnetic resonance (NMR) spectrometry. It is
demonstrated that, from a practical point of view, rewarding results can be obtained, since much more information is needed to deal with the most complex analytical systems such as those samples from herbal medicines [25].

1.7 Role of Markers in the Standardization of Herbal Drugs

Quality control of herbal medicine aims to ensure its quality, safety and efficacy. Manufacturer has to develop specific quantitative assay methods of phytochemical analysis of herbal medicine to monitor the quality of the product. Phytochemical markers are pivotal in the current practice of quality control since they are used as indicator at various stages of development and manufacturing of herbal medicine, such as authentication and differentiation of species, collecting, harvesting, quality and stability assessment and discovery of lead compounds. Lack of chemical markers remains a major problem in the quality control of herbal medicines. In many cases, sufficient chemical and pharmacological data of phytochemical marker is not available. Furthermore, there are many technical challenges in the production of chemical markers. For example, temperature, light and solvents often cause degradation and/or transformation of purified components; isomers and conformers may also cause confusions [26].

In the present study emphasis is made on the isolation, characterization, analysis and antidiabetic study of flavonoids and secoiridoid glycosides from *Enicostemma littorale* (Gentianaceae) and phenolic compounds from *Phyllanthus emblica* (Euphorbiaceae).

The specific aim of the study is --

- To evaluate the use of HPLC/HPTLC as analytical techniques for flavonoids, secoiridoid glycosides and phenolic compounds.
- To develop commercially viable method for the isolation and purification.
- To study acute, repeated dose toxicity and antidiabetic activity of extracts of *Enicostemma littorale* and *Phyllanthus emblica*.