I. INTRODUCTION

In general, humans have been using many species of plants medicinally for centuries. Through personal experience and knowledge passed down for generations, indigenous people have learned which species of plants may help alleviate certain ailments\(^1\). Many patients look for complementary and alternative medicine options in coping with this debilitating disease. Research has indicated that people suffering from chronic pain, as in RA, and those dissatisfied with current treatment are very likely to seek alternative treatments\(^2\).

The commonly used anti-inflammatory agents are becoming less acceptable due to serious adverse reactions such as gastric intolerance, bone marrow depression and water and salt retention, resulting from prolonged use. This necessitates the continued search for potent anti-inflammatory agents with reduced or no side-effects. Studies based on the ethnobotanical use of plants have often proved to be a more efficient method of drug discovery than random plant screening. Some plant secondary metabolites such as alkaloids, phenols, tannins, glycosides, terpenoids, saponins, flavonoids and steroids have been implicated in their ability to inhibit the formation of pro-inflammatory signalling molecules such as prostaglandin or leukotrienes\(^3\).

Recently, there is a greater global interest in non-synthetic, natural medicines derived from plant sources due to better tolerance and minimum adverse drug reactions as compared to synthetic medicines. Herbal products are also commonly used by the patients with certain chronic medical conditions, including breast cancer, liver disease, human immunodeficiency, asthma and
rheumatological disorders. WHO estimates that about three-quarters of the world's population currently uses herbs and other forms of traditional medicines for the treatment of various diseases. The herbs are formulated in different modern dosage forms, such as Tablets, Capsules, Topical cream, Gel, Ointment and even some novel drug delivery forms, like extended release, sustained release, and microencapsules dosage forms. Patenting of herbal formulations has increased over the past few years and scientific evidence of therapeutic activity has been reported by performing various in-vitro and in-vivo experiments\(^4\).

The recent global resurgence of interest in herbal medicines has led to an increase in the demand for them. Commercialization of the manufacture of these medicines to meet this increasing demand has resulted in a decline in their quality, primarily due to a lack of adequate regulations pertaining to this sector of medicine. The need of the hour is to evolve a systematic approach and to develop well-designed methodologies for the standardization of herbal raw materials and herbal formulations.

Various methods of phytochemical standardization, such as preliminary phytochemical screening, fingerprint profiling, and quantification of marker compound(s) with reference to herbal raw materials and polyherbal formulations will remain important in case of herbal drug technology\(^5\).

The revival of interest in natural drugs, especially those derived from plants, started in the last few decades mainly because of the widespread belief that green medicines are healthier and safer than the synthetic ones. The modern allopathic system makes no claims of complete cure for many difficult to treat diseases. The answer for their cures may lie within the herbal medicinal systems\(^6\).
Standardization is an important aspect for maintaining and assessing the quality and safety of the herbal formulation as these are combinations of more than one herb to attain the desire therapeutic effect. Standardization minimizes batch to batch variation; assure safety, efficacy, quality and acceptability of the herbal formulations.

WHO stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and / or chemical markers and the fingerprint profiles. If a principle active component is known, it is most logical to quantitate this compound. Where active ingredients contributing to therapeutic efficacy are known botanical preparations should be standardized to these compounds. Where the active ingredients are not yet known a marker substance which should be specific for the botanical could be chosen for analytical purpose \(^{(7)}\).

GACP is a technical guideline on the production of medicinal plant materials as starting materials for crude drugs and finished crude drugs, and deal with the following areas:

- Cultivation and collection of medicinal plants and production of medicinal plant materials;
- Post-harvest processing required for medicinal plant materials;
- Quality control of medicinal plant materials \(^{(8)}\).

Crude drugs are the starting raw material for manufacturing herbal medicines in all the indigenous systems of medicine. Efficacy and safety of any
medicine depends on the genuineness and quality of the raw materials used in its preparation. Because of wide-spread belief that herbal medicines are safer than synthetic drugs, demand of medicinal plants has increased many folds in the national and international markets to meet the needs of traditional and modern medicines (9).

Phytochemical standardization encompasses all possible information generated with regard to the chemical constituents present in an herbal drug. Hence, the phytochemical evaluation for standardization purpose includes the following:

1. Preliminary testing for the presence of different chemical groups.
2. Quantification of chemical groups of interest (e.g., total alkaloids, total phenolics, total triterpenic acids, total tannins). Establishment of fingerprint profiles.
3. Multiple marker-based fingerprint profiles.
4. Quantification of important chemical constituents

Phytotherapeutic agents are normally marketed as standardized preparations in the form of liquid, solid (powdered extract), or viscous preparations. They are prepared by maceration, percolation or distillation (volatile oils). Ethanol, water, or mixtures of ethanol and water are used for the production of fluid extracts. Solid or powered extracts are prepared by evaporation of the solvents used in the process of extraction of the raw material. Some phytotherapeutic agents are greatly concentrated in order to improve their therapeutic efficacy.
Standardization of herbal formulation requires implementation of Good Manufacturing Practices (GMP). In addition, study of various parameters such as pharmacodynamics, pharmacokinetics, dosage, stability, self-life, toxicity evaluation, chemical profiling of the herbal formulations is considered essential. Heavy metal contaminations, Good Agricultural Practices (GAP) in herbal drug standardization are equally important (10).

Quality control and the standardization of herbal medicines also involve several other steps like source and quality of raw materials, good agricultural practices and good manufacturing practices. These practices play a pivotal role in guaranteeing the quality and stability of herbal preparations. The quality of a plant product is determined by the prevailing conditions during growth, and accepted Good Agricultural Practices (GAP) can control this. These include seed selection, growth conditions, fertilizers application, harvesting, drying and storage. In fact, GAP procedures are integral part of quality control.

Factors such as the use of fresh plants, age and part of plant collected, period, time, method of collection, temperature of processing, exposure to light, availability of water, nutrients, drying, packing, transportation of raw material and storage, can greatly affect the quality, and hence the therapeutic value of herbal medicines. Apart from these criteria, factors such as the method of extraction, contamination with microorganisms, heavy metals, and pesticides can alter the quality, safety, and efficacy of herbal drugs. Using cultivated plants under controlled conditions instead of those collected from the wild can minimize most of these factors. Sometimes, the active principles are destroyed by enzymic processes that continue for long periods from collection to marketing, resulting in
a variation of composition. Thus, proper standardization and quality control of both the raw material and the herbal preparations should be conducted (11).

Inflammation is one common and major cause of sufferings now and every time past. Those drugs that are available are known as NSAID, that is, non-steroidal anti-inflammatory drugs, act by inhibiting the function of prostaglandin. Prostaglandin is an autocoid that is released extracellularly and initiate pain. Anti-inflammatory agents block this autocoid synthesis by either inhibiting COX enzyme or protecting lysosomal membrane from breakdown (12).

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs. Because existing synthetic molecule like non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors that increase the incidence of adverse cardiovascular thrombotic effects. So, in order to overcome, there is need to focus on the scientific exploration of herbal drugs having fewer side effects (13).

Rheumatoid Arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder that causes pain, swelling, stiffness and loss of function in joints. It occurs more frequently in women than in men and its prevalence depends upon age. In humans, RA is the most common inflammatory joint disease where skeletal complications start with focal erosion of cartilage initially followed by marginal and sub-chondral bone loss. Extended joint destruction with alkalosis and generalized bone loss are characteristic for late complications.
The steroidal and non-steroidal anti-inflammatory drugs are used in the treatment of the disease, but they offer only temporary relief and produce severe side effects including gastrointestinal bleeding and cardiovascular toxicity. Consequently, there is a need to develop new long acting anti-inflammatory agents with minimum side effects.

Herbal medicine is a form of alternative treatment for several ailments and plant derived drugs are gaining popularity both in developing and developed countries due to their natural origin and less side effects in the last few years. WHO has listed 21,000 plants which are used for medicinal purposes around the world and India is known as the “Emporium of Medicinal plants” due to availability of several thousands of medicinal plants in the different bioclimatic zones.

Plant derived secondary metabolites in the plant extracts are the important source of drugs with desired pharmacological activity. Although herbal drugs are effective in treatment of various ailments, very often these drugs are unscientifically exploited and/or improperly used. Therefore, a detailed pharmacological evaluation and documentation of plants used in local health tradition is needed (14).

The therapeutic potentials of plant and animal origin are being used from the ancient times by the simple process without the isolation of the pure compounds. The pharmacological action of crude drug is determined by the nature of its constituents. Thus the plant species may be consider as a biosynthetic and for the chemical compounds example proteins, carbohydrates, and fats that are
utilized as food by the animals and humans, but also for a huge number of compounds including alkaloids, terpenoids, flavonoids, glycosides etc. which exert definite physiological effects. These chemical compounds are mostly responsible for the desired beneficial properties (15).

Authentication is especially useful in cases of drugs that are frequently substituted or adulterated with other varieties which are morphologically and chemically indistinguishable. Several herbal drugs in the market still cannot be identified or authenticated based on their morphological or histological characteristics. Use of wrong drugs may be ineffective or it may worsen the condition. A study cannot be considered scientifically valid if the material tested is not authenticated and characterized such that the material can be reproduced.

Authenticated raw material is the basic starting point in developing a botanical product. In addition, each step of harvest, storage, processing and formulation may dramatically alter the quality and consistency of final product. Therefore methods to ensure quality control in manufacturing and storage are requisite tools to ensure optimal efficacy and safety of these products. Furthermore, such controls are critical for the evaluation of pharmacological, toxicological or clinical studies involving botanical products (16).

Aflatoxins are a group of highly toxic secondary metabolites known to be produced by Aspergillus flavus and Aspergillus parasiticus. These are ubiquitous in nature, associated with the spoilage and toxin production of stored, agricultural commodities. Considerable importance is associated with the presence of aflatoxins in food and feed because of their carcinogenic, mutagenic and teratogenic effects.
Approximately 300 to 400 substances are recognized as mycotoxins, comprising a broad variety of chemical structures. It has also been known that mycotoxins are toxic secondary metabolites produced by various mold species growing on many agricultural commodities and processed food, either in the field or during storage.

Mycotoxins have been ranked as the most important chronic dietary risk factor, higher than synthetic contaminants, plant toxins, food additives or pesticide residue. Lately, additive and synergistic have been observed concerning the health hazard posed by mycotoxins \(^{(17)}\).

It is estimated that about 70-80 % of the world's population relies on non-conventional medicine, mainly of herbal origin. However, owing to the nature and sources of herbal medicines, they are sometimes contaminated with toxic heavy metals such as lead, arsenic, mercury and cadmium, which impose serious health risks to consumers. It is critical to analyze source materials for heavy metals in order to ensure that their concentrations meet the related standards or regulations limiting their concentrations in herbal medicines \(^{(18)}\).

The residue analysis of pesticides has developed in recent years into a comprehensive methodology for the detection of many hundreds of potential contaminating compounds. A multi-residue method for herbal products is faced with additional challenges from the worldwide origin of the products and the complex matrix of the dried materials. In the due quality control of raw materials, the unknown or undeclared local plant protection treatments must be taken into account with a wide variety of potential pesticide contaminations.
Dried leaves, fruits or seeds and other herbal products of medical use deliver highly complex extracts from the sample preparation due to the rich content of active ingredients, essential oils and the typical high boiling natural polymer compounds from broken cells, leaves or fruit skins.

A thorough cleanup of the extracted sample can lead to losses of critical analytes of interest. A complete characterization of pesticide, and other residue, contamination is done by both LC and GC-MS/MS to cover the complete range of functional groups\(^{(19)}\).

Herbal drug technology involves conversion of botanical materials into medicines where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is employed.

Conventional pharmaceutical products, herbal medicinal products may vary in composition and properties, and increasing reports of adverse reactions has drawn the attention of many regulatory agencies for the standardization of herbal formulations. In this context, correct identification and quality assurance is an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy.

“Standardization” expression encompasses the entire field of study from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations\(^{(20)}\).
Standardization of Herbal drugs

- Color, Odour, Taste, Texture and Fracture
- Microscopical studies (Fresh parts of the Plant) and Powder studies
- Loss on Drying and Foreign organic matter
- Macroscopy
- Microscopy
- Physical
- Botanical
- Chemical
- Microbiological
- Toxicological
- Biological
- Qualitative and Quantitative Analysis
- Microbiology and Aflotoxin
- Pesticide residues and Heavy metals
- Pharmacological evaluation

**Figure – 1:** A schematic representation of herbal drug standardization