Traditional medicine is also known as indigenous or folk medicine, that comprises of knowledge systems that was developed before the era of modern medicine. As per World Health Organization (WHO) traditional medicine is defined as: *"Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness."* Medicinal herbs constitute an important source for medicaments in traditional systems of medicine (Ayurveda, Siddha, Homoepathy, Chinese medicine, Herbalism) and also modern system of medicines for health care [1].

Over the past two decades, in developed and developing countries, the interest in traditional and herbal medicines has increased significantly. For primary ailments, WHO estimates that 80% of the world's population (approx. 4 billion people) use herbal medicine. As per the estimates of WHO, 74% of the plant derived pharmaceutical medicines are used in similar way as they were used in traditional system of medicine [2].

India has cultural usage and traditional wisdom of using herbs, processed herbs and formulation prepared from herbs/processed herbs. These Herbs have place in trade and commerce and are now a day used for a variety of diseases.

The herbal formulation comprises of dosage form of one/more herbs or processed herbs in specified quantities. These formulations are used for specific nutritional and cosmetic benefits, and/or other benefits that can be used to diagnose, treat, and alleviate diseases of human beings or animals and/or to alter the structure or physiology of human beings or animals. For internal and external usage, the herbal formulations may also be prepared from the dosage form of traditional medicines [3].

Unfortunately, the reports of patients experiencing negative health consequences caused by the use of herbal medicines have also been encountered. Analysis and studies have showed that a number of reasons are responsible for such problems. The poor quality of herbal medicines including raw materials of medicinal plants have been reported to be major cause of adverse events. This reveals that insufficient attention has been paid to the quality assurance and control of herbal medicines [2, 4].

The latest World Health Assembly resolution medicine (WHA56.31) requested WHO "*To provide technical support for methodology development to monitor or ensure product quality, efficiency, safety and preparation of guidelines and promotion of exchange of information."* With respect to the quality control of herbal medicines series of technical guidelines has been developed by WHO. Some guidelines are on good agricultural and collection practices (GACP) for medicinal plants. A detailed description of the techniques and measures are provided by WHO guidelines. During processing of medicinal plants, it required for the appropriate cultivation and collection of medicinal plants and also the recording and documentation of necessary data and information [5].

The discrepancy exists between knowledge and implementation despite of availability of such guidelines. The efficacy and safety of herbal medicinal products are directly affected by quality control strategy [6]. The poor quality of raw medicinal plant materials results in to poor quality of finished products. The medicinal plants which are collected from the wild population are mostly contaminated by other verities of plants or other plant parts through misidentification, intentional adulteration or accidental contamination, all of which may have unsafe consequences [6].

1.1) WHO Guidelines for Quality Assessment of Herbal Medicines

WHO has recently defined that traditional medicine are the medicine that have been in existence, over hundreds of years, before the development and spread of modern medicine and are still in use at present. The traditional preparations include medicinal plants, minerals, organic matter, etc. According to WHO, "Herbal Medicines" are the, "Finished, labelled medicinal products that containing active ingredients aerial or underground parts of plants or other plant material or combinations thereof either in crude state or as plant preparations. Plant material includes essential oils, gums, fatty oils, juices and any other substance of this nature [7].

Every herbal formulation must be standardized according to World Health Organization (WHO) guidelines. WHO is involved in collaborating and assisting health ministries in establishing mechanisms for the introduction of traditional plant medicines into primary healthcare programmes, in assessing safety and efficacy and ensuring proper supply and the quality control of raw and processed plant materials. According to WHO guidelines less stringent selection procedures could be applied for

the screening, chemical analysis, clinical trials and regulatory measures but the procedure for pure phytochemicals for quality control should be identical to that of synthetic drugs according to WHO guidelines**.** Some of the important parameters are stability testing, safety assessment, specific therapeutic activity, analysis and estimation of the active constituents in plant raw material and finished products. The aim of WHO guidelines is to define basic criteria for the evaluation of quality, safety and efficacy of herbal medicines and to assist national regulatory authorities, scientific organizations and manufacturers to undertake an assessment of the documentation in respect of herbal products [7].

A method of identification and quantification of the plant material in the finished product should be defined. If the identification of an active principle is not possible, it is sufficient to identify a characteristic substance or mixture of substances (e.g., "chromatographic fingerprint") to ensure consistent quality of the product [8]. Polyherbal formulations can be standardized with modern techniques such as high pressure thin layer chromatography (HPTLC), liquid chromatography-mass spectroscopy (LC-MS). The animal testing for establishing safety and toxicity for values are also critical the botanicals used in herbal medicines prepared using drugs with a narrow therapeutic index. All the critical pharmacopoeial tests like dissolution time, ash values, particulate matter, microbial, pesticide and heavy metals contamination etc. must be also in accordance with global standards and all the Ayurvedic medicines manufactured must be in accordance with current good manufacturing procedures for herbs [9]**.**

1.2) Analysis of Raw Materials from Plants

Raw material can be defined as starting material or any intermediate which are used for further processing. The specifications for impurities and other related substances must be established with suitable test methods, purity, identity and quality of finished pharmacopoeial dosage forms. Pharmacopoeias and formularies of various countries provide standardized test methods for the most common and widely used materials in their monographs [10].

1.3) Quality Control of Herbal Drugs

Quality control for efficacy and safety of herbal products is of paramount importance. Quality can be defined as *"the status of a drug that is determined by identity, purity,*

content and other chemical, physical or biological properties or by the manufacturing processes." Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. For the quality control of a traditional medicine, the traditional methods are procured and studied and documented and the other information about the identity and quality assessment is interpreted in terms of modern assessment [11, 12].

In general, all medicines, whether they are of synthetic or of plant origin, should fulfil the basic requirements of being efficacious and safe and this can be achieved by suitable clinical trials. In medicine, Natural products constitute a vast range of "*raw plant materials*," making clear definitions. Quality criteria are based on clear scientific definitions of the raw material. The term *"herbal drugs"* denotes the conversion of plants or plant parts into phyto-pharmaceuticals by means of simple techniques involving drying, harvesting and storage conditions. So they are causes of variation in plant species. The differences in growth, geographical location and time of harvesting results in variability. Derived or isolated compounds in the processed state like extracts or even isolated purified compounds or mixtures of compounds are not included in the definition. Isolated constituents or active substances or their combinations and plants containing homeopathic preparations are not considered as herbal medicines. Their production is based on satisfactory quality control of the respective starting materials [13, 14].

According to Pharmacopeia, the quality control of herbal drugs is based on:

• Identity: Is the herb the one it should be?

• Purity: Are there contaminants, e.g., in the form of other herbs which should not be there?

• Content or assay: Is the content of active constituents within the defined limits?

The assessment of content is most difficult because the active constituents of herbal drugs are unknown. Markers are oftenly used which are defined as chemical constituents of interest, independent of any therapeutic activities that are used for control purposes. To prove identity and purity several criteria have to be checked such as sensory properties, physical constants, moisture, ash content, solvent residues, adulteration, contaminants and type of preparation. To establish the quality control of

herbal drugs, the correct identity of the crude herbal material or the botanical quality is of prime importance [15].

Identity can be checked by morphological and microscopical examinations. Reliable reference sources are the voucher specimens. Diseases among plants cause changes in physical appearance of the plant that lead to incorrect identification. An incorrect botanical quality of the plant species with respect to the labelling can be a problem. Purity is closely related to the safe use of drugs and deals with factors like contaminants, heavy metals and ash values. The application of advanced analytical methods involves the evaluation of purity due to aflatoxins, microbial contamination, pesticide residues and radioactivity [16].

Some problems that influence the quality of herbal drugs and are not applicable to synthetic drugs include:

• Herbal drugs are mostly mixtures of many constituents.
• The active principles, in most cases are unknown.
• Selective analytical methods or reference compounds may not be commercially available.
• Chemically and naturally variable plant materials.
• Existence of chemo-varieties and chemo cultivars.
• Variability in the source and quality of the raw materials.
• The methods of drying, harvesting, storage condition, processing and transportation also affect the quality.

For the successful production of quality herbal drugs, strict guidelines have to be followed. Some are proper botanical authentication, phytochemical screening and standardization. Several steps are involved in quality control and the standardization of herbal medicines. For the quality control of herbal medicines, the essential steps involve the source and quality of raw materials, good agricultural practices and manufacturing processes. These play a pivotal role in maintaining the quality and stability of herbal preparations [17].

The prevailing conditions that determine the quality of plant products and are controlled by Good Agricultural Practices (GAP) include seed selection, growth conditions, and use of fertilizers, harvesting, drying and storage. The GAP procedures are an integral part of quality control of herbal drugs. Factors that affect the quality

and therapeutic value of herbal medicines are the use of fresh plants, age and part of plant collected, time period and method of collection, exposure to light, temperature of processing, availability of nutrients, water, drying, packing, transportation and storage of raw materials. The method of extraction, contamination with microorganisms, heavy metals and pesticides also alter the quality, safety and efficacy of herbal drugs. These factors can be minimized by the use of cultivated plants under controlled conditions instead of those collected from the wild. The variation of compositions results from the destruction of active principles by enzymatic processes due to long periods from collection to marketing. Thus proper standardization and quality control of the raw material and the herbal preparations should be conducted [18].

1.4) Finger-printing of Herbal Drugs

Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration and variation in composition. So, quality control of herbal medicines is a challenging task. A marker is a chemical entity of in the plant material which serves as a characteristic fingerprint for that plant. We can visualize the presence of marker compound in the plant through various analytical techniques like HPLC, TLC and HPTLC and also quantify it to ascertain the limits. A biomarker is a chemical component which in addition of being unique for that plant material also correlates with the biological efficacy. Batch to batch variations start from the collection of raw material itself in the absence of any reference standards for proper identification, and multiply during storage and further processing. So the need arises to lay standards by which the right material is selected and incorporated into the formulation. DNA fingerprinting these days is considered the final tool to identify and differentiate among different genes of a species [19].

1.5) Parameters for Quality Control of Herbal Drugs and Herbal products

The quality control of starting material is very important step to get a good quality of final product. The WHO guidelines are suggested some points which are needed to be considered in the quality control of starting material.

1.5.1) Microscopic Evaluation

Quality control of herbal drugs is based on appearance and microscopic evaluation viz is indispensable in the initial identification and detection of crude or powdered herbs

and in detection of foreign matter and adulterants. A simple magnifying lens can be used to ensure that the plant is of the required species and that the right part of the plant is being used. Microscopic analysis is required to determine the correct species and/or the correct part of the species is present. For example, pollen morphology may be employed to identify the species of flowers and the presence of certain microscopic structures such as leaf stomata can be used to identify the plant part used. Such identification is of prime importance, especially when different parts of the same plant are used for different treatments [20].

1.5.2) Determination of Foreign Matter

Herbal drugs should be devoid of other parts of the same plant or other plants. They should be free from moulds or insects including excreta and visible contaminant such as sand and stones, poisonous and harmful foreign matter and chemical residues. Animal matters such as insects and "invisible" microbial contaminants which can produce toxins, are also among the potential contaminants of herbal medicines. Morphological examination can be used to determine the presence of foreign matter but microscopy is indispensable in certain special cases (for e.g., starch deliberately added to dilute the plant material). Also Thin Layer Chromatography is often needed to detect the contaminants such as chemical residues [21].

1.5.3) Determination of Ash

The WHO prescribed the limits of the inorganic contents depending upon the properties of the plant material. If the inorganic content is found to exceed the limits, it may be concluded that the plant material contains sand and siliceous material in excess which may be due to improper method of cultivation and harvesting. For determination of ash contents, the plant material is initially burnt and the residual ash is measured as total ash. It is the measure of the total amount of material left after burning and includes ash derived from the part of the plant itself. The acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid (HCl) and burning the remaining insoluble matter. The later procedure measures the amount of silica present especially in the form of siliceous earth and sand [20].

1.5.4) Determination of Heavy Metals

Herbal medicines can be contaminated by toxic metals either accidentally or intentionally. Sometimes, heavy metals like mercury, lead, arsenic and cadmium are

present in herbal remedies. They produce environmental pollution and can pose clinically relevant dangers for the health of the user and should therefore be limited. The potential intake of the toxic metal can be estimated on the basis of the level of its presence in the product and the recommended or estimated dosage of the product. When the heavy metals are present in trace quantities, in admixture or in quantitative analysis, the instrumental analyses have to be used. There are three different methods generally used are Inductive Coupled Plasma (ICP), Atomic Absorption Spectrophotometry (AAS) and Neutron Activation Analysis (NAA) [22, 23].

1.5.5) Determination of Microbial Contaminants and Aflatoxins

Herbal plants may be involved with a wide variety of microbial contaminants mainly bacteria, fungi and viruses. Inevitably, this microbiological background depends on various environmental factors and exerts an important impact on the overall quality of herbal products and preparations. Medicinal drugs normally carry a number of bacteria and molds which are often originating in the soil. Poor methods of cleaning, harvesting, drying, handling and storage conditions may also cause additional contamination, as may be the case with or *Salmonella* species or *Escherichia coli* [24].

The well-known pharmacopoeias and also in the WHO guidelines are mentioned the procedures for determining the microbial contaminations along with its limit values. Generally, a complete procedure consists of determining the total fungal count, the total aerobic microbial count, and the total Enterobacteriaceae count, together with tests for the presence of *Staphylococcus aureus*, *Escherichia coli*, *Shigella* and *Salmonella* species and *Pseudomonas aeruginosa* [25].

Some common species produce toxins, especially aflotoxins. Therefore the presence of fungi should be carefully investigated and/or monitored. When minute amounts of aflatoxins which is present in herbal medicines, is absorbed by human beings is dangerous to health. Aflatoxin producing fungi sometimes build up during storage. The WHO published the procedures for the determination of aflatoxin contamination in medicinal herbal drugs. Thin Layer Chromatography is also used for confirmation of aflotoxins [26].

1.5.6) Determination of Pesticide Residues

It is very important that herbs and herbal containing products are free of pesticides and fumigants or at least they are controlled for the absence of unsafe levels. Even though there are no serious reports of toxicity due to the presence of these chemicals

[27]. Herbal drugs are liable to contain pesticide residues which accumulate from agricultural processes such as treatment of soils during cultivation, spraying and administering of fumigants during storage. However, it may be desirable to test herbal drugs for broad groups in general, rather than for individual pesticides. Many pesticides contain chlorine in the molecule which can be measured by analysis of total organic chlorine. In a parallel way, insecticides containing phosphate can be detected by measuring total organic phosphorus. The various samples of herbal plant material are extracted by a standard procedures mentioned in Pharmacopoeias. The impurities present in herbal products are removed by partition and/or adsorption and individual pesticides are measured by Gas Chromatography (GC), Mass Spectroscopy (MS) or GC/MS [28].

1.5.7) Determination of Radioactive Contamination

The verities of sources for radioactive contaminations are ionization radiation including radio nuclides which are occurring in the environment. Therefore a certain degree of exposure is to be expected. Dangerous contamination however may be responsible for the consequence of a nuclear accident. The World Health Organization has developed guidelines in close co-operation with several other international organizations, in the event of a widespread contamination by radionuclides resulting from major nuclear accidents. Now a day, no limits are anticipated for radioactive contamination [24].

1.5.8) Analytical Methods

Published monographs in a pharmacopoeia are the most practical approach for quality control of herbal drugs. When pharmacopoeial monographs are unavailable, validated of analytical procedures should be developed. The best strategy is to follow properly the pharmacopeial definitions of identity, purity and content or assay. In the pharmacopoeias and in guidelines published by the WHO, valuable sources for general analytical procedures are included. Additional information, mainly in chromatographic or spectroscopic methods can be found in the general scientific literature. Determination of pharmacological activity, potency & toxicity is done by evaluating plant or plant extract by using various biological methods.

A simple chromatographic technique such as TLC may provide valuable additional information to ascertain the identity of the plant material. This is particularly important for those species that contain different active constituents. Qualitative and

quantitative information can be gathered regarding the presence or absence of metabolites or breakdown products. TLC fingerprinting is important for herbal drugs made up of essential oils, resins and gums which are complex mixtures of constituents that no longer have any organic structure. It is a powerful and comparatively rapid solution to distinguish between chemical classes where macroscopy and microscopy will be failed. Chromatograms of essential oils are widely published in the scientific literature and very useful in identification. The instruments for UV-visible determinations are easy to operate, and validation procedures are simple but at the same time precise. Although measurements are completed quickly, work well only for less complex samples, and those compounds with absorbance in the UV-visible region. HPLC is one of the preferred methods for quantitative analysis of more complex mixtures. However the separation of volatile components such as essential and fatty oils can be achieve by HPLC, it is best performed by GC or GC/MS. Recent developments in analytical instrumentation have made the quantitative determination of constituents very easy. Recent advances in the isolation, purification, and structure clarification of naturally occurring metabolites have made it possible to establish suitable strategies for the determination and analysis of quality and the process of standardization of herbal preparations.

Categorization of plants and organisms by their chemical constituents is referred as chemotaxonomy. To determine the homogeneity of a plant extract TLC, HPLC, GC, quantitative TLC (QTLC) and high performance TLC (HPTLC), Over-pressured layer chromatography (OPLC), infrared and UV-VIS spectrometry, MS, GC, liquid chromatography (LC) used alone or in combinations such as GC/MS, LC/MS, and MS/MS, and nuclear magnetic resonance (NMR), electrophoretic techniques, mainly by hyphenated chromatographies are powerful tools often used for standardization and to control the quality of both the raw material and the finished product. The results from these sophisticated techniques provide a chemical fingerprint as to the nature of chemicals or impurities present in the plant or extract [29].

Based on the concept of phyto-equivalence, to address the issue of quality control the chromatographic fingerprints of herbal medicines can be used. Methods based on information theory, similarity estimation, chemical pattern recognition, spectral correlative chromatograms (SCC), multivariate resolution, the combination of chromatographic fingerprints and chemometric evaluation for evaluating fingerprints are all powerful tools used for quality control of herbal Products [30].

1.5.9) Validation

Fake business selling adulterated herbal medicines are common both in developed and resource-poor countries. Hence, the validation of herbal products is of utmost importance. The existing guidelines in some individual countries and those outlined by the WHO need to be followed. It is necessary to ensure scientific validation and periodic monitoring of the quality and efficacy by drug control administrators if these herbal products are to be marketed as therapeutic agents. Introduction of scientific validation helps to control the production of impure or adulterated herbal products and ensure their rational use. Only qualified physicians and health providers should be allowed to prescribe the medication. Several of the principal pharmacopoeias contain monographs outlining standards for herbal drugs. The major advantage of an official monograph published in a pharmacopoeia is that well defined data complying standards and validated analytical procedures are available. Validation is defined as the process of proving that an analytical method is acceptable for its intended purpose for pharmaceutical methods. Guidelines from the United States Pharmacopeia (USPC, 1994–2001), the International Conference on Harmonization (ICH) and the US Food and Drug Administration (FDA) provide a framework for performing such validations. Studies on specificity, linearity, accuracy, precision, range, detection and quantitative limits must be included in validation, depending on whether the analytical method used is qualitative or quantitative. Availability of standards is also important. Reliable reference samples of the plant must be available for macroscopic and microscopic procedures. A defined botanical source (e.g. voucher specimens) will normally solve this problem. Standards for chromatographic procedures are difficult to obtain. Characteristic plant constituents, active or markers, are difficult to make available commercially. Characterization can be carried out by LC/MS. Going one step further, after isolation of such a compound, elucidations to prove its definite structure will not be easy. The method often employed is to use readily available compounds that behave similarly in the chosen chromatographic systems, and retention values and/or times towards these compounds as a standard are calculated. TLC and HPLC are the main analytical techniques commonly used to detect and isolate the active ingredient (s). The quality of plant extracts can be assessed by a "fingerprint" chromatogram in cases where active ingredients are not known or too complex [31, 32].

1.6) Toxicity of Herbal Drugs

Absolute safety standards for herbal preparations are difficult to establish based solely on epidemiological studies. Firstly, these types of studies would be expensive. Second is the lower availability of published data in countries where the major use of medicinal plants occurs and hence standards would be based on limited number of reports. Third, the exact identification of the products implicated in side effects claimed for medicinal plants is usually lacking. In spite of all such inadequacies, there are some general statements that can be deduced with regard to avoiding potential serious side effects from herbal medicines [33].

The definition of "toxic" is ultimately a matter of viewpoint. Traditionally, herbs and herbal products have been considered to be nontoxic and have been used by the general public and traditional medicinal doctors worldwide to treat a range of ailments. If something is natural it does not necessarily imply that it is safe or effective. The active ingredients of plant extracts are chemicals that are similar to those in purified medications, and they have the same potential to cause serious adverse effects. On many occasions the potential toxicity of herbs and herbal products has not been recognized in spite of the literature that documents severe toxicity of herbal drugs. Toxic adverse effects, serious allergic reactions, adverse drug interactions can be caused by herbs and herbal products, and can also interfere with laboratory test [34].

Herbal medicines are shown to have two side effects. One, considered to be intrinsic to herbal drugs themselves, is mainly related to predictable toxicity due to toxic constituents of the herbal ingredients and over dosage, and the second is allergy. Many cases of allergic reactions have been reported for herbal drugs. It is impossible to completely eliminate the possibility of any substance, including prescription drugs, herbal remedies, or cosmetics, producing an allergic response in people exposed to them. Perhaps the major problem with regard to the safety of herbal medicines is related to the manufacturing practice, including contamination, substitution, incorrect preparation and dosage, intentional addition of unnatural toxic substances, interactions involving synthetic prescriptions, drugs and herbal medicines, either intentional or unintentional mislabelling, and the presence of natural toxic contaminants [35].

In this context herbs can be broadly classified into three major categories:

• The *food herbs* – medicines such as ginger, garlic, peppermint, hawthorn, lemon, nettles, and balm are mild in action, are less toxic, and are unlikely to cause any adverse effects. They can be consumed in acceptable quantities over long periods of time without any acute or chronic toxicity. However they may bring about allergic reactions in certain individuals.

• The *medicinal herbs* – these cannot be used daily and need to be used with greater knowledge (dosage and rationale for use) for specific conditions (with a medical diagnosis) and for a limited period only. There are higher chances of drug interactions and adverse drug reactions.

They include comfrey, aloe vera, senna, echinacea, black cohosh, ginkgo biloba, ephedra, kava kava, milk thistle and ginseng.

• The *poisonous herbs* -- possess a strong potential for either acute or chronic toxicity and should only be prescribed only by trained clinicians who understand their toxicology and appropriate use. But these drugs are not freely available to public or sold in market like *Digitalis, Datura species, Arnica species, Atropa belladonna, Aconite, Male fern, Veratrum and Gelsemium are* typical examples [36-38].

1.7) Screening of Herbal Drugs

After establishing the botanical identity of herb, the next step is phytochemical screening which includes bioassays, extraction, purification, and characterization of the active constituents of pharmaceutical importance. Active substance is the herb or herbal drug preparation in its entirety. These constituents are either of known therapeutic activity or are chemically defined substances or a group of substances generally accepted to contribute substantially to the pharmacological activity of a herbal drug. Some type of pharmacological screening, or evaluation, must obviously be done in any program in which the end product is to be a drug. Pharmacological screening programs are not devoid of problems. Bioassay guided isolation ideally the active principles should be isolated, preferably using bioassay guided isolation processes, which can be problematic. The ideal pharmacological screen would be to identify those extracts or pure compounds that are highly active and nontoxic. There are many pharmacological screening tests available. An extension of this procedure is to isolate metabolites or “active compounds” from the plant that had shown most promising activity and subject them to pharmacological tests. In another approach, plants containing specific types or classes of chemical compounds, for example

alkaloids, are tested. Simple tests such as colour reactions are carried out on various parts of the plant in the field, and assays are carried out in the laboratories.

In vitro screening methods though restricted to the detection of defined activities, are simpler and more useful [39]. Recently, biochemical and receptor- ligand binding assays have gathered momentum. This has been made possible by the increasing availability of human receptors from molecular cloning, and extracts and compounds can be tested for binding directly to the presumed therapeutic target protein [40].

1.8) Standardization of Herbal Formulations

Standardization means to adjust the herbal drug preparation to a defined content of a constituent or a group of constituents with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations. Standardized extracts contain consistent levels of specified compounds, and they are subjected to rigorous quality controls during various phases of the growing, harvesting, and manufacturing processes. A specific regulatory definition does not exist for standardization of dietary supplements. As a result, the term "standardization" may mean different things. The term standardization is incorrectly used by some manufacturers to refer to uniform manufacturing practices; following a recipe is not sufficient for a product to be called standardized. So, the presence of the word "standardized" on a supplement label does not necessarily indicate product quality. The marker substance(s) should be established for analytical purposes and standardization, when the active principles are unknown.

Marker substances are chemically defined constituents of a herbal drug that are important for the quality of the finished product.

Ideally, the chemical markers chosen can also be responsible for biological effects in the body. There are two types of standardization: In the first class, "true" standardization, a definite phytochemical or group of constituents is known to have activity. These are highly concentrated products and no longer represent the whole herb, and are now considered as phytopharmaceuticals. In many cases they are much more effective than the whole herb.

The other type of standardization is based on manufacturers guaranteeing the presence of a certain percentage of marker compounds; these are not indicators of therapeutic activity or quality of the herb.

In order to ensure that the concentration and ratio of components in an herbal mixture are present in reproducible levels in raw materials, manufacturing intermediates and in the final dosage forms, one can use single or multiple markers. In this way, multiple markers or chromatographic fingerprints give information assisting manufacturing control and thus assure batch-to-batch consistency [41].

1.8.1) Chemical marker for herbal standardization [19]

It is important to understand that a plant extract consists of established classes of chemical compounds, which include the primary metabolites, secondary metabolites and inorganic salts and metals. Compounds like carbohydrates, proteins, lipids which are essential for the plant physiology are primary metabolites. Secondary metabolites are compounds which are not essential for the plant physiology as such but are formed as byproducts in the biochemical pathways. Selection of chemical markers is critical for the quality control of herbal medicines, including authentication of genuine species, harvesting the best quality raw materials, evaluation of post-harvesting handling, assessment of intermediates and finished products, and detection of harmful or toxic ingredients. Ideal chemical markers should be the therapeutic components of herbal medicines. However, the therapeutic components have not been fully elucidated or easily monitored for most herbal medicines. Bioactive, characteristic, synergistic, correlative, toxic and general components may be selected. Various classes of compounds like alkaloids, flavonoids, coumarins, terpenoids, anthocyanins, etc and these secondary metabolites can be utilized for the identification of plant material. Through sophisticated analytical techniques these compounds can be measured qualitatively and quantitatively.

Liquid chromatography like HPLC which is useful when a marker compound is known and can be used as reference compound and finally planar chromatography like TLC and more advanced version like HPTLC which accounts for all the variations found in TLC. The extracts obtained similarly from each batch are loaded onto a TLC plate and are developed in suitable solvent system. Different solvent systems can be used to develop a better TLC pattern. This developed plated is then studied under different conditions in the scanner which utilizes a laser beam and detects and quantifies distinct spots due to different compounds on the plate. The different conditions which can be used for detecting spots on plate include UV at different wavelength, derivatization of plate to obtain distinct coloured spots which

are then detected by the software program and a characteristic graph obtained. There is a very unique graphic pattern for each plant material and the extracts from different batches usually show overlapping graphic patterns. Patterns for each fractionated plant part gives us a distinct HPTLC profile.

1.9) Need for standardization

About 1000 single drugs and 1500 formulations are present in the Indian system of medicine. These drugs are fully documented in traditional text for their therapeutic properties. However extensive and unpredictable variation in the quality and efficacy results due to the difficulties in identification of appropriate plant species, their geographical source, time of collection, drying and storage conditions, microbial contamination and presence of xenobiotics. There is a need to develop method for standardization of raw materials. Moreover, for pharmaceutical purposes, the quality of the medicinal plant material must be as high as that of other medicinal preparations.

Most of the herbal formulations, especially the classical formulation of the traditional medicine, are Polyherbal. Each formulation comprises of 10 to 20 ingredients, a few have even 50 to 75. Many preparations are either liquids or semi solids. It is very difficult to establish parameters for quality control for such formulations, moreover, the unique processing method followed for the manufacture of drugs turn single drug in to very complex mixture, from which separation and identification, along with the analysis of components is a tedious task [42].

1.10) Problems in Standardization

Complex chemical composition of herbal drugs can cause problems in standardization. Standardisation of certain marker compound of herbal drug in general does not serve the purpose of standardisation since activity of drug may depend upon one or more components. In most of the cases, it is the result of concerted activity of several active compounds as well as inert accompanying substances. Though these inert components do not directly affect the activity of drug, they might have an influence on the bioavailability and excretion of active component/s. Moreover, these inert components may play a role in the stability of the active component and minimise the rate of side effects. If there are several active components in the herbal drug, they may have synergistic or potentiating effect. The quantity of active constituents may be influenced by several factors such as age and origin, harvesting

period and so on. To eliminate at least some of the causes of inconsistency, in terms of active ingredients it was suggested that cultivated plants should be used rather than wild plants which are often heterogeneous with respect to the above factors and consistently in their content of active constituent [43].

1.11) Traditional Ayurvedic formulations and Modern dosage forms

Ayurveda is one of the traditional medicinal systems with an established history of many centuries. The meaning of *Ayuh* is life and *veda* means knowledge. Ayurveda may be defined as the science of life or the true knowledge of life. *Ayurveda* involves the use of natural elements to eliminate the root cause of the disease by restoring balance, at the same time to prevent recurrence of imbalance so as to create a healthy life-style. In *Ayurveda,* single or multiple herbs or polyherbals are used for the treatment of various diseases. The *Ayurvedic* literature '*Sarangdhar Samhita'* emphasized on the concept of polyherbalism to achieve superior therapeutic efficacy. To achieve the desirable therapeutic effects, the active phytochemical constituents of individual plants are insufficient. Therefore, combining the multiple herbs/plants in a particular ratio will give a greater therapeutic effect and simultaneously reduce the toxicity also.

Herbal formulations are the Pharmaceutical dosage forms prepared from parts like leaves roots, rhizomes, wood, bark, fruits, seeds, tubers, corms, flowers and flowering buds used to combat diseases.

Table 1.1: Some common Dosage forms

Ayurvedic dosage forms	Modern dosage forms
Churna	Powder
Bhasma	Tablet
Vati/gutika	Capsule
Ark	Liquid/Suspension/Emulsion
Asava/Aristha	Ointment/Cream
Avaleha/Pak	Transdermal patch
Kwatha	Lozenge
Ghrita	Suppository
Lepa	Eye, ear, nose drops
Taila	Gel

A variety of novel herbal formulations are also available like polymeric nanoparticles, naocapsules, nanoemulsions, phytosomes, microsphere, transferosomes, liposomes, ethosomes and hydrogels [44].

1.12) Modern approach based on Design of Experiments (DoE)

It is a well known fact that quality cannot be tested or inspected into a finished product, but rather that quality, safety, and effectiveness must be "designed" and built into a product and its manufacturing process. To encourage new initiatives and provide guidance to pharmaceutical process developers, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use introduced the quality by design (QbD) concept. ICH-Q8 defines it as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management"

DoE is a structured, organized method for determining the relationships among various factors that affect a process and its output. DoE can offer returns that are four to eight times greater than the cost of running the experiments in a fraction of the time that it would take to run one factor at a time experiments [45].

1.13) Immunity

Immunity means "Free from burden". It is defined as *"Ability of an organism to recognize and defend itself against specific pathogens or antigens"*

Immunity is said to be the body's ability to identify and resist large numbers of infectious and potentially harmful microorganisms, enabling the body to prevent or resist diseases and inhibit organ and tissue damage. The immune system is not restricted to any one part of the body. Immune stem cells, formed in the bone marrow, may remain in the bone marrow until maturation or migrate to different body sites for maturation. Consequently, most immune cells circulate throughout the body and producing specific biological effects [46, 47].

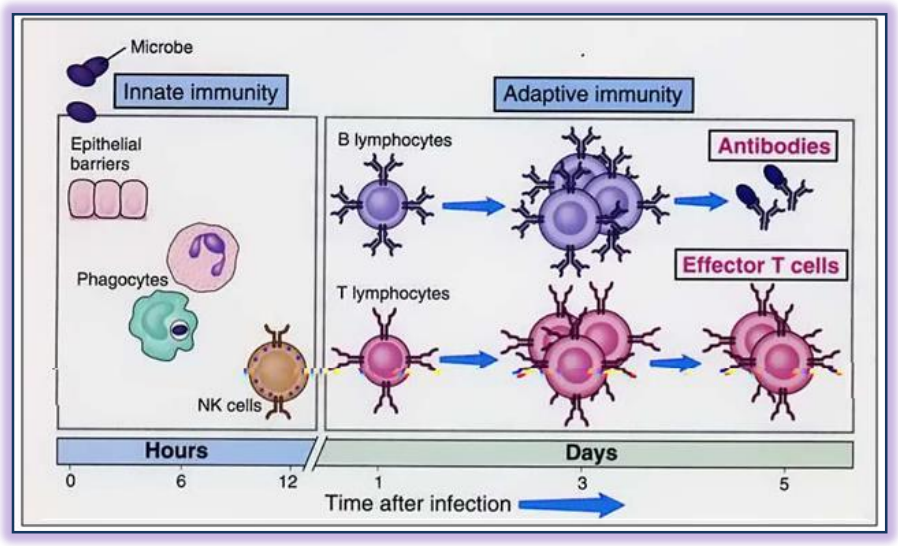

Fig. 1.1: Types of immunity

1.13.1) Immune System

It consists of biological structures and processes within an organism that protects against diseases. The immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue in order to protect it.

There are two distinct but overlapping mechanisms with which immune system fights to invade organisms, the antibody mediated defence system (humoral immunity) and the cell-mediated defence system (cellular immunity) [48, 49].

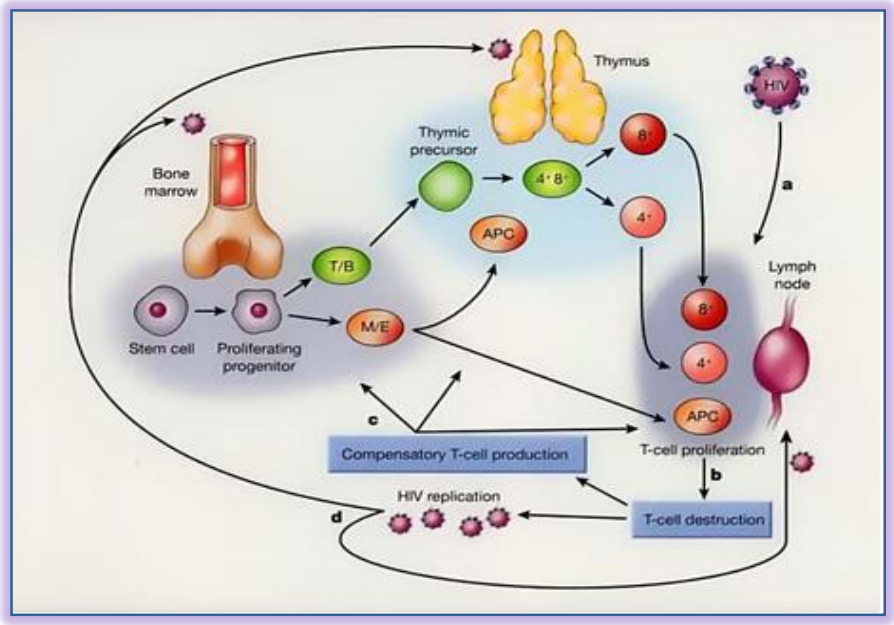

Fig 1.2: An overview of Immune system

1.13.2) Immunomodulation

Immunomodulation means that one can modulate immunity using various synthetic or natural substances. Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases, specifically in situation of autoimmune disorders, when the host defence mechanism has to be activated under conditions of impaired immune response, or when a selective immunosuppressant is desired. The immune system works normally through its own modulation by factors usually synthesised by the immune cells. The immune response requires timely interplay of multiple cell types within specific microenvironments in order to restore immune homeostasis. The differential distribution and regulated expression of cytokines and their receptors provide the selectivity and flexibility that is necessary to regulate cell traffic under homeostatic and diseased conditions. As a result, cytokines are responsible for the development of phenotypes and are, therefore, logical targets for therapeutic immune modulation. Use of immunomodulators for enhancing host defence responses is one of the most promising recent alternatives to classical antibiotic treatment. Several types of immunomodulators have been identified, including substances isolated and purified from natural sources such as plants including microorganisms. An immunomodulator may be defined as a substance, biological or synthetic, which can stimulate, suppress or modulate any of the components of the immune system including both innate and adaptive arms of the immune responses [50].

1.13.3) Plants as Immunomodulators

There are several medicinal plants used in the Indian traditional system that have attracted the attention of scientists' world-wide. These are known as Rasayana (devoted to enhancement of the body's resistance). These medicinal plants possess not only immunomodulatory activity but also a wide range of antioxidant, anti-arrhythmic, cardiotonic, anti-asthmatic, anti-inflammatory hepatoprotective, hypocholesterolemic, antifungal, diuretic and other medicinal activities. An important source of natural potent immunomodulator is Uncaria genus (Rubiaceae), particularly for alkaloids and triterpenes, triterpenoidal saponin called pulcherrima from the leaves of *Calliandra puleherrima.* This saponin exhibited remarkable immunomodulating efficacy similar to the potent adjuvant QS 21 saponin. Increase in the delayed type of hypersensitivity against leishmanial antigen was seen. It could be deduced from the

safety analysis and effect on humoral and cellular immune responses that this saponin can serve as a potential candidate for a vaccine adjuvant. The effect of immunosuppressive action of the ethanol extract of *Pollen typhae* consisting of a mixture of flavonoids, steroids and volatile oils was studied on the immune response in mice. Concanavalin A (Con A) and lipopolysaccharide (LPS) was significantly supressed and *in-vitro* splenocyte proliferation was stimulated in a concentration-dependent manner. The immunomodulation activity of methanolic stem bark extract of *Pouteria cambodiana* on BALB/c mice was studied. The extract showed a good dose dependent response in the peritoneal macrophage phagocytosis and lysosomal enzyme activity was also potentiated. The immunosuppressive activity on mice using ethanolic extract of *Spica prunellae* consisting of a mixture of triterpenoids, flavonoids, tannins and polysaccharide, suggesting that the extract could suppress the cellular and humoral responses in mice. The methanol extract of rhizomes of *Curculigo orchioids* showed the immunostimulatory effect on immunosuppressed mice, which holds potential as a protective agent against cytotoxic drugs in a dose dependent manner. Immunomodulatory activity in mononuclear cells of normal individuals and rheumatoid arthritis patients was seen using the methanolic extract of *S. anacardium*. The extract inhibited the spontaneous and lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines but displayed no effect on TNF-α and IL-6 production, neither at protein nor at mRNA level. The immunomodulatory activity of the ethanol and aqueous extracts of *Caesalpinia bonducella* was studied which suggested that this plant could be a promising immunostimulatory agent. Cellular and humoral immune responses could be stimulated by Extracts derived from *C. bonducella* seeds. The extract not only potentiated non-specific immune response, but also improved humoral as well as cell-mediated immunity effectively. The macrophage immune responses were activated by the botanical polysaccharides, subsequently leading to immunomodulation, wound-healing, anti-tumor activity and other therapeutic effects. A significant immunostimulant effect was evoked by oral administration of ethanolic and water extracts of *Capparis zeylanica* leaves , in a dose dependent manner in mice. The efficacy of ethanolic extract of the roots and the sapogenin of the *Chlorophytum borivilianum* were tested for any immunomodulatory action. The ethanolic extract of *C. borivilianum* was found to be more pronounced as compared to sapogenin. The potent therapeutic potential and immunomodulatory activities of *Stevia rebaudiana* ethanolic leaf extract for the prevention of

immunological disorders was studied. Both cellular and humoral immune responses could be stimulated by the ethanolic leaf extract of *S. rebaudiana* [51].

1.13.4) Rasayana drugs as antioxidants

In order to control the oxidative stress, plants produce several antioxidants. Hence, they can represent a source of new compounds with antioxidant activity. Ayurveda, which is the oldest traditional health care system Indian traditional health care system in the world, exploits the potential of various herbs in Polyherbal formulations as drugs. A number of plants and plant isolates have been reported to protect free radical induced damage in various experimental models. Rasayana is one of the clinical specialities of Ayurveda. Rasayana is not only a drug therapy but is a specialized procedure practiced in the form of rejuvenating recipes, dietary regimen promoting good habit. The strong antioxidant activity of the rasayana has been reported with regard to the rasayana drug therapy. Antioxidant properties have been clained with some drugs like *Embilica officinalis, Tinospora cordifolia, Asparagus racimosus, Tribulus terristris, Withania somnifera, Mangifera indica*. These compounds were found to be 1000 times more potent than ascorbic acid, α-tocopherol and probucol. Rasayana are said to promote physical and mental health, improve defence mechanisms of the body and enhance longevity of life. These attributes are similar to the modern concept of adaptogenic agents. These are well known to impart protection of the human physiological system against diverse stressors. The importance of antioxidants and their great benefits in achieving optimum health has been now accepted by even the most conservative medical fields. The endogenous antioxidant defences from reactive species (ROS) are potentiated by natural antioxidants and they restore the optimal balance by neutralizing such reactive species. Thus, by the virtue of their critical role in disease prevention, herbal drugs containing antioxidants are gaining immense importance [51].

1.14) Commercial herbal formulations for Immunity

- Septilin (The Himalaya Drug Company, Bangalore, India) [52]
- Guard Sandar (Pradhan herbal Company, Bangalore) [53]
- Saya Churnam (Ayurvedic formulation) [54]
- Bharangyadi (Ayurvedic formulation) [55]
- Immunocare (Bacfo, Noida, New Delhi)
- Imunocin (Gufic Biosciences Limited, Mumbai)
- NRZTCDP (Natural Remedies, Bangalore) [56]

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