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Modern Quality evaluation and Standardization of Herbal Medicine

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ABSTRACT

Modern herbal medicinals are building a bridge between traditional healing methods and conventional medicine.

Modern Medicine was built around the model of running tests on sick patients to determine which drug or medical procedure would best deal with some illness. This makes Modern Medicine more precise in determining the diagnosis and how to treat this specific disease.

Parameters of evaluation of herbal drugs includes; Microscopic Evaluation,Determination of Foreign Matter ,Determination of Ash , Determination of Heavy Metals. , Determination of Microbial Contaminants and Aflatoxins. ,Determination of Pesticide Residues., Determination of Radioactive Contamination.

Method for herbal drugs evaluation includes; The analysis of herbal preparations is mostly done by running high performance liquid chromatography (HPLC) or gas chromatography (GC) and thin layer chromatography (TLC) methods, quantitative determinations by UV visible spectroscopy or combinations of these.

Modern medicine so important because , Prescription medicines and advances in medical treatment have helped people avoid disability and death caused by disease, lowered overall treatment costs, and has lowered death rates for heart disease, stroke, cancer, and other deadly diseases for several decades.

Medication use evaluation (MUE) is a performance improvement tool that can be used when there is uncertainty regarding whether a medication will be beneficial. It is particularly useful when limited evidence is available on how best to choose between two or more medications.

Major challenges to modern medicines may be , illnesses can be analysed at cell level, Personalised medicine is the future , Longer lifespan equals more people with dementia, Ageing is still mystery, The obesity epidemic is still a great threat, Maybe epigenetics makes us fat.

Keywords : Herbal Medicines**,** Standardization, Quality Control test.

Introduction

Different chromatographic and electrophoretic techniques commonly used in the instrumental inspection of herbal medicines (HM) are first comprehensively reviewed. Chemical fingerprints obtained by chromatographic and electrophoretic techniques, especially by hyphenated chromatographies, are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the "chemical integrities" of the herbal medicines and therefore be used for authentication and identification of the herbal products. Based on the conception of phytoequivalence, the chromatographic fingerprints of herbal medicines could be utilized for addressing the problem of quality control of herbal medicines. Several novel chemometric methods for evaluating the fingerprints of herbal products, such as the method based on information theory, similarity estimation, chemical pattern recognition, spectral correlative chromatogram (SCC), multivariate resolution, etc. are discussed in detail with examples, which showed that the combination of chromatographic fingerprints of herbal medicines and the chemometric evaluation might be a powerful tool for quality control of herbal products. "Despite its existence and continued use over many centuries, and its popularity and extensive use during

the last decade, traditional medicine has not been officially recognized in most countries. Consequently, education, training and research in this area have not been accorded due attention and support. The quantity and quality of the safety and efficacy data on traditional medicine are far from sufficient to meet the criteria needed to support its use world-wide. The reasons for the lack of research data are due to not only to health care policies, but also to a lack of adequate or accepted research methodology for evaluating traditional medicine"

QUALITY CONTROL AND STANDARDIZATION OF HERBAL MEDICINES –

CONCEPT AND SCOPE Generally, all medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being safe and effective (EMEA, 2005; WHO, 2002c, 1998c, 1996, 1991a,b, 1990, 1988).

The term “herbal drugs” are plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage (EMEA, 1998), Hence they are capable of variation.

This variability is also caused by differences in growth, geographical location, and time of harvesting. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant

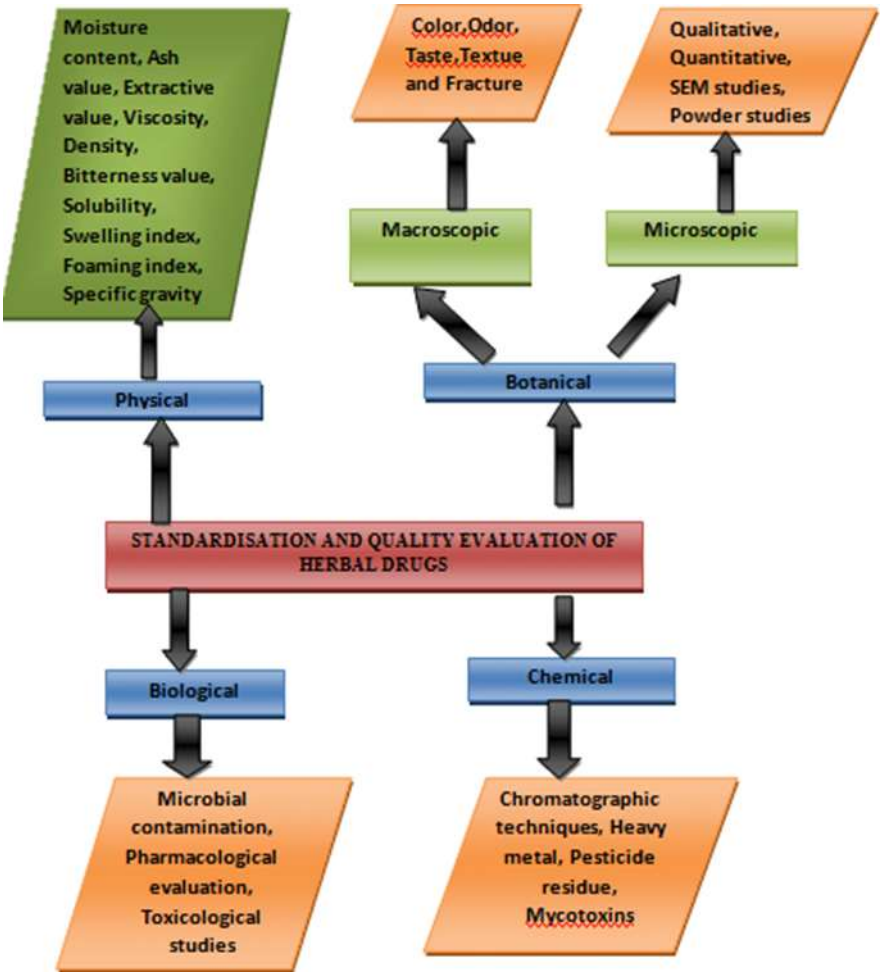
parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility.

It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine.

Hence standardization is a tool in the quality control process. Several problems not applicable to synthetic drugs often influence the quality of herbal drugs.

For instance:

1. Herbal drugs are usually mixtures of many constituents.
2. The active principle(s) is (are), in most cases unknown.
3. Selective analytical methods or reference compounds may not be available commercially.
4. Plant materials are chemically and naturally variable.
5. Chemo-varieties and chemo cultivars exist.
6. The source and quality of the raw material are variable.



Standardization and quality control of herbal crude drugs – Processes and procedures

According to WHO (1996a and b, 1992), standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Attention is normally paid to such quality indices such as:

1. Macro and microscopic examination: For Identification of right variety and search of adulterants.
2. Foreign organic matter: This involves removal of matter other than source plant to get the drug in pure form.
3. Ash values: These are criteria to judge the identity and purity of crude drug – Total ash, sulphated ash, water soluble ash and acid insoluble ash etc.
4. Moisture content: Checking moisture content helps reduce errors in the estimation of the actual weight of drug material. Low moisture suggests better stability against degradation of product.

The specific aims of such investigation in assuring herbal quality are as varied as the processes employed.

Physical evaluation

Each monograph contains detailed botanical, macroscopic and microscopic descriptions of the physical characteristics of each plant that can be used to ensure both identity and purity. Each description is accompanied by detailed illustrations and photographic images which provide visual documentation of accurately identified material.

Microscopic evaluation

Full and accurate characterization of plant material requires a thorough physical examination.

Microscopic analyses of plants are invaluable for assuring the identity of the material and as an initial screening test for impurities.

Chemical evaluation

This covers screening, isolation, identification and purification of the chemical components. Chemical analysis of the drug is done to assess the potency of vegetable material in terms of its active principles. The chemical screening or tests may include colour reaction test, which help to determine the identity of the drug substance and possible adulteration.

Biological evaluation Pharmacological activity of certain drugs has been applied to evaluate and standardize them. The assays on living animal and on their intact or isolated organs can indicate the strength of the drug or their preparations. These assays are known as Biological assays or Bioassay.

Purity determination

Each monograph includes standards for purity and other qualitative indices already mentioned above. Analytical methods Critical to compliance with any monograph standard is the need for appropriate analytical methods for determining identity, quality, and relative potency. There are a plethora of analytical methods available.

However, it is often difficult to know which is the most appropriate to use, but critical among know analytical tools in monograph standardization is chromatography. Chromatography Chromatography is the science which studies the separation of molecules based on differences in their structure and/or composition. In general, chromatography involves moving a preparation of the materials to be separated, “the "test preparation”, over a stationary support. The molecules in the test preparation will have different interactions with the stationary support leading to separation of similar molecules.

Test molecules which display tighter interactions with the support will tend to move more slowly through the support than those molecules with weaker interactions. In this way, different types of molecules can be separated from each other as they move over the support material.

Chromatographic separations can be carried out using a variety of supports, including immobilized silica on glass plates (thin layer chromatography), very sensitive High Performance Thin Layer Chromatography (HPTLC), volatile gases (gas chromatography), paper (paper chromatography), and liquids which may incorporate hydrophilic, insoluble molecules (liquid chromatography).

High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time.

With HPTLC, the same analysis can be viewed collectively in different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific type of analysis.

Quantitative analysis The most appropriate quantitative analytical method with accompanying chromatograms is desirable. The primary goal of the methods is to provide validated methods to be used to quantify the compounds most correlated with pharmacological activity or qualitative markers

(Wani, 2007). Control of starting material Control of the starting materials is essential in order to ensure reproducible quality of herbal medicinal products (De Smet, 2004; Gaedcke and Steinhoff, 2003; WHO, 2002b; Phillipson, 1993).

The following points are to be considered in the control of starting materials:

Authentication and reproducibility of herbal ingredients The problems associated with unregulated herbal products highlight the major public health issues that can arise when their herbal ingredients have not been authenticated correctly. Herbal ingredients must be accurately identified by macroscopic and microscopic comparison with authentic material or accurate descriptions of authentic herbs (Houghton, 1998). It is essential that herbal ingredients are referred to by their binomial Latin names of genus and species; only permitted synonyms should be used. Even when correctly authenticated, it is important to realise that different batches of the same herbal ingredient may differ in quality due to a number of factors such as: 1. Inter- or intra-species variation: The variation in constituents is mostly genetically controlled and may be related to the country of origin. 2. Environmental factors: The quality of a herbal ingredient can be affected by environmental factor like climate, altitude and other conditions under which it was cultivated. 3. Time of harvesting: For some herbs the optimum time of harvesting should be specified as it is known that the concentrations of constituents in a plant can vary during the growing cycle or even during the course of a day.

4. Plant part used: Active constituents usually vary between plant parts and it is not uncommon for a herbal ingredient to be adulterated with parts of the plant not normally utilised. In addition, plant material that has been previously subjected to extraction and is therefore 'exhausted' is sometimes used as adulterants to increase the weight of a batch of herbal ingredient.

Good agricultural/Manufacturing practices:-

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 (WHO, 2004, 2003, 2000, 1992, 1988b; EMEA, 2002; Blumenthal et al., 1998; Roberts and Tyler, 1997). 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 and 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.

Contaminants of herbal ingredients Herbal ingredients of high quality should be free from insects, animal matter and excreta. It is usually not possible to remove completely all contaminants, hence specifications should be set in order to limit them:

1. **Ash values:**

Incineration of a herbal ingredient produces ash which constitutes inorganic matter. Treatment of the ash with hydrochloric acid results in acid-insoluble ash which consists mainly of silica and may be used to act as a measure of soil present. Limits may be set for ash and acid-insoluble ash of herbal ingredients.

2. **Foreign organic matter:**

It is not possible to collect a herbal ingredient without small amounts of related parts of plant or other plants.

Standards should be set in order to limit the percentage of such unwanted plant contaminants.

3. **Microbial contamination:**

Aerobic bacteria and fungi are normally present in plant material and may increase due to faulty growing, harvesting, storage or processing. Herbal ingredients, particularly those with high starch content, may be prone to increased microbial growth. Pathogenic organisms including Enterobacter, Enterococcus, Clostridium, Pseudomonas, Shigella and Streptococcus have been shown to contaminate herbal ingredients. It is essential that limits be set for microbial contamination and the European Pharmacopoeia now gives non-mandatory guidance on acceptable limits (Barnes et al., 2007).

4. **Pesticides:**

Herbal ingredients, particularly those grown as cultivated crops, may be contaminated by DDT (dichlorodiphenyltrichloroethane) or other chlorinated hydrocarbons, organophosphates, carbamates or polychlorinated biphenyls.

Limit tests are necessary for acceptable levels of pesticide contamination of herbal ingredients. The European Pharmacopoeia includes details of test methods together with mandatory limits for 34 potential pesticide residues (Barnes et al., 2007).

5. **Fumigants:**

Ethylene oxide, methyl bromide and phosphine have been used to control pests which contaminate herbal ingredients. The use of ethylene oxide as a fumigant with herbal drugs is no longer permitted in Europe (Barnes et al., 2007).

Standardization of Herbal Medicines

This involves 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. Botanical extracts made directly from crude plant material show substantial variation in composition, quality, and therapeutic effects. Standardized extracts are high-quality extracts containing consistent levels of specified compounds, and they are subjected to rigorous quality controls during all phases of the growing, harvesting, and manufacturing processes. No regulatory definition exists for standardization of dietary supplements.

As a result, the term “standardization” may mean many different things. Some manufacturers use the term standardization incorrectly to refer to uniform manufacturing practices, but following a recipe is not sufficient for a product to be called standardized. Therefore, the presence of the word “standardized” on a supplement label does not necessarily indicate product quality.

The potency and quality of an individual herbal product may be unclear because of lack of regulation. It is obvious that for a given plant product its quality will also be determined by the prevailing conditions during the growth cycle of the plant. Therefore, for cultivated plants the good agricultural practice (GAP) system has been introduced, under which each step, including seed selection, growing conditions, use of fertilizers, and optimization of harvest time, harvesting, and drying, has to adhere to a set of criteria. It is likely that GAP procedures will become an integral part of quality control in the near future.

Critical Factors Affecting The QUALITY CONTROL OF HERBAL DRUGS

Microscopic evaluation Quality control of herbal drugs has traditionally been based on the appearance and today microscopic evaluation is indispensable in the initial identification of herbs, as well as, in identifying small fragments of crude or powdered herbs, and detection of foreign matter and adulterants.

A primary visual evaluation, which seldom needs more than 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. At other times, microscopic analysis is needed to determine the correct species and/or that the correct part of the species is present. For instance, pollen morphology may be used in the case of flowers to identify the species, and the presence of certain microscopic structures such as leaf stomata can be used to identify the plant part used.

Although this may seem obvious, it is of prime importance, especially when different parts of the same plant are to be used for different treatments. Stinging nettle (Urtica urens) is a classic example where the aerial parts are used to treat rheumatism, while the roots are applied for benign prostate hyperplasia (AOAC, 2005). Foreign matter Herbal drugs should be made from the stated part of the plant and be devoid of other parts of the same plant or other plants.

They should be entirely free from moulds or insec, 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 (WHO, 2004, 2003; EMEA, 2002).

Macroscopic examination can easily be employed to determine the presence of foreign matter, although, microscopy is indispensable in certain special cases (for example, starch deliberately added to “dilute” the plant material). Furthermore, when foreign matter consists, for example, of a chemical residue, TLC is often needed to detect the contaminants (AOAC, 2005; WHO, 1999a, 1998a).

Ash content To determine ash content, the plant material is burnt and the residual ash is measured as total and acid-insoluble ash. Total ash is the measure of the total amount of material left after burning and includes ash derived from the part of the plant itself and acid-insoluble ash. The latter is the residue obtained after boiling the total ash with dilute hydrochloric acid, and burning the remaining insoluble matter.

The second procedure measures the amount of silica present, especially in the form of sand and siliceous earth (AOAC, 2005). Heavy metals Contamination by toxic metals can either be accidental or intentional. Contamination by heavy metals such as mercury, lead, copper, cadmium, and arsenic in herbal remedies can be attributed to many causes, including environmental pollution, and can pose clinically relevant dangers for the health of the user and should therefore be limited (AOAC, 2005; WHO, 1998c; De Smet, 1992).

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. This potential exposure can then be put into a toxicological perspective by comparison with the socalled Provisional Tolerable Weekly Intake values (PTWI) for toxic metals, which have been established by the Food and Agriculture Organization of the World Health Organization (FAO-WHO) (De Smet, 1999; WHO, 1981, 1979).

A simple, straightforward determination of heavy metals can be found in many pharmacopoeias and is based on colour reactions with special reagents such as thioacetamide or diethyldithiocarbamate, and the amount present is estimated by comparison with a standard (WHO, 1988a).

Instrumental analyses have to be employed when the metals are present in trace quantities, in admixture, or when the analyses have to be quantitative. Generally, the main methods commonly used are atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP) and neutron activation analysis (NAA) (Watson, 1999).

Microbial contaminants and aflatoxins Medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi, and viruses. Inevitably, this microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations.

Risk assessment of the microbial load of medicinal plants has therefore become an important subject in the establishment of modern Hazard Analysis and Critical Control Point (HACCP) schemes.

Detection of Phytoconstituents

Chemical Test For Alkaloids

Sr. No.	Name of the test	Procedure	Observation	Inference
1	Mayers Test	Filtrates + Mayer's reagent (Potassium Mercuric Iodide)	Yellow precipitate	Presence of alkaloids
2	Wagner's Test	Filtrates+Wagner's Reagent Iodine in potassium Iodide)	Brown reddish precipitate	Presence of alkaloids
3	Dragendroff's Test	Filtrates + Dragandroff's reagent (solution of potassium Bismuth Iodide)	Red precipitate	Presence of alkaloids
4	Hager's Test	Filtrates + Hager's Reagent (Saturated Picric acid solution)	Yellow Precipitate	Presence of alkaloids

Detection of Carbohydrates

Sr. No.	**Name of Test**	**Procedure**	**Observation**	**Inference**
1	Molisch's Test	Filtrates + Two drops of alcoholic α-naphthol solution	Formation of violet ring at junction	Presence of Carbohydrates
2	Benedict's Test	Filtrate + Bendict's Reagent (Heat)	Orange red precipitate	Reducing Sugar
3	Fehling's Test	Filtrates + Dil. HCL Neutralized with alkali and heated with fehlings A & B solution	Red precipitate	Reducing Sugar

Detection of Phytosterols

Sr. No.	Name Of Test	Procedure	Observation	Inference
1	Salkowskis's Test	Extracts + Chloroform and filtered filtrates + few drops of conc. Sulphuric acid Shaken.	Appearance of golde.n yellow.	Presence of Triterpenes
2	Liebermann Burchard Test	Extracts + Chloroform and filtered filtrates + few drops of Acetic anhydride boiled and cooled conc. H2SO4	Brown ring at the Junction	Presence of Phytosterols

Detection Of Glycoside

Sr. No.	Name of Test	Procedure	Observation	Inference
1	Modified Borntrager's Test	Exstracts + Ferric Chloride and Immersed in boiling water for about five minute cooled and add equal volumes of benzene.	Benzene layer + Ammoni Sol^{n}. Give pink colour	Presence of Anthranol Glycosides.
2	Legal's Test	Extracts + Sodium nitropruside in pyridine and sodium hydroxide.	Pink colour	Cardiac Glycosides.

3	Froth Test	Extracts + Distilled Water to 20ml and Shaken for 15min.	Formation of 1cm layer of foam	Presence of Saponias.
4	Foam Test	0.5gm of Extract + Shaken with 2ml of Water	If foam produced persists for 10min.	Presence of Saponias.

Detection of Tannins

Sr. No	Name of Test	Procedure	Observation	Inference
1.	Gelatin Test	Extract + 1% Gelatin Solution Containing Sodium Chloride	White Precipitate	Presence of Tannins

Chromatographic evaluation

Thin Layer Chromatography TLC is one of the most important tool for separation of compound. It is widely used technique of chromatography. It is based on principle of adsorption [18]. In this method stationary phase is a finely divided solid and it is applied as a thin layer on supporting plate and the mobile phase is a liquid which is allowed to flow on the surface of the plate by capillary action.

HPTLC:

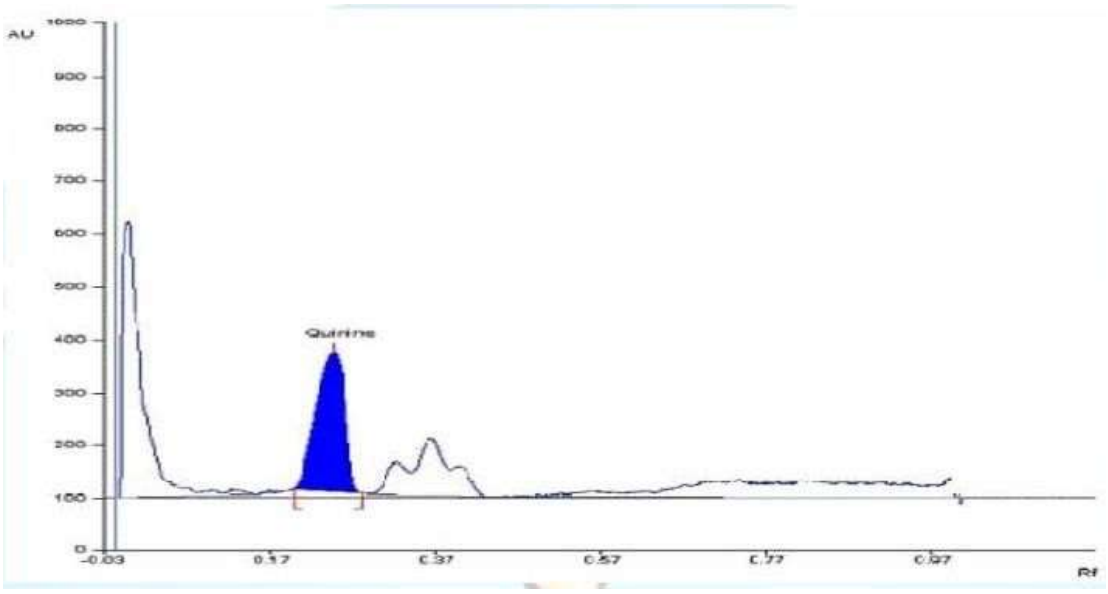
In HPTLC a layer thickness of 100-150micron is used to achieve separation. HPTLC uses open layers of adsorbents

on plates or foils to separate component of samples.

Significance of HPTLC:

Identification and detection of adulterants in herbal product and it is also important in identification of pesticide content, mycotoxins and in quality control of herbs and health foods.

Example:- Chromatogram of Quinine



Ultra Violet Spectroscopy:-

UV spectroscopy is important technique in the analysis of herbal as well as synthetic drugs. It gives idea about purity of the substance. Its detection capability depends on Beer-Lamberts Law that is absorbance is directly proportional to the concentration and path length. [25]

Examples of drugs with their wavelengths

Drug	UV range (nm)
Morphine	284
Aloe-emodin	225,258,279,287,430
Caffeine	243,326
Scopholine	227,250,288,33

Conclusion / Review Outcomes:-

- To understand the modern quality evaluation and standardization test for herbal medicines.
- Study of different parameters related to Pharmacognostic and phytochemical
- Study on different traditional plant such as
  - I. Duranta Erecta
  - II. Datura
  - III. Vinca
  - IV. Ashwagandha
- Study of different evaluation parameter such as,
  - I. Determination of ash value
  - II. Determination of moisture content.
  - III. Determination of pesticides residue.
  - IV. Determination of heavy metals.
- Analysis of herbal preparations mostly done by,
  - I. U.V Visible spectroscopy
  - II. Thin layer chromatography
  - III. HPTLC

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