



INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ADVANCED HERBAL TECHNOLOGY

Author

Dr. Babasaheb Ambedkar Technological University Lonere Raigad Maharashtra India

Fabtech College of pharmacy sangola

Name of Guide – Dr. J.P. Lavande

Principal -Dr.S.K.Bais

Name of Author - Pawar N.D.

• ADVANCED HERBAL TECHNOLOGY



1.ABSTRACT:-

Due to their numerous advantages, people have recently started to show increased interest in herbal medicines. Today, many people successfully treat a range of conditions with herbal medicines. More than 80% of the world's population relies on herbal medications and goods for a healthy lifestyle, despite the fact that the majority of these operations are unorthodox. The rising use of herbal goods has also resulted in a range of inventive abuses and product manipulation, which have angered buyers and producers and, in some cases, had disastrous outcomes. The development of actual logical systems that can precisely define the phytochemical composition, including quantitative studies of marker/bioactive composites and other essential elements, is a serious challenge for scientists. Setting guidelines is a crucial first step in creating a Standardization is an essential initial step in the creation of a harmonious natural effort, a harmonious chemical profile, or even just a quality assurance programme for the production of herbal medicines. Both colourful old designs and more recent innovations are described in the current review composition. The fields of DNA characteristics, metabolomics fashion, discrimination palpitation paleography, chemometrics, X-ray diffraction, etc. have all seen recent advancements. Additionally, it has been claimed that chromatographic and capillary electrophoresis techniques are useful for standardising herbal remedies.

*Important Words Standardization, natural remedies, DNA traits, and optimal chromatographic methods

1. Recognize raw materials as the origin of herbal medicines from civilization to finished products

2. Be familiar with the WHO and ICH standards for evaluating herbal remedies.

3. Understand natural sweeteners, nutraceuticals, and herbal cosmetics

4. Support GMP and the patenting of herbal medicines.

2.INTRODUCTION:-

Herbs, herbal ingredients, herbal preparations, or herbal finished goods can all be used to make herbal remedies. Traditional herbal medicines in some cultures may contain natural, organic, or inorganic active ingredients that are not sourced from plants (e.g. animal and mineral materials). Herbs are unprocessed plant parts, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes, or other plant parts, that can be whole, split up, or ground up. Herbal materials also include fresh juices, gums, fixed oils, essential oils, resins, and dry powders of various herbs in addition to the actual herbs themselves. Local cuisines in various countries may prepare these materials by steaming, roasting, or stir-baking them with honey, alcoholic beverages, or other ingredients.

The building blocks for finished herbal medicines are herbal preparations, which can include comminuted or powdered herbal components as well as extracts, tinctures, and fatty oils of herbal materials. They are produced using a variety of physical or biological techniques, including as extraction, fractionation, purification, and concentration. They also include mixtures made by steeping or boiling herbal ingredients in alcoholic drinks, honey, or other substances.

Finished herbal goods are herbal remedies made from one or more herbs. If more than one plant is utilised, the term "mixed herbal product" may also be used. Along with the active ingredients, finished herbal products and herbal blends may also contain excipients. Completed products or herbal combinations, however, to which chemically defined active elements, such as synthetic chemicals and/or botanical compounds, have been added, are not considered to contain their separated ingredients [5]. To be regarded as rational drugs, phytopharmaceuticals must maintain constant efficacy and safety, therefore they must be standardised and have their pharmaceutical quality certified [6]. The World Health Organization (WHO) highlights the importance of utilising both qualitative and quantitative methods to characterise samples, quantify biomarkers, and/or chemical analyse fingerprint profiles and genetic markers. If the main active component of a chemical is known, it makes the most sense to quantify it. Standardization of botanical preparations to known active ingredients that contribute to their medical efficacy is necessary. In cases where both of the active ingredients are still unknown, a marker substance unique to each should be utilised.

3.DIFFERENT METHODS OF IDENTIFICATION OF PLANTS:-

1. Expert Determination: Expert decision is the most dependable and precise way of identification. The relevant group has typically been treated by professionals (monographs, revisions, synopses), and it is likely that the taxa used by specialists are included in more current floras or manuals. Experts are frequently found in botanical gardens, herbaria, museums, colleges, and universities, among other places. Although very effective, this procedure has limitations in that it delays identification and takes up professionals' important time.

2. Recognition: It has a reliability similar to that of expert opinion. Based on the identifier's extensive past knowledge of the questioned plant group, this conclusion was reached.

3.Comparison: A third method compares an unidentified object to known specimens, images, drawings, or descriptions. Although this is a reliable method, the lack of sufficient comparable materials may make it time-consuming or nearly impossible.

4. Making Use of Keys and Similar Instruments (Synopses, Outlines, etc.) This strategy—by far the most widely used one—needs neither the time nor the resources nor the knowledge required for comparison and recognition. [1]

3.1.AUTHENTICATION OF PLANTS :-

A quality control process called herb authentication ensures that the correct plant species is being used. Additionally, plant components are used as the primary basic ingredients in herbal therapies. The proper verification of herbal raw materials has a significant impact on the safety and efficacy of herbal medications. [1] Verifying herbal medications Genuine raw materials serve as the essential building block for producing a botanical product. Additionally, the quality and homogeneity of the final product can be considerably altered during each stage of formulation, including harvest, storage, processing, and processing. To ensure the greatest efficacy and safety of these products, processes for guaranteeing quality control throughout production and storage are crucial instruments. The efficiency of such controls is also crucial for the evaluation of pharmacological, toxicological, or therapeutic studies that use botanical chemicals.

1.Taxonomic method: The initial step in identifying and verifying botanical materials entails gathering and recording the plant at its source using conventional botanical techniques. This method identifies the botanical source of the medicine and establishes its scientific Latin binomial name (i.e., genus species). The basis for authentication is laid forth there [8]. Information like the botanical name, colloquial names, location of the plant material collection, collector details, habitat, season of collection, altitude, and the section obtained are required even before verification. [8] 2. The material sample should be stored as a voucher sample in a herbarium or research facility for potential use in the future. [8]

3. Macroscopic approach: Based on criteria like organoleptic traits that are compared to a standard reference material, such as shape, size, colour, texture, surface characteristics, fracture characteristics, aroma, and taste, botanical materials can be macroscopically identified. [9]

aterial, such as shape, size, colour, texture, surface characteristics, fracture characteristics, odour, and taste [9]

4.Microscopical approach: Botanicals' structural, cellular, and interior tissue characteristics can be ascertained via microscopy. It is typically used to distinguish between two herbals that are similar [10, 11] and to identify them. This method is the most popular since it is simple, quick, and it also works with patented medications [12]. Star anise is an illustration of a plant that can benefit from microscopic methods to help with its identification (*Illicium verum* Hook.f). Originally from southern China, star anise is a star-shaped fruit with an anise-like flavour that has spread to the tropics and eastern subtropics. Asia. The food is mostly used in China and India as an aromatic spice to enhance foods and confections[13]. It is renowned in traditional Chinese medicine for its medicinal

effectiveness in the treatment of hernias, back pain, and rheumatism. Unfortunately, more and more instances of newborns experiencing abrupt neurological symptoms such as seizures, vomiting, and fast eye movement after drinking star anise herbal tea have been documented in western nations and the United States [14]. The adulteration of Chinese star anise with Japanese or “Bastard” anise was thought to be the cause of these many incidents. It is generally known that Japanese star anise (*Licium anisatum*) contains dangerous sesquiterpenes.

5. Physical Chemistry: Total ash, water soluble ash, acid insoluble ash, and sulphated ash are all physical chemistry metrics. By comparing these values to the normative values of the Indian pharmacopoeia, it is possible to identify the specific pharmaceuticals or proprietary medications [15].

6. Chemometric and Spectral Methods: Initially, employing an infrared (IR) spectroscopy method, only the structural elucidation of isolated compounds from herbal matrices is feasible. It has been demonstrated to be useful in phytochemical research as a “fingerprinting” tool for comparing a natural with a synthetic sample [16]. As a result of developments in computer technology, chemometric approach has become a crucial tool in the scientific community for accelerating analysis and shortening the time needed for product creation [17]. Unsupervised pattern recognition methods like Principal Component Analysis (PCA) are the most widely used method for managing multivariate data without prior knowledge of the study samples [18]. While Soft Independent Modeling of Class Analogy (SIMCA), which is based on building a PCA model to assign unknown samples into the predefine class model, has also been applied in the analysis of infrared spectra [19]. A study using FTIR transmission spectroscopy in conjunction with the appropriate chemometric techniques (PCA and SIMCA) was carried out to categorise *Orthosiphon stamineus* Benth (commonly known as Java tea for treating urinary tract infections, kidney stone disease, and bladder stone disease) based on its geographical origin and varieties from the obtained characteristics infrared spectrum. Using chemometrics, analysis of The process of acquiring spectra is quick and simple because materials do not require chemical processing [20].

7. chromatographic techniques: High Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE), and Thin Layer Chromatography are the most common analytical methods for herbal compounds (TLC). [21] Chemical analysis of herbal remedies must pay close attention to the gas chromatography examination of volatile components. [22]

a) Thin Layer Chromatography (TLC): Thin layer chromatography (TLC) was the method of choice for the examination of herbs before instrumental chromatography techniques like GC and HPLC were created. TLC is still extensively employed for the investigation of herbal medicines nowadays because several pharmacopoeias still use it to offer the initial characteristic fingerprints of herbs [23], [24]. TLC has the advantage of offering a wide range of detecting options. TLC is simple to use and can be utilised for multiple sample analysis. Each plate can include up to 30 sample spots that can be analysed all at once [25]. Overall, the simplicity, adaptability, high velocity, special sensitivity, simple sample preparation, and economy of using TLC to produce the fingerprint of herbal medicines are its advantages. TLC is a useful method to evaluate the quality and potential for adulteration of herbal items [26].

b) High Performance Liquid Chromatography (HPLC): HPLC is a widely used method for the examination of herbal medicines since it is straightforward to learn and use and is not limited by the volatility or stability of the sample component. In general, HPLC may be used to analyse almost all of the compounds found in herbal medications [27].

c) Gas chromatography (GC): The GC of the volatile oil creates a trustworthy fingerprint that can be used to identify the plant. The extraction procedure may be standardised and made quite straightforward, and the components of the volatile oil can be quickly identified using GC-MS analysis. The advantages of GC are most shown in the great sensitivity of detection for almost all volatile chemical compounds. [22]

d) Capillary Electrophoresis (CE): It is quite efficient to use capillary electrophoresis (CE) to assess a sample's purity and complexity. It can handle almost all charged sample components, including DNA and even the most basic inorganic ions. CE shows promise for the separation and analysis of active ingredients in herbal remedies because it can evaluate samples and only needs small amounts of standards. [22]

8. Chemical fingerprinting:- is a characteristic pattern that allows the identification of the different chemical markers found in a sample [28]. Regardless of whether they have any therapeutic impact, chemical markers are categorised by the European Medicines Agency (EMA) as interesting compounds or groupings of constituents of herbal medical products. The concentration of a chemical marker can be used as a gauge for the effectiveness of a herbal medicine. Chemical marker research can be useful for a variety of research areas, including the confirmation of genuine species, the search for novel raw material or alternative sources, the development of extraction and purification methods, structure elucidation, and purity evaluation

9. Molecular markers: These biochemical components, also referred to as molecular markers, include primary and secondary metabolites, as well as other macromolecules including nucleic acids. DNA markers are dependable for finding useful polymorphisms since each species' genetic makeup is distinct and unaffected by age, physiological conditions, or environmental influences. Additionally, DNA can be extracted from live or dried organic plant tissue, therefore detection is not restricted by the sample's physiological form. [29],[30] Numerous DNA-based molecular techniques are employed to evaluate DNA polymorphisms. These techniques rely on polymerase chain reaction (PCR), hybridization, and sequencing [31], [32], [33].

*Two hybridization-based methods are restricted fragment length polymorphism (RFLP) and variable number tandem repeats. 48 Labeled probes, such as those for micro satellite” [34] and mini satellite sequences, are hybridised to filters containing DNA that has been digested with restriction enzymes, such as random genomic clones, cDNA clones, and probes. Polymorphisms are discovered by evaluating the presence or absence of bands following hybridization

***PCR-based techniques:** To in vitro amplify particular DNA sequences or loci, PCR-based techniques use the heat stable DNA polymerase enzyme and specified or random oligonucleotide primers. PCR-based techniques that employ random primers include Randomly Amplified Polymorphic DNA (RAPD) [35, 36], Arbitrarily Primed PCR (AP-PCR) [37], and DNA Amplification Fingerprinting [38, 39]. AFLP, or amplified fragment length polymorphism, is a more recent technique that relies on the detection of amplification [40, 41].

10. Genomic restriction fragments by PCR: a) Multiplex technologies called DNA micro arrays are employed in molecular biology and medicine. It is made up of millions of tiny DNA oligonucleotide patches, or features, that are organised in an array and contain Pico moles (10–12 moles) of a specific DNA sequence, or probes. This could be a brief gene fragment or other DNA piece that is used under controlled circumstances to hybridise a cDNA or CRNA (referred to as the target). Targets that have been fluorescent, silver, or chemiluminescence-tagged are commonly employed to detect and quantify probe-target hybridization in order to gauge the relative abundance of nucleic acid sequences in the target. This objective can be met by a micro array experiment because an array can hold tens to thousands of probes. This objective can be met by a micro array experiment because an array can hold tens to thousands of probes. Due to the fact that an array can include tens to thousands of probes, a micro array experiment can run several genetic tests at once. [42] For the purpose of authenticating plant species with therapeutic value, DNA-based approaches are frequently applied. This is especially helpful when it comes to species or variants that are commonly mixed in or contaminated with others that are identical morphologically or phytochemically [43]. RAPD markers were used to distinguish *Lycium barbarum*'s dried fruit samples from those of similar species [44]. The RAPD technique has also been used to pinpoint the constituents of the Chinese herbal treatment Yu-feng-san. Three plants (*Astragalus membranaceus*, *Ledebouriella seseloides*, and *Atractylodis macrocephala*) were detected in the formulation in this study using a single RAPD primer.

3.2. ADVANCES IN HERBAL EXTRACTION

1. Supercritical fluid extraction: This extraction system is the most advanced. Gases, frequently CO₂, are employed in Super Critical Fluid Extraction (SFE) and compressed into a thick liquid. The to-be-removed material is subsequently forced through a cylinder that contains this liquid. The extract-containing liquid is then forced into a chamber for separation, where the extract and gas are split apart and the gas is collected for later use. The pressure and temperature that one works at can be changed in order to manage and adjust the solvent characteristics of CO₂. The benefits of SFE include its versatility in identifying the ingredient you want to extract from a particular material and the near absence of solvent residues in the final product (CO₂ totally evaporates). The drawback of this technique is its high cost. When under pressure, very effective extraction solvents can be used for a variety of additional purposes and liquids, including:

a) Coupled SFF-SFC- System that uses a supercritical fluid to extract a sample before putting the extracted material in a supercritical fluid chromatographic system's intake. After that, a supercritical fluid chromatography is performed directly on the extract.

A sample is extracted using a supercritical fluid, which is then depressurized to deposit the extracted material in the intake component or a column of a gas chromatographic system or a liquid chromatographic system, as appropriate. b) Coupled SFE-GC and SFE-LC. High yield, reliable, robust sample preparation, and the potential to be linked with a number of chromatographic techniques are all present.

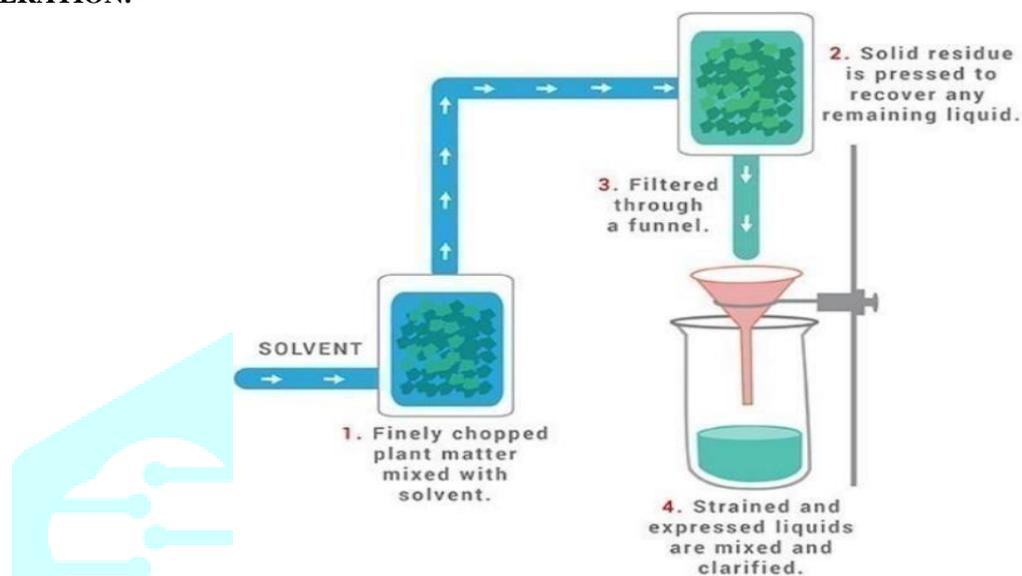
2. Microwave-Assist Extraction:-In 1975, Abu-Samra et al. made the first reference of using microwave energy. For the treatment of biological materials for metal trace analysis, they employed household ovens in the lab. Then, in 1986, Ganzler et al. research .s made microwave irradiation a viable method for extracting chemical molecules. The first extraction-related patent of a natural product using microwaves was filed by Pare in 1995

3. Solid phase extraction:-The same ideas that maintain molecules on stationary phases in chromatography are also used here to keep solutes from a liquid medium from adhering to a solid adsorbent. These adsorbents are similar to chromatographic media. arrive in the form of beads or resins that can be used in column or batch fashion. They are usually utilised in the form of syringes loaded with medium that are readily accessible on the market and can be gently pressed with the plunger or by vacuum. The amount of medium in these syringes ranges from a few hundred milligrammes to a few grammes. Solid phase extraction medium examples include reverse phase, normal phase, and ion-exchange media. This sample purification method concentrates and isolates the analyte from a solution of raw Cartridge for phases by adsorption onto a temporary solid surface. The analyte is typically maintained on the stationary phase, cleaned, and then evaluated with different mobile phases. If an aqueous extract is passed down a column containing reverse phase packing material, everything that is somewhat nonpolar will hind, but everything polar will flow through.

4. Extraction with ultrasound assistance:-Extraction has been used at least since that fire was detected. The Maya and Aztecs, Egyptians and Phoenicians, Jews and Arabs, Indians and Chinese, Greeks and Romans, and even Jews and Arabs devised novel extraction and distillation processes. There isn't a production line in the current world that involves an extraction process in the food, pharmaceutical, cosmetic, nutraceutical, or bioenergy industries (maceration, solvent extraction, steam or hydrodistillation, cold pressing, squeezing..). It is difficult for the food and plant-based chemical industries to achieve legal criteria for emissions, product/process safety and control, cost reduction, and enhanced quality without creating new technologies. This is a result of increased energy prices and efforts to decrease greenhouse gas emissions. Taking current extraction technologies as an example, they frequently account for up to 50% of the costs of a new plant and more than 70% of the total energy used in the food industry. They must also overcome significant technological and scientific challenges. The past 20 years have seen consideration given to more efficient and automated extraction techniques, including ultrasound-assisted extraction. Shorter extraction times. The main objectives were to reduce the consumption of organic solvents while saving energy and money. These goals have fueled advancements in ultrasound-assisted extraction, leading to a number of cutting-edge techniques such as ultrasound-assisted Soxhlet extraction, ultrasound-assisted Clevenger distillation, and more. [55]

4.GENERAL ISOLATION TECHNIQUE:-

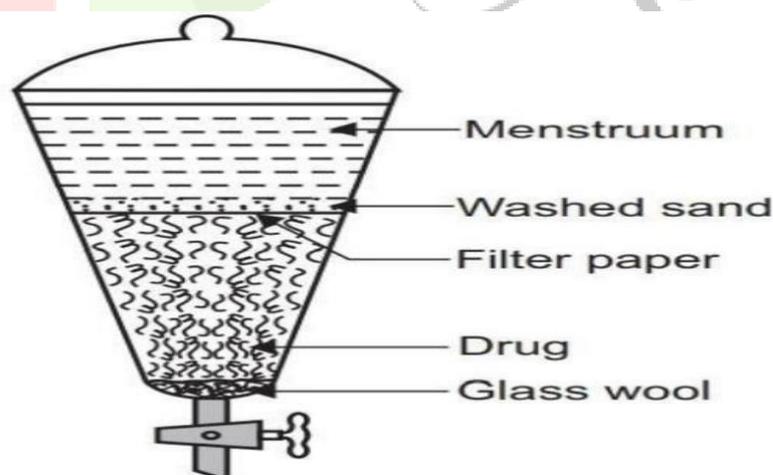
- 1.Maceration
- 2.Percolation
- 3.Decoction
- 4.Reflux extraction
- 5.Sohxlet extraction

1.MACERATION:-**Fig 1.1 Maceration**

The disadvantages of this incredibly simple extraction method include a lengthy extraction time and subpar extraction efficacy. It may be used for the extraction of a thermolabile component.

*maceration types include:

1. Simple maceration
2. haphazard maceration
3. Repeated maceration

2. PERCOLATION:-**Fig 2.1 Percolation**

Percolation is more efficient than maceration because it is a continuous process in which the saturated solvent is continuously replaced by fresh solvent.

1. To create extract, a bed of raw drug material is continuously displaced downward by the solvent.
2. The method most frequently used to extract active ingredients when tinctures and fluid extracts are being made.
3. It is the short successive maceration method or displacement process.
4. In most cases, a percolator—a thin, conical vessel open at both ends—is utilised. [47]

3.DECOCTION:

The procedure is mostly employed for vegetable medications with thermostable water soluble components that are hard and woody in nature.

The decoction extract contains a significant amount of water-soluble pollutants. Decoction is ineffective for the extraction of In this process, the uncooked drug is boiled in a precise amount of water for a certain period of time, after which it is cooled and filtered. Components that can be extracted using heat and water should be done so.

4.REFLUX EXTRACTION:-

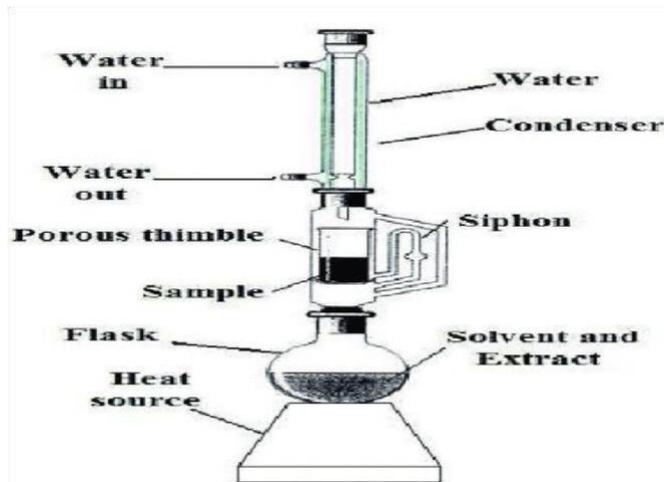


Fig 4.1 Reflux Extraction

Compared to percolation or maceration, reflux extraction is more effective and uses less solvent and extraction time. It cannot be used to extract naturally thermolabile products.

5.SOXHELT EXTRACTION

The Soxhlet extraction method is an automatic continuous extraction process with superior extraction efficiency as compared to maceration or percolation. Given the high temperature and extended extraction time of the Soxhlet extraction, thermal degradation is more likely. [48,49]

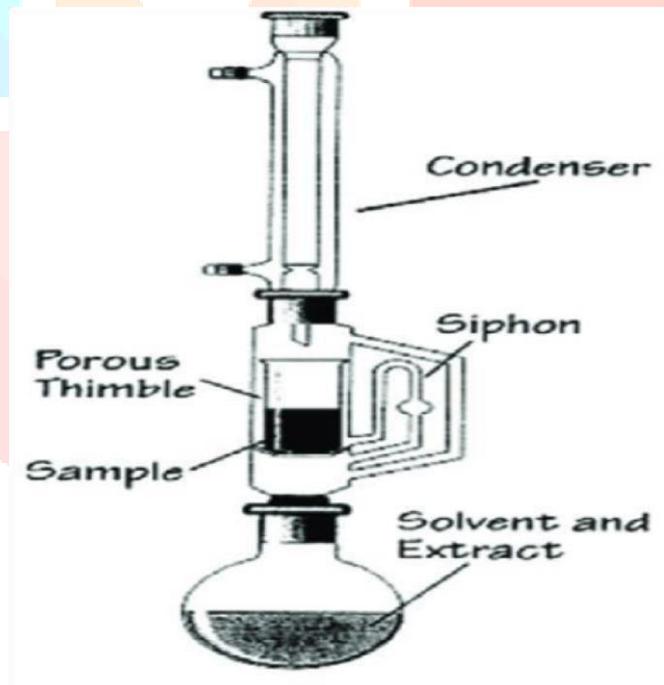


Fig 5.1 Soxhelt Extraction

6.PRESSURIZEDLIQUIDEXTRACTION

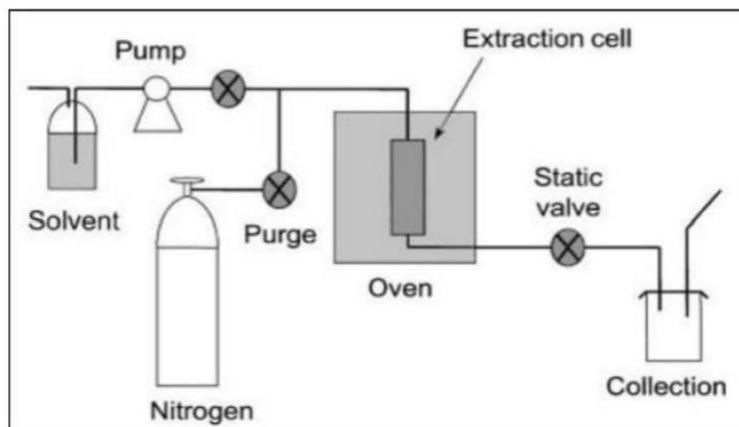


Fig.6.1 Pressurized liquid extraction

Pressurized liquid extraction (PLE) is also known as high pressure solvent extraction, accelerated solvent extraction, enhanced solvent extraction, pressurised fluid extraction, and accelerated fluid extraction. PLE uses high pressure for extracting. High pressure keeps solvents in a liquid state above their boiling point, which boosts the solubility and diffusion rate of lipid solutes in the solvent and the solvent's ability to permeate the matrix [50].

7.SUPERCritical FLUID EXTRACTION:-

In supercritical fluid extraction, supercritical fluid (SF) is used as the extraction solvent (SFE). Due to its similar solubility to liquid and similar diffusivity to gas, SF can dissolve a variety of natural substances. Their solvating properties substantially changed close to their critical points as a result of small pressure and temperature changes. Supercritical carbon dioxide (S-CO₂) was commonly used in SFE due to its appealing properties, such as its low critical temperature (31 °C), selectivity, inertness, low cost, non-toxicity, and ability to extract thermally labile compounds. Due to its low polarity, S-CO₂ is the ideal option for the extraction of non-polar natural substances like lipids and volatile oils. [51]

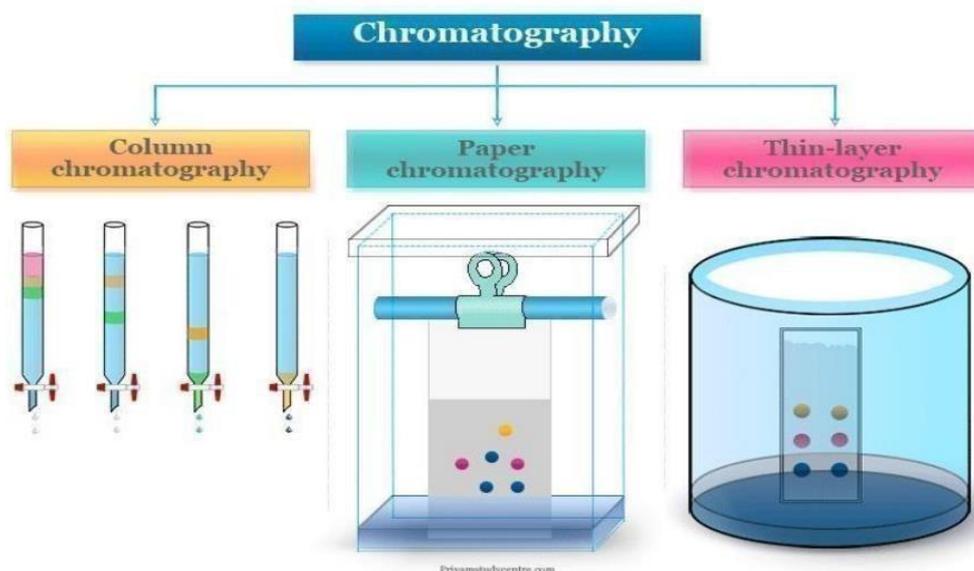
8.MICROWAVE ASSISTED EXTRACTION :-

Ionic conduction and dipole rotation are the two mechanisms by which microwaves interact to produce heat with polar substances such as water and some components of the organic plant matrix. The simultaneous occurrence of mass and heat fluxes during MAE has the effect of accelerating extraction and boosting extraction yield. The use of MAE has various benefits, including improving extract yield, reducing thermal deterioration, and selectively heating vegetal material. Because MAE uses less organic solvent, it is also rated as a green technology.

Two categories of MAE methodologies exist:

- non-solvent extraction (usually for volatile compounds)
- (Typically used for non-volatile substances) Solvent extraction [51].

4.1.CHROMATOGRAPHIC TECHNIQUES:-



Chromatography is a method for separating, purifying, and testing different chemicals. The roots of the word “chromatography” are the Greek words chroma, which means “colour,” and graphein, which means “to write.” In this process, the mixture to be separated is applied to a stationary phase (solid or liquid). The stationary phase is then allowed to be slowly traversed by a pure solvent, such as water or any gas, which transports the components separately according to their solubility in the pure solvent.

• **Chromatography Principle:-**The basis of chromatography is the idea that molecules move between the fixed phase and the mobile phase. It happens as a result of the molecules in the mixture that we want to separate absorbing or partitioning. Individual solute molecules move through a column or thin layer at different rates. It has a direct bearing on how molecules are distributed between the mobile phase and fixed phase.

4.2 TYPES OF CHROMATOGRAPHY:-

1. Column Chromatography Method
2. Thin Layer Chromatography Method
3. Adsorption Chromatography Method
4. Partition Chromatography Method

1. Column Chromatography:-

Column chromatography is a method for separating the components of a mixture using a suitable adsorbent packed in a glass tube, as shown in the image below. The combination is placed on top of the column and an appropriate eluent is made to gradually flow down the column. Depending on how much of each component has been adhering to the adsorbent column wall, the components are separated. While the other elements flow downward to different heights in accordance, the element with the highest absorptivity is retained at the top.

2. Thin Layer Chromatography-

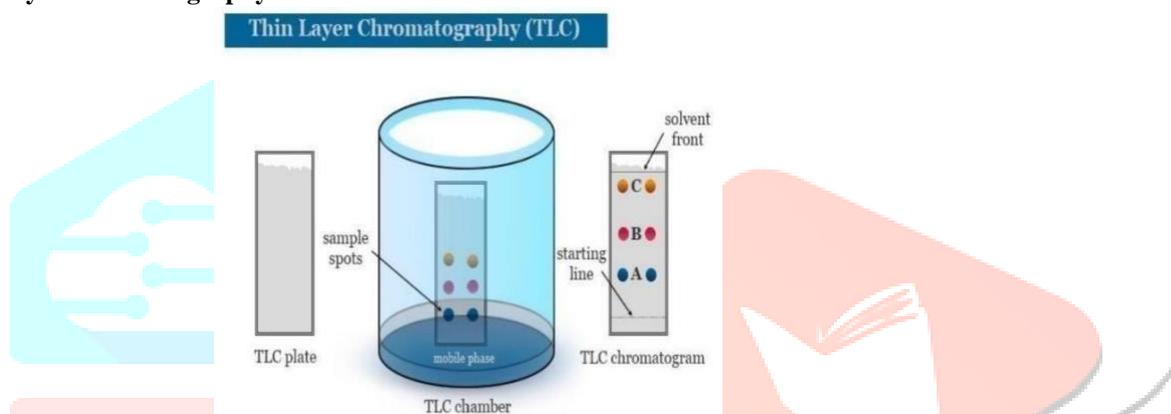


Fig 2.1 Thin Layer Chromatography

Using a glass plate coated in a very thin layer of an adsorbent, such as silica gel or alumina, the thin-layer chromatography (TLC) procedure separates the chemical mixture into its component elements. The plate used in this method is known as chrome plate. To start the separation process, a small area of the mixture's solution is placed 2 cm above one end of the plate. The plate is then placed into a container that is tightly closed and filled with an eluent, which causes the plate to rise and raise the various mixture components to different heights. [52]

3. High performance liquid chromatography-

High-performance liquid chromatography, or HPLC, is an analytical technique for separating, identifying, or quantifying each component in a mixture. The mixture is separated using the principles of column chromatography, and it is identified and measured using spectroscopy.

4..High performance thin Layer chromatography-

Similar physical TLC (adsorption chromatography) concepts, namely the adsorption principle of separation, are used in HPTLC. The solvent from the mobile phase passes through due to capillary action. The components migrate in accordance with their affinities with the adsorbent. The component moving more slowly is the one that is more drawn to the stationary phase. The element that is more fast moving has a lesser attraction for the stationary phase. The components are subsequently separated using a chromatographic plate.

*Application of chromatography :-

1. Pharmaceutical Analysis.
2. Herbal Analysis.
3. Quality Control.
4. Forensic Science.
5. Preparative studies.
6. Bioequivalence studies.
7. Biomarker analysis. [53]

4.3.PURIFICATION TECHNIQUES FOR ISOLATED PHYTOCONSTITUENTS

The separation of phytochemicals is the method of isolating the elements of plant extracts or active sections one at a time and purifying them into monomer compounds using physical and chemical techniques. Traditional isolation methods include solvent extraction, precipitation, crystallisation, fractional distillation, salting out, and dialysis still have a wide variety of modern applications. However, the employment of modern separation methods such high performance liquid chromatography, ultrafiltration, and high performance liquid drop counter current chromatography is also advantageous for the separation of phytochemicals. This section describes common methods and their particular applications for isolating phytochemicals. Acidic and basic solvent method.

1.solvent method

The different amounts of acidity and alkalinity present in each component of the mixture are taken into consideration. Alkaloids and other insoluble in water alkaline organic compounds may react with inorganic acids to form salts that can be used to distinguish them from non-alkaline and water-soluble compounds. Acid components with carboxyl or phenolic hydroxyl groups can be salined by bases and then dissolved in water. Before isolating components with lactone or lactam substructures from other water-insoluble components, it is possible to saponify and dissolve those components in water. Complete extracts can be extracted using acid water or alkali water, and then dissolved in lipophilic organic solvents, respectively (ethyl acetate is frequently employed). Neutral, alkaline, and acidic components. Of course, after adjusting the pH, the entire extract can also be dissolved in water and extracted with organic solvents. The fractions can be further separated by using a pH gradient extraction due to differences in the alkalinity or acidity of the fractions. In order to avoid structural changes of some compounds under harsh conditions or the inability of the chemical structures to be returned to their original state, it is crucial to pay attention to the strength of the acidity or alkalinity, the contact time with the separated components, the heating temperature, and the time when using the acid and basic solvent method.

2.Polarity gradient extraction method

Using this technique, the separation goal is accomplished based on the various polarities of the various plant extract constituents and the various partition coefficients in two-phase solvents. The polarity of the components in plant extracts is typically taken into account when choosing between different two-phase solvent systems. For instance, a water system containing n-butanol can be used to separate components with strong polarity, a water system containing ethyl acetate can be used to separate components with medium polarity, and a water system containing chloroform (or ether) can be used to separate components with weak polarity. The plant extract must first be dissolved in water before the extraction process can begin. A separate organic solvent that is not miscible with water due to polarity differences is then used to extract the solution or suspension in a separating funnel. As shown in Figure 1, the extract was commonly extracted using petroleum ether (also known as cyclohexane), ethyl acetate (often known as chloroform), and water-saturated n-butanol in that order. The petroleum ether layer contains low polarity, lipid-soluble compounds. The ethyl acetate layer contains medium-polar compounds such monoglycerides, flavonoids, and chemicals with more polar functional groups. The n-butanol layer contains oligoglycosides and other highly polar components that are water soluble. Chemicals in the water layer, such as glycosides with more glycosyl groups, carbohydrates, amino acids, proteins, and other water soluble compounds, exhibit the strongest polarity.

3.Precipitation method

It is a method that depends on interactions with certain reagents to produce certain phytochemicals as precipitates or on the addition of specific reagents to precipitate certain components from solutions, which can reduce the solubility of certain components in solutions. The precipitation process must be reversible if the target components are required for precipitation to develop. If the components are nontarget, the precipitation reaction may be irreversible since they will destroy the precipitation. Depending on the addition of chemicals or solvents, the following classifications may be assigned to this strategy: The components in the mixed component solution can be changed so that they can precipitate out of the solution by adding a specific solvent that is mutually soluble with the solution. The progressive precipitation brought on by altering the polarity or quantity of solvent supplied is known as fractional precipitation. When using water as an extracting solvent to extract phytochemicals, for example, ethanol is added to the water extracting concentrate to raise its alcohol content to more than 80%, which causes polysaccharides, proteins, starch, gum, and other substances to precipitate and be removed after filtration. The prior procedure is known as water extraction and ethanol precipitation. Crude plant polysaccharides are routinely isolated using this method [1].

4.4 IDENTIFICATION AND CHARACTERIZATION OF BIOACTIVE CONSTITUENTS:-

The separation of plant extracts remains a major challenge in the identification and characterization of bioactive compounds because they typically comprise a mixture of different kinds of phytochemicals or bioactive substances with varied polarities.

In order to obtain pure molecules, it is common procedure to extract these bioactive substances using a number of separation techniques, such as TLC, column chromatography, flash chromatography, Sephadex chromatography, and HPLC. The pure molecules are then used to determine the structure and biological activity. Additionally, nonchromatography techniques such as Fourier-transform infrared spectroscopy (FTIR), phytochemical screening assay, and immunoassay, which uses monoclonal antibodies (MAbs), can be used to obtain and facilitate the identification of bioactive molecules.

Table 1: A brief summary of the experimental conditions for various methods of extraction for plants material [54]

	Soxhlet extraction	Sonification	Maceration
Common Solvents used	Methanol, ethanol, or mixture of alcohol and water	Methanol, ethanol, or mixture of alcohol and water	Methanol, ethanol, or mixture of alcohol and water
Temperature (°C)	Depending on solvent used	Can be heated	Room temperature
Pressure applied	Not applicable	Not applicable	Not applicable
Time required	3–18 hr	1 hr	3-4 days
Volume of solvent required (ml)	150–200	50–100	Depending on the sample size
Reference	Zygmunt and Namiesnik, 2003; Huie, 2002	Zygmunt and Namiesnik, 2003; Huie, 2002	Phrompittayarat et al., 2007; Sasidharan et al., 2008; Cunha et al., 2004; Woisky et al., 1998

TECHNIQUES:-

1) Chromatographic techniques, including thin layer chromatography (TLC) and bioautographic techniques

High-performance liquid chromatography

2) Non-Chromometric Methods

1..Immunoassay

Immunoassays, which use monoclonal antibodies against drugs and low molecular weight naturally occurring bioactive compounds, are becoming more and more used in the analysis of bioactive chemicals. For enzyme assays, receptor binding studies, and qualitative as well as quantitative analytical procedures, they demonstrate good sensitivity and specificity. Enzyme-linked immunosorbent tests (ELISA) based on MAb are usually more sensitive than conventional HPLC methods. Monoclonal antibodies are made using hybridoma technique, which uses specialised cells. The steps for creating monoclonal antibodies against plant-based medicines using hybridoma technology are as follows:

In order to induce the production of a specific antibody, which is made possible by the proliferation of the targeted B cells, a rabbit is immunised using repeated injections of specific plant-based drugs.

- A mouse or a rabbit can develop tumours. In addition, spleen cells, which are rich in T and B cells, are grown separately from these two species of animals.
- The independently grown spleen cells produce specific antibodies against the plant remedy as well as the myeloma cells that cause tumours.
- Polyethylene glycol is utilised to cause the fusion of myeloma and spleen cells, which results in the creation of hybridoma (PEG). The hybrid cells are grown on selective hypoxanthine aminopterin thymidine (HAT) medium
- The desired hybridoma is selected for cloning and antibody production against a plant medication. Making single cell colonies that may be used in this method and will proliferate will aid in the screening of hybridomas that produce antibodies
- Massive amounts of monoclonal antibodies directed against the chosen plants and drugs are created by cultivating the chosen hybridoma cells.
- Monoclonal antibodies are used to find equivalent medications in a mixture of plant extracts using enzyme-linked immunosorbent tests (ELISA).

2. Phytochemical screening assay

The many secondary metabolic products that are found in plants are commonly referred to as "phytochemicals," which refers to molecules derived from plants. The phytochemical screening assay, a crucial instrument in the investigation of bioactive compounds, is an easy, quick, and affordable process that provides the researcher with an immediate response to the various phytochemical types in a combination. A brief explanation of the experiment's steps Labie illustrates one of the numerous phytochemical screening techniques using secondary metabontes. To determine the kinds of phytochemicals contained in the extract mixture or fraction, phytochemical screening can be done without using the crude extract or acve iracton from the plant material. A list of the appropriate tests for this circumstance is provided

3. Fourier-transform infrared spectroscopy (FTIR)

FTIR has shown to be a powerful technique for the investigation and identification of compounds or functional groups (chemical bonds) present in an unidentified mixture of plant extracts (Eberhardt et al., 2007; Hazra et al., 2007). A molecular "fingerprint" can also be thought of as a result of how distinctive the FTIR spectra of pure compounds are usually. It is possible to determine the spectrum of the majority of common plant chemicals by comparing the spectrum of an unknown compound to a library of known compounds. There are many ways to get samples ready for FTIR. For liquid samples, sandwiching two plates of sodium chloride with one drop of the sample is the most straightforward procedure. The drop deposits a thin layer between the plates. Solid materials can be ground up using potassium bromide (KBT), compressed into a thin pellet, and then examined. In the absence of a solvent, solid samples can be dissolved in a solution such as methylene chloride and then applied on a single salt plate. A thin film of the original substance is then left on the plate once the solvent has evaporated. [54]

5.METHODS FOR STANDARDIZATION OF HERBAL DRUGS:-**5.1.IMPORTANCE OF STANDARDIZATION****5.1.1.STANDARDIZATION OF HERBAL FORMULATION**

Application of Good Manufacturing Practices is required for standardising herbal formulation (GMP). Additionally, it is deemed crucial to research a variety of parameters, including pharmacodynamics, pharmacokinetics, dose, stability, self-life, toxicity evaluation, and chemical profiling of herbal formulations. Aflatoxin level, heavy metal contamination, and Good Agricultural Practices (GAP) in herbal medication standardisation are a few additional factors that are equally important.

5.1.2.STANDARDIZATION OF POLYHERBAL FORMULATION

As polyherbal formulations combine more than one herb to achieve the desired therapeutic effect, standardisation is crucial for maintaining and evaluating the product's quality and safety. Standardization reduces batch-to-batch variation and guarantees the polyherbal formulations' acceptability, safety, efficacy, and quality. The standardisation of several commercially available herbal and polyherbal Madhumehari Churna (Baidynath) formulations, which contain a blend of eight herbs. A traditional remedy called dashamularishta is used to restore physiological processes to normality following childbirth. To determine the identification, purity, and potency of the polyherbal formulation as well as to fix criteria for this Ayurvedic formulation, TLC and HPTLC fingerprint profiles were used. [1]

5.2.STANDARDIZATION AND QUALITY CONTROL OF HERBAL CRUDE DRUGS**• PARAMETERS**

According to WHO (1996a and b, 1992), standardisation and quality control of herbals is the process involved in the physicochemical evaluation of crude drugs. It includes aspects like the choice and handling of crude material, the assessment of the finished product's safety, efficacy, and stability, the documentation of safety and risk based on experience, the distribution of product information to consumers, and product promotion. Usually, quality metrics like these are taken into consideration:

1. Morphology and organoleptic assessment: In the case of whole drugs, morphological traits are essential for discrimination. Typically, it includes attributes like colour, aroma, flavour, form, and size. Among other things, detail qualities include venation, roughness, and fractures.

2. Microscopic and histologic evaluation: Both whole and powdered forms of these are advantageous. Trichomes, calcium oxalate crystals, vascular bundle patterns, stomata, fibres, and parenchyma are mainly examined in this study.

3. Quantitative microscopic analysis: counting the number of vein terminations, stomatal index, palisade ratio, and fibre size. These studies help to differentiate closely related species.

4. Physical evaluation: analysis of a number of physical factors, such as ash content, viscosity, refractive index, melting point, and solubility of extractives and foreign organic materials. The separation of closely related species is aided by this research.

5. Physical evaluation: investigation of a wide range of physical characteristics, including ash values, extractives, solubility, viscosity, refractive index, melting point, and foreign organic components.

6. Qualitative chemical evaluation: This includes identifying and classifying crude drugs according to their phytochemical components. It uses various analytical methods to find and isolate the active ingredients. Identification of the botanical components, extraction with the appropriate solvents, purification, and characterisation of the active components of medicinal value are all steps in phytochemical screening approaches.

7. Quantitative chemical analysis: Calculate the ratios of the major ingredient classes using quantitative chemical analysis.

8. Toxicological studies: These help determine whether potentially harmful microorganisms, pesticide residues, possibly deadly substances, safety testing in animals like LD50, and microbial assays are present or absent.

9. Microbiological parameters: It contains the entire amount of viable, the entire mould count, and the entire coliforms count. Limiters are a quantitative or semiquantitative tool that can be used to measure and limit the level of impurities, such as solvents, reagents used in the extraction of various herbs, and contaminants that are sent directly from the production process. Issue with modern herbal technology

Although herbal medicine has a very strong history of traditional applications and a global restructuring, there are still many obstacles to its promotion, particularly in wealthy countries. Prior to the dissemination of traditional herbal knowledge throughout the world, the following issues must be resolved.

10. Quality issues: The main issues that diminish the effectiveness of herbal preparations and can be regarded as important variables impacting the quality and purity of herbal medicines include adulteration, misidentification of plants, poor collecting and preparation, and inappropriate formulation processes.

11. Processing and harvesting issues: Inadequate pre and post harvest processes, indiscriminate harvesting, poor agriculture and propagation methods, and a lack of processing techniques all contribute to the inferior quality of herbal medications.

12. Quality control-related issues: Standardization, inadequate quality control procedures, and a lack of Good Manufacturing Practices are the largest barriers to preserving the quality of herbal medications (GMP). In small and medium-sized companies, it is also common for farmers and manufacturers to be unaware of the guideline, and for the guideline to not be implemented or regulated.

13. Toxicological studies: Lack of effective monitoring and controlling, as well as a lack of regulatory and governing power in the herbal sector, are necessary necessities for the quality of medicines.

14. Infrastructure-related issue: The primary problems are the absence of processing expertise, skilled employees, complex equipment, use of modern procedures, and nearby instrument production facilities.

15. Pharmacovigilance: In order to discover toxicological information and adverse drug reactions of herbal medications, proper pharmacovigilance in the herbal sector is currently required. It is important to thoroughly monitor adverse responses, contraindications, combinations with other medications, foods, and traditional drugs.

16. Clinical trial: Because safety is still a big worry when utilising herbal remedies, clinical trials are necessary to demonstrate the safety and efficacy of these treatments before introducing them into the global market.

17..IPR and biopiracy: The main obstacle to the promotion of herbal traditional medicine is biopiracy. Thus, recording traditional knowledge is crucial for the future.

18. Use without reason: It's a common misconception that herbal products have no interactions or side effects, but sadly, this is untrue. Therefore, the inappropriate use of these pharmaceuticals can result in a number of issues that could impede their promotion.

19.R&D: Although it is far less in the herbal sector than in allopathic medicine, research and development on dosage, processing, and methods are the most important requirements for any drug. However, in recent years, the trend has altered. In order to better/create monographs and reference standards for marker-based analysis, as well as to understand the mode of action and pharmacokinetics phenomena, research is necessary. The substantial gap between existing ethnopharmacological and modern medicinal plant research is another problem for a sustainable, socio-culturally equitable, and safe supply of herbal medicines.

20.Other issues:Other difficulties preventing the global promotion of herbal medicine include unreliable and erroneous information, a shortage of qualified physicians, a lack of finance, a lack of targeted marketing and branding, and a lack of knowledge exchange. The absence of protection for traditional medical herbs and biodiversity is a serious problem as well. [1]

5.3.SELECTION CRITERIA FOR HERBAL ORIGIN SUBSTANCES APPLICABLE TO STANDARDIZATION AND QUALITY CONTROL OF HERBAL MEDICINE

General characteristics of herbal resources, herbal preparations, and herbal medicines standardisation and quality control:

Herbal ingredients, herbal concoctions, and herbal products in their completed forms are highly complicated. This can make it exceedingly difficult to identify and quantify herbal medicines and make it very difficult to detect adulteration. It should be made clear that utilising markers to identify herbal medicines and measuring the amount of marker compounds present in herbal medicines do not, by themselves, ensure the quality of herbal medicines.

Quality control, which must be applied to every stage of production, must be backed up when needed by good agricultural and collection practises (GACP) and good manufacturing practises (GMP). The choice of references and supervision of standards of excellence It is vital to keep in mind that various herbal medicine components may influence their final quality, safety, and efficacy to variable degrees. This calls for the following principles to be followed when choosing the chemicals for identification and quantification. Components that have been recognised as having therapeutic activity (activities) should be used as indicators. If situation number one is not true but known constituents with established pharmacological activity (activities) should be employed as markers. If none of the aforementioned scenarios occur, it is possible to identify and quantify herbal materials, preparations, and pharmaceuticals by looking at the manufacturing method and marker substance(s) that contain other distinctive constituents (s). To be aware of, microscopic, macroscopic, or DNA analytical methods may be used to identify herbal constituents, as well as, to a lesser extent, herbal preparations and finished herbal products, if the proper reference materials and descriptions are employed. [1]

6.DRUGS FOR HERBAL TECHNOLOGY:-

1.Hibiscus /Shoeblack plant –



In Ayurvedic teas that help decrease blood pressure, hibiscus is frequently utilised. Additionally, it helps with hair loss, hypertension, cough, piles, diarrhoea, and haemorrhaging. It can be effective as a contraceptive as well. [56]

2.Jasmine -



Jasmine has been used to treat hepatitis, cirrhosis-related discomfort in the liver, and severe diarrhea-related stomach pain (dysentery). Additionally, it is used as a sedative to relax, an aphrodisiac to increase sex desire, a stroke preventative, and a cancer treatment. [58]

3..Golden shower Tree –



These are chains of yellow blooms that dangle long and drooping from their tree. It is effective in the treatment of earaches, jaundice, constipation, indigestion, and even skin and heart conditions. [57]

4.Shankpushpi-



The plant was given the name shankpushpi because of its shankh or conch-shaped blooms. Additionally, it aids in improving focus, learning potential, mental tiredness, sleeplessness, stress, anxiety, sadness, etc. Due to its antidepressant effect, it enhances mental wellness and could assist in controlling depression. According to Ayurveda, Shankpushpi relieves tension and anxiety while calming the brain. Its Medhya (improves intelligence) characteristic also helps memory by functioning as a brain tonic. Shankpushpi powder can be consumed with warm milk or water to assist improve focus and memory. Additionally, shankpushpi pills and capsules can enhance cognitive abilities. Ayurvedic Shankpushpi Syrup is a memory and mental acuity enhancer. It helps with mental acuity, forgetfulness, memory loss, low retention power etc. [59]

7.CONCLUSION –

Since the beginning of human history, plants, herbs, and ethnobotanicals have been used for illness prevention and treatment in many different parts of the world. Plants and other natural resources provide the basis of modern medicine, and they also have a big impact on how commercial drug preparations are created today. Around 259% of medicines prescribed worldwide are derived from plants. However, as opposed to pharmaceuticals, plants are commonly used in the healthcare industry. Some individuals favour employing herbal medicines as a kind of treatment. Some people use herbal remedies in addition to conventional medicine. However, in many undeveloped countries, traditional medicine, of which herbal medicine is an essential part, is the only available or affordable type of healthcare. Anytime someone buys herbal treatments, they should feel confidence that the products they buy are safe and contain what they are supposed to, regardless of whether they contain a specific herb or a precise amount of a given herbal component. Consumers should also be given information that is supported by science on dosage, contraindications, and effectiveness. To do this and to guide the ethical manufacture and distribution of herbal medicines, there must be global legislative harmonisation. A herb should be promoted if there is sufficient scientific evidence to support its use in order to enjoy the benefits for the enhancement of public health and the treatment of disease. [60]

8.REFERENCE:-

[1]<https://1drv.ms/b/s!AhmmDyw7Dow-gTL-TN1h8ZcWhmdS>

[2] Calixto J.B. Barz J.. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents), *Med Biol Res*, 2000, 33: 179-189.

[3] Kinghorn A.D. The role of pharmacognosy in modern medicine, *Expert Opin. Pharmacother.* 2002, 3(2) 77-79.

[4] Farooqi A.A. and Sreeramu B.S. Cultivation of medicinal and aromatic crops. University press, Delhi, 2001, 9-10.

[5] General guidelines for methodologies on research and evaluation of traditional medicine. Geneva, World Health Organization, 2000 (WHO/EDM/TRM/2000.1)

[6] Bauer R, Czygan F-C, Franz G, Ihrig M, Nahrstedt A, Sprecher E. Demands on the quality of rationally applicable phytopharmaceuticals (in German). *Dtsch Apoth Ztg.* 1993; 133:41054108

[7] Dixit V.K., Yadav N.P. Recent approaches in herbal drug standardization. *Integr Biol*, 2008; 2 (3): 195-203.

[8] Smille TJ and Khan IA, A Comprehensive approach to Identifying and authenticating Botanical Products, *Clinical Pharmacology and therapeutics* 2010; 87(2): 175-186.

[9] Quality control methods for Medicinal Plant materials, WHO, Geneva, 1998

[10] Cheng XX, Kang TG and Zhao ZZ. Studies on microscopic identifying of animal drugs remnant hair (3): identification of several species of Cauda cervi. *J. Nat. Med* 2007; 57:163171. [11] Lau. PW, Peng Y and Zhao ZZ. Microscopic identification of Chinese patent medicine- Wu Zi Yan Zong. *Wan J.Nat. Med* 2004; 58:258-265.

[12] The Ayurvedic Pharmacopoeia of India. The controller of Publications Civil lines, Ministry of Health and Family welfare of India. Delhi (India): 2001; 3.

[13] Duke JA, Bogenschutz-Godwin MJ, duceulier J and Duke PK. *CRC Handbook of Medicinal Spices* Boca Raton (FL) CRC Press; 2003;187-189.

[14] Ize-Ludlow, Rafone D, Bruck S, Berstein IS, Duchonomy JN and Pena BM.

Neurotoxicities in infants seen with the consumption of star anise tea. *Pediatrics* 2004; 114:

E 653-e656.

[15]. Indian pharmacopoeia, Controller of Publications, New Delhi, India; 1995: 2: A 54.

[16] Horborne JB, *Phytochemical methods, A guide to Modern techniques of Plant Analysis*, Chapman and Hall, UK, Edition 3, 1998.

[17]. Seashottz MB, *Chemometrics and Intelligent Laboratory Systems*, 45; 1999:55. [18]. Miller JN and Miller JC. *Statistics and Chemometrics for Analytical Chemistry*, Prentice Hall Edition 4; 2000.

[19]. Lotrim AR, Ferraz A, Gancalves AR, Silva FT and Bruns RE, Identifying the origin of lignans and monitoring their structural changes by means of FTIR PCA, *SIMCA Bioresource Technology*; 1999; 68: 29-34.

[20] Chow Oon Sim et al., Assessment of Herbal Medicines of chemometrics-Assisted FTIR spectra, *Analytica clinica Acta* 2004;

[21]. Baralcat HH, Sahar Hussin AM, Mohamed Marzouk S, Merfort I, Linscheid M, Nawwar Mohamed AM. Polyphenolic metabolites of *Epilobium hirsutisam*. *Phytochemistry* 1997; 46: 935-941.

[22] Yi-Zeng Liang et al., Quality control of herbal medicines, *Journal Of Chromatography B* 2004; 812: 53-70.

[23]. Li N, Lin G, Kwan YW, Min ZD, Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography. *J Chromatogr A* 1990;849(2):349-55.

[24] Wagner A, Blatt S, Rick! V, *Plant Drug Analysis: A thin Layer Chromatography Atlas*, Springer Berlin, Edition 2, 1996.

[25] Baerheim Svendsen A, *Modern TLC 2. J. Planar Chromatogr.* 1989; 8.

[26]. Chau FT, Chan TP, Wang J. TLCOA: quantitative study of thin-Layer chromatography. *Journal of Bioinformatics* 1998; 14(6):540-541.

[27]. Lin G, Li P, Li SL, Chan SW, Chromatographic analysis of Fritillaria is steroidal alkaloids, the active ingredients of Beimu, the antitussive traditional Chinese medicinal herb.

Chromatograph 2001; 935: 321-38

[28]. Songlin Li et al., Chemical markers for the quality control of Herbal medicine: an overview, *Chinese medicine* 2008; 3:7.

[29]. Warude D, Chavan P, Kalpana J and Pat wardhan B, DNA Isolation from Fresh and Dry Plant Samples with highly Acidic Tissue Extracts. *Plant Mol Biol. Rep* 2000; 21:1.

[30]. Singh M, Bandana and Ahuja PS, *Plant Mol. Biol. Rep.*1999;17:171.

[31]. Joshi SP, Ranjekar PK, Gupta VS. Molecular markers in Plant genome analysis. *Curr. Sci.* 1999;77: 230-240.

[32] Powell W, Morganite M, Andre C, Hanafly M, Vogel J, Tingey S and Rafalski A, *Mol. Breed* 1996; 2: 225-238.

[33] Botstein D, White RL, Skolnick M and Davis RW, Construction of a Genetic Linkage

Map in Man Using Restriction Fragment Length Polymorphisms. *Amt. J. hum. Genet* 1980; 32: 314-331. [34]. Jeffrey AJ. Wilson V and Thein SL, Hypervariable

'minisatellite' regions in human DNA. *Nature* 1985; 314: 67-73.

[35]Williams JGK, Kubelik AR, Livak J, Rafalski JA and Tingey SV, DNA Polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic acids* 1990; 18: 65316535.

[36]Welsh J and Mcchelland M, Fingerprinting genomes using PCR With arbitrary primers. *Nucleic Acids Res* 1990; 18(24): 7213-7218.

[37]Welsh J and Mcchelland M, Genomic fingerprinting using AP- PCR and a matrix of pair wise combination of primers. *Nucleic Acids Res*, 1991; 19:5275-5279.

[38]Caetano Anolles G, Bassam BJ and Gresshof PM, DNA Amplification fingerprinting using very short arbitrary oligo Nucleotide primers. *Biotechnology (NY)* 1991; 9:553-557. [39]Caetano Anolens G, Bassam BJ. DNA amplification fingerprinting using arbitrary oligo nucleotide primers. *Appl. Biochem. Biotechnol* 1993; 42:189-200.

[40]Zabeau M, European Patent Application, Publication no 0534858A 1, 1993

[41]Vos. P, Hoggers R, Blecker M, Reijans M, Vande Lee T, Hornes M Et al., AFLP a new technique for DNA fingerprinting. *Nucleic Acids Res* 1995; 23(11): 4407-4414.

[42]Hao Da Chang, CHEN shi-lin, XIAO pei-gen, PENG Yong, - Authentication of medicinal plants by DNA based marker(4): 250-261.

[43]Kalpana Joshi et al., Molecular markers in herbal drug Technology, *Current Science* 2004; 87(2): 159-165.

[44]Zhang KY, Laung HW, Yeung HW and Wong RN, Differentiation of Lycium barbarum from its related Lycium species using random amplified polymorphic DNA. *Planta Med* 2001; 67:379-381.

[45] <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5905184/>

[46] <https://www.newdirectionsaromatics.com/blog/articles/how-essential-oils-are-made.html>

[47] <https://pharmacvscope.com/percolation-extraction/L>

[48] https://www.researchgate.net/figure/Soxhlet-apparatus-adapted-from-Mitra-2003_fig1_236229671

[49]https://www.google.com/search?q=soxhlet+extraction&rlz=1C1YTUH_enIN950IN950&sxsrf=ALiCzsbrYRMtdYL1FtaVyV5whDQWh8JA:1669450576398&source=lnms&tbm=isch&sa=X&ved=2ahUKEwid4s2EtMv7AhXWytGgGHcgfDGcQUAoAXoECAIQAw&biw=1536&bih=714&dpr=1.25#imgrc=QH1_e5jwD527E9M

[50]https://www.researchgate.net/figure/Scheme-of-pressure-liquid-extraction-PLE-apparatus_fig2_270706324

[51]<https://byjus.com/chemistry/differential-extraction-chromatography/>

[52]<https://www.priyamstudycentre.com/2021/11/thin-layer-chromatography.html>

[53]Application of chromatography (Instrumental method of analyses) Nirali prakashan.

[54]<https://1drv.ms/b/s!AhmmDyw7Dow-gXC9yBJFAc8xVPHi>

[55] <https://1drv.ms/b/s!AhmmDyw7Dow-gTopt5fTvzsCx4Yg>

[56] <https://images.app.goo.gl/nuRVLnEAMzEdivaP9>

[57]https://live.staticflickr.com/65535/49356195152_3c378919c6_b.jpg

[58]<https://images.app.goo.gl/8hWVo4YPtCh7iNzW6>

[59]<https://www.google.com/search?q=shankhpushpi%20fruit&tbm=isch&authuser=3&hl=enGB&sa=X&ved=0CBoQtI8BKAFqFwoTCKjh8b2eivwCFQAAAAAdAAAAABAG&biw=360&bih=693#imgrc=gVxh0sM7U8xxeM>

[60]<https://1drv.ms/w/s!AhmmDyw7Dow-gT5OnfXFgfBVXAaB>

