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A Review on Isolation Techniques and Methods for Active Constituents of Herbal Crude Drug.

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ABSTRACT:

For thousands of years, natural remedies have been the only way to prevent and treat human ailments. Natural substances derived from therapeutic plants, whether in the form of pure chemicals or standardised extracts, present countless possibilities for the creation of novel medications. There are extremely few active compounds in therapeutic plants. Today, it is imperative to develop the most effective methods to extract and isolate these bioactive natural products. The purpose of this white paper is to provide a comprehensive overview of various chromatographic techniques and methods for isolating botanicals from raw herbal medicines. This article also presents some modern chromatographic techniques used to separate active plant constituents. The methods include Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Supercritical Fluid Extraction, Gas Chromatography-Mass Spectrometry, Multidimensional Chromatographic Separations, Gel Filtration Chromatography, High Performance Thin Layer Chromatography. (HPTLC), Flash Chromatography, And Capillary Electrophoresis., Column Chromatography, Ion Exchange Chromatography, Etc.

Keyword: *HPLC, TLC, HPTLC, Column chromatoghaphy Planner Chromatography.*

1. INTRODUCTION-

buIn recent years, various drugs derived from plants and their metabolic activities have been developed. Plants produce natural compounds with powerful antibacterial, antifungal, antiparasitic, antioxidant, antibacterial, antidiarrheal, analgesic, anticancer and antitumor properties, and therefore the most important ingredients of modern medicine are plants. is. Plants have been used as medicines since ancient times, but today, medicinal plants are used by extracting their active ingredients from plants [1]. Secondary plant matter is a biologically derived plant component. Botanicals are the direct source of therapeutic compounds derived from many parts of the plant, such as the seed coat, flowers, roots, leaves, bark, seeds, and pulp of the plant [2]. The active ingredients are highly effective not only in pharmaceuticals, but also in various flavoring protocols, some being used as flavorings and preservatives in food [3]. According to the World Health Organization (WHO), there are approximately 20,000 medicinal plants in 91 countries, including 12 countries with enormous biodiversity. According to the World Health Organization (WHO), such essential oils are used by more than 80% of the world's population.

Population depends on traditional medicine for primary health care[4]. The chemistry of herbal remedies has been thoroughly investigated, our expertise on the minor ingredients is far from enough. Even from the most popular plants like Panax ginseng (ginseng) and Glycyrrhiza uralensis (licorice), new ingredients are revealed practically each year. Recently, a number of minor compounds extracted from Chinese herbal remedies were shown to contain unique chemical structures and considerable biological activity [5]. Phytoconstituent accessible in plant are crude form thus it is vital to isolated them and undertake various purification tests. There are different procedures for isolation and extraction, purification, characterization of phytoconstituents from plant.

Methods For Isolation and Purification 0f Phytoconstituents-

1. Gas Chromatography - Mass Spectroscopy
2. Supercritical Fluid Extraction
3. Stimulated Moving Bed Chromatography
4. Multi-Dimensional Chromatographic Separation
5. Gel Filtration Chromatography
6. Thin Layer Chromatography (TLC)

7. High Performance Thin Layer Chromatography (HPTLC)
8. Flash Chromatography
9. Capillary Electrophoresis
10. High performance liquid chromatography (HPLC)
11. Column chromatography
12. Ion-exchange chromatography

2) Methods and Materials: -

2.1) Gas Chromatography- Mass Spectroscopy

Numerous pharmacologically potent components in herbal medicinal plants are known to be volatile chemical molecules. Gas chromatography is essential for the analysis of volatile chemical compounds in herbal treatments as a result. In this examination, each component of a compound combination is separated using gas chromatography, and the components are then analysed using mass spectrometry. A wide range of volatile and nonvolatile chemicals can be component-qualified, component-separated, and quantified using GC-MS in a single run. Several connections can be examined at once. On each compound in a sample, GC-MS offers both qualitative and quantitative information. A technique for dissolving mixes of mixtures into their constituent parts is gas chromatography, often known as gas-liquid chromatography [11-12].

Gas chromatography is employed to isolate and separate greater amounts of target chemicals with high purity. On each compound in a sample,

GC-MS offers both qualitative and quantitative information. A technique for dissolving mixes of mixtures into their constituent parts is gas chromatography, often known as gas-liquid chromatography. This method depends on the redistribution of components, sometimes known as support materials, between stationary phases. For GC separations, there are two different kinds of stationary phases. In gas chromatography, the species to be separated are dispersed in mobile gas phase and stationary liquid phase (GLC, or simply GC). In gas chromatography, the species to be separated are dispersed between the mobile gas phase and the stationary liquid phase. The samples are first vapourised at high temperatures. The vapourised samples are then injected into the chromatographic column's head, where The inert gaseous mobile phase carries them. There is liquid stationary phase in the column itself and is adsorbed on inert solids surface. The distribution of chemical species in the gas phase is used to examine the rate of migration for those species. The species that completely disperses itself into the mobile gas phase will travel at the same gas phase flow rate.[13-18].

Column is most important component of GC. The columns used in GC chromatography are divided into two types :-

1) Wall coated open tubular column

2) Support coated open tular column

In GC, separation columns such as open tubular columns, capillary columns, and packed columns are employed. Wall-coated open-tube columns (WCOT) are the first type of capillary columns, and support-coated open-tube columns (SCOT) are the second type. The WCOT column's wall is covered with a thin coating of stationary phase. An adsorptive material, such as diatomaceous earth, is initially applied in a thin coating to the SCOT column's wall, a material consisting of the skeleton of single-celled marine plants. A liquid stationary phase is then applied to the adsorbed solid. SCOT columns have a higher sample capacity and can contain more stationary phases than WCOT columns. While SCOT columns may contain more stationary phase than WCOT columns because to their larger sample capacity, WCOT columns still offer higher column efficiency. One of the most widely utilised types of capillary columns is coated fused silica open tube columns. With the help of an external cryoptrap and a 15m x 0.32mm i.d. PB column, several medicines have been isolated. an cryotrap Deans Switch gadget gained flow switching between cryotrap and detector (FID). The Deans switch gadget boosts the GC's resolving power. Deans Switch dependably directs effluent to a chosen Column for rapid and simple compound separation. The distribution of chemical species in the gas phase is used to analyse the rate of chemical species migration. Species that disperse perfectly in the mobile phase of gas will travel the same gas phase flow rate. The species that disperses into the stationary phase will migrate gradually and elute gradually.

2.2) Supercritical Fluid Extraction

The most efficient method to obtain priceless herbal ingredients. This is the procedure for using CO2 to separate one component from another as an extraction solvent. Critical pressure of 74 bar and critical temperature of 31^{0}C are the extract conditions for supercritical CO_2.

The analysis of complex materials requires separation of analysis form sample matrix. It is simple, inexpensive, rapid which gives quantitative analytes without degradation [19-20].

Process: -

These systems have a pump for CO2 and a pressure cell that maintains pressure in the collection tank and system. Liquids fed into the heating zone are heated. A heating zone is used to warm the liquid that is pushed there. Then passing in extraction process. These matrix ranges form extraction cell in separator at low force. This extracting material is out. Then CO_2 be It is cooled, compressed and released into the airspace.

2.3) Simulated Moving Bed Chromatography

Simulated moving bed chromatography technology was created in the 1960s to purify sugars from molasses. Simulated moving bed method is improvement the traditional chromatography to continuous the chromatography process. Stimulated moving bed process used in petrochemical industry in the 1940s [21-22].

Simulated moving bed chromatography, a process, continuous separation method, is a strong large-scale tool separations of natural pharmaceuticals, with the advantage of reduced solvent usage in a short period of time. Simulated moving bed process it's numerous application and theoretical description has recently summarised in reviews. The simulated moving bed system consists of his two types of multiple chromatographic columns and multiple positive valves to actually simulate the flow of liquids and solids. In simulated moving bed technoloav. the feed throat takes the place of the moving bed. The positions of the constant movement of the eluent inlet, the desired product outlet, and the undesirable product outflow gives the appearance of a moving bed in which the solid particles are continuously flowing with the liquid particle stream.

Process :-

Simulated moving bed chromatography based on liquid flow (that is, providing a constant flow of solids in mobile is an impractical manufacturing process. This solid is packed into a high-pressure column. Simulated In moving bed chromatography, the column is ring-shaped and consists of four divisions, each having one or more columns. The sections are show the different roles they are

❖ **SECTION I-**

Regeneration of adsorption (desorption of A and B, still present, form the solid)

❖ **SECTION II-**

Desorption of B and adsorption of A (that are extract, rich in A, is not contaminated with B)

❖ **SECTION III-**

Adsorption of A and adsorption of B (that are Raffinate, rich in B, is not contaminated with A)

❖ **SECTION IV-**

Regeneration of eluent (adsorption of B and A still present, form the fluid).They are directed alternately and form a ring of columns. These columns cannot be moved. The positions of the entrance and exit are periodically reversed. [23-24]

2.4) Multidimensional Chromatography

Multidimensional chromatography is a chromatographic technique capable of separating complex and difficult substances. Multidimensional chromatography preserves separation by passing samples through two stages of separation. Multiple columns, each with a unique stationary phase, are used to achieve this.

By arranging the columns that are parallel to one another, you can To divide components from one another, choose some from the first column and move them to the rule column. Complex chemicals that are unable to be separated in the first column stop can be separated in the second column.

Gas chromatography and liquid chromatography both benefit from multidimensional chromatography. This chromatographic technique requires the use of a remote separation phase on each column.

For both qualitative and quantitative analysis, there are a variety of exceedingly complex mixes that need to be separated but can't be done so because of their complex mixing. One-dimensional chromatography can provide some separation, but under certain conditions it is not possible to completely separate high-efficiency columns from single-column chromatography. However, these mixtures can be separated more effectively by combining two different chromatographic separation steps with different selectivities for the analytes of interest. For the best results, keep the sample after the initial separation and move it to the second dimension for additional analysis.[25].Multidimensional chromatography can be used for water ID identification including:

*Identification of proteins and peptides

*Identification of DNA fragments

*Determination of biomarkers in petroleum and oil

*Drug isolation in urine

*Draw a solution in plasma

2.5) Gel Filtration Chromatography

Gel filtration chromatography is a kind of partition chromatography used to separate molecules of differing molecular sizes. [26]

This technique is also referred as:

*gel permeation

*gel exclusion

*molecular sieve chromatography

Gel filtration is a particularly attractive technique for separating substances according to their relative molecular size [28-29]. In this process, the dissolved material is passed through a chromatographic column consisting of porous gel particles. In the absence of adsorption processes, this type of Chromatography is a process that uses distribution of dissolved molecules between the phase within the gel granules and the moving external phase. Due to the size of the molecules and the fact that their rates of passage through the column are inversely correlated with the fluid volume that is available to them within the column, it is thought that fractionation happens when diffusion into the gel pores is restricted but not prohibited [30-31]. In order to construct each column, a thin slurry of gel particles in buffer solution was poured into a vertical tube that had already been partially filled with buffer, while simultaneously allowing more liquid to percolate through the developing gel bed.

The rationale for this process is to separate the two using a substance that contains dextran micromolecules depending on variations in molecule size. The beds that make up the stationary phase in these chromatography methods are porous, sponge-like materials that operate as molecular dimensions and tiny size ranges. If a column made of molecular sieves is allowed to pass through an aqueous solution containing a variety of herbal medications with molecules of various sizes. Larger molecules can easily pass through the column because they are smaller than the filtration media. Smaller molecules can only move slowly over the column because they become trapped in gel pores. It is important to highlight that the molecules in the sample solution are isocratically eluted, therefore separate buffers are not required for the separation. They are eluted sequentially while having smaller molecules. The molecules may appear in the effluent in decreasing order of size when a combination of molecules is passed through a column of porous gel granules. [32-33]

2.6) Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a commonly used technique in the plan herb industry. The advantage of using this technique; Such as speed, versatility & low cost make it one of the leading techniques used for locating & analysing bioactive Components from herbal crude drug. TLC is additionally used to distinguish between different chemicals in a combination by comparing their RF readings to known substances. Spraying reagents for phytochemical screening that cause a colour change corresponding to phytochemicals present in the raw herbal medicine, or observing viewing the plate with UV light Additionally, it is employed to confirm the identity and purity of isolated compounds. [34]

Using either silica gel-G or silica gel-GF (which exhibits fluorescence under UV illumination). Exactly 30 g of silica gel were weighed and distributed in 60 ml of distilled water as a homogenous suspension for several minutes, after which this homogenous suspension was evenly spread on a TLC plate and the plate was air-dried.. These TLC after 30 minutes of hot air oven drying at 110 °C, plates were stored in a dry environment until being utilised as needed. The crude extracts were then diluted to create samples with appropriate solvents or mobile phases. Samples were then spotted onto TLC plates (1-10 µl volume) from below using a capillary tube 2 cm above the plate [35]. The movement of active substance was expressed as retention factor (R). Phytochemical Rf values reveal the polarity of the substance and segregation of this phytochemicals in TLC separation process. Different Rf values of botanical compounds are provide information on their polarity through the usage of various solvents systems. The TLC plate was lightly UV light at 254 nm was used to find spots that had dried (low wavelength) and 366 nm (high wavelength).. Rf = Distance travelled by solute / Distance travelled by solvent[35].

2.7) High Performance Thin Liquid Chromatography (HPTLC):

Herbal medicines are combinations of active ingredients. According to the European Pharmacopoeia, herbal medicines are mainly whole plants or herbal medicines are shattered plant pieces that haven't been handled, usually in dried form. Oxazolidinone derivative rivaroxaban, is the first bioavailable medication taken orally that directly inhibits the anticoagulant factor Xa (Cstuart-Prowers factor). High-performance thin layer chromatography (HPTLC) is mainly used to analyze plant active substances.

HPTLC is used for component identification, impurity determination, and bioactive compound quantification. it is more accurate. Reproducibility and easy documentation of results compared to TLC. The versatility of using high-resolution mixing particle size sorbents or chemically altered plates with other it is a perfect TLC approach for analytical purposes thanks to the equipment. [38-39]

***Plant Collection and Preparation for Extraction**

The traditional medical applications of this plant led to its selection for investigation. Water was used to clean and maintain the freshness of the plant material. For the study, various plant components including leaves, stems, bark, flowers, fruits, and roots were gathered and preserved in sterile, airtight containers for 7 days at room temperature. You must make sure the plant material is really healthy and pest-free. A certified botanist must certify the

plant material samples that were collected. The freshly harvested plant material was dried outdoors in the shade. Avoid exposing this plant matter to sunlight as it will lose potency and volatile constituents. The dried plant material was then ground and separated according to particle size. The powder was then processed with different solvent methods such as the Soxhlet method [41], Microwave, ultrasonic, supercritical fluid extraction, solid phase microextraction, etc. Commonly used polar solvents and extractable secondary metabolites are listed in Table 1. 45. Methanol is rarely used for extraction due to its propensity to blind animals in bioassays. Acetone is volatile and miscible with water, and it dissolves a variety of hydrophilic and lipophilic components from plant materials low toxicity and less polar solvents are employed to extract materials such as ethyl acetate, dichloromethane, chloroform, and dichlorobenzene less polar components. Hexane and petroleum ether are two typical non-polar solvents used to destroy plant matter[42].

2.8) Flash Chromatography

Flash Chromatography In 1901, the Russian batanist Mikhail Tsvet invented this technique. 1978, American organic chemist Dr. W. Clark, Columbia University flashes his chromatographic techniques. Flash chromatography is a purification method that uses air pressure for quick separation as compared to gravity chromatography's lengthy, ineffective process. Flash chromatography is a method of chemical separation used to clean up chemical combinations. Flash chromatography shows different types of [43-45]:

*Adsorption chromatography

*Partition chromatography

*Ion exchange chromatography

*Molecular exclusion chromatography

*Affinity chromatography

Medium pressure chromatography, commonly referred to as flash chromatography, is used to split molecular mixtures into their constituent molecules components.

There are two ways that flash chromatography varies from traditional techniques -

*Littler silica gel debris 250-400cross segment are utilised.

*Second due to confined flow of dissolvable introduced approximately via way of means of the little gel debris, pressurised fueloline Ca. (pressurised fueloline at 50-200psi).

❖ **Various components of flash chromatographic systems:**

A) Sorbent system -

The most used in chromatography is silica for the stationary phase. Flash chromatography uses alumina (A1203) and silica gel (SiO2) as adsorbents. They were sold in different sizes, with bottle labels bearing numbers such as "Kieselgel 60" and "Kieselgel 230-400". There are show the some adsorbents in mostly used in the flash chromatography technique they are show the follows: [45-46]

*Silica- Silica is a used in slightly acidic medium.

*Florisil- It is used in mild, neutral medium.

*Alumina- They are used in basic or neutral medium.

*Reverse phase silica- The highly polar compounds.

B) Solvent system -

Flash chromatograph is performed mixture of two solvent first is polar and second is non polar components. [45-47]

1] One-component solvent system-

The one components solvent used the follow:

*Hydrocarbons: Pentane, petroleum ether hexanes

*Ether and dichloromethane

*Ethyl acetate

2] Two components solvent system-

 # Ether/ petroleum ether, ether/ hexane, and ether/pentane:

Hydrocarbon components were dependent on availability and boiling requirements range. Petroleum ether has a low boiling point, hexane is commercially available, and pentane is expensive.

 # Ethyl acetate/hexane:

The suitable for the standard of ordinary compounds and best for the difficult in the separation.

*10% ammonia in methanol solution:

*If basic (nitrogen group), it may be necessary to add approximately 0.1% pyridine to the solvent mixture.

*A tiny amount of acetic acid can be used to separate acidic substances..

C) Column Selection-

Choose columns with 10,20and 40 mm id according to the given requirements. Thomson Flash Columns are available in various sizes from 4g to 300g based on silica gel for simple scaling of chemical processes. Thomson Flash offers other packaging materials such as Flash columns can be used for a variety of reactions thanks to amine and C18 flash columns, which are available to end users. [48]

D)solvent selectivity

Solvent selectivity is defined as a solvent that selectively affects retention of one compound in a mixture over others, thus affecting RH and column capacity. Solvent selectivity should be adjusted so that RH > 0.20. TLC separation under typical conditions suitable for effective flash chromatography separation. The column volume difference CV predicts the column volume that can be successfully separated with a single column load. The higher the CV, the greater the effective capacity of the column.

2.9) Capillary Electrophoresis

Capillary electrophoresis has been used for decades as an important analytical method for drug characterization. H. Used for determination of active ingredients and impurities, chiral separation, analysis of pharmaceutical excipients, etc. The main advantage is that depending on the substances and samples to be separated, the analyst can use a variety of separation techniques analyzed. Capillary electrophoresis has several advantages, including faster rate, great efficiency, ultra-small sample volume, and minimal solvent consumption.Capillary electrophoresis is favoured over HPLC in the evaluation of numerous plant raw material components due to its better resolution [49-53].

Capillary electrophoresis separates analytes based on changes in their size to charge ratios. The choice of an appropriate separation solution is crucial for producing a significant difference in the electrophoretic mobility of the analytes while preserving an ideal and steady electroosmotic flow [54]. The electric field is important for separations in capillary electrophoresis. The capillary tube's liquid flow is caused by potential difference. The charge, size, shape, and resistance of electrically charged particles all affect their speed. The studied compounds with an electric charge possess electric mobility, causing them to split apart. The total charge in the matrix and capillary walls influences capillary electrophoresis resolutions. The capillary is the most important component of the device. The capillary is the most critical component of equipment. Their size and substance (fused silica or quartz) may have an impact on the analytical conditions. The usage of quartz capillaries has the benefit of also serving as a measuring cell for UV/Vis or a fluorescence detector [55-56].

- **Free Solution Capillary Electrophoresis (FSCE) :-**

Most drugs are basic and thus ionize at low pH. Free-solution capillary electrophoresis was used with a low-pH buffer system to separate a variety of basic pharmaceuticals. The size and quantity of the analytes' positive charge are used to separate them. It is possible to distinguish combinations of acidic and basic medicines using FSCE at high pH (cationic or anionic). Anions over pH 7.0 can be swept to the detector by the electroosmotic flow created by the applied current. Charge type and solute density have an impact on how quickly an analysis migrates. [57-58].

- **Non-aqueous capillary electrophoresis (NACE):-**

Since it is challenging to separate water-insoluble parent medicines using FSCE, the NACE method can be applied. Organic solvents like acetonitrile or methanol are employed in place of water. [59-62].

- **Capillary zone electrophoresis with UV detector:-**

Analytes can be measured directly or indirectly using UV detectors. This approach is used when the analyte does not absorb UV light well. Chromophore ions such as phthalate and 2,3- pyrazinedicarboxylic acid were added to the buffer to separate the chemicals [63].

2.10) High Performance liquid Chromatography (HPLC)

Separation identification technology. Used for estimation of biological and pharmaceutical samples. HPLC is modern form of column chromatography. In HPLC, purification takes place on a separate column between mobile and stationary phases. Retention time as a function of the interaction between the stationary phases on which the molecule is analyzed. This sample is added to a tiny volume of mobile phase flow after being physically or chemically delayed by the stationary phase. The parameters of the analyte, the makeup of the stationary phase, and the mobile phase all affect lag time. Retention time is the term for this period. The mobile phase composition is sieved to achieve separation. during an analysis called gradient rise. Gradients separate analyte mixtures [64-66].

❖ **HPLC analysis**

Combining there are two alternatives for detecting plant material using HPLC separation along with different spectroscopic detection techniques as PDA, MS, and NMR. On the one hand, chemotaxonomic applications can make use of particular marker molecules. Rosa genus flavonol acyl glycosides include 3-hydroxy-3-methylglutaric acid, and pattern recognition analysis combined with HPLC fingerprinting can be used to identify the source plant from extracts. [67]

In a study on the chemical phylogeny of Taxus. By using principal component analysis (PCA) and hierarchical cluster analysis (HCA), the LC-PDA-MS fingerprint chromatograms were examined to separate the eight researched species into six well-supported groupings, with most species correctly assigned [68] .A combination of PCA and pharmacological fingerprinting of two LC and 1 H NMR fingerprints was used to comprehensively characterize a commercial extract of willow bark (Salix spp) [69]. Combining HILIC (see 4.2.5) with a reversed-phase column enabled the identification of six species of Reishi, a fungus used in traditional Chinese medicine [70]. Phytochemical analysis, such as the identification of unprocessed plant extracts, still relies heavily on HPLC analysis for CH3. [71,75-76]

Apart from biological identification purposes, the main application of HPLC methods is dereplication. H. Ideally, identify known metabolites in the extract early in the fractionation process. 14 chemicals were definitively detected using his UHPLC-PDA-TOF-MS apparatus at Lippia spp., while an additional 28 compounds were potentially identified[73]. When used for analysis, UHPLC (UPLC), reviewed by i. H. Eugster et al., showed that a stationary phase with a particle size of less than 2 µm was combined with an instrument capable of high-speed elution and high backpressure to achieve The analysis of complex mixtures has been greatly improved. It is clearly outlined in al [74].

We were able to significantly improve the sensitivity of NMR analysis using microcoil NMR. This has been Sturm and Seger recently examined LC-NMR techniques. [75]

2.11) Column chromatography

In 1950 and 1970, are toxicologists routinely used column chromatography for isolation and purification of herbal drug form biological fluids and separation of chemical compounds form mixture. Column chromatography are used in herbal extraction plants for the separation of products and chemical process. The engineering are manufactures high quality chromatography columns and also leading and manufacture of herbal extraction plant and herbal extraction plant machinery in India. In the column chromatography used, solid and liquid samples are separated and washed. Column chromatography presents an adsorbed stationary solid phase and a separate compound, the liquid mobile phase.

Principal:

The migration of the mixture's distinct components happens at various speeds depending on where in the column the mobile phase is added after the mixture to be separated. When compared to substances with strong adsorption and affinity to the stationary phase, poor adsorbers move more quickly. Eluted last are slow-moving components, which are removed earlier in the process.

The rate component movement is expressed as:

RF= The distance travelled by solute /the distance travelled by the solvent

RF is the retardation by the solvent .

The parts of a typical system in column chromatography use liquid and gas as mobile phases are two phases and include:

1)Stationary Phase-

This phase is generally separated from the solid and suitable for analysis due to its good adsorption properties. Do not cause mobile phase flow.

2)Mobile phase-

This phase consists of a solvent-complementary stationary phase. This phase acts as a solvent for the developer and eluent. The mobile phase acts as a solvent.

***Column:-**

For liquid chromatography

1) In liquid chromatography-

They are developer and eluent. Columns used in liquid chromatography have a length of 2 to 50 cm, internal diameter of 4 mm, and are made of stainless steel.

2) For gas chromatography-

Gas chromatography uses glass or stainless steel columns having an interior diameter of 2 to 4 mm and a length of 1 to 3 m.

3) Injector system.

The injector system serves to reproducibly feed the test sample to the top of the column.

4) Defector & chart recorder

As the analytes in the effluent leave the column, a detector and chard recorder continually record their concentrations. The measurement of physical properties forms the basis of detection. Each separated analyte in the recorder is represented by a peak[77-79].

2.12) Ion Exchange Chromatography

To separate charged biomolecules, ion exchange chromatography is frequently used**.** such as proteins, peptides, amino acids and nucleotides are separated by using this technoique. The method utilised for the most fundamental and powerful interactions is ion-exchange chromatography, which is regarded as a reasonably cost-effective and efficient sample purification medium.

Cation exchangers always feature negatively charged groups, and these draw in a positively charged cation. Because the acidic groups become ionised and provide a negative charge, these exchangers are also referred to as "acidic ion-exchange materials.". [80-81]

Principle:-

The ion exchanger, which is the stationary phase in this type of chromatography, and the analyte are attracted to each other by opposite charges.

1) The basic component of lon exchangers is a charged group covalently bonded to the surface of an insoluble matrix.

2) Positively or negatively charged groupings can be found in the matrix. The matrix's charged groups are surrounded by ions with the opposite charge when it's suspended in an aqueous solution.

3) Ions can be exchanged reversibly in this "ion cloud" without altering the composition or characteristics of the matrix.

Positively charged cations are retained in cation exchange chromatography because the stationary phase comprises functional groups that are negatively charged.

R-X-C++ M BR-X M++ C++B

To keep anions in solution, anion exchange chromatography employs positively charged functional groups:

R-X+ A+M+ BR-X+ B+ M++ A-

3. Conclusion-

Plants, herbs, and their phytochemicals have been used since ancient times and are used all over the world to effectively treat a variety of ailments. It is very difficult to separate the active ingredient from the plant material. As bioactive components from plant materials consist of multicomponent mixtures, their separation and isolation presents a great challenge. Most of them have been purified and isolated by various chromatographic techniques and various extraction and purification techniques**.**

REFERENCES:-

[1]. Sasidharan, S.; Chen, Y.; Saravanan. D.; Sundram, K.M. and Latha, L.Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines, 8(1): 1-10.

[2]Azwanida, N.N. (2015). A review on the extraction methods use in medicinal plants. principle, strength and limitation. Med Aromat Plants, 4(196): 2167-0412

[3.] Altemimi, A.: Lakhssassi, N.; Baharlouci, A.; Watson, D.G. and Lightfoot. D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants, 6(4): 42

4. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complementary Altern Med. 2006;6:35–41. [PMC free article] [PubMed] [Google Scholar]

5. WHO traditional medicine strategy: 2014–2023; 2013. http://www. who.int/medicines/publications/traditional/trm_strategy14_23/en/. Accessed 29 Dec 2017.

6. Akinyemi K O, Oladapo O, Okwara C E, Ibe C C, Fasure K A. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicilin resistant Staphylococcus aureus activity. BMC Complement Altern Med. 2005;5:6. [PMC free article] [PubMed] [Google Scholar]

7. Cai Z, Lee F S C, Wang X R, Yu W J. A capsule review of recent studies on the application of mass spectrometry in the analysis of Chinese medicinal herbs. J Mass Spectrom. 2002;37:1013-1024. [PubMed][Google Scholar]

8. Cannell RJ P. Natural Products Isolation. New Jersey: Human Press Inc; 1998. pp. 165-208. [Google Scholar]

9. Chanda S V, Parekh J, Karathia N. Evaluation of antibacterial activity and phytochemical analysis of Bauhinia variegate. L bark Afr J Biomed Res. 2006;9:53-56. [Google Scholar]

10. Cosa P, Vlietinck A J, Berghe D V, Maes L. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept' J Ethnopharmacol. 2006;106:290-302. [PubMed] [Google Scholar]

11.Ylinen M. Naaranlahti T, Lapinjoki S. Huhtikangas A. Salonen ML, Simula LK, Lounasmaa M. Tropane alkaloids from Atropa elladonna part-L capillary gas chromatographic analysis, Planta Med. 1986, 52, 2, 85-87.

12. Malliard MP, Wolfender JL. Hostettmann K. Use of liquid chromatography thermospray mass spectrometry in phytochemical analysis of crude plant extract. J. Chromatogr., 1993, 647, 147–154.

13. Grob RL, Barry EF (eds) (2004) Modern prac- tice of gas chromatography, 4th edn. Wiley, New Jersey.

14. Cazes J (ed) (2005) Eving's analytical instrumentation handbook. 3rd edn, vol 23, Marcel Dekker, New York.

15. Zhang A, Sun H, Wang X (2018) Mass spectrometry-driven drug discovery for development of herbal medicine. Mass Spectrom Rev 37(3):307-320

16. Wei D, Chang X, Cao Y, Yan H, Shang E, Qian D, Duan JA (2019) GC-MS based metabolomics to evaluate three commercial products of Chrysanthemun mori. https://doi.org/10.20944/preprints201902.0116.v1

17. K. S. Banu and L. Catherine, "General Techniques Involved in Phytochemical Analysis.," International Journal of Advanced Research in Chemical Science., vol. 2, pp. 25-3, 2015.

18. W. A. König and D. H. Hochmuth, "Enantioselective Gas Chromatography in Flavour and Fragrance Analysis.," J.Chromatogr. Sc., vol. 42, pp. 423-439., 2004.

19. Edit Székely, Supercritical Fluid Extraction, Budapest University of Technology and Economics. http://sunny.vemt.bme.hu/sfe/angol/supercritical.html. Retrieved 2007-11- 20. 20. Supercritical Fluid Extraction, Density Considerations, http://eng.ege.edu.tr/~otles/ SupercriticalFluidsScienceAndTechnology/ We488d76f2c655.htm. Retrieved 2007- 11-20.

21. Okinyo-Owiti DP, Burnett PGG, Reaney MJT. Simulated moving bed purification of flaxseed oil orbitides: unprecedented separation of cyclolinopeptides C and E. J Chromatogr B. 2014;965:231–7.

22. Kang SH, Kim JH, Mun S. Optimal design of a tandem simulated moving bed process for separation of paclitaxel, 13-dehydroxybaccatin III, and 10-deacetylpaclitaxel. Process Biochem. 2010;45(9):1468–76.

23. Ruthven, D.M. and C.B. Ching, “Counter-current and simulated moving bed adsorption separation processes,” Chem. Eng. Sci., 44, p. 1,011 (1989).

24. Storti, G., M. Mazotti, M. Morbidelli and S. Carra, “Robust design of binary countercurrent adsorption separation processes,” A.I.Ch.E. J., 39, p. 471 (1993).

25.https://www.chromatographytoday.com/news/gc-mdgc/32/breaking-news/what-is-multidimensional-chromatography/34426.

26. E. Uliyanchenko, "Size-exclusion chromatography-from high-performance to ultra-performance.," Anal Bioanal Chem., vol. 406, pp. 6087-94, 2014.

27. E. Uliyanchenko, "Size-exclusion chromatography-from high-performance to ultra-performance.," Anal Bioanal Chem., vol. 406, pp. 6087-94, 2014.

28.J. PORATH AND]'. FLODIN, Nature, 183 (1959) I657.

29. S. HJERTEN AND R. MOSBACH, Anal. Biochem., 3 (1962) lO9.

30. J. F'ORATH, Advan. Protein Chem., 17 (1962) 209.

31. A. TISELIUS, J. PORATH AND P. A. ALBERTSSON, Science, 141 (1963) 13.

32. Walls D, Sinéad T. Loughran. Protein Chromatography: Methods and Protocols, Methods in Molecular Biology.vol. 681;2011.

33. Helmut D. Gel Chromatography, Gel Filtration, Gel Permeation, Molecular Sieves. A laboratory Hand book, Springer-Verlag;1969.

34. Rahalison, L., Hamburger, M., Hostettmann, K., Monod, M. and Frenk, E. (1991). A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. Phytochem. Anal. 2: 199–203.

35. M Sonam, RP Singh, S Pooja (2017) “Phytochemical Screening and TLC Profiling of Various Extracts of Reinwardtia indica,” 9 (4): 523-527.

36.“CAMAG®Automatic TLC Sampler 4 (ATS 4) | CAMAG.” https://www.camag.com/product/camag-automatic-tlc-sampler-4-ats-4#technical-specifications (accessed Dec. 07, 2020).

37. P N Preethi, G J Jothi, A Rajasekaran, R Arivukkarasu (2020) “Rapid Densitometric Method for Quantification of Gallic Acid and Coumarin in the Leaf of Medicinal Plant Pouzolzia Bennettiana From the Nilgiri Hills Using Hptlc,” Int. Res. J. Pharm., 11 (3): 40-44. doi: 10.7897/2230-8407.110328.

38. K Gopalasatheeskumar, G Ariharasivakumar, V K Kalaichelvan, T Sengottuvel, V S Devan, V Srividhya (2020) “Antihyperglycemic and antihyperlipidemic activities of wild musk melon (Cucumis melo var. agrestis) in streptozotocin-nicotinamide induced diabetic rats,” Chinese Herb. Med., 12 (4): 399-405. doi: 10.1016/j.chmed.2020.02.005.

39. M Attimarad, K K Mueen Ahmed, B E Aldhubaib, S Harsha (2011) “High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery,” Pharm. Methods, 2(2): 71-75. doi: 10.4103/2229-4708.84436.

40. “Detection - densitometric evaluation (TLC/HPTLC)| Thin Layer Chromatography (TLC/HPTLC).”https://lpp-equipment.com/Products/Product-line-PDF/productline/1263/productgroup/1066/productgroupl2/1113 (accessed Dec. 06, 2020).

41. G Km (2019) “Significant Role of Soxhlet Extraction Process in Phytochemical Mintage Journal of Pharmaceutical & Medical Sciences Significant Role of Soxhlet Extraction Process in Phytochemical,”.

42. T M Bandiola (2018) “Screening of medicinal plants for,” Int. J. Pharm., 8(1): 137-143

43. https://en.wikipedia.org/wiki/W_Clark_Still”

44.William CSand Hill DC. General methods for flash chromatography using disposable

column. Mol. Divers, 13(2), 2009, 247-252.

45.Still WC. Kahn M., Mitra A, Flash chromatography, J.Org.Chem. 43(14),1978; 2923-2925.

46. www.sorbeadindia.com

47. www.chem.rochester.edu/how to flash.html Kahn M., Mitra A, Fl

48. www.saiadsorbants.com

49.Altria, K., Marsh, A., Sa¨nger-van de Griend, C., Electrophoresis 2006, 27, 2263–2282.

50.Barcelo-Barrachina, E., Moyano, E., Puignou, L., Galceran, M. T., Electrophoresis 2007, 28, 1704–1713.

51.Qi S. D., Li Y. Q., Deng Y. R., et a/., J. Chromatogr. A, 2006. 1109, 300

52.Hsieh S. C., Huang M. F., Lin B. S., et al., J. Chromatogr A, 2006. 1105

53. Yang, Z.; Li, Z.; Zhu, J.; Wang, Q.; He, P.; Fang, Y. Use of different buffers for detection and separation in determination of physio-active components in oolong tea infusion by CZE with amperometric detection. J. Sep. Sci. 2010. [CrossRef] [PubMed]

54. Harstad RK, Johnson AC, Weisenberger MM, Bowser MT. Capillary electrophoresis. Anal Chem. 2016;88(1):299–319.

55. Liu, H.; Gao, Y.; Wang, K.; Hu, Z. Determination of active components in Cynanchum chinense R. Br. by capillary electrophoresis. Biomed. Chromatogr. 2006. [CrossRef]

56. Pang, H.; Wu, L.; Tang, Y.; Zhou, G.; Qu, C.; Duan, J.A. Chemical analysis of the herbal medicine salviae miltiorrhizae radix et rhizoma (Danshen). Molecules 2016, 21, 51. [CrossRef]

57. Hudson, J., Golin, M., Malcolm, M., and Whiting, C. (1998) Capillary zone electrophoresis in a comprehensive screen for drugs of forensic interest in whole blood: an update. Can. Soc. Forens. Sci. J. 31, 1.

58. Altria, K. D., Bryant, S. M., and Hadgett, T. (1997) Validated capillary electrophoresis method for the assay of a range of acidic drugs and excipients. J. Pharm. Biomed. Anal. 15, 1091–1101

59. Esaka, Y., Okumura, N., Uno, B., and Goto, M. (2001) Non-aqueous capillary electrophoresis of p-quinone anion radicals. Anal. Sci. 17, 99–102.

60. Cherkaoui, S., Varesio, E., Christen, P., and Veuthey, J.-L. (1998) Selectivity manipulation using nonaqueous capillary electrophoresis. Application to tropane alkaloids and amphetamine derivatives. Electrophoresis 19, 2900–2906.

61. Hansen, S., Tjornelund, J., and Bjornsdottir, I. (1996) Selectivity enhancement in capillary electrophoresis using non-aqueous media. Trends Anal. Chem. 15, VII.

62. Bjørnsdottir, I. and Hansen, S. H. (1995) Determination of opium alkaloids in crude opium using non-aqueous capillary electrophoresis. J. Pharm. Biomed. Anal. 13, 1473–1481.

63. Liu, Q.; Wang, L.; Hu, J.; Miao, Y.; Wu, Z.; Li, J. Main organic acids in rice wine and beer determined by capillary electrophoresis with indirect UV detection using 2, 4-Dihydroxybenzoic acid as chromophore. Food Anal. Methods 2017. [CrossRef]

64. Martin M., Guiochon, G. Effects of high pressures in liquid chromatography. J. Chromatogr. A, 2005; (1-2)7: 16-38.

65. Liu Y, Lee M.L. Ultrahigh pressure liquid chromatography using elevated temperature. Journal of Chromatography. 2006; 1104 (1-2): 198-202,

66. Abidi,S.L. High-performance liquid chromatography of phosphatidic acids and related polar lipids. J. Chromatogr. 1991; 587: 193-203

67. E. A. Porter, d. B. A. A. van, G. C. Kite, N. C. Veitch and M. S. J. Simmonds, Phytochemistry, 2012, 81,90–96.

68.G.-B. Ge, Y.-Y. Zhang, D.-C. Hao, Y. Hu, H.-W. Luan, X.-B. Liu, Y.-Q. He, Z.-T. Wang and L. Yang, Planta Med., 2008, 74, 773–779.

69. S. Agnolet, S. Wiese, R. Verpoorte and D. Staerk, J. Chromatogr., A, 2012, 1262, 130–137. 33 Y. Chen, W. Bicker, J. Y. Wu, M. Y. Xie and W. Lindner, J. Chromatogr., A, 2010, 1217, 1255–1265.

70. High performance liquid chromatography in phytochemical analysis, M. Waksmundzka-Hajnos and J. Sherma, eds., CRC Press, Boca Raton, 2011

71. J.-L. Wolfender, Planta Med., 2009, 75, 719–734.

72. C. S. Funari, P. J. Eugster, S. Martel, P.-A. Carrupt, J.-L. Wolfender and D. H. S. Silva, J. Chromatogr., A, 2012, 1259, 167–178.

73. P. J. Eugster, D. Guillarme, S. Rudaz, J.-L. Veuthey, P.-A.CarruptandJ.-L.Wolfender,J.AOACInt.,2011,94,51–70.

74. J. Zhao, G.-P. Lv, Y.-W. Chen and S.-P. Li, J. Chromatogr., A, 2011, 1218, 7453–7475.

75. S. Sturm and C. Seger, J. Chromatogr., A, 2012, 1259,50

76.Chromatographic ngerprint analysis of herbal medicines. Thin-layer and high performance liquid chromatography of Chinese drugs, 2nd edn, ed. H. Wagner, R. Bauer, D. Melchart, P.-G. Xiao and A. Staudinger, Springer, Wien, New York, 2011.

77.http://www.rnlkwc.ac.in/pdf/study-material/physiology/column%20chromatography.pdf

78. https://www.biomadam.com/types-of-column-chromatography

79.https://microbenotes.com/column-chromatography (https://microbenotes.com/column-chromatography/)/.

80. Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (8 eds.). Cambridge University Press: New York.

81. https://www.biochemden.com/ion- exchange-chromatography/