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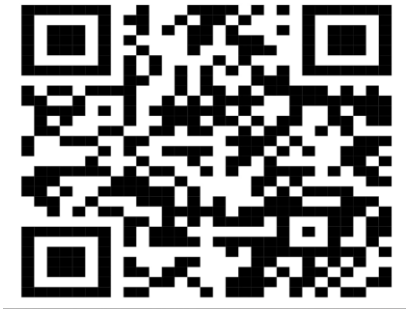

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SEPARATED CHEMICALS SHOULD BE CHARACTERIZED BY USE OF SEVERAL CHROMATOGRAPHIC AND SPECTROSCOPIC TECHNIQUES.

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ABSTRACT

Pharmaceutical items have caused a major upheaval in human health in the modern era. Only when supplied in sufficient doses and free of contaminants will these medications have any effect on patients. Impurities may be found in pharmaceutical goods at different stages of their development or production, storage, and transportation. Analytical methods play a vital role in the detection and quantification of contaminants at various phases of their development. Spectroscopic techniques such as UV-Visible, IR, and NMR are discussed here as well as chromatographic techniques like HPLC and TLC along with their accompanying procedures, concepts, and instruments that have been used in the examination of numerous pharmaceutical goods. As a last conclusion, we will look at the most extensively used and modern analytical method for medicines analysis.

KEYWORDS: Analytical, techniques, Pharmaceuticals, Spectroscopy, Chromatography.

INTRODUCTION: -

The first step in the creation of a new medicine is the discovery of a therapeutically effective drug molecule. "Active Pharmaceutical Ingredient" refers to the medication molecule that exhibits some therapeutic activity (API). Analysis of bulk drug material, intermediate drug products and samples containing pharmaceuticals and their metabolites is very vital to the pharmaceutical industry's success. Analytical procedures such as titrimetric, spectrometry, and electro-analytical methods are used to ensure the quality of a product.

Many bioactive chemicals found in plants have a substantial impact on the treatment of a wide range of illnesses. These compounds are full of bioactive secondary metabolites that may be used in a variety of therapeutic contexts. The structures and physicochemical characteristics of these secondary metabolites vary widely. Lantana camara (Verbenaceae) is a robust, evergreen, fragrant flowering shrub with a unique scent.

Throughout the globe, the plant is found in tropical and subtropical climates. 3 Locally called as "Yewef kollo" in Ethiopia, L. camara is often utilized in traditional medicine there as well. 4 It was first brought to Ethiopia as an ornamental plant because of the lovely scent and vibrant colors of its flowers. Although it may reach a height of 4 meters without assistance, L. camara can reach a height of 13 meters. It thrives in a wide range of climatic settings.

LITERATURE REVIEW

Weisheng Feng (2019) Monomeric compounds must be extracted and isolated prior to identifying the structure of a plant's chemical ingredients and testing their bioactivity. In recent years, novel extraction, isolation, and structural identification technologies and procedures have emerged, allowing for faster extraction and analysis of phytochemicals. For further research into the bioactivities, structure-activity interactions, metabolisms in vivo, structural modification, and synthesis of active chemicals from plant molecules, chemical structures must be found or understood. Classical techniques are sometimes unable to conduct out structural research on plants because of the little number of chemical ingredients that can be extracted. As a result, the most used technique is spectrum analysis. This chapter explains how phytochemicals are isolated and identified during research.

Most. Chand SultanaKhatun (2021) An in vitro and in silico strategy was used in this work to identify and describe several secondary metabolites that have anticancer, bacterial and antioxidant characteristics by employing repeated chromatography, spectroscopic techniques. Using spectroscopic techniques, the molecules 5-O-caffeoyl quinic acid, syringin, luteolin, apigenin, jhanol, and jhanidiol were all shown to be the result of thin layer chromatographic extraction and analysis. Dose-dependent cytotoxicity made it clear that compound 1 had a greater anti-proliferative impact than the others (IC50 = 181.3 g/ml) (compound 2, 4, 5, and 6). With an antibacterial zone of inhibition of between 12 and 15 millimeters, compound 1 was shown to be the most promising antibacterial agent. Compound 3 on the other hand, had the best ability to scavenge DPPH free radicals. Molecular docking investigations further confirmed the in vitro bioactivities of the drug. As a result of this computational investigation, the isolated compounds showed a greater affinity for DHFR, GSHR, and UROA binding sites than the usual medications.

Adane Adugna Ayalew (2020) The goal of this research was to discover the chemical composition of Lantana camara leaf oil. Soxhlet extraction was used to extract the essential oil from dried leaf samples. Extraction of the oil and identification of bioactive components were performed using GC-MS and Fourier transform infra-red spectroscopy (FTIR) (FTIR). The mass spectra's detected peaks were cross-referenced with the NIST library's database to ensure accuracy. Alkanes, phenols, carboxylic acids, phenolic acids, ketone acids, and primary amine compounds were all found in the FT-IR analysis. Ninety-five percent of the total essential oil components were found in the 43 compounds discovered by GC-MS. It was found that pyrrolizine, 1,2-nonadecane-diol, phytonadecene, 9-methyl-4-undecenes (Z), 1-eicosanol and imidazole were some of the most important molecules that had been synthesized and purified. An extract of the oil has recognized pharmacological and insecticidal properties, as well as being utilized in the food, beverage, and cosmetic sectors. This extract is very beneficial in the treatment of a wide range of medical conditions.

Ammar Altemimi (2017) Synthetic phenolic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been linked to a number of health issues, including cancer and cardiovascular disease. Antioxidant extractions from diverse foods have so been recommended as an alternative for these synthetics. Phenolic chemicals, of which there are more than 8000 known, may be found in fruits and vegetables. Researchers employ a number of techniques and methodologies to extract, quantify, and identify bioactive chemicals in a broad range

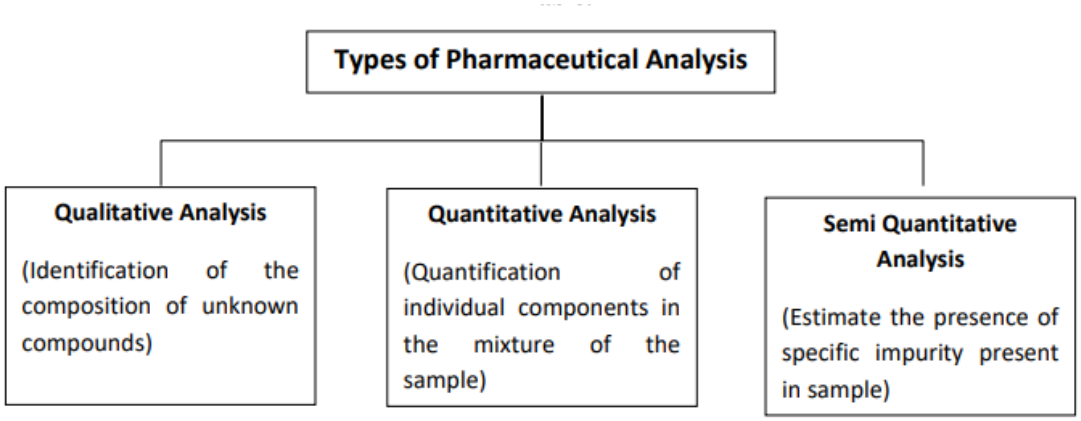
of fruits and vegetables. In this overview, a broad variety of tests are briefly described. These natural compounds from fruits and vegetables also have antibacterial and anticancer capabilities.

Vivek K. Bajpai (2016) Additionally, the identification of new and creative pharmaceutical and biological molecules is greatly aided by the use of chromatographic methods. To demonstrate fractionation and separation of physiologically active plant secondary metabolites utilizing column-chromatographic methods, this work employed step-by-step visual demonstrations. Preparation, packing, pouring, and elution of fractions are all steps in column-chromatographic extraction of bioactive chemicals. Thin-layer-chromatography is used to analyze each fraction. Compounds can be further purified using HPLC and NMR spectrum analysis, however this depends on the research's specific focus and objectives.

PHARMACEUTICAL ANALYSIS: -

Compounds are tested at both the qualitative and quantitative levels in pharmaceutical analysis, a field of chemistry. Methods for determining medication purity and safety as well as separating the components of pharmaceuticals and determining their chemical structures are included.

TABLE 1



Analytical Techniques: -

Techniques for analyzing materials and chemical states are known as analytical methods. Analytical methods enable us to know the composition of materials and chemical states qualitatively and/or quantitatively. Numerous methods have been used to analyze medicines, such as: -Chromatography; Electrochemistry; Titrimetric; Spectroscopy; Electrophoresis; and others. Spectroscopic and chromatographic methods of analysis, as well as their use in pharmaceutical analysis, are briefly discussed.

Spectroscopy:

Light and electromagnetic radiation interact with matter in spectroscopy, which is a discipline of science. To determine the qualitative and quantitative composition of materials, this technique employs electromagnetic waves with a certain wavelength or range of wavelengths.

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Principle: The interaction of electromagnetic radiations with matter is the basis for spectroscopy research. Samples (atoms and molecules) absorb, reflect, transmit, or scatter electromagnetic energy. An examination of the spectrum is then performed.

Spectrum: - This is a graph that shows the relationship between light frequency and electromagnetic radiation wavelength.

Spectrophotometer: - It is a spectrometer that is used to measure the spectrum**.**

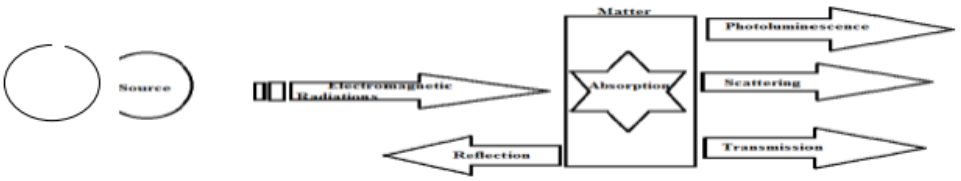


Figure: 1 Principle of Spectroscopy

For example, UV visible spectroscopy techniques require less time and use a smaller number of resources than other approaches. This approach has remarkable accuracy. Over the last several years, the usage of UV-Visible spectrophotometers in the examination of pharmaceutical dosage forms has grown significantly.

1.1.1(a) UV-Visible spectroscopy:

Principle: Measurement of light attenuation following reflection off a sample surface is called photometry. The transfer of electrons inside a molecule from a lower to a higher-level result in the UV absorption spectrum. Absorption occurs only if conjugated pie electrons are present in the wavelength range of 200-800 nm.

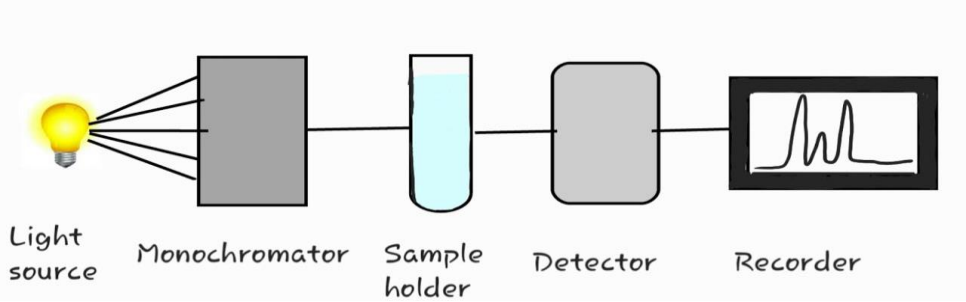


Figure: 2 Instrumentation of UV-Visible Spectroscopy

Applications:

- Analysis of medicinal compounds in terms of their quantity.
- The existence of functional groups in a sample was discovered.
- Qualitative research.
- Illumination of contaminants in colorful and organic materials.

Infra-Red Spectroscopy (IR):

Principle: Based on the idea of absorption. Vibrational and rotational stimulation of atom groups in molecules is caused by absorption of low-energy radiations.

Identification of a functional group is simple thanks to their ability to absorb distinctive information.

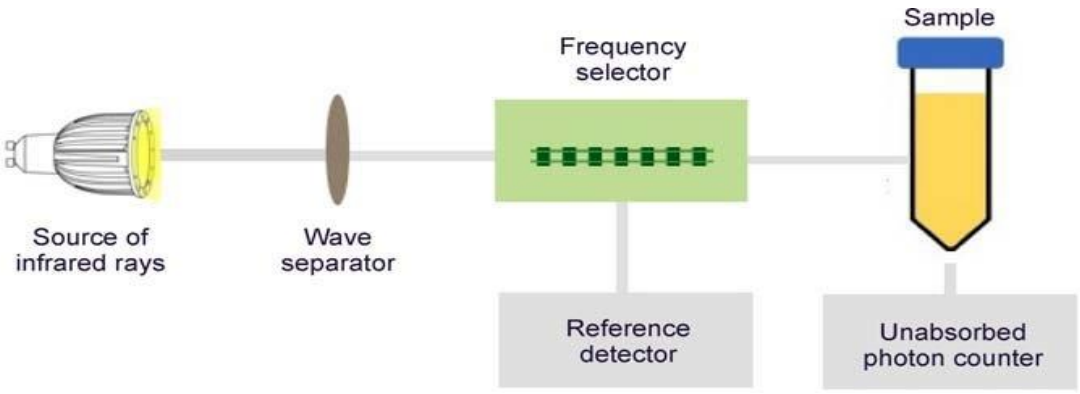


Figure: 3 Instrumentation of Infra Red Spectroscopy

Applications:

- Identifying the functional group and discovering the structure.
- Detection and quantification of narcotics.
- Polymer research.
- cis/trans isomer ratio in a chemical combination.
- Intermolecular or intermolecular hydrogen bonding is the focus of this research.

Mass Spectroscopy:

Using an electron bombardment to turn the material into fast-moving positive ions, the charged particles are then sorted by mass in a mass spectrometer.

Principle: It measures the mass-to-charge ratios of charged molecules or molecule fragments generated by ionizing chemical substances.

Applications:

- Phyto chemical analysis: The capacity of mass spectroscopy to detect and analyze metabolites with low molecular weight at very low concentrations makes it commonly used in phytochemical studies..
- Detection of impurities: Additional peaks, which have the largest mass peaks than the compounds themselves, may reveal the presence of impurities.
- Structure elucidation: Compound structure elucidation relies heavily on mass spectrometry.
- Clinical studies: In the clinical laboratory, mass spectroscopy has led to substantial improvements.

Nuclear magnetic resonance spectroscopy (NMR):

Principle: Radiation in the radio frequency range between 4 and 900 MHz is absorbed by the nuclei of an atom. It is a strong method for determining the amount and kind of atoms in a molecule. The spin state of atoms is excited when they are absorbed in the low-energy radio frequency range.

Applications:

- Organic compound structure clarification.
- Optical purity determination.
- Molecular interactions are examined.
- Investigation of dynamic characteristics.

Chromatography:

Using chromatography, you may physically separate the constituents of a mixture. An inert fluid known as the mobile phase is used to dilute the combination (the sample). Chromatography may be used for both analytical and preparative purposes. To identify and separate the many components in a mixture, preparative chromatography is a method of purification. Compositions of analytes may be determined by using this technique. Chromophore is a combination of stationary phase and mobile phase that is used to separate a mixture into its constituent parts. An inert, usually solid or liquid, phase sustained on a solid or gel surface during standstill. Gas isn't the culprit here. Liquid or gaseous, yet not solid.

Principle of Chromatography: Adsorption and partitioning are the two fundamental concepts upon which most chromatography methods are based. Between the two phases of differing polarity and strength in chromatography, components of a mixture may be separated. One of these phases is the mobile phase (gas/liquid) in which this mixture was dissolved, whilst the other is the stationary phase (solid).

Classification of Chromatography:

1. Based on the principle of separation:

- **Adsorption chromatography:** Adsorption chromatography uses a solid stationary phase, whereas the mobile phase may be either liquid or gas.
  Example: Thin layer chromatography (TLC), Column chromatography, High performance liquid chromatography (HPLC), Gas- liquid chromatography.

- **Partition chromatography:** Liquids and gases may be used as fixed and mobile phases in this system, which uses partitioning for separation.
  Example: Gas- liquid chromatography, Paper partition chromatography, Column partition chromatography.

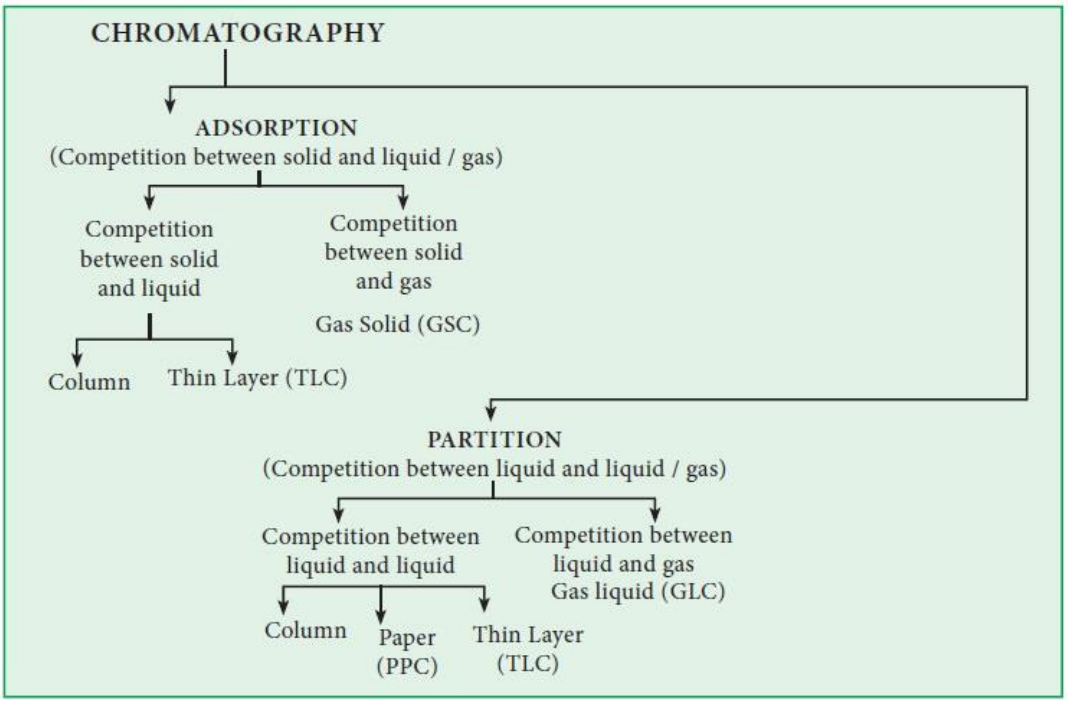
Based on the nature of stationary & mobile phase:

- Gas- Solid chromatography
- Gas – liquid chromatography
- Solid – liquid chromatography

Based on the mode of chromatography:

- Chromatography in the normal phase: Polar phase: mobile phase; nonpolar phase: stationary phase
- Reverse phase chromatography: Mobile Stationary phase: Non-Polar; Polar phase: Polar.

Table: 2 Types of Chromatography



Column Chromatography:

In this form of chromatography, the column is used to separate components from a mixture of other components.

Principle:

Adsorption-based separation is the foundation of column chromatography. Mobile phase is liquid in column chromatography, while the stationary phase is solid, and the process may be reversed. A mixture dissolves in the mobile phase and is delivered into a column at varied speeds based on the affinities of the separate components. As a result, the chemical that has a lower affinity for the stationary phase elutes from the column first, and vice versa.

Requirements:

Stationary phase: - Solid (Silica gel), 100-200 mesh size, 60–200-micron partial size. Mobile phase: - Liquid (petroleum ether, Acetone, ether, toluene, esters, chloroform etc.)

Applications:

- Separation of a chemical combination.
- Purification or removal of contaminants.
- Metabolite isolation from biological fluids.
- Preparation or crude drug extracts may be estimated.
- Extracting the active ingredients.

1.1.2 (b) Thin layer chromatography (TLC):

The term "thin layer chromatography" refers to a technique that uses a finely split adsorbent (silica gel) distributed on a glass plate as a mobile phase and liquid to separate or identify a mixture of components into distinct components...

Principle:

Adsorption chromatography is the basis for this method. On a TLC plate, the compound mixture is detected. Capillary action allows the solvent in the mobile phase to pass through. Compounds travel in accordance with their affinity for adsorbent, which determines their location. Adsorbent-affinity-increasing compounds move more slowly and vice versa .

Applications:

- Separation of chemical or biologically derived medications, plant extracts, and other mixtures.
- Detection of similar chemicals in a medication.
- Identification of drug degradation by-products.
- Find out whether the medications you're taking include any unidentified additives.
- Drug identification is an important part of any drug testing program.

1.1.2 (c) Paper Chromatography:

Chromatography technology that relies on the passage of liquids through a properly constructed filter paper to analyze unknown compounds.

Principle:

This technique uses partition rather than adsorption since the filter paper's cellulose layer acts as a stationary phase while organic solvents or buffers are employed as a mobile phase in the paper chromatography process.

Paper used: Thickness, flow rate, purity, method, and other factors all play a role in filter paper selection. Various grades of filter paper, such as NO.1, NO.3MM, and NO.17, are used.

Applications:

- Drug and impurity detection and analysis.
- Separation of polar and non-polar molecules from a combination.
- In order to ensure the purity of medications, it is utilized.

Gas Chromatography:

Both gas solid and gas liquid chromatography are techniques in the broad field of gas chromatography. gas and solid/liquid are both employed as stationary phases in both kinds of systems.

Principle:

Separation in gas-liquid chromatography is achieved by partitioning a liquid-coated solid support as a stationary phase. The mixture to be separated must be vaporized and then mixed with the mobile phase. More soluble components are eluted later, whereas less soluble components are eluted sooner, based on their partition coefficients.

Carrier gas: Components to be separated are combined with these in gas chromatography, where they are utilized as mobile phases. Hydrogen, helium, nitrogen, and argon are only a few examples.

Application:

- Compounds including clove oil, atropine sulphate, and stearic acid may be purified.
- Control and analysis of medicinal products including antibiotics, general anesthesia, antivirals, and so on.
- To find out how many metabolites are present in various bodily fluids, such as plasma, serum, and urine.

1.1.2 (e) High performance liquid chromatography (HPLC):

Column chromatography is an advanced technology. High-pressure liquid chromatography is another name for this technique. Each component of a mixture may be separated, quantified, and identified using this analytical approach.

Principle:

Adsorption is the principle of HPLC separation. High pressure is used to deliver the combination of components in the liquid solvent, which is blended and injected into the column. Solid adsorbent material fills the column. The flow rates of each component of the mixture will be different because of the unique interactions between each sample component. Consequently, the components of the mixture migrate in accordance with their affinities to adsorbents in the mixture.

Type of HPLC: - HPLC may be classified into the following kinds based on the stationary phase employed.

- **Normal phase chromatography**: - stationary phase (silica gel) is polar, whereas mobile phase (liquid) is nonpolar (diethyl ether, chloroform). Non-polar samples are first to elute from the column, whereas polar samples remain on it.
- **Reverse Phase HPLC**: - Mobile phase is polar, stationary phase is nonpolar. Thus, the polar sample is eluted more often.
- **Size –exclusion HPLC**: - Substrate in the column has been properly calibrated. Separation of mixture components happens as a result of molecule size differences.
- **Ion- exchange HPLC**: - The stationary phase has an ionically charged surface that is in direct opposition to the sample's charge. The aqueous buffer used to adjust ionic strength and pH is the mobile phase.

Applications: -

- Maintain the stability and quality of the medicine.
- For example, determining the amount of dopamine in a levodopa medicine dose form.
- The amount of medication in the bloodstream. a good example of this would be the amount of blood glucose.
- Mercury and phenol in sea water analysis for natural contamination.

Conclusion:

Overall, this research shows that HPLC, UV-Visible, and Nuclear Magnetic Resonance Spectroscopy are reliable and adaptable methods for estimating pharmaceuticals. They may be used in a broad range of industries to measure the quantity and quality of active compounds.

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