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Review Article

Various Methods of Phytoconstituent Estimation

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ABSTRACT

This review explores the diverse analytical methods used to assess phytoconstituents in medicinal plants, focusing on their unique characteristics and therapeutic potential. Key techniques such as UV-VIS spectroscopy, Paper, thin-layer, high-performance liquid, and gas chromatography are examples of chromatography, and various spectroscopic methods (FTIR and mass spectrometry) are highlighted for their roles in the detection, isolation, and measurement of bioactive substances. The efficacy of these methods in evaluating antioxidant properties and quality control in phytopharmaceuticals is underscored, with a particular emphasis on developing a validated UV-visible spectrophotometric approach for routine laboratory analysis. This work aims to improve the accuracy and efficiency of phytochemical analysis, facilitating better understanding and utilization of medicinal plants in healthcare.

INTRODUCTION

Medicinal plants include a variety of phytoconstituents, including trepans, alkaloids Inorganic acids, amino acids, carboxylic acids, phenol and tannins. These *phytoconstituents* offer plants a unique identity and set of characteristics. [1] Finding phytocomponents is simple, quick, and inexpensive with the UV-VIS spectroscopic test. UV-visible spectroscopy uses light in the visible or nearby visible bands. The color of the chemicals involved has a direct effect on the absorption in the visible spectrum. Electronic changes of molecules occur in these bands of the electromagnetic spectrum. [2] Review paper Plant ingredients are primarily separated and purified through the use of one or more of the following chromatographic technologies, either alone or in combination High Performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC), Gas Chromatography, and Paper Chromatography (PC). The properties of solubility and volatility of the substances that need to be separated have a major role in the procedure selection. Chromatography is a popular analytical technique for identifying, separating, and figuring out the constituent chemicals in complicated mixtures. It

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is made up of two phases: one stationary and the other movable .The mixture's components are transported via the gaseous or liquid mobile phase through the stationary phase, with the sample components being separated according to their varying rates of migration.[3] Antioxidants are vital substances that can protect the body from oxidative stress caused by free radicals. Numerous antioxidants found in the body scavenge free radicals, and many of them are derived from foods including tea, fruits, and vegetables (Sauria, 2007) [4]. Chromatographic techniques employed in phytopharmaceuticals (5) A literature review indicated that several analytical techniques, including UV-Visible, HPTLC, and HPLC, have been established for their analysis, although in plasma and urine[6]. Since there are formulations accessible without any combinations, it is possible to estimate curcumin in pure form and in pharmaceutical preparations using an analytical method that is straightforward, sensitive, quick, and accurate [7]. Therefore, the current work's goal is to create and validate an easily-adaptable UV-visible spectrophotometer analysis method that may be used on a regular basis in quality testing labs. This has not only helped us address the inaccuracy resulting from incomplete extraction and application of internal standards, but it has also allowed us to shorten the overall analysis time. To monitor the trans esterification procedure and determine the amounts of the biodiesel blend, spectroscopic methods such as nuclear magnetic resonance (NMR) spectroscopy and infrared spectroscopy (FTIR) are frequently used [8–11].

Evaluation of phytoconstituents qualitatively: `

1]Alkaloids.

a) **Mayer's Test**: Extracts were treated with the reagent, potassium mercuric iodide. Alkaloids are present when a precipitate with an ammonia hue occurs.

b) **Wagner's Test**: The extracts were subjected to Wagner's reagent, iodine in potassium iodide. When a brown or reddish precipitate appears, alkaloids are present.

c) **Dragendroff's Test**: a) Extracts were exposed to the Dragendroff's reagent, a potassium bismuth iodide solution. The development of red precipitate indicates the presence of alkaloids.

d) **Hager's Test**: Hager's reagent, a saturated picric acid solution, was applied to the extracts. The precipitate's yellow hue indicates the presence of alkaloid

2]**Flavonoids**:

a) **Alkaline Reagent Test**: The extracts were mixed with a small amount of sodium hydroxide solution. A brilliant yellow tint that goes colorless when diluted acid is added is a sign of flavonoids.

b)**Lead Acetate Test**: The extracts were mixed with a few drops of lead acetate solution. The development of a yellow precipitate indicates the presence of flavonoids.

3]**Amino-Acids**:

Ninhydrin test: Following the addition of 2 mL of ninhydrin reagent and a brief period of boiling, the creation of a blue color shows the presence of aminoacids.

4]**Phenols**:

Test: The extracts were mixed with three to four drops of ferric chloride solution. The formation of a bluish-black tint indicates the presence of Ferric Chloride phenols.

5] **Tannin**: 4 mL of extract and 4 mL of FeCl3 were combined to generate

6] **Terpenoids**: 2 mL of the extract, 2 mL of chloroform, and 3 mL of Conc were mixed together, then sulfuric acid was added gradually to create a layer. The presence of terpenoids was indicated by the interface turning reddish-brown.[12] Finding the total amount of phenol The amount of phenolic compounds in the extracts was determined using the Folin Ciocalteu Colorimetric technique (McDonald et al., 2001) and a

calibration curve created using catechol as a standard. Five milliliters of Folin Ciocalteu reagent and four milliliters of aqueous sodium carbonate were added to 0.5 milliliters of extract (1:10g/ml). After being allowed to sit at room temperature for fifteen minutes, the absorbance was measured at 765 nm using a UV-visible spectrophotometer. The phenol content was expressed as mg/g. [12]

Evaluation of phytoconstituents quantitative:-

Calculating the total flavonoid content

The aluminum chloride colorimetric technique was used to identify flavonoids (Chang et al., 2002). One milligram per milliliter of ethanol was used to make each extract. Each sample received 2.8 milliliters of distilled water, 0.1 milliliters of 10% aluminum chloride, 0.1 milliliters of 1M potassium acetate, and five milliliters of ethanol. The mixture was then allowed to rest at room temperature for 30 minutes. At 415 nm, the reaction mixture's absorbance was then determined. The calibration curve was created using quercetin. It was mentioned how much flavonoids there were per milligram.[13]

Calculating the tannin content

Pompei and Peri's (1971) method was used to measure the total tannin content. One milliliter of the sample extract, at a concentration of one milligram per milliliter, was placed in a test tube. The volume was created using 1 milliliter of distilled water, while the blank was made using 1 milliliter of water. 5 ml of 35% sodium carbonate was added after 0.5 folin phenol (1:2) reagent was introduced. This was let to sit at room temperature for five minutes. The color turned blue. The color intensity was ready at 640 nm.The tannin content of the extract was determined using the standard tannin graph, which was tannic acid concentration (1 mg/ml). The reference's total tannin content is expressed in milligrams per kilogram.[14].

Estimation Method

Chromatography on paper

In paper chromatography, the inert phase is a sheet of paper. One advantage of paper chromatography is that it makes it simple to carry out separations on sheets of filter paper, which act as the process's medium and support. Another advantage is that retention factor (Rf) values obtained on paper are highly repeatable. In paper chromatography, filter paper serves as the solid phase, also known as the inert phase. A sample is placed near the bottom of the filter paper. This filter paper is then placed in a chromatographic chamber that is filled with solvent. The solvent moves forward with soluble molecules by means of capillary action. Paper with poor porosity will allow the solvent to move more slowly.[15] Chromatography using thin layers (TLC) Demonstrated the first practical application of thin layer chromatography. The speed, sensitivity, and adaptability of TLC make it superior to paper chromatography. TLC is a type of adsorption chromatography in which samples are separated based on the interactions between thin adsorbent layers that are attached to a plate. Low molecular weight compounds are typically separated using this technique.[16]

HPLC stands for high performance liquid chromatography.

High-performance liquid chromatography (HPLC) is an analytical technique for separating and identifying both organic and inorganic solutes, particularly for biological, pharmaceutical, food, environmental, and industrial samples. The process known as high-pressure liquid chromatography, or HPLC for short, separates substances based on their interactions with the solid particles in a densely packed column and the solvent of the mobile phase. Modern HPLC uses a non-polar solid phase, such C18, and a polar liquid phase, typically a mixture of water and another solvent. For analytes to flow through a diode array detector (DAD), they must be rinsed via a column at high pressures of up to 400 bars. To help identify the analytes, a DAD looks to how well they are

absorbed. A DAD looks at the absorption spectra of the analytes to help identify them. HPLC has numerous applications.[18]

Chromatography by gas (GC)

Gas chromatography is one method for separating volatile compounds [16]. In this process, species are dispersed between a liquid and gas phase. While the gas phase moves, the liquid phase remains motionless. The rate of migration for a chemical species can be inferred from its distribution in the gas phase. A species that distributes itself 100% into the gas phase will migrate at the same rate as the flowing gas, while a species that distributes itself 100% into the stationary phase will not migrate at all. Partial phase division species will migrate at a modest rate.[17].

Uv-Vis Spectroscopy:

A molecule or ion will exhibit absorption in the visible or ultraviolet spectrum when radiation causes an electronic change in its structure. Consequently, the electronic state of the molecules within a sample is altered when it absorbs visible or ultraviolet light. The energy from the light will push electrons from their ground state orbital to a higher energy orbital, like an excited state orbital or an anti-bonding orbital. There could be three types of ground state orbitals involved.[19–20]

Wavelength by Region

The far-infrared (or vacuum) ultraviolet wavelength is between 10 and 200 nm.

The near ultraviolet wavelength is between 200 and 400 nm.

Perceptible 400–750 nm

The near infrared wavelength is between 0.75 and 2.2 μm.

The mid-infrared wavelength measures 2.5 to 50 μm.

Far-infrared 50–1000 μm.

Fourier-transform infrared spectroscopy (FTIR)

Fourier-transform infrared spectroscopy is a practical technique for determining the functional groups present in a plant extract. It facilitates the identification and structural characterisation of the molecule [21, 22]. FTIR samples can be prepared in a number of ways. Sandwiching a single drop of a liquid sample between two sodium chloride plates is the most straightforward technique. The drop forms a thin layer between the plates. Solid materials can be ground with potassium bromide (KBr), and the thin pellet that is produced can be compressed for examination. Alternatives include adding a tiny drop of the solution to a single High Attenuated Total Reflectance (HATR) plate after dissolving solid components in a solvent, like methylene chloride. and transmittance percentage was utilized to Take note of the spectrum. The Varian FTIR instrument guide [23] states that the peaks at specific wave numbers were assigned based on bonding and functional group.

Mass spectrometry, or (MS):

Mass spectrometry is a powerful analytical technique that can be used to identify new compounds, quantify existing compounds, and ascertain the structure and chemical properties of molecules. The MS spectrum can be used to identify the sample's molecular weight. This technique is mainly used for peptide or oligonucleotide sequencing, the structural elucidation of organic compounds, and the high-specificity monitoring of the presence of previously characterized compounds in complex mixtures. It does this by simultaneously determining the molecular weight and a diagnostic fragment of the molecule.

CONCLUSION:

This review emphasizes the importance of various analytical methods in assessing the phytochemical profile of medicinal plants. By developing standardized techniques such as UV-visible spectrophotometry, the study aims to enhance quality control and improve the reliability of phytochemical analyses in the healthcare sector.

Further exploration of these methods can significantly contribute to the therapeutic potential and application of medicinal plants.

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