

Phytochemical Analysis of Bioactive Components of Medicinal Plants

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Abstract

In the past, natural substances and their structural analogs have made significant contributions to drug treatment. Several drugs come from plants, including antiepileptics, emetics, antibacterial drugs, diarrhea drugs, antioxidants, and anti-cancer drugs. Many herbs are said to have been used by indigenous people around the world for their therapeutic properties as well as being valuable in traditional medicine. The research focuses on assessment and characterization of different plant species and plant components for a range of ailments. The identification and evaluation of biologically active substances in plants has always been a difficult task. It is very important to improve qualitative and quantitative analysis techniques of medicinal plants for quality assessment in the herbal pharmaceutical industry. Many scientific and technological advances, such as sophisticated tools for deep analysis, genome extraction strategies, and advances in microbial culture, have addressed these challenges and opened up fresh opportunities.

Characterization, including phytochemical screening tests that provide information for the presence of secondary metabolites, is most significant phase in analysing bioactive chemicals contained in natural plant extracts. Chromatography techniques such as HPLC/HPTLC/GC-MS as well as FTIR Spectroscopy, nuclear magnetic resonance (NMR) are highly sophisticated techniques for determining the structure of the bioactive fraction lead. This review explains how to determine the most common bio active component both qualitatively as well as quantitatively using easy and reliable approaches.

Keywords: herbal medicine, medicinal plant, analysis, screening for phytochemical, estimation, bioactive constituents.

INTRODUCTION

In the past, natural substances and their structural analogs have made significant contributions to drug treatment. It is accepted worldwide that natural herbs are used in treatment of many diseases.

Plants are known to provide a variety of important metabolites including carbohydrates, lipids, and nucleic acids, as well as a variety of secondary metabolites like terpenoids, alkaloids, and phenolic chemicals which possess activity against cancer allergies inflammation as well against microorganisms, as well as hepato & cardioprotective, effects. [1]

Herbal plantation acts as source for many medicinal preparations, namely anticonvulsants, antibacterial drugs, diarrhea drugs, antioxidants, and anticancer drugs. There is increased Interest of researchers in study. Various Plant constituents which are very useful as cosmetics, food additives perfumes, mouth fresheners as well as home remedy for many medical diseases. Many fine chemicals extracted and purified from secondary metabolites from plant are used as drug, dye, favour, fragrance, insecticide

Medicinal plants and herbal preparations because of its pharmacological action, easy availability, affordability and ability to treat many diseases. It is an established fact that many plants possess valuable properties in folk medicine. They are being widely utilised by indigenous peoples around the world.

As per WHO[2] herbal preparations from plants obtained after identification, separation decontamination or by other processes which manufacture nutraceutical compounds to be consumed on immediate basis. Some authors outlined medicative plants as plants containing bioactive component which can be utilised to treat any ailment or for pain relief. [3]

Medicinal Plants are a reservoir of natural compounds, they have historically generated many significant novel medications. Herbal products, natural compounds possess high level of structural diversity that is not typically observed in synthetic molecules. Around 50,000 [4] different species of various herbs are in used in treating variety of ailments. Medicinal plants provide variety of natural bioactive compounds with variety of biological from various families of molecules having vast bioactivities in humans. As per estimates as little as 6% of 300,000 species (approximately) of plants with medicinal properties are investigated scientifically for their pharmaceutical properties while on 15 % of these medicinal plants have been examined phytochemically [5][6]

For the existing system of herbal and natural medication identification, herbal plants having excellent medicinal properties are important in many ways because as they can utilized as source of bioactive and medicinal agents directly. Moreover these bioactive agents are often used as a basis for formulating more complex semisynthetic chemical compounds based on herbs.

In many developing countries around world Usage of medicinal plant have assumed significant importance especially in primary healthcare for both communities as well individuals. Demand of medicinal plants have been increased in trade because of easy availability, low cost, appears to be effective, negligible apparent aftereffects and premise that plant based products are more safer as compared to allopathic medicines.

Discovery of drugs is appearing as a difficult job to discover vigorous & suitable leads which is actually process from examining medicinal plant to identification of bioactive constituents that required skill and experience.[7] In any case, notwithstanding their compound design variety, diversity, improvement of advancements has brought revolution in preliminary analysis of regular items while finding new medications.

Analytical Methodologies

It is most important part natural herbal drug discovery, and with out analytical methodologies. Spectroscopic depiction remains backbone of natural medication discovery and its understanding is essential to develop new lead, from which new molecules with brief modification can be designed. The essential steps are the extraction, isolation, and depiction of bioactive substances from herbs. [8] The most vital step in evaluation of biological active ingredients present within herbal extracts is biodepiction, which includes chemical screening test for estimation of phytochemicals that provides indicators regarding presence of consequential metabolites utilized in treating various medical ailments. Ultra modern analytical methods such as Chromatography (HPLC/GC/HPTLC) Mass Spectrometry, NMR, are utilised to identify structure of main bioactive molecule.

The bioactive compounds alongwith concentrations of its ingredients determine a plant's therapeutic value. The initial step in isolating the sections of herbs having medicinally activity elements from the other ingredients is to use extraction methods. Plant ingredients can come from the bark, leaves, flowers, roots, fruits, seeds, and other parts of the plant. Some plant components may have a higher concentration of active ingredients than others. Secondary metabolites can be extracted from fresh or dried plant sources. Before extraction, plants are normally dried in air otherwise plants can be kept in oven for 3 days at a temp of 40-50 degree celsius. [9]

The essential premise is to effectively grind finely the material obtained from plants, increasing area of surface of fine particle for extraction and resultant extraction effectiveness. According to some studies optimal solvent to sample ratio, is 10:1 ratio of solvent & dry weight [10]. Researchers have utilised solvent homogenization to homogenise plant tissue. Plant ingredients (Fresh/dried) are ground in a blender and made to powder, mixed with a solvent in specific quantity and vigorously stirred for 10 minutes or left for 2 hours before its filtration. Some investigators [11] used a sonicator for half an hour and repeated the procedure many times. One more technique to extract bio active compound is to use a series of solvents with increasing polarity so as compounds with wide polarity can be covered. Soxhlet methods using some organic solvents was also used by many investigators. This procedure can't be utilised with heat sensitive compounds since they may degrade because of heat [9]. Many persons involved in research defat the herbal material before performing analysis by using petroleum ether at temperature of 60–80 degrees Celsius [12].

Isolation, Identification, And Characterization Of Phytochemicals

Various plant extract usually contains a blend of different sorts of bioactive compound or phytochemicals. These diverse bioactive mixtures have various polarities. Complicated procedure are available so as to identify and characterize bioactive compounds.

Phytochemical examination is a simple, less time consuming inexpensive procedure that confirms presence of various phytochemicals in a mixture in bioactive compound analyses. Chemical examination of all herbal extracts was performed as per scientifically accepted [13]

Identification of Carbohydrates

Molisch's test: In 2 ml of plant sample, 2 ml of α -naphthol solution are added in alcohol. Test tube is shuddered later 5 ml of sulphuric acid is added. Violet ring formation confirms presence of carbohydrates

Benedicts Test: In 1ml plant extracts 1ml of Benedicts solution is added. It is gently heated. Orange red precipitate confirms carbohydrate presence.

Fehling's test: In a test tube 1 ml each of Fehling's A & B solutions are gently mixed. For 1 min Test tube is boiled for 1 minutes & then 2ml of test solution is added and kept on heat for some times. Yellow precipitate formation confirms presence of sugar.

Tests for proteins

Biuret test : In 2 ml extract, 2ml of Sodium hydroxide(4%) and 2 ml of 1% Copper Sulphate solution are added, violet or pink color Indicates proteins presence

Detection of Alkaloids:

Mayers Test: Potassium mercuric iodide is added to test solution .Yellow precipitate confirms presence of alkaloids

Hagers test: Test solution from extract is treated with picric acid(saturated).Yellow precipitate confirms presence of alkaloids

Detection of Glycosides:

Legals Test: Test solution is treated with sodium nitropruside. Pink blood red color shows presence of glycosides

Modified Borntragers Test: Ferric chloride solution is added to test solution and the test tube is placed in boiling water. After cooling of mixture benzene(equal volume) is added. Presence of glycosides is indicated by rose pink color in ammonia layer

Detection of Saponins:

Froths Test: with distilled water test solution is diluted . For 10-15 minutes test tubes are shaken. Presence of saponins is indicated by foam layer formation.

Foams Test: in 0.5 of test solution 2ml of water is added and shaken vigorously. Persistence of foam for 10 min provides indication that saponins are present

Detection of Phytosterols:

Salkowski's Test: In Test solution chloroform is added and filtered. In filtrates few drops of conc H₂SO₄ is added. Presence of phytosterols if indicated with formation of golden yellow color

Libermann Burchards Test: Chloroform is added to test solution and then filtered. 2-3 ml of ethanoic anhydride is mixed to filtrates, later concentrated sulphuric acid is added. Presence of phytosterols is indicated on formation of brown ring.

Detection of Phenols

Ferric Chloride test: In test solutions 3-4 ml of FeCl₃ is mixed. Appearance of bluish black colour indicates presence of Phenol.(14)

viii) Identification of Tanins:

Gelatin Test: In test solution 1% solution of Gelatin is mixed with. Precipitate with white colour confirms existence of tanins in solution.

Identification of Flavanoids:

Alkaline reagent Test: 2-3 drops of H₂O₂ is added to test solution. Deep yellow color is formed and becomes colorless once dilute acid is added thereby indicating presence of flavanoids

Chromatography Techniques

Chromatography is a vital biophysical technique that basing on their charge, size & shape allows the identification, isolation as well and decontamination of elements of any combination for measure of quality as well as quantity. In plant extract, hundreds of unknown components exists in very low amount Chromatography is analytical technique basing on premise that the modicum of combination are administered to any surface/ solid and that the stationary phase of the liquid (the stable phase) separates from each other during movement using the mobile phase. [15] Usually variability may exists within same herbal material depending upon its geographical distribution . As a result, reliable results obtained using sophisticated chromatographic techniques confirming presence of chemically and active components of herbal medicine is critical. Chromatography Techniques such as TLC, HPTLC, GC-MS/ CC are some of the techniques routinely used for identification and characterization of bioactive constituents from herbal extracts

TLC(Thin Layer Chromatography)

TLC is fastest, easiest economical, chromatographic techniques and is used to quantitate as well as qualitative analysis to separate organic compounds and determine compound pureness.

As a liquid chromatographic technique TLC consists of two phases namely mobile as well as stationary phase. Experimental analysis is done at room temperature at surface which is flat.

TLC is used when surface is non volatile or substance are strongly polar or where large number of samples are analysed simultaneously. TLC enables quantification of chemical constituents [15][16]

HPTLC

HPTLC (High Performance Thin Liquid Chromatography) uses this technique in a more advanced manner thus it is an essential separation tool for quantitative analysis. It is automated, sophisticated form improved version of TLC. HPTLC is simple, time saving and a very effective tool, with improvement so as to increase the resolution of the compounds to be separated and to allow analysis of bioactive compound such as identification & quantification of constituents impurities as well as of active substances. HPTLC is widely utilized analytical techniques in drug & medicinal industries, analysis of water, vitamins, food dyes as well as other packed food items because of its many advantages such as economical, simple, rapid results, simultaneous analysis of many samples. It is very much popular as it provided visual chromatogram, simultaneous processing of many sample, and enables complicated separation easily, and highly efficient resulting in reduction in analysis time [17]

Gas Chromatography(GC)

GC is a widely used analytical technique which used to analyze volatile substances in the gas phase. In GC, the constituents of test sample are mixed in a solvent and subsequently vaporize so as to separate the analytes. In chromatographic column firstly vaporized sample is injected. Along with inert gas, as sample moves in column, separation of various sample component occurs. As the leave it gets recorded as a peaks. Various sample constituent get separated at specific interval which is termed as retention time. The quantity of peaks corresponds to the quantity of constituents present in the given sample where as characteristic retention times helps in identification of the constituents & areas under peak determines the quantity of the constituent in the given sample GC is used as analytic tool in pharmacology, environmental analysis, Food analysis, as well as catalysis. It is used for quantification of drugs and metabolites for both medical and forensic applications. [17]

Column Chromatography(GC)

Column chromatography is chromatographic approach widely employed to identify and separate each chemical compound from any mixture. Through the column compounds move at various speeds, and then permitting these compound to be released in fractions. GC widely utilised because a wide variety of adsorbents and solvents can be utilised. The approach may be applied on scales from mgs to kgs. Advantages of column chromatography is economical and the availability of the stationary phase used in the process. Column chromatography can be performed using gravity to act on the solvent or using pressurized gas to force the solvent through the column. [18][19][20]

HPLC

HPLC is a special form of chromatographic technique commonly employed in identification, isolation and quantification of biologically active components. HPLC uses a column containing packing material, a pump detector displaying molecules retention times. [19] Various types of HPLC are NP-HPLC RP- HPLC, [20][21] HPLC provides information regarding identification, quantification, and resolution of a compound [22][23].

Methods of Detection

FTIR

Fourier transform infrared spectroscopy (FTIR) established on the reaction between various molecular groups present in the test samples with EMR (electromagnetic radiation) that leads to vibrational energy levels. FTIR spectroscopy has been proved to be a reliable fast, non-destructive analytical technique to be used in authentication analysis. [22]. The infrared (IR) range comprises wave numbers ($1/\lambda$) which can be divided into 03 ranges, namely, near Infra red the mid infra red and the far Infra red [23][24] FTIR is a quick time saving, nondestructive, technique this is used to pick out wide variety of purposeful compounds and is touchy to modifications in molecular structure. FTIR offer facts on the premise of chemical composition and bodily country of the entire sample

Mass Spectrometry

Mass spectrometry imaging (MSI) is very useful method which permits directionless investigations in spatial distribution in numerous samples. MS is based on principal of ionization and fragmentation of sample molecules in the gas phase. As molecules tends fragment in unique way thus resultant patterns of fragmentations of Ion-ion may be used to procure detailed

information regarding structure of given molecule. Mixture of facts received from mass spectrometry (MS) makes it as useful evaluation device for characterisation of specimen. Mass Spectrometry is routinely used in medicinal discovery, as well as quality control and protocol for food safety [25][26]. Main utility of MS is that minimal quantity of sample is required for testing.

NMR

It is important powerful instrument to study setting of polymer as well as for determination of polymer settings and existence of important group of polymer chain. NMR spectroscopy is based upon absorption of electromagnetic radiation by nuclei of atom. NMR is very much useful for identification and quantification of metabolite structure. Spectroscopy using nuclear magnetic resonance provides information about the chemical, physical, and biological characteristics of a sample. C-13 NMR identifies various carbons present in a compound. H-1 NMR identifies the various hydrogen occurring in a compound. Carbon 13 Spectroscopy provides details pertaining to molecules carbon skeleton. Combination of results of 13C-NMR spectroscopy and proton NMR provides reliable information for identification of unknown compound.[27]

Conclusion

Increasing interest in the development of herbal drugs with minimum side effects has created a lot of opportunities to explore previously inaccessible natural products for their medicinal and biological properties.

In order to assess quality in herbal medicine industry, improvement in techniques to assess quality and quantitation of medicinal plants is important. An essential step in establishing its benefits is to visualize and identify unused herbal plants throughout the world, then to extract, isolate, and characterize phytochemicals, a gift to the world from nature. Phytochemical analysis also provides good insight into the seasonal changes in active constituents, as well as during cultivation and harvest, helping to collect the substantial quantity of bioactive ingredients. In order to boost the use of herbs as the low cost readily available raw material in the pharmaceutical industry, further research must be done on phytochemical analysis.

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