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A Review on Role of chemical and biological markers in and standardization evaluation for herbal formulation

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ABSTRACT:
Chemical markers are specific compounds present in herbal formulations that can be used to assess their quality and authenticity. These markers can be identified and quantified using advanced analytical techniques such as chromatography, spectroscopy, and mass spectrometry. Chemical markers provide valuable information about the presence and concentration of bioactive compounds, which are responsible for the therapeutic effects of herbal formulations. They serve as reference points for quality control, ensuring consistency and reproducibility of herbal products.

Biological markers, on the other hand, are indicators of biological activity and can provide insights into the pharmacological properties of herbal formulations. These markers can be accessed through in vitro and in vivo experiments, including cell culture studies, animal models, and clinical trials. By evaluating biological markers, researchers can determine the efficacy, safety, and mode of action of herbal formulations. These markers are essential for establishing the pharmacological profile and therapeutic potential of herbal products.

Standardization of herbal formulations involves the development and implementation of quality control measures to ensure uniformity and reliability. Chemical and biological markers play a pivotal role in this process by providing objective criteria for assessing the identity, purity, strength, and consistency of herbal products. By establishing appropriate marker profiles, manufacturers can monitor the composition and quality of their formulations, enabling batch-to-batch consistency and minimizing variations in the final products.

I. INTRODUCTION:

Chemical and biological markers play a pivotal role in various scientific fields, including medicine, environmental science, forensic investigations, and biological research. These markers serve as valuable indicators, providing valuable insights into the presence, concentration, or activity of specific compounds or biological processes. By detecting and measuring these markers, scientists can gain a deeper understanding of complex systems, make informed decisions, and develop effective strategies for diagnosis, treatment, and prevention of diseases, as well as environmental monitoring and biological studies.

Chemical markers, also known as biomarkers or chemical indicators, are substances or compounds that can be measured and analyzed to provide information about a biological or chemical process. These markers can exist in various forms, including small molecules, proteins, nucleic acids, metabolites, and even specific isotopes. They are often associated with specific physiological or pathological conditions and can be detected in various biological samples such as blood, urine, saliva, tissues, or environmental samples. Chemical markers have revolutionized the field of diagnostics, allowing for the early detection of diseases, monitoring of treatment effectiveness, and assessment of disease progression.

On the other hand, biological markers, commonly referred to as biomarkers, encompass a broader range of indicators, including not only chemical compounds but also biological characteristics, genetic variations, cellular and molecular changes, and physiological responses. These markers are often specific to certain biological processes, such as inflammation, oxidative stress, immune response, or genetic mutations. Biomarkers can be found in different biological materials, including blood, urine, tissues, and even breath. They serve as powerful tools for disease identification, risk assessment, and monitoring of therapeutic interventions.

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Chemical and biological markers have become indispensable in various areas of research and application. In medicine, they aid in the early detection of diseases, guiding treatment decisions, and monitoring patient response to therapies. For instance, cancer biomarkers can help identify specific tumor types, assess disease stage, and monitor treatment efficacy. In environmental science, markers provide valuable information about pollution sources, exposure levels, and ecological impacts. Forensic investigations rely on the identification of unique markers to link suspects to crime scenes or establish the presence of illicit substances. Furthermore, in biological research, markers facilitate the study of complex cellular and molecular processes, enabling a better understanding of physiological and pathological mechanisms.

In this article, we will explore the significance of chemical and biological markers in various scientific disciplines. We will delve into their applications, methodologies for detection and measurement, and their potential impact on advancements in diagnostics, therapeutics, environmental monitoring, and scientific research. By understanding the role of these markers, we can harness their power to improve human health, protect the environment, and deepen our knowledge of the intricate workings of life[6,7,8,9].

Chemical and biological markers type:

There are several types of chemical and biological markers, each serving different purposes and providing unique insights into specific processes or conditions. Here are some common types of markers:

1. Chemical Biomarkers:

These markers are specific chemical compounds or molecules found in biological samples that can indicate a particular physiological state or disease. For example, cholesterol levels can serve as a biomarker for cardiovascular health, while glucose levels can indicate diabetes. Other examples include neurotransmitters, hormones, enzymes, and metabolites

2. Genetic Biomarkers:

Genetic markers involve variations or mutations in an individual's DNA sequence that can be associated with certain diseases or conditions. These markers can be detected through techniques like genetic testing or sequencing. Genetic biomarkers have played a significant role in personalized medicine and the identification of genetic predispositions to diseases such as cancer, Alzheimer's disease, and cystic fibrosis.

3. Protein Biomarkers:

Proteins are essential molecules involved in various biological processes. Protein biomarkers refer to specific proteins or their altered levels, patterns, or modifications that can indicate a particular disease or physiological state. For example, prostate-specific antigen (PSA) is a protein biomarker used in the detection and monitoring of prostate cancer. Other protein biomarkers include C-reactive protein (CRP) for inflammation and troponin for heart damage.

4. Cellular Biomarkers:

These markers involve specific characteristics or changes at the cellular level that can be indicative of a particular condition or process. Examples of cellular biomarkers include the presence of specific cell surface receptors, changes in cell morphology or structure, or abnormal cell proliferation rates. These markers are often used in cancer research and diagnosis

5. Environmental Biomarkers:

Environmental markers are indicators used to assess the quality of the environment, the presence of pollutants, or the impact of environmental changes. For instance, the presence of certain heavy metals in water or soil samples can serve as environmental biomarkers for pollution. Similarly, the presence of specific organisms or their characteristics in an ecosystem can indicate environmental health.

6. Physiological Biomarkers:

These markers involve measurements or assessments of physiological parameters to evaluate an individual's health or response to interventions. Examples include heart rate, blood pressure, body temperature, respiratory rate, and electroencephalogram (EEG) readings. Physiological biomarkers are often used in clinical settings to monitor patients, assess treatment efficacy, or predict outcomes[2,3].

Role of chemical and biological markers in and standardization:

Chemical and biological markers play a crucial role in various fields, including medicine, environmental science, and forensic science. These markers are used to measure and identify specific

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substances or characteristics in samples, providing valuable information for diagnosis, monitoring, research, and quality control. Standardization is essential in ensuring the accuracy, reliability, and comparability of marker measurements across different laboratories and studies.

Here are some key points regarding the role of chemical and biological markers and the importance of standardization

1. **Diagnosis and Monitoring:**

Chemical and biological markers can be used to diagnose diseases or conditions and monitor their progression or response to treatment. For example, specific biomarkers in blood samples can indicate the presence or severity of certain diseases, such as cardiac enzymes for heart attacks or prostate-specific antigen (PSA) for prostate cancer

2. **Research and Drug Development**:

Biomarkers are widely used in biomedical research and drug development processes. They can help identify potential therapeutic targets, evaluate drug efficacy, and assess safety profiles. By measuring specific markers, researchers can gain insights into disease mechanisms, evaluate treatment outcomes, and identify patient subgroups that may benefit from specific interventions

Environmental Monitoring: Chemical markers are employed to assess environmental contamination, pollution levels, and the impact of human activities on ecosystems. For instance, the presence of specific pollutants or toxins in water, air, or soil samples can be determined through chemical markers, enabling scientists to evaluate environmental health and develop appropriate mitigation strategies

3. **Forensic Investigations:**

Chemical and biological markers play a significant role in forensic investigations, assisting in identifying suspects, establishing timelines, and determining causes of death. DNA profiling is one of the most well-known biological markers used in forensic science, allowing for the identification of individuals based on unique genetic patterns.

Standardization of chemical and biological markers is crucial for ensuring consistency and comparability across different laboratories and studies. It involves the establishment of protocols, guidelines, and reference materials to ensure accurate and reliable measurements. Here's why standardization is important:

4. **Quality Control:**

Standardization ensures that laboratories follow uniform procedures, leading to consistent and reliable results. It helps identify and minimize measurement errors, equipment variations, and operator biases, thereby improving the overall quality and reproducibility of marker measurements.

5. **Comparability:**

Standardization enables the comparison of data obtained from different sources or studies. When markers are measured using standardized methods, it becomes easier to pool and analyze data from multiple studies, leading to more robust conclusions and generalizable findings.

6. **Clinical Decision Making:**

Standardized markers provide a basis for clinical decision making, as healthcare professionals can rely on consistent measurements to guide diagnosis, treatment, and patient management. Consistency in marker measurements is particularly crucial when establishing cutoff values for diagnostic tests or assessing treatment responses.

7. **Regulatory Compliance:**

Standardization is often necessary to meet regulatory requirements in fields such as pharmaceuticals, diagnostics, and environmental monitoring. Regulatory agencies often mandate adherence to standardized methods and reference materials to ensure the safety and efficacy of products and to facilitate harmonization and global acceptance of data[1,2].

The Need for Standardization of Herbal/Traditional Medicine

The main reason for standardizing herbal extracts is to achieve the greatest possible control in double-blind clinical studies. According to herbalist Bob Bruce, standardization has advantages. It produces a constantly strong product with guaranteed components. When you consider the quality of most commercial herbs, standardization will at least ensure that they contain something and that the right herb is used. Many herbalists look to the brighter side of standardized herbal products than to the quantum intake of more people, including doctors and pharmacists, who are accustomed to the consistency and percentage of active ingredients.

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Morphological Evaluation:

Herbal drugs evaluation by size, shape color, odor, taste and particular characteristics like touch, texture etc. This is a technique of qualitative evaluation related to the study of morphological and sensory report of whole Drugs[6].

Microscopical evaluation

Microscopic evaluation of raw herbal medicines is necessary to identify crushed or powdered materials. It includes a detailed evaluation of herbal medicines and is used to identify medicines arranged on the basis of their known histological features. Measurement of leaf constants Surface constants can be measured, such as number of stomata, stomatal index, number of venous islets, number of vein terminations, Proportion of palisades. The number of stomata, the index of the stomata, is present in the upper and lower epidermis and is made by peeling the epidermal layer and then the clear layer is slowly held on a microscope slide, cut with a slide and then a drop of chloral hydrate is added to remove any chlorophyll. Microscope 45X. The Stomata are taken using a lucid camera attached to a microscope and the number of bronchi is calculated using formulas. The venous islet, venous end, palisade ratio is determined by cooking the leaf pieces in chloral hydrate for 15-20 minutes and then the leaf fragment is placed on a microscope slide and observed 45X for the venous islet, vein end and 5X for the palisade ratio[7].

1. Physical qualitative evaluation

- Acid-insoluble ash
- Determination of total ash
- Acid-insoluble ash
- Water soluble ash
- Moisture content (loss on drying)
- Volatile oil content
- Chemical Evaluation
- Biological Evaluation

2. Toxicological Evaluation

- Determination of pesticides
- Radioactive contamination
- Aflatoxins determination
- Pharmacological Evaluation

3. Analytical Evaluation

- Thin Layer Chromatography
- High Performance Liquid Chromatography
- Gas Chromatography and Mass Spectrometry
- Supercritical fluid chromatography (SFC)
- Capillary electrophoresis (CE)

- **Acid-insoluble ash: -**

The ash remained in the total ash is taken in 25 ml of dil HCL and it is filtered, the residue remained on filter paper is Acid insoluble ash and the percentage is calculated with the dried crude drug[9]

- **Determination of total ash: -**

About 2 grams of the drug is weighed and placed in the china dish and keep in incinerator and kept it for about 5-10 min at 450°C The remained ash is cooled and weighed and percentage of ash is calculated with dried drug[2].

- **Water soluble ash: -**

The total ash is dissolved in 25 ml of distilled water and filters the ash solution; the remained ash is subtracted from the total ash gives the water-soluble ash and percentage is calculated to the dried drug. Determination of alcohol soluble extractive Weigh 5 gm of the drug and keep in contact with 100 ml of alcohol and kept for 24 hrs for maceration with intermittent shaking and it is filtered after 24 hrs and filtrate is evaporated to dryness and percentage of alcohol soluble extractive is calculated with the dried drug. Determination of water-soluble extractive It is same with the alcohol soluble extractive but the alcohol is replaced with water with chloroform as preservative Determination of ether soluble extractive weigh 75 gm of the drug and prepare a thimble and the extracted with petroleum ether in soxhlet apparatus for 6 hrs and then the extract is allowed to evaporate the extract and calculate the percentage of drug Water soluble ash[3].

- **Moisture content (loss on drying): -**

Weigh 5 gm of the drug and place in the china dish and dried in the oven at 105°C for 5 hrs and weigh the drug continuously, with an interval of 1 hour until the two successive weights was not more than 0.01 gm.

- **Volatile oil content: -**

Efficiency of several drugs is due to their odorous principle (volatile oils). Such crude drugs are standardized on the basis of their volatile oil contents. Weighed quantity of the drug is boiled with water in a round bottomed flask fitted with clevenger apparatus. The distillate collected is graduated into volatile oil. The amount thus obtained is recorded from the tube[4].

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- **Chemical Evaluation: -**

This includes the identification and Characterization of the crude drug in relation to the phytochemical component. It uses various analytical techniques to detect and isolate the active ingredients. Phytochemical screening techniques include botanical identification, extraction with suitable solvents, purification and characterization of active ingredients of pharmaceutical importance.

- **Biological Evaluation: -**

Swelling Index test is very useful for materials with swelling properties, especially gums and mucilage, pectin and hemicelluloses. Hemolytic index- Saponin have characteristics of frothing property and have ability to cause haemolysis when added to suspension of blood. The plants from caryophyllace, Aralaceae, Sapindaceae, Primulaceae contain saponin. it is determined by comparing with reference material saponin which have haemolytic activity in 1000 unit per gram. Bitterness value- Bitter properties of the plant's materials are determined the comparing the threshold bitter concentration of the materials with that of a dilute solution of quinine HCL. It stimulates the Gastric secretion. Foam index- Saponin are high molecular weight containing phytoconstituents having detergent activity. The foaming ability of an aqueous decoction of plant materials is measured in term of foaming index. Total tannins value -Tannins are present in the cell sap. It has astringent property. Tannin binds with proteins and turns into water insoluble materials and are resist to proteolytic enzymes[5]

- **Determination of pesticides: -**

WHO and FAO (food & agricultural Organisation) set limits of pesticides, which are usually present in the herbs. These are mixed with the herbs during the time of cultivation. Mainly pesticides like DDT, BHC, toxaphene, aldrin cause serious side effects in human beings. Determination of arsenic and heavy metals Arsenic and heavy metals are even in trace amounts but they are dangerous removed from herbal drugs. Amount is estimated by matching the depth[6].

- **Radioactive contamination: -**

Dangerous contamination, however, may be the consequence of a nuclear accident. The WHO, in close cooperation with several other international organizations, has developed guidelines in the event of a wide spread contamination by radionuclides resulting from major nuclear accidents. These publications emphasize that the health risk, in general, due to radioactive contamination from naturally occurring radio nuclides is not a real concern, but those arising from major nuclear accidents such as the nuclear accident in Chernobyl and Fukushima may be serious and depend on the specific radionuclide, the level of contamination, and the quantity of the contaminant consumed. Taking into account the quantity of herbal medicine normally consumed by an individual, is unlikely tobe a health risk. Therefore, at present, no limits are proposed for radioactive contamination[8,9;10].

- **Aflatoxins determination:** -Aflatatoxins produced by the growth of mold Aspergillus flavus and have carcinogenic properties. B1, B2, G1 and G2 are highly dangerous contamination in any material of the plant origin[9]
- **Pharmacological Evaluation: -**Some drugs have specific biological and pharmacological activity which is utilized for their evaluation. Actually, this activity is due to specific type of constituents present in the plant extract. For evaluation the experiments were carried out on both intact and isolation organs of living animals. With the help of bioassays, strength of drug in its preparation can be evaluated

Thin Layer Chromatography: -

Thin layer chromatography is a simple versatile method used in pharmaceutical analysis for both qualitative and quantitative evaluation of chemical constituents. Compared to other chromatographic methods, TLC has been associated with many advantages including use of simple equipments, short development time of 15 min to 1h, wide choice of stationary phases and quick recovery of separated constituents. Moreover, easy visualization of separated components by UV light makes TLC a method of choice for simple quick and easy analysis[10].'

High Performance Liquid Chromatography: -

High performance liquid chromatography is one of the modern-day applications highly utilized in separation and isolation of natural pharmaceutically active compounds including alkaloids and glycosides whose role in modern conventional medicine is undisputable. It is the most preferred method[12].

TLC is the common fingerprint method for herbal analysis. Fourspecies of herbal

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medicines were identified easily by TLC of the resins. With this technique, authentication of various species of Ginseng and Radix Puerariae is possible, as well asthe evaluation ofstability and consistency of their preparations from different manufactures. HPTLC fingerprint is mainly used to study the compounds with low or moderate polarities, but Di et al. established a fingerprint of fungal polysaccharide acid hydrolyzates by using automated multiple development. HPTLC technique is widely employed in pharmaceutical industry in process development, identification and detection of adulterantsin herbal product and helpsin identification of pesticide content,mycotoxins and in quality control of herbs and health foods . HPTLC technique was reported for simultaneous determination of Withaferin A and beta-sitosterol-dglucoside in four Ashwagandha formulations. SyzygiumJambolanum was quantitatively evaluated in terms of stability, repeatability, accuracy and phytocconstituentssuch as glycoside (jamboline), tannin, ellagic acid and gallic acid by HPTL. HPTLC was used for detection, monitoring and quantification of bacoside A & B in Bacopa monnieria and its formulations.The standardization of Cannabis stavia was done by estimating the content of cannabinoidses in urine sample using HPTLC. HPTLC was used to estimate Withaferine A, a constituent of Withaniasomnifera in herbal extract and polyherbalformulations. HPTLC method has been reported for quantitative estimation ofswetiamarin in differentmarketed polyherbal formulations and small fruits, big fruits and fresh fruits variety of E. littorale.Chandanasava known to be effective in karsya (malnutrition) was standardised by organoleptic study, physico-chemical analysis, TLC and HPTLC110. Ultra-performance liquid chromatography (UPLC) was used to evaluate decocting-induced chemical transformations and chemical consistency between traditional and dispensing granule decoctions. Combined chromatographic fingerprinting with metabolomics enables the working mechanism of traditionalChinese medicine (TCMs) and to further control their intrinsic quality. In addition, the intensive study of chromatographic fingerprinting coupled with multivariate analysis tools developed in bioinformatics and chemometrics strengthened the working mechanisms of TCMs and to further control and strengthen TCMs' intrinsic quality in a comprehensive manner[13,14].

Gas Chromatography and Mass Spectrometry: -

Many biologically active chemical compounds are volatile there by making gas chromatography an important tool in quality control of herbal medicine. It has high sensitivity of detecting almost all the volatile and thermostable chemical compounds. HPTLC-High-performance thin-layer chromatography (HPTLC) has been emerged as an important tool for the qualitative, semi quantitative, and quantitative phytochemical analysis of the herbal drugs and formulations. This includes developing TLC fingerprinting profiles and estimation of biomarkers. This review has an attempt to focus on the theoretical considerations of HPTLC and some examples of herbal drugs and formulations analyzed by HPTLC.

GC-MS instruments have been used for identification of large number of components present in natural and biological systems. The identification and quantification of chemical constituents present in polyherbal oil formulation (Megi) consisting of nine ingredients, mainly Myristica fragrans, Eucalyptus globulus[14],

Gaultheria procumbens and Mentha piperita was analyzed by GCMS method. A headspace solid-phase microextraction method was reported for analysis of the volatile compounds in a traditional Chinese medicine (TCM), RhioxmaCurcumae Aeruginosa. Thirty-five volatile compounds were separated and identified. An effective, fast.

Accurate capillary gas chromatography method was employed for determining organochlorine pesticide residues in Scutellariabaicalensis, Salvia miltiorrhiza, Belamcanda chinensis, Paeoniaelactiflora, Angelica dahurica, Arisaema erubescent, Fructus arctii, Anemarrhenaasphodeloides and Platycodon grandiflorum[15].

Supercritical fluid chromatography (SFC): -

Researchers evaluated the importance of CE for quality control of herbal medicinal products. Several CE studies dealing with herbal medicines have been reported and two kinds of medicinal compounds i.e., alkaloids and flavonoids have been studied extensively. The methodology of CE was established to evaluate one herb drug in terms of specificity, sensitivity and precision, and the results were in agreement with those obtained by the HPLC method. Furthermore, the analysis time of the CE method was two times shorter than that in HPLC and solvent consumption was more than 100-fold less. A characteristic fingerprint of

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Flos carthami established usingCE, simultaneously contributed to several objects in a study: identifying the raw herb, helping distinguish the substitute or adulterant and further assessing the differences of Flos carthami grown in various areas of China. Comparison of the CE and HPLC fingerprints of Radix scutellariae showed a decrease in analysis time from 40 to 12min for CE, but also a decrease in detected peaks. The hyphenated CE instruments, such as CE-diode array detection, CE-MS and CE-NMR, have been utilized, however,some limitations ofCEhyphenations with respect to reproducibility were reported[16].

II. CONCLUSION:

Chemical and biological markers play a crucial role in the evaluation and standardization of herbal formulations. These markers provide objective criteria for assessing the quality, authenticity, and biological activity of herbal products. By incorporating these markers into quality control protocols, manufacturers can ensure uniformity, consistency, and reliability in their formulations.

Chemical markers serve as reference points for quality control, allowing manufacturers to monitor the presence and concentration of bioactive compounds responsible for therapeutic effects. They enable the identification and quantification of specific compounds using advanced analytical techniques. This information helps ensure batch-to-batch consistency and reproducibility of herbal products.

Biological markers, on the other hand, provide insights into the pharmacological properties of herbal formulations. By evaluating these markers through in vitro and in vivo experiments, researchers can determine the efficacy, safety, and mode of action of herbal products. This knowledge is essential for establishing the pharmacological profile and therapeutic potential of herbal formulations.

Standardization of herbal formulations is crucial to ensure their quality, safety, and efficacy. Incorporating chemical and biological markers into quality control measures enables manufacturers to assess the identity, purity, strength, and consistency of their products. By establishing appropriate marker profiles, manufacturers can minimize variations and maintain high standards, providing reliable healthcare options to consumers.

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