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A Review on Role of Markers in Standardization of Herbal Products

Dinesh Kawade, Mohit Bakshe, Mohammad Qutub, Mayank Pandey, Neha Gotmare, Nikita Shukla

PRIYADARSHINI J. L. COLLEGE OF PHARMACY, ELECTRONIC ZONE BUILDING, MIDC, HINGNA ROAD,

Nagpur-440016, Maharashtra, India.

Corresponding Author

Dr. Dinesh P. Kawade

Associate Professor

Department of Pharmaceutical Chemistry

Priyadarshini J. L. College of Pharmacy,

Electronic Zone Building, MIDC, Hingna Road, Nagpur- 440016, Maharashtra, India.

Abstract**:** Plant based medicine have acquired notoriety worldwide because of their practically immaterial incidental effects. In India, the three customary therapeutic frameworks, specifically homeopathy, Ayurveda and Siddha depend vigorously on plants, for restorative details. To forestall the aimless assortment of these important therapeutic plants and for their appropriate validation and preservation, it is basic to go for supported endeavors towards legitimate germplasm listing and contriving protection systems. For this reason, atomic markers play a huge part, as they give data going from variety at nucleotide level (single nucleotide polymorphisms) to quality and allele frequencies (genotype data), the degree and circulation of hereditary variety, and populace structure. Throughout the course of recent years, the atomic marker field has totally changed the importance of preservation hereditary qualities which has risen up out of a hypothesis based field of populace science to an undeniable realistic discipline. In this survey, we have investigated the change and change of atomic marker advances over time.

Keywords – Biomarkers, Chemical markers, Molecular Markers, Applications, Authentication

1. INTRODUCTION [1] [2]

Natural restorative items for the most part contain complex combinations of dynamic synthetic compounds, accordingly the choice of trademark synthetic constituents for insightful testing is helpful for ensuring and showing satisfactory and reliable quality. The significant impediment in the worldwide acknowledgment of home-grown drugs is absence of appropriate normalization. Because of the notoriety of natural medications, their contaminations or replacements are getting more significance industrially. Replacement alludes to consider expansion of different types of the plant to build the mass as well as intensity or to diminish the expense of medications.

Contaminations are caused generally at assortment focuses, in some cases at broker level and seldom at the producer level, hence influencing the adequacy of the definition. Natural medication makers for the most part depend on nontechnical people from provincial or woods region for rough plant materials prompting expanded events of contaminated/replacement. Disappointment in the legitimate recognizable proof of the handled plant material is additionally a vital variable for corruption/replacement.

Thus, it is exceptionally fundamental to take on required recognizable proof methodology prior to continuing for the assembling of home-grown plants. Normalization guarantees that each prescription has required dynamic standards in the fitting fixation to deliver wanted remedial action. Normalization of plant-based prescriptions becomes compulsory for their worldwide acknowledgment.

In numerous natural arrangements, a huge number together are answerable for organic action. There are not many spices for which the restorative action is appointed because of single constituent or set of distinct constituents. These constituents called as bioactive parts. They are both artificially distinguished and known to add to the helpful movement of plant material or

arrangement. Assuming parts which are explicitly answerable for the organic action are not known, explicit constituent or gathering of constituents present in the plant material are picked also, are utilized for quality control reason. These constituents are known as marker compounds or Biomarker.

2. TYPES OF MARKERS

2.1 Chemical Markers

For the most part allude to biochemical constituents, including essential and auxiliary metabolites and other macromolecules, for example, nucleic acids which are of interest for quality control purposes in any case whether they have any helpful movement. The amount of a substance marker can be a pointer of the nature of a home grown medication. The investigation of substance markers is appropriate to many exploration regions, including confirmation of authentic species, look for new assets or substitutes of crude materials, enhancement of extraction and cleansing strategies, structure clarification and immaculateness assurance.

2.2 Molecular or DNA Markers

They are reliable for informative polymorphisms as the genetic composition is unique for each species and is not affected by age, physiological conditions as well as environmental factors. DNA can be extracted from fresh or dried organic tissue of the botanical material; hence the physical form of the sample for assessment does not restrict detection

As per the definition by the EMEA, insightful markers are the constituents or gatherings of constituents that fill exclusively for logical needs, though dynamic markers are the constituents or gatherings of constituents that add to remedial exercises.

There are different arrangements of substance markers.

3. CHEMICAL MARKERS AND THEIR TYPES: - [3]

An aggregate of 282 substance markers are recorded in the Chinese Pharmacopeia (2005 version) for the quality control of Chinese home grown medications. As examined in the monographs of the American Herbal Pharmacopeia (AHP), the utilization of single or various synthetic markers was essential to quality control. Researchers and administrative offices certainly stand out to the choice of compound markers in quality control.

As indicated by EMEA (European Medicines Agency) rules, substance markers are comprehensively arranged into two classifications

1) **Scientific Markers**: Analytical markers need restorative movement and are exclusively utilized for quality control purposes.

2) **Dynamic Markers**: Active markers are constituents or gatherings of constituents which are answerable for the restorative movement.

• Srinivasan proposed following 4 classes

A. General Components (Active standards)

General parts are normal yet they are available specifically species, class or family. These parts might be utilized with 'fingerprints' to separate plant from its substitutes furthermore, defilements.

B. Dynamic markers

Dynamic standards have known clinical exercises Job of Markers in Standardization of Herbal Products. Scientific markers need helpful movement and are exclusively utilized for quality control purposes. Dynamic markers add to clinical viability.

C. Insightful markers

Insightful markers need restorative movement, for example they have no clinical or pharmacological exercises and are exclusively utilized for quality control purposes.

D. Negative markers.

They display allergenic or poisonous properties and their levels in spices are used to screen poisonous parts in natural medications.

All markers may contribute to the evaluation, standardization and safety assessment of herbal medicines. Group chemical markers have similar chemical structures and/or physical properties. The pharmacological activities of individual components are not necessarily known. Polysaccharides are classified under this category. This type of markers is not necessarily specific and can be easily masked by other components especially in proprietary products. 'Phantom' markers are constituents that have known pharmacological activities; however, they can be undetectable in some herbal medicines due to low quantities. Special care should be taken when 'phantom' markers were selected as chemical markers for quality control. While group chemical markers have a lower resolving power in qualitative analysis, chemical fingerprinting cannot provide adequate quantitative information.

• Hongxi Xu et al. proposed eight new categories of chemical markers: - [4]

a. Therapeutic components

Therapeutic components have medicinal constituents which exhibit a direct therapeutic benefit. They can be employed in both qualitative and quantitative assessments as chemical markers.

b. Bioactive components

While individual components may not have direct therapeutic benefits, the combination of their bioactivities contributes to the therapeutic effects of a herbal medication. Bioactive components can be utilized as chemical indicators to check quality and quantity.

c. Synergistic components

Synergistic components don't directly contribute to therapeutic benefits or bioactivities. They do, however, work together to reinforce the bioactivities of other components, regulating the herbal medicine's therapeutic benefits. Chemical markers for qualitative and quantitative assessment can be employed with synergistic components.

d. Characteristic components

While distinctive components may contribute to therapeutic effects, they must be specific and/or unique herbal medicine constituents.

e. Main components

In an herbal medication, the main components are the most plentiful (or much more abundant than other components). They aren't typical components, and it's possible that their bioactivities are unknown. The main components of herbal medications can be used for both qualitative and quantitative examination, particularly for differentiation and stability evaluation.

f. Correlative components

Herbal medicine's correlative components have a close link with one another. Correlative elements can be utilized as chemical indicators to assess the quality of medicinal herbs that come from various geographical regions and have been preserved for varying amounts of time**.**

g. Toxic components

Herbal medicines which does not contribute to therapeutic benefits, but instead causes toxicity to the body are included under the toxic components

h. General components

The common and specific components found in a certain species, genus, or family are referred to as general components. For quality control, these components could be combined with 'fingerprints.'

Sr.No.	Categories of chemical markers	Examples of the markers
1.	**Therapeutic components**	Isosteroidal alkaloids of *Bulbus Fritillariae*, including verticine, verticinone and imperialine, were identified as the major therapeutic components that account for the antitussive effect.
2.	**Bioactive components**	Isoflavonoids, saponins and polysaccharides of *Radix Astragali* showed pharmacological actions in immune and circulatory systems, which were consistent with the Chinese medicine indications.
3.	**Synergistic components**	Rutin, a common flavonoid found in many natural products, was found to have synergistic antidepressant properties in St John's wort (*Hypericum perforatum* L.)
4.	**Characteristic components**	Characteristic components of *Ginkgo biloba* L. leaf extract EGb 761, is a well-defined product for the treatment of cardiovascular diseases, memory loss, and cognitive impairments linked to age-related dementia. The therapeutic effects of EGb 761 are attributed to flavonoid and terpene lactones.
5.	**Main components**	Radix et Rhizoma Ginseng (Renshen), Radix et Rhizoma Ginseng Rubra (Hongshen), Radix Panacis Quinquefolii (Xiyangshen), and Radix et Rhizoma Notoginseng (Sanqi), all of which are derived from the genus Panax, include tri-terpenoid saponins such as ginsenoside Rg1, Re, Rb1 & notoginesenoside R1 as their main component.
6.	**Correlative components**	Only psoralen and isopsoralen are used as chemical markers for assessing the quality of *Fructus Psoraleae* (Buguzhi).
7.	**Toxic components**	Radix Aristolochiae Fangchi (Guangfangji), Caulis Aristolochiae Manshuriensis (Guanmutong), and Radix Aristolochiae (Qingmuxiang) are examples of herbal remedies that contain Aristolochic Acids [AAs]. AAs are increasingly being employed as markers to monitor nephrotoxic herbs and

		herbal products.
8.	**General components**	In thin-layer chromatography, lobetyolin, a polyacetylene molecule, is employed as a marker for Radix Codonopsis (Dangshen) (TLC)

Table 3.1 Examples of Hongxi Xu et al. proposed eight new categories of chemical markers

4. DNA MARKERS AND THEIR TYPES: - [5]

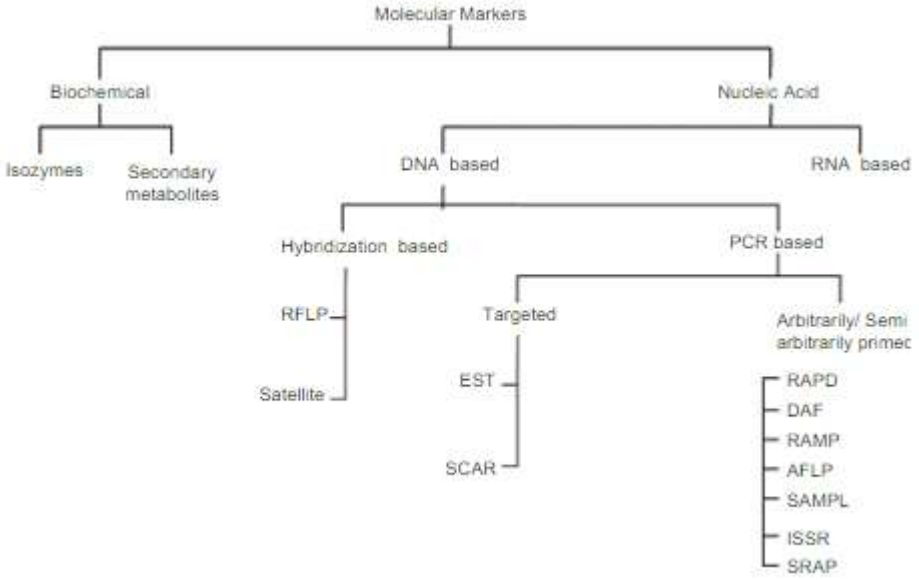

Fig. No. 1 Types of DNA Markers (Molecular Markers)

4.1 BIOCHEMICAL INDICATORS

Isozymes and, to a lesser extent, the utilization of secondary metabolites are examples of biochemical markers. Both of these categories are reliant on the environment and developmental stage because they are based on gene expression. Furthermore, epistatic and pleiotropic interactions have an impact on these indicators. These factors function as substantial impediments to their widespread use in germplasm analysis.

4.2 MARKERS BASED ON DNA (NUCLEIC ACID BASED)

DNA-based markers are unaffected by the environment and can be used at any developmental stage (temporally and spatially independent).Depending on the sort of test used, DNA-based molecular markers can be divided into two broad types. It's possible that the assay will be based on hybridization or amplification. DNA probes are visible in the first method by combining restriction enzyme-digested DNA with a tagged probe, which is a DNA or RNA fragment of known origin or sequence. PCR-based markers use locus-specific or arbitrarily chosen oligonucleotide sequences (primers) and a thermostable DNA polymerase enzyme to amplify specific DNA sequences or loci in vitro.

Before going any further, a few key characteristics of probes are highlighted. The number of loci detected at the same time, whether by hybridization or PCR, is determined by the probe's source. A probe that is derived from or targeted at a single or low copy region detects only one or a few loci, and so is co-dominant. A marker developed from or aimed towards high copy areas, on the other hand, detects many copies and is thus dominant in nature.

The former kind of probes are most suited for recombinational mapping due to their co-dominant nature, whereas the latter types are best suited for genetic diversity research due to their capacity to simultaneously screen many loci. In therapeutic plant biotechnology, nearly all sorts of molecular markers have been used.

- **Amplification-based markers or Hybridization-based markers**

The target DNA is hybridized to a probe in this test. A single or low copy zone, as well as a high copy region, may be probed. Hybridization-based markers are frequently referred to as RFLP-based markers, which is incorrect because several PCR-based assays (such as AFLP or CAPS) also look for RFLPs (Restriction fragment length polymorphism**).**

Point mutations, insertion, deletion, and inversion are known to cause RFLPs to form in the genome, resulting in the creation, abolition, or rearrangement of restriction sites. Botstein et al. were the first to use RFLP markers in the development of genetic maps (1980). They're a popular tool for creating genetic maps because they're co-dominant in nature. Co dominant RFLP markers are of limited utility in the context of diversity study unless dominant multi-loci RFLP probes such as microsatellites are used. There are various limitations of using based probes to identify diversity or for genetic mapping are linked, such as the need for vast amounts of very pure DNA and the usage of radioactivity because of the low rate of polymorphism detection, the necessity for highly skilled people, and the low rate of polymorphism detection. Because of these characteristics, hybridization-based markers are less effective than PCR-based markers. There are markers based on microsatellites. Microsatellites (1–10 nucleotides) and minisatellites (> 10 nucleotides) are subclasses of tandem repeats (TRs) that make up genomic repetitive areas, together with the prevalent interspersed repeats (or remnants of transposable elements). TRs are important in evolution because of their volatility.

They mutate at a rate of 103 to 106 each cell generation, which is up to 10 times faster than point mutations. Prokaryotes and eukaryotes both have microsatellites, Simple Sequence Repeats (SSR), Short Tandem Repeats (STR), and Simple Sequence Length Polymorphisms (SSLP). They're found all over the genome, especially in eukaryotes' euchromatin, as well as coding and non-coding nuclear and organellar DNA.

- **Markers based on PCR**

PCR-based markers, in contrast to hybridization-based probes, are extremely simple to employ and have thus gained appeal for both mapping and genetic diversity research. As previously stated, PCR-based markers can be directed to a specific locus (co-dominant) or can amplify a large number of loci at random (dominant marker). The former forms are more beneficial for genetic diversity analysis, while the former types are more commonly employed for the production of genetic maps.

a) Targeted PCR

Target PCR is based on the availability of prior sequencing information, which allows for the creation of locus-specific primers. Expressed sequence tag (EST), cleaved amplified polymorphic sequence (CAPS), sequence characterized amplified region (SCAR), and sequence tagged sites are examples of this type of marker (STS). Each of these will be briefly discussed. EST (expressed sequence tag): Partially sequencing random cDNA clones yields these results. Once sequenced, the cDNA ends can be used successfully for mapping by (Adams et al., 1991; Cooke et al., 1996). To name a few, ESTSSRs have been used to create genetic linkage maps for *Trifolium repens* (Barrett et al., 2004), *Hubus idavus* (Graham et al., 2004), *Secale cereale* (Khlestkina et al., 2004), and *Lolium perenne* (Faville et al., 2001). Among the medicinal plants, genes for ginsenoside biosynthesis have been characterized through analysis of ESTs derived from *ginseng* (Jung et al., 2003).

It has also been used for the characterization of medicinal plants (*Epimedium sagitation*, Zeng et al. 2010) by Sequence characterized amplified region (SCAR). Because EST and SCARS are locus-specific and co-dominant, they can be utilised for mapping directly by following their segregation in a mapping population. PCR products can be restricted using an enzyme for polymorphism that may be present in the amplified region if they fail to detect polymorphism or discriminate alleles across genotypes. A cleaved amplified polymorphic sequence, or CAPS, is one type of marker (Konieczny and Ausubel, 1993).

b) Arbitrarily primed PCR

Arbitrarily-primed PCR has become one of the most attractive tools for genetic diversity analysis because it simultaneously tests numerous loci for polymorphism, arbitrary-primed PCR has become one of the most appealing techniques for genetic diversity study. Randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), selectively amplified microsatellite polymorphic loci, and variations of these technologies are only a few examples of these types of markers.

i. Randomly amplified polymorphic DNA (RAPD)

One of the first modifications of PCR for genome scanning and analysis was the RAPD approach (Welsh and McClelland, 1990: Williams et al., 1990). Using a single primer of any nucleotide sequence, this technique detects nucleotide sequence polymorphisms in DNA. A single species of primer anneals to genomic DNA at two distinct places on the complementary strands of the DNA template in this process.

Through thermal cyclic amplification, a distinct DNA product is generated if these priming sites are within an amplifiable range [i.e. 300BP (Base Pairs) to 2.5 Kb]. Polymorphisms are formed when primer binding sites are present or absent, and they can be spotted using agarose gel resolution. Each primer, on average, guides amplification of up to fifteen distinct loci in the genome. Bulk segregant analysis (BSA) was developed as a method for locating genes of interest using RAPD markers (Michelmore et al., 1991).

BSA has been utilized to find markers connected to genes that cause disease resistance. Because of its simplicity, efficiency, ease of use, and lack of demand for prior sequence information, RAPD has grown in popularity. RAPD has been altered in a number of ways, the most notable of which being the introduction of a distinct class of random primers.

ii. DNA Amplification Fingerprinting (DAF)

DAF was developed by Caetano Anolles et al. (1991) and uses short oligonucleotide primers with a length of 5 to 8 BP for amplification. It differs from traditional PCR reactions in that it uses a two-step cycle protocol rather than the standard three. The PCR products are resolved on a poly-acryl amide gel followed by silver staining, with high or low stringency conditions chosen depending on the needed complexity. Various changes, such as fluorescence tagging of primers and predigesting of templates, can be used to customize reaction conditions.

iii. Arbitrarily Primed PCR (AP-PCR)

Welsh and McClelland (1991) created this approach, which uses longer oligonucleotide primers of 20 or more BP. Low stringency conditions are present for the first two cycles, followed by 30-40 cycles of extreme stringency. [32P) dCTP is employed in the last 20-30 cycles to help with product resolution on poly-acryl amide gels and autoradiography viewing. However, by separating the fragments on agarose gels and using ethidium bromide for visibility, this method has been simplified.

iv. Randomly amplified microsatellite polymorphisms (RAMP)

RAMP was created as a result of the existence of random non-repeat sequences flanking a basic microsatellite repeat. PCR is performed with two distinct primers to detect the variability. To accommodate for the differing Tm of the two oligos, an asymmetric thermo cycling programme toggles between high and low annealing

temperatures using a random decamer and 5'-anchored mono, di, or tri-nucleotide repeat primer. RAMP combines the finest aspects of RAPD and ISSR by using random decamers in combination with SSR primers to generate vast numbers of bands that may be modified by various combinations of the two primers.

v. Amplified Fragment Length Polymorphism (AFLP)

Zabeau and Vos (1993) and Vos et al. (2003) created this approach, which combines RFLP and RAPD (1995). It is based on restriction enzyme-digested DNA fragments being amplified selectively. In theory, AFLP involves restriction of genomic DNA using two restriction enzymes, a rare cutter (Eco RI or Pst I) and a frequent cutter (Mse I or Taq 1), followed by enzyme-specific adaptor ligation. This adapter is pre-amplified using primers unique to enzyme specific adapters bearing an additional nucleotide at the 3 end of ligated DNA fragments with differential ends. The pre-amplification procedure produces a preamplified library, which is a library that contains a subset of an initial set of DNA fragments. Finally, adapter-specific primers are used to do selective amplification. for specificity, bearing +3 selected nucleotides at the 3 end One of the selective primers has either 32P or 33P radioactivity.

The reaction products are electrophoresed on poly-acryl amide gels, then autoradiography is performed to determine their identity detection. Each amplifier produces many bands ranging from 50 to 100 in number reaction. Because primers are employed under strict reaction conditions, the AFLP approach is dependable annealing. It has a high multiplex ratio, is highly sensitive, and is repeatable. Several AFLP modifications have been conducted depending on the necessity and genomic complexity, such as using three restriction enzymes instead of two, and using primers with 14 instead of 13 selected nucleotides for selective amplification. AFLP is frequently used for DNA fingerprinting and genetic diversity analysis, genetic map creation, genetic relatedness evaluation, population genetic structure and mating systems, phytochemical marker prediction, species identification, and molecular tagging.

vi. Inter Simple Sequence Repeats (ISSR)

Also called ISA (inter SSR amplification) it was first introduced by Zietkiewicz et al. (1994) where 3 anchored primers are utilized to amplify the inter SSR sequences or sequences flanking SSRS. ISSRs are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16-18 BP)

vii. Sequence Tagged Microsatellite Markers (STMS)

STMS markers, also known as simple sequence repeat (SSR) markers, are sequence-specific markers in which the primers are designed to complement the flanking sections of the microsatellite locus, which are usually preserved among genotypes of the same species. STMS offer several advantages over other markers in that they are co-dominant, locus specific, and highly repeatable, despite the fact that their production is complex, time demanding, and expensive.

viii. Sequence-related amplified polymorphism (SRAP)

SRAP was developed to overcome some of the drawbacks of the approaches stated above and to produce a marker system that may be used to separate certain bands for sequencing, particularly for the development of new markers for gene tagging (Li and Quiros, 2001). SRAP combines simplicity, reliability, a moderate throughput ratio, and straightforward band sequencing. It also focuses on coding sequences in the genome, resulting in a moderate amount of co-dominant markers. Its purpose is to amplify open reading frames (ORFs) using a two-primer amplification method. The following components are found in primers that are 17 or 18 nucleotides long. Core sequences are 13 to 14 bases long, with the first 10 or 11 bases starting at the 5′ end being sequences of no particular constitution ("filler" sequences), followed by the forward and reverse primer sequences CCGG and AATT. At the 3′ terminus, three selected nucleotides follow the core. The forward and reverse primers' filler sequences must be distinct from one another and can be 10 or 11 bases long. The annealing temperature was set at 35°C for the first five cycles, and 50°C for the remaining 35 cycles. Autoradiography was used to detect amplified DNA fragments on denaturing acryl amide gels.

5. ISOLATION OF PHYTOCHEMICAL MARKERS: - [6] [7] [8] [9]

The nature of the marker should be known before continue with its isolation. Solubility, acid-base properties, charge, stability, and molecular size are all characteristics of a marker compound that are important for isolation. It is more difficult to isolate an unknown compound than a known compound. Isolating one or more chemicals from a crude extract or fractions of an extract can be a time-consuming and costly operation that often necessitates specialized knowledge and numerous separation steps involving various chromatographic techniques.

The chromatographic processes used for isolation are broadly classified into two categories: -

• **Classical Techniques**

➢Thin Layer Chromatography (TLC), a "planar" or "flat-bed" chromatography, is the simplest of all of the widely used chromatographic methods to perform. In TLC studies, a finely divided solid coated on a rigid support like glass or aluminum acts as stationary phase and the mobile phase (pure solvent or mixture of solvents) is allowed to run across the stationary phase by capillary action. TLC studies are also helpful in isolation process to have an idea about the number components present. TLC is also used to determine initial column chromatography conditions. Based on the nature of compound and general separation techniques, isolation of compound can be done easily and quickly by TLC.

➢ Flash chromatography is a low-cost method that combines gravity column chromatography and traditional HPLC to separate synthetic and natural compounds. The sample is put into a column that has been dry filled with adsorbent, and the mobile phase is circulated through it under the pressure of compressed air or nitrogen. Short columns with particle sizes of 40-63 m are employed here. To identify chemical markers, flash chromatography is used alone or in combination with other chromatographic procedures.

• **Modern Methodologies**

➢Solid Phase Extraction(SPE)

SPE is a sample preparation technique that is used for sample concentration as well as isolation and clean-up. It makes use of solid material to retain a certain component from a solution. The solution to be processed is run through a tiny column, cartridge, or disc containing a particular amount of finely divided solid material. Interfering chemicals can be selectively retained and analyte is allowed to pass, or the analyte can be retained in solid phase while interfering molecules are washed away. Following the removal of interfering chemicals, the analyte is eluted with a separate solvent and collected.

➢High Performance Thin Layer Chromatography (HPTLC)

When properly done by well-trained analysts, HPTLC offers for a quick and economical method of analysis, and in many analytical settings, it can be more advantageous than HPLC. Automated sample application, an automatic development chamber, and densitometric scanning are all key features of modern HPTLC techniques. HPTLC is a very sensitive and dependable technology that may be used for both qualitative and quantitative research.

6. APPLICATIONS OF CHEMICAL MARKERS [10]

1. Adulterants are detected.
   Identification of distinctive markers of adulterants was proposed as an effective method for detecting adulteration of edible oil, which could give a solution for multispecies adulteration detection. In this study, chemical markers such as four isoflavones, trans-resveratrol, and sinapic acid were quantified using liquid chromatography tandem mass spectrometry (LC-MS/MS) combined with solid phase extraction (SPE) to develop a simple method of multispecies adulteration detection for camellia oil (adulterated with soybean oil, peanut oil, rapeseed oil) .
2. Differentiation of herbal medicines with multiple sources.
   A key theoretical advantage of botanicals over single-component medications is the presence of many active components that, when combined, can give a potentiating effect that a single molecule may not be able to provide. For the separation and identification of active elements, this advantage poses a distinct challenge.
3. Determination of the best harvesting time.
   November to January after 8–9 months of sowing is the optimum time for harvesting ginger as it can give highest yield of active constituents, such as phenolic and terpene compounds. The phenolic compounds in ginger are mainly gingerols, shogaols, and paradols. In fresh ginger, gingerols are the major polyphenols, such as 6-gingerol, 8-gingerol, and 10-gingerol.
4. Confirmation of collection sites
   Because some herbs grow under specific conditions and chemicals contained in soil, collection sites have a significant impact on the major compounds present in an herb.
5. Assessment of processing methods
6. Quality evaluation of herbal parts
7. Identification and quantitative determination of proprietary products
8. Stability test of proprietary products
   Stability test is used to evaluate product quality over time and determine recommended shelf life.
9. Diagnosis of herbal intoxication
   Toxic components may be used as chemical markers in screening methods. E.g. Rapid diagnosis of acute hidden aconite poisoning in urine samples by HPLC-MS
10. Lead compounds for new drug discovery
    The component responsible of therapeutic effects may be investigated as lead compound for new drug discovery.

7. APPLICATIONS OF MOLECULAR OR DNA MARKERS: -

1. Genetic Variation/Genotyping:
   Differentiating various accessions of *Taxus wallichiana*, neem, *Juniperus communis* L., *Codonopsis pilosula*, *Allium schoenoprasum* L., and *Andrographis paniculata* collected from different geographical regions has been proven to be possible using RAPD-based molecular markers. Interspecies variation in Glycerrhiza, Echinacea, Curcuma, and Arabidopsis has been examined using RFLP and RAPD. RAPD has also been used to find variability in Jojoba (*Simmondsia chinensis* L. Schneider), *Vitis vinifera* L., and tea (*Camellia sinesis*).
2. Authentication of Medicinal Plants:
   Sequence SCAR, AP-PCR, RAPD, and RFLP have all been used to successfully differentiate these species as well as detect substitution by other closely related species. Medicinal plant species that are uncommon and expensive are

frequently falsified or swapped with morphologically similar, readily available, or less expensive species. *Swertia chirata*, for example, is routinely falsified or replaced with the less expensive *Andrographis paniculata*.

3. Marker Assisted Selection of Desirable Chemo Types:
   DNA profiling has been utilised to discover the evolutionary link of *Acorus calamus* chemo types differing in their essential-oil composition. AFLP analysis has been found to be beneficial in predicting phytochemical markers in cultivated *Echinacea purpurea* germplasm and several associated wild species.
4. Medicinal Plant Breeding:
   Molecular markers have been used as a tool to verify sexual and apomictic offspring of intraspecific crosses in *Hypericum perforatum*, a well-known anti-helminthic and diuretic.
5. Applications in Foods and Nutraceuticals:
   Roundup ready soybeans, maize and cecropin, capsicum have been successfully discriminated from non-GM products using primers specific for inserted genes and crop endogenous genes.
6. DNA Markers as New Pharmacognostic Tool:
   These markers have shown remarkable utility in quality control of commercially important botanicals like *Ginseng*, *Echinacea*, and Atractylodes.

8. CONCLUSION

Biomarkers are essential in the drug discovery and development as well as in the greater field of biomedical research. Understanding the link between quantifiable biological processes and clinical outcomes is crucial to improve our treatment options for all disorders and improving our knowledge of normal, healthy physiology. Biomarkers have been widely explored as surrogate outcomes in large trials of important diseases such as cancer and heart disease since at least the 1980s. The FDA continues to promote biomarker use in basic and clinical research, as well as research into new biomarkers which could be used as surrogates in future trials.

However, for all their potential to do well to speed drug development, to reduce exposure to ineffective experimental treatments, and so on biomarkers present substantial risks when trial designers confuse them with clinical endpoints. Only if we fully comprehended the normal physiology of a biological process, the pathophysiology of that process in the disease state, and the impacts of a pharmaceutical, device, or other intervention on these processes could biomarkers serve as meaningful replacements for clinically relevant endpoints.

Biomarkers as surrogate endpoints must be reevaluated on a regular basis because we rarely, if ever, have a complete picture of those types of processes, and there are always more details we don't know or comprehend. Clinical outcomes should always be the final measure of biomarker correlation success in studies using biomarkers, at least for retrospective analysis of biomarker correlation success. Without regular reevaluation of the link between surrogate endpoints and genuine clinical endpoints, we risk approving entire classes of medications that provide no additional benefit or, worse, cause harm to the patients.

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