ORIGINAL ARTICLE

Significance of Molecular Markers and DNA Based Technology in Research and Standardization of Medicinal Plants: A Review

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ABSTRACT

BACKGROUND: There is a global as well national resurgence and revival of faith in drugs of plant origins. WHO has played a significant role in recognizing the relevance of these in today's world. Herbal formulations and traditional medicines are generally polyherbal consisting of numerous bio-molecules because of which their identification and characterization is difficult. Also there are similar looking herbal materials that can often vary greatly in their medicinal properties. Thus the correct identification and quality is an essential prerequisite in quality assurance, safety and efficacy of the drug. Along with the morphological, pharmacognostical and chemical examination, molecular marker analysis has gained considerable importance in the standardization of herbs owing to many added advantages over the chemical markers. Molecular markers, DNA based technology, biostatistics and bioinformatics are an indispensible part of herbal drug research and standardization. DNA fingerprinting is one of the most promising tools for genome analysis as it not only identifies alterations in the genotypes of plant species, but is also used for the betterment of drug-yield by tissue culturing. This review provides a brief account of various molecular markers, DNA based technology and bioinformatics tools that are useful in herbal drug research and standardization with relevant examples.

Key Words: Molecular Marker, DNA polymorphism, Random fragment length polymorphism, Amplified fragment length polymorphism, Microarray, Bioinformatics tools.

INTRODUCTION

There is a global as well national resurgence and revival of faith in drugs of plant origins including traditional medicines. In spite of great advances of modern scientific medicine, traditional or alternative medicine is still the primary and reliable system than the modern medicinal system, especially in developing countries like India and is gaining popularity and acceptance globally[1]. The use of medicinal plants has long been practiced under the indigenous systems of medicine like *Ayurveda, Siddha and Unani* for the reasons of availability, effectiveness, diversity and affordability. According to an estimate of the World Health Organization (WHO), about 80% of the world populace still uses herbs and other traditional medicines for their primary health care necessities[2]. Herbal formulations have reached widespread acceptability as therapeutic agents for not only in acute diseases like cough, diarrhoea, vomiting etc. but also in chronic disorders like diabetes, arthritis, liver diseases, neurological disorders, cardiac disease, obesity because of the encouraging features like diversity, flexibility, easy accessibility, broad continuing acceptance, relative low cost, low levels of technological input, relative low side effects and growing economic importance[3,4]. Moreover, in the recent years they have gained worldwide popularity as health supplements, nutraceuticals, cosmetics and energy boosters, which emphasizes that the universal demand for herbal medicine is not only large but is also growing.

Total global herbal drug market is estimated as US $62 billion and is expected to grow to US $5 trillion by the year 2050. India has a great wealth of

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traditional knowledge and wisdom. Ayurveda contributes Rs 3500 crores (US $813 million) annually to the domestic market. The Indian medicinal plants-based industry is growing at the rate of 7–15% annually. As quoted by N.N. Singh, Vice chancellor of Birsa Agriculture University, India ranks fourth in Asia and tenth in the world in plant biodiversity[5]. About 15,000-20,000 plants have good medicinal value, out of which in India traditional communities use 7000- 7,500 for their medicinal values [6]. In addition, there are at least 120 distinct chemical substances derived from plants that are among the important drugs currently in use globally, while several other drugs are simple synthetic modifications of the natural products[7].

Therefore it is extremely important to know the available molecular markers as well as the molecular techniques employed to identify the plant based drugs.This review provides a brief account of various molecular markers, DNA based technology and bioinformatics tools that are useful in herbal drug research and standardization with relevant examples.

Factors influencing quality of drugs of plant origin:

Herbal formulations are generally polyherbal and consist of dried or processed part of herbal materials with numerous bio molecules because of which the identification and their characterization is difficult. Also there are similar looking herbal materials that can often vary greatly in their medicinal properties. Along with the classical methods of herb identification, molecular markers and DNA based technology are now an indispensible part of herbal drug research and standardization of the herbs due to the many added advantages. As per the WHO guidelines herbal medicines can be classified into four categories, based on their origin, evolution and the forms of current usage as shown in table no.1[8]. Plants synthesize various secondary metabolites having medicinal and therapeutic value. However the quantity and quality of these metabolites vary due to several environmental and geographical factors. Besides, drugs of plant origin and herbal formulations generally are polyherbal and consist of dried or processed part of herbal materials with numerous biomolecules because of which the identification and their characterization is difficult. This is true for similar looking herbal materials that can often vary greatly in their medicinal properties and market value. Several criteria influence the identification and quality of herbal drugs as given under:

- Drug of plant origin are usually mixtures of many constituents.
- The source and quality of the raw material are variable.
- The active principle(s) is (are), in some cases is unknowne. g. *Ginkgo biloba* L. isstandardized to contain 24% flavonoid glycosides and 6% terpenoids in commercial herbal products but the active constituent is unknown[9,10].
- Selective analytical methods or reference compounds may not be available commercially.
- Plant materials are chemically and naturally variable. Chemo-varieties and chemo cultivars existse. g. The medical use of marijuana is limited and the dosage cannot be fixed because the different cultivars and different crops of marijuana plants have variable amounts of the active constituents[11].
- The required herb is deliberately or in-deliberately substituted with the other low quality and morphologically similar medicinal or non-medicinal plants e. g. *Belladonna* leaves are substituted with *Ailanthus* leaves, papaya seeds in place of *Piper nigrum* L., mother cloves and clove stacks are added to clove, beeswax is adulterated with Japan wax[9].
- The exact time and methods of harvesting, drying, storage, transportation, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) have an effect, eg. *Datura strumarium* L. leaves should be collected during the flowering stage and wild cherry bark in autumn[9].
- Common vernacular name for different plants or different vernacular names in

different states of the same medicinal plants. e. g. *Centella asiatica* L and *Bacopa monnerri* L. are interchangeably known as Brahmi and Mandukparni.

MATERIALS AND METHODS

Literature related to the DNA based technologies, markers, herbs and their uses have been compiled and reviewed. The source of information was the online research papers, books, websites and online magazine.

Plant based molecular markers: Each medicinal plant manufactures a wide variety of different bioactive compounds and it is not possible to do quantitative and qualitative analysis of all these. Therefore, specific marker compounds have been identified that are analyzed to evaluate for the standardization botanical preparations. Markers are chemically defined constituents or group of constituents which are analyzed for the qualitative and quantitative estimation of the herbal substance. A marker can be either therapeutically active compound, a portion of DNA, a bio molecule like protein or enzyme or one of the analytically significant major constituent of the herb. Several DNA based techniques have been developed to define and study the molecular markers for identification, research and toxicological studies in herbal drugs. Transcriptomics, proteomics and metabolomics are among the principle 'omics' helpful in the analysis of food science, alternative and complementary medicine. Just as a sound background of 'omics' (epigenomics, pharmacogenomics, and ayurgenomics) plays a vital role in the development of personalized medicine similarly molecular markers and DNA based technology are an indispensible part of herbal drug research and standardization[12].

Classification of Plant Based Markers:

A. Chemical Markers: They are located in a particular part of the plant like root, stem or leaves. These include biochemical constituents like primary and secondary metabolites, proteins, glycoprotein, steroids, glycosides etc. Various techniques have been employed to access these marker compounds such as chromatographic techniques like High performance thin layer chromatography (HPTLC), Thin layer chromatography (TLC), gas chromatography (GC) and column chromatography; volumetric analysis; gravimetric analysis, spectrophotometry and different types of microscopy. The chemical marker profiling is frequently used for the identification of the plants. Example: In *Nardostachys jatamansi* DC. (Sans. Jatamansi), the major constituent and chemical marker is jatamansone. In *Derris indica* L.(Sans. Karanj) although the major constituent is Fisetin tetramethyl ether, the chemical marker is Kanugin[13].

B. Biochemical markers: Biochemical markers are either proteins or isoenzymes. Isoenzymes, also known as alloenzymes are the enzymes which are functionally similar but differ from each other in the location, structure, net charge, electrophoretic mobility, types of activators and inhibitors and heat stability. The isoenzyme pattern has been used in many population genetics studies; including measurements of out crossing rates, sub population structure and population divergence. These are particularly valuable to distinguish closely related species and therefore useful to study diversity and identification of crops[14]. Example: The isoenzyme pattern of glucose 6 phosphate dehydrogenase has been used for the identification of *Eclipta prostrate* L [15]. The peroxidase isoenzyme pattern is used to distinguish the three *Gastrodia elata* Bl.variants[16].

C. Genetic Markers: DNA markers are easily recognizable pieces of DNA that flag the location of particular genes. It is a particular gene or the portion of a genome, which is highly polymorphic and can be used to differentiate between the plant species in view with the fact that each living form has a unique genetic makeup. An ideal DNA marker to be used as tool for analysis should have the properties like high polymorphic nature, Co- dominant inheritance, easy and fast assay, frequent occurrence in genome, stable towards physiological and environmental changes etc.[4] Various types of DNA-based molecular techniques are utilized to evaluate DNA polymorphism. These are

hybridization-based methods, polymerase chain reaction (PCR)-based methods and sequencing-based methods.

Some of the DNA markers are:

i. Short tandem repeat (STR)
ii. Simple sequence repeats (SSR)
iii. Variable number tandem repeat (VNTR)
iv. Single nucleotide polymorphism (SNP)
v. Micro satellite polymorphism
vi. Random fragment length polymorphism (RFLP)
vii. Random amplified polymorphic DNA (RAPD)
viii. Amplified fragment length polymorphism (AFLP)
ix. Inter-Simple Sequence Repeat (ISSR)
x. Selectively Amplified Microsatellite Polymorphic Loci (SAMPL)

Some of the general applications of molecular markers have been enlisted below:

a. For genomic profiling or genotyping of medicinal plants.
b. In pharmacodynamics for the discovery of new diagnostic and prognostic indicators.
c. In pharmacogenomics for prediction of potential side effects of the herbal drug during preclinical activity and safety studies brought to light by DNA studies.
d. In pharmacognosy for correct botanical identification and authentication of crude plant materials as part of standardization and quality control.
e. In toxicogenomics.
f. For the presence of adulterants in the medicinal plants. Sequence characterized amplified region (SCAR), Arbitrarily primed – polymerase chain reaction (AP-PCR), RAPD and RFLP have been used to identify the biological adulterants in the herbal products. Example, *Actinidia macrosperma* CF Liang is a well known traditional Chinese medicine used for the treatment of cancer and is often adulterated by the roots of other species of *Actinidia valvata* Dunn. In medicinal plant breeding technique. Molecular markers have been used to verify sexual and apomictic offspring of intraspecific crosses in *Hypericum perforatum*L., a well known anti helminthic and diuretic drug[17].
g. For genetic bar coding of the medicinal plants.
h. For the conservation of the endangered or rare medicinal plants. Example, *Commiphora wightii* (Arnott.) or Guggul-rare, endangered and threatened (RET) species, which is used in Ayurvedic medicines as a remedy to reduce cholesterol, obesity and in the treatment of arthritis.

DNA based techniques for analysis of Plant Genome and Molecular Markers:

Various types of DNA-based molecular techniques are utilized to evaluate DNA polymorphism. These are

i. Hybridization based methods,
ii. Polymerase chain reaction (PCR) based methods and
iii. Sequencing-based methods.

Based on the development of these techniques in the last three decades, classified into three classes[18]:

a. The first generation molecular markers, including RFLPs, RAPDs and their modifications;
b. The second generation molecular markers, including SSRs, AFLPs and their modified forms,
c. The third generation molecular markers including ESTs and SNPs.

Microarray technology is one of the most promising molecular biology techniques for investigating gene expression. It is a very useful tool in the study of molecular markers and development of personalized medicine. This technique has been developed in the last one and a half decade. By using this technology the expression levels of thousands of genes can be determined in a single experiment. Microarray experiments generate a huge amount of data. The handling, storing, sharing, and distribution of the data can be quite complex. As a result a variety of database tools have been developed for assisting in this aspect of microarray studies. There are a number of database tools with highly different utility and platform requirements. Genetic markers mentioned in the previous section can be identified by the use of DNA based

techniques[19,20]. Table no. 2 gives an account of DNA based techniques used in identification of some known plants having medicinal value[12,21-67].

RESULTS AND DISCUSSION

In developed as well as developing countries, herbs and their preparations are widely used as home remedies, over the counter drug products and raw material for the pharmaceutical industries and represent a substantial proportion of the global drug market. To promote the popularity of the herbal formulations amongst the people and to ensure the proper health benefits, the quality of the drug should be maintained at every step of the drug preparation starting from the correct herb identification to the final product dispatch. To provide the standard procedure, WHO has published the book Quality control methods for medicinal plant materials in 1998 in order to support WHO Member States in establishing quality standards and specifications for herbal materials, within the overall context of quality assurance and control of herbal medicines.

Role of World Health Organization:

WHO strategy for traditional medicine (2002–2005, 2005–2010) has been developed which allows the widespread use of traditional medicine (TM). It is not just restricted to a particular region but has its roots in various countries throughout the globe. WHO is directing and harmonizing with health authorities in respective countries to provide leadership on global health matters, outline the health research agenda, set norms and standards, articulate evidence-based policy options, make available technical support to countries, monitor and evaluate health trends. The Government of India has already recognized Ayurveda as one of the official systems of medicine to be practiced in this country. Rules for education and practice of Ayurveda in India have been laid out in the Indian Medicine Central Council Act, 1970. WHO has persuaded the department of AYUSH, Ministry of health and family welfare, government of India, to implement a comprehensive pharmacovigilance programme for Ayurveda, as a means to ensure safety and efficacy of Ayurvedic medicines which was launched nationally on 29 September 2008. This program is running successfully at present [8].

The role for WHO has been identified which is as follows:

1. To facilitate the efforts of interested member states in formulating national policies and regulations on traditional, complementary and alternative medicine, promoting the exchange of information and collaboration of national policy and regulation among member states;
2. To provide technical support for the development of methodologies to monitor or ensure product quality, efficacy and safety, preparation of guidelines and promotion of exchange of information; and
3. To provide technical support to member states in defining indications for the treatment of diseases and conditions by means of TM.

Advantages of DNA markers over chemical markers:

Analysis of DNA markers is more reliable than the chemical markers. They provide an efficient and accurate means of testing the authenticity of several hundreds of samples simultaneously whereas the conventional chemical based methods usually takes several days for verification. In order for any compound to act as a chemical marker, it should be unique to that particular species. Not all plants have a unique chemical compound and also the same chemical marker is used for the identification of two or more plants. Moreover the concentration of secondary metabolites and other biochemical markers may change due to environmental factors and hence correct identification of the botanicals is difficult, whereas genetic markers are unique and are not affected by age, physiological conditions and environmental factors.

Table 1: Criteria for classification of herbal medicines according to WHO guidelines

Category Name	Criteria	Examples
Indigenous herbal medicines	This category of herbal medicines is used in the folklore of a local community or region and is very well known through ages by the local population in terms of its composition, treatment and dosage.	*Azadirachta indica* A. Juss, *Curcuma longa* L, *Piper nigrum* L, *Ocimum sanctum* L,etc.
Herbal medicines in systems	Medicines in this category have been used for a long time and are well documented with their special theories and concepts, and are duly accepted by the respective countries. For example, Ayurveda, Unani and Siddha fall into this category.	Ayurveda: *Ficus religiosa* L, *Acalypha indica* L, *Andrographis paniculata* (Burm. f.) etc. Unani: Arq nelofer, Habb-e- Jawahar, Arq makoh etc. Siddha: Aswagandha Balalakshadhi thailam, Narasimha legiyam etc.
Modified herbal medicines	These are herbal medicines as described above in categories 1 and 2, except that they have been modified in some way or the other with respect to their shape, dosage form, mode of administration, ingredients and methods of preparation or medical indications. They have to meet the national regulatory requirements of safety and efficacy of herbal medicines.	*Datura metel* L, *Convolvulus pluricaulis* Choisy, *Digitilis purpurea* L , *Plantago ovata* Forssk., etc.
Imported products with a herbal medicine base	This category covers all imported herbal medicines including raw materials and products. Imported herbal medicines must be registered and marketed in the countries of origin. The safety and efficacy data have to be submitted to the national authority of the importing country and also need to meet the requirements of safety and efficacy of herbal medicines in the recipient country.	*Argemone mexicana* L, *Withania coagulans* Stocks, etc.

Adapted from WHO guidelines: Criteria for classification of herbal medicines: Chaudhary A, Singh N. Contribution of world health organization in the global acceptance of Ayurveda. J Ayurveda Integr Med, 2011; 2(4): 179–186.

Table 2: DNA based techniques and molecular markers in identification of some plants having known medicinal value.

Botanical name of medicinal plant (Hindi name)	Therapeutic uses	Adulterants/ Substitutes	Chemical marker	DNA based techniques and molecular markers	Purpose of using molecular marker
*Abrus precatorius*L(Ratti, Ghungchi)	Parturition, Dysuria	Liquorice, *Glycyrrhiza glabra* L	Abruquinones	RAPD , ISSR	To study genetic diversity
Andrographis paniculata(Burm. f.) (Kalmegh, charayetah,mahatela, kalpanath)	Hepatoprotective, antipyretic, dermatosis	*Andrographis echioidea* (Burm. f.)	Andrographolid	RAPD	To study genetic diversity
*Azadirachta indica*A. Juss (Nimb, neem)	Helminthiasis, pyrexia, diabetes mellitus, pruritis	---	Epicatechin	AFLP, SAMPL, RAPD	To find out intra-population genetic variation, for ascertaining clonal fidelity during micropropagation, genetic diversity
Actinidia macrosperma CF Liang*	Anticancer, leprosy, abscess, rheumatism, arthritis, jaundice, abnormal leucorrhea.	This drug is often adulterated by the roots of other species like *Actinidia valvata*Dunn and *Actinidia melanandra* Franch	--	RAPD, RFLP	To find out adulterants, for authentication, to obtain phylogenetic relationship
*Acorus calamus*L (Bach, Gora- bach)	Epilepsy, dyspnoea, colicky pain	*Alpinia galangal* (L) Willd, *Aconitum species, Costus speciosus* Koen ex Rutz	*Beta- asarone, methyl isoeugenol*	RAPD, ISSR, chloroplast microsatellite marker	To develop population genetic structure, and study genetic diversity
*Amomum subulatum*Hook f. (Bari elaichi)	Dyspnoea, Pruritis, nauseation	---	1,8- cineole	ISSR	To study genetic diversity
*Bacopa monnieri*L (Brahmi)	Anti leprotic, antipyretic, antidiabetic, anti-inflammatory, anxiolytic	*Centella asiatica* (L) Urban	Bacoside A	SCAR, RAPD, ISSR	For authentication, genetic variability
Betula utilis D. Don (Bhojpatra)	Wound healing, systemic tumours	---	Betulic acid	AFLP	To obtain phylogenetic relationship
Carthamus tinctorius L(Kusum, karrah)	Constipation	---	Carthamin	EST-SSR, RAPD, AFLP, ISSR	To carry out genetic analysis, and genotyping
*Chenopodium ambrosioides*L(Khatua)	Helminthiasis	---	Z-& E-ascaridoles	RAPD	To find out genetic relationship
Botanical name of	**Therapeutic uses**	**Adulterants/**	**Chemical**	**DNA based**	**Purpose of using**

Medicinal plant (Hindi name)		Substitutes	marker	techniques and molecular markers	molecular marker
Commiphora wightii(Arnott.) (Gugglu, Guggul)	Reduces cholesterol, obesity and in the treatment of arthritis	*Boswellia serrata* Roxb. (Salai gum) and*Hymenodictyon excelsum* (Roxb.) Wall		RAPD	To study polymorphism, for sex determination, to obtain genetic diversity
Cucurbita pepo L (Kaddu, Kohada)	taenicide	---	Methyl oleate	AFLP, SSR, ISSR , SRAP, RAPD,	To find out genetic relationship, genetic diversity, genetic mapping,
Cyamopsis tetragonoloba (L) Taub (Goovaar, Gowar)	Diabetes	Locust bean gum from *Ceratonia siliqua* L	Galactose, mannose, glucose	RAPD	To study genetic diversity.
Cymbopogon Citratus (DC) Stapf (Ghandhatrina, Harichaya)	Helminthiasis, dyspnoea, cold and cough	---	Citral	RAPD	To find out genetic diversity and species relationship.
Derris indica L (Karanj)	Dermatopathies, pruritis	---	Kanugin	RAPD	To find out genetic variability, phylogenetic affinities
*Matricaria chamomilla*L(Babunphul)	Anti inflammatory, spasmolytic, antimicrobial, vulnerary	Stems of *M.chamomilla* L, flower heads of *Anthemis cotula* L, *A.nobilis* L and *S.chamaecyparissus*	Umbelliferone	RAPD	To find out genetic diversity
Moringa oleifera Lam.(Sahjana, Sahijana)	Inflammatory condition, obesity, splenomegaly, cervical adenitis	---	Niazinin B	AFLP, RAPD	To find out genetic variability, out crossing rate.
Ocimum tenuiflorum L(Tulasi)	Dysuria, burning micturition, strength promoting	---	Linoleic acid	RAPD, AFLP, ISSR	Genetic diversity, phylogenetic relationship.
Oroxylum indicum(L) Benth e Kurz (Sonapatha, Shyonak, Tentoo)	Inflammatory condition, arthritis	---	Baicalein	RAPD	Genetic diversity
Piper nigrum L (Kali mirch)	Constipation, flatulence, anti tumourigenic, immunostimulatory, carminative, anticholesterolemic, tooth ache	Seeds of *Carica papaya* L	Piperine	RAPD, SCAR, microsatellite	Adulteration, Genetic diversity, to know phytophthora resistance, Genetic diversity and conservation, genetic fingerprinting.
Raphanus sativus L (Muli, Muri)	Cough, coryza, haemorrhoids	---	Chlorogenic acid	RFLP, RAPD, SCAR, AFLP, SSR	Genetic relationship, Ogura cytoplasmic male sterility, construction of molecular linkage map.
Ricinus communis L (Erand, Rendee, Andu)	Root: inflammatory disorders, abdominal colic. Leaf: Lower abdominal colic,dysuria, anthelmintic.Seed: Purgative, hepatic and spleenic disorders,sciatica	---	Rutin, Ricinoleic acid	EST- SSR, microsatellite ,RAPD, ISSR	Genetic purity testing of hybrids, Genetic diversity and population structure.

Limitations of DNA marker analysis:

Although DNA analysis is a reliable, reproducible and currently considered as cutting- edge technology, it has certain limitations. There are approximately 25 000 and 50 000 genes in plants. Finding if a desirable gene is present can be very cumbersome. Besides isolation of good quality DNA suitable for analysis from semi processed or processed drugs is a challenge as DNA is highly fragile and processing leads to breaking of DNA into unequal small pieces with loss of complete information. Also some portion of DNA may get damaged or broken so that its identification becomes impossible. Where more than one herb is used in a drug, the complexity and difficulty in the analysis increases. DNA fingerprinting does not reveal the contents of the active constituents of the plant. For every new herb analyzed, a suitable DNA primer has to be screened and standardized, which is a time consuming and costly.

Bioinformatics and data analysis software tools for plant genome analysis:

Bioinformatics is an interdisciplinary science that combines the power of computers, mathematical algorithms and

statistics for storing, retrieving, organizing and analyzing biological data. Bioinformatics and biostatistics are excellent tools for the plant genome analysis. All the biological information and data obtained by using various molecular biology techniques can be analyzed and integrated with the help of various bioinformatics software available from multiple experiments. The information is currently being used for designing hybrid varieties, harness genetic diversity, developing pest resistance crops, obtaining phylogenetic relationship between the closely resembled species and for identification and authentication of crops and medicinal plants. There are many programs in bioinformatics with many functions to suit the needs available for genome analysis which has been listed in table no. 3 [68].

Table 3: Bioinformatics programs and their applications

Program	Application
MAPMAKER/QTL	A linkage analysis package designed to help construct primary linkage maps of markers segregating in experimental crosses.
QTL CARTOGRAPHER	A suite of programmes for mapping quantitative trait loci onto a genetic linkage map.
SRS	A tool for bio sequences.
TASSEL	Trait analysis by association, evolution and linkage. This application evaluates linkage disequilibrium, nucleotide diversity and trait associations.
QGENE	A Macintosh software application written for the visualization and rapid statistical analysis of molecular marker and phenotypic trait data.
GDPC	Provides access to genomic diversity data such as SNP, SSR, sequences etc. and phenotypic data that may be collected in field, genetic or physiological experiments.
STRUCTURE	It is a free software package for multi locus genotype data to investigate population structure.
MAPDISTO	A program for mapping genetic markers in experimental segregating populations like backcross, doubled haploids, single- seed descent.
GDPDM	Captures both molecular and phenotypic diversity data.
DISTRUCT	Is a program that can be used to geographically display results produced by the genetic clustering program STRUCTURE.

***Adapted from:** www.gramene.org/resources

Table no 4 : List of data analyses softwares

Software	Application	Website
Expression profiler	Expression Profiler is a set of tools for cluster analysis, pattern discovery, pattern visualization, study and search for gene ontology categories. The tool also generates sequence logos, extracts regulatory sequences, studies protein interactions, and links analysis results to external tools and databases.	http://ep.ebi.ac.uk/.
Gene pattern	Multidisciplinary genomic research programs and designed to encourage rapid integration of new techniques;	http://www.broad.mit.edu /cancer/software/genepattern/index.html
GeneXPress	A visualization and analysis tool for gene expression data, integrating clustering, gene annotation and sequence information.	http://genexpress.stanford.edu/.
Gene expression pattern analysis suite (GEPAS)	It is an integrated web-based tool for the analysis of gene expression data.	http://gepas.bioinfo.cipf.es/
High-dimensional biology statistics (HDBStat)	HDBStat is a free java application that allows for the normalization, transformation, and statistical analysis of expression.	http://www.ssg.uab.edu/hdbstat/
SAM (significant analysis of microarrays)	SAM can be used on any type of array data: oligo or cDNA arrays, SNP arrays, protein arrays, and so forth. Both parametric and nonparametric tests are available for correlating expression data to clinical parameters including treatment, diagnosis categories, survival time, paired data, quantitative (e.g., tumor volume), and one-class.	http://www-stat.stanford.edu/ tibs/SAM/.

*Adapted from: Page GP, Coulibaly I. Bioinformatic Tools for Inferring Functional Information from Plant Microarray Data: Tools for the First Steps. Int J Plant Genomics 2008:1-9.

Most statistical packages such as SAS, SPSS, JMP, and R can be used to analyze microarray data and will do most of the functions the following tools will do, for there are few statistical methods that are 100% unique to expression studies. Some other tools which may be utilized in interpreting the information obtained from Microarray analyses are as shown in table no. 4[69].

CONCLUSION

Along with the morphological, pharmacognostical and chemical examination, molecular marker analysis should also be included as a parameter for the standardization of herbs as the genetic markers have many added advantages over the chemical markers.In India several agricultural universities and research institutes are carrying out active research in DNA-based techniques in genotyping of medicinal plants. DNA based technology, biostatistics and bioinformatics are an indispensible part of herbal drug research and standardization. DNA fingerprinting is one of the most promising tools for

genome analysis as it not only identifies alterations in the genotypes of plant species, but is also used for the betterment of drug-yield by tissue culturing. Also as an added advantage, DNA of interest can be stored as germplasm, which is then used for future cultivation and for the conservation of selected plant species, which are endangered. It is an urgent need of the nation to train more and more hands in this area.

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