MOLECULAR BIOLOGICAL TECHNIQUES: Application in herbal medicine

Mansoor AhmadI, Zehral, Muniral and Shakeel Ahmad Khan2 J Research
Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Faculty of
Pharmacy, University of Karachi, Karachi-75270, Pakistan 2 Department of
Microbiology, University of Karachi, Karachi-75270, Pakistan

ABSTRACT: Herbal medicine, also called botanical medicine or phytomedicine, are plant derived medicines that contain, an active ingredients, in the aerial or underground parts of the plants, or other plant materials or combinations thereof, whether in crude state or as plant preparations. Long practiced, outside of conventional medicine, herbalism is becoming more mainstream. As up-to-date analysis and research show their value in the treatment and prevention of diseases. Herbal drugs are obtained from different plant sources, therefore, should be analyzed for their safety, toxicity, usefulness, purity, mechanism of action etc. before their use in humans. Their authentication and standardization by controlling the quality is necessary. Uptill now, this was achieved by traditional methods like plant morphology, histology, various chromatographic techniques and spectrophotometric analysis. Recently scientists observed that although these traditional methods are very efficient and reproducible for chemoprofiling and analyzing their purity but it cannot determine the absolute chemical characteristics and genetic variation in chemical composition of same species or genotype of medicinal plants. Therefore, for authentication and standardization of medicinal plants, more sophisticated tools are required. Advancements in molecular biology, now provide more standardized and reliable methods in identifying the quality, standard, selection and analysis of genetically pure medicinal plants. The aim of this review is to delineate some molecular biological techniques, which are utilized in the field of herbal medicines. These techniques are useful in authentication of herbal drugs, their standardization, analyzing their effectiveness, usefulness, mechanism of action etc. Although these techniques are quite reliable, there is still a need for better more precised standardization of herbal medicines.

KEY WORDS: Herbal drugs, Molecular biological techniques, DNA markers, chemoprofiling.

INTRODUCTION

Following WHO recommendations of traditional medicines, many developed countries in the world use herbal drugs in the form of nutraceuticals or as the primary source of medicinal compounds. Hence, thus there is an ever increasing use of herbal drugs worldwide. Their safety and efficacy effectiveness must be determined prior to their use.

For quality control and standardization of herbal drugs, traditional methods are used like macroscopic observance (size, shape, color, texture, smell, taste etc.) and microscopic features which are compared with its standard herbal compounds (Blumenthal, M. 1997). These macro and microscopic features are not enough for identification and standardization of herbal drugs because of possibility of substitution of standard herbs with their adulterants. Therefore, for better selection of standard herbal drugs and for their chemoprofiling, chemical and chromatographic techniques are used. These techniques include: Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Gas Chromatography (GC), Spectrophotometric methods etc. HPLC-Mass Spectrometery is used to profile lead substances and to evaluate the pharmaceutical quality of the products. In this method, active constituents can be identified (Tolonen et al 2002).

In TLC/Densitometry, selective separation and simultaneous determination of few drugs can be analyzed even in traces.

Capillary GC with electron captures detection is used for recovery from fortified fragrance products at several concentrations (Wisneski et al2001).

These techniques are routinely used for quality control analysis of herbal medicines. Although these techniques are valuable tools for determination of impurities but it cannot determine the absolute chemical characteristics of medicinal plants for example in-process artifacts and overlapping of certain chemicals like sterols, phenolics. Similarly, in many herbal species, changes in environmental conditions from region to region and growing conditions may
cause variation in chemical composition (and variation in active components) in plants of same specie or genotype, which reduces the reliability of these methods. In such conditions, these variations are observed at genetic level.

On the other hand, better quality medicinal herbs (which are expensive and rarely available) can be adulterated with morphologically similar and easily available drug. Therefore, for the authentication and standardization of medicinal plants, more sophisticated tools are required (Chan et al 2000, Blumenthal, 1997).

Advancement in molecular biology, now provide more standardized and reliable methods of identifying the quality, effectiveness, standard, selection and analysis of genetically pure herbal plant.

The aim of this review is to describe the use of these techniques for authentication, standardization and quality control analysis of herbal drugs. Besides this, how these techniques are utilized for observing the effectiveness of herbal drugs and their mechanism of action.

MOLECULAR BIOLOGICAL TECHNIQUES UTILIZED FOR AUTHENTICATION AND STANDARDIZATION OF HERBAL MEDICINES

Polymerase chain reaction (PCR)

PCR is the technique used for amplification of defined DNA sequences in the course of standardization of herbal medicine. Scientists mainly utilize this technique for identifying true plant species, genetic purity and differentiating it from its adulterants. On the other hand, for analyzing their mechanism of action, expression level of different genes are analyzed with the help of this technique (Mullis et al. 1986).

Following PCR based techniques (single or combined) are commonly utilized for authentication of herbal medicines:

- Random amplified polymorphic DNA (RAPD),
- Microsatellite technique,
- Amplification refectory mutation system-PCR (ARMS-PCR),
- Mutant allele specific amplification-PCR (MASA-PCR),
- Reverse transcription-PCR (RT-PCR).

Frequently used PCR based technique is RAPD, which is this simplest form of PCR technique in which complex genomic DNA is used as a template and random single decamer primer is used to amplify
pieces of this complex DNA template (Welsh et al. 1996).

Many scientists utilized this technique for detecting different plant species, which were not detected earlier, genetic variability between closely related plant species, for identification of components in herbal prescriptions and for differentiating standard herb from its adulterant plants.

**RAPD analysis for genetic variability and species detection**

Scientists utilized RAPD technique to search high quality *Panax ginseng* among ginseng populations. Along with RAPD analysis, PCR-RFLP analysis was also utilized for authentication of *Panax ginseng* species (Tochika-Komatsu et al. 2001).

Methods have been developed for the identification of *ginseng* drugs at DNA level by using RAPD and PCR-RFLP analysis. RAPD analysis was carried out to authenticate *Panax ginseng* among *ginseng* populations. By using RFLP-PCR very different fingerprints were obtained within Korean ginseng plants. Their results supports that these methods are able to authenticate the concerned *Panax* species (Um et al. 2001).

RAPD analysis was also performed to check the genetic variability of *Astragalus* medicinal material. Along with RAPD analysis, SSCP analysis was also performed on PCR products from ITS-1 region of ribosomal DNA to differentiate plant species. In their study, Cheng et al., 2000, suggested that the major factor, which affected the reproducibility of RAPD is the quality of the extracted DNA and thus considered that contaminated DNA very easily introduced variable RAPD patterns. They observed that the polymorphic patterns among the *Astragalus membranaceus* samples in RAPD analysis showed genetic variations in the samples they collected. They could not differentiated between various ITS-1 PCR products therefore, they successfully utilized SSCP technique for better discrimination and suggested that SSCP analysis will be frequently performed as an additional strategy to the RAPD technique and thus concluded that the RAPD technique provided a sensitive and fast method for identification of large number of herbs (Cheng et al. 2000).

RAPD method also contributed in quality control and scientific analysis of traditional Chinese medicines like *Atractylodes* plants because of its efficiency and sensitivity and thus is also used to investigate phylogenetic relationships among closely related species (Chen et al. 2001).

Genus *Echinacea* are perineal herbs and are used traditionally as medicinal plants. In order to determine the genetic relationship, level of genetic diversity between the germplasm and to compare accessions of each commercially important species from different sources, RAPD analysis was done and thus found that most of the variation occurred within accessions of the same species (Kapteyn et al. 2002).
RAPD marker technique was utilized in combination with two-way pseudo-testcross mapping strategy to differentiate the species of Eucalyptus at genomic level. It was suggested that using this combination of technique, one could construct single individual genetic linkage maps. With crosses of genetically divergent individuals and mitigating the problem of linkage equilibrium between marker and trait loci for the application of marker assisted strategies in tree breeding (Grattapaglia et al. 1994).

With the help of RAPD technique, Jojoba clones were discriminated at the genetic level. The data showed that RAPD technique generated multiple amplified fragments. Some DNA fragments stained as single band contained different DNA species of the same size and that cloned RAPD products of known sequence that do not target repetitive DNA can be used as hybridization probes in RFLP to detect a polymorphism among individuals (Amarger et al. 1995).

Epimedium species including E. Sagittaum, E. Koreanum, E. Grandiflorum var. higoense, E. Trifoliatobinalum, E. Trifoliatobinalum, E. Koreanum, E. Sempervirens and E. Diphyllum were analyzed for genetic characterization and variation using RAPD analysis and RFLP technique (Nakai et al. 1996).

For detecting genetic variation in Neem, which is one of the most useful plants with huge medicinal usage, RAPD technique was employed and the results suggested that Neem might have narrow genetic base (Farooqui et al. 1998).

Researchers also evaluated highly potent antimalarial compound artemisinin, obtained from Artemisia annua L. plant, was sole attention for scientists. Therefore, in order to analyze and select the plant with maximum content of artemisinin, RAPD marker technique was utilized and clearly differentiated genetic variation among these plants and thus could be useful tool for selection of better and superior species (Sangwan et al. 1999).

RAPD analysis was performed for differentiation of 3 species of medicinal plants in genus Scutellaria (S. galericulata, S. Lateriflora and S. baicalensis). RAPD markers were generated by two specific primers that were capable of differentiating members of the 3 species of Scutellaria. Thus, these markers were found to be useful for identification of the three species of medicinally important Scutellaria plants (Hosokawa et al. 2000).

RAPD analysis was performed on Kenaf (Hibiscus cannabinus L.), a is very useful herb. In order to conserve useful Kenaf germplasm, the study of their genetic diversity and to identify different Kenaf variability was important. With the help of RAPD analysis and by analyzing Argonomic characters, their different varieties and genetic relationships were identified (Cheng et al. 2002).

**RAPD analysis for differentiating adulterants from standard herbs**

Pharmacognostical identification of American and Oriental ginseng roots was conducted by genomic fingerprinting using arbitrarily primed PCR (APPCR). They amplified DNA from dried or fresh roots of three medicinal Panax species and their adulterants by AP-PCR or RAPD. They found consistent fingerprints for P. ginseng or P. quinquefolius but very different fingerprint from adulterants and thus confirmed that P. ginseng is closely related to P. quinquefolius than to P. notoginseng. For authentication of concerned Panax species, PCR approach might be used (Cheung et al.1994).

RAPD analysis was done to differentiate three medicinal Panax species from their adulterants and this technique was also helpful in authentication of these three Panax species (P. quinquefolius, P. ginseng and P. notoginseng) (Shaw et al. 1995).

DNA fragment obtained by RAPD analysis of Panax quinquefolius was converted to a sequence characterized amplified region (SCAR) marker and primers synthesized on this sequence were used in authentication of Panax species and their adulterants (Wang et al. 2001).

To search high quality Panax ginseng among ginseng populations, RAPD technique was utilized. Along with RAPD analysis, PCR-RFLP analysis was also utilized for authentication of Panax ginseng species (Tochika-Komatsu, Y. et al. 2001).

Genomic fingerprints were also used to differentiate *herba elephanto* (Ku-di-dan) from its adulterants. DNA fingerprinting and polymorphism among *herba
elephantopi was demonstrated by AP-PCR and RAPD (Cao et al. 1996).

By using RAPD analysis, AP-PCR and Genomic fingerprints, Taraxacum mongolicum (species of Compositae) were differentiated from their adulterants (Cao et al. 1997).

**RAPD analysis for identification of components in herbal prescriptions**

The utilization of RAPD technique was studied for determination of components in 3 Chinese herbal prescriptions such as the dried root of Astragalus membranaceus (Fisch.) Bge., the dried root of Ledebouriella seseloides Wolff, and the dried rhizome of Atractylodes macrocephala Koidz and thus concluded that this technique prove the identification of components in Chinese herbal prescriptions (Cheng et al. 1998).

Microsatellites also called short tandem repeats (STR) are simple sequence repeats (SSRs) and are wide spread over the genome of living organisms. Therefore, microsatellite alleles are co-dominant markers. Microsatellite are polymorphic in nature and provide more information per assay than any other technique. Using this technique, one can detect the difference at multiple loci between strains. This technique can be applied for specie and strain identification, genome mapping etc. Internal transcribed spacer regions (ITS-1 and ITS-2) are the small sequences of nuclear ribosomal gene utilized extensively in differentiation, examination and diagnosis of taxonomic status of species (Wright et al. 1994, Rong Wen et al. 1991, Porter et al. 1991).

This is one of the most effective and reliable techniques for differentiating species of herbal medicines and their adulterants.

**Microsatellite technique for differentiating species of herbal medicines and their adulterants**

Phylogenetic relationship was constructed for 12 species of Panax using internal transcribed spacers (ITS) and the 5.8S coding regions of the nuclear ribosomal DNA repeat and it was found that discrepancy between the sequence divergence pattern and phylogenetic pattern in Panax suggest that sequence divergence data alone is not enough in inferring bio-geographical patterns (Wen et al.1996). Studied have been conducted on the molecular differentiation of Atractylodes drugs by using PCR-RFLP and PCR-Selective Restriction analysis (PCRSR) on the 18S-5.8S rDNA Intra-transcribed spacer region 1 (ITS 1) gene. They amplified 18S-5.8S rDNA Intra-transcribed spacer I-gene regions from Atractylodes japonica, A. lancea and A. ovata rhizomes. They made distinction between Atractylodes species by cloning and sequencing of the PCR products. Thus it was concluded that both techniques such as PCR RFLP and PCR- SR analysis on the ITS 1 gene were useful tools for authentication between Atractylodes rhizomes (Cheng et al. 1997).

Researchers also utilized another way, which might be a useful tool for authentication and differentiation of medicinal herbs mainly medicinal Dendrobium species from one another and there adulterants is the sequences of the internal transcribed spacers 2 (ITS 2) of ribosomal DNA and perhaps these regions could be adopted as a molecular marker for differentiating medicinal herbs from adulterants (Lau et al. 2001).

Chemical analysis and differentiation of three different samples of Cannabis sativa L, were classified as tetrahydrocannabinol (THC) and cannabidiol (CBD) chemotypes. HPLC patterns could not differentiate the two samples of CBD but inter-simple sequence repeat (ISSR) fingerprinting clearly differentiated polymorphic DNA patterns of these samples which were undifferentiated by HPLC analysis (Kojoma et al. 2002).

In order to differentiate medicinal Codonopsis species (Codonopsis pilosula, Codonopsis tangshen, Codonopsis modesta and Codonopsis nervosa var. macrantha) from similar adulterants Campanumoea javana and Platycodon grandiflours, the sequence difference between medicinal species from adulterants was evaluated using PCR-RFLP and ITS. These techniques were found to be effective and reliable (Fu et al. 1999).

Other PCR based technique used is ARMS-PCR. This technique is used to detect point mutations, deletions/insertions, heterozygosity in genomic DNA sequence and genotyping. Through this technique, single nucleotide polymorphisms can be analyzed (Newton et al. 1989).
This technique is mainly used for differentiating different species of medicinal herbs. This technique was also applied for the conserved genes of medicinal plants for their authentication.

**ARMS-PCR for differentiating species of medicinal herbs and their authentication**

Five *Panax* species (*P. ginseng*, *P. japonicus*, *P. quinquefolius*, *P. notoginseng* and *P. vietnamensis*), were differentiated by using multiplex amplification refractory mutation system (MARMS). In their study they extracted total DNA from 5 *Panax* species and amplified by means of PCR and thus concluded that the method they utilized could give more reliable results for identification of 5 *Panax* species as well as for corresponding Ginseng drugs by simultaneous detection of 4-site nucleotide differences on 2 completely different genes (Zhu et al. 2003).

Scientists also studied the sequence analysis of Chinese and Japanese *Curcuma* drugs such as *Curcuma longa*, *C. phaeocaulis*, the Japanese population of *C. zedoaria*, *C. kwangsiensis*, *C. wenyujin*, and *C. aromatic* based on a comparison of their 18S rRNA gene and trnK gene and for their authentication they applied amplification-refractory mutation system (ARMS) analysis. They observed that by using ARMS methods, together with information on producing areas, the identification of *Curcuma* plants was accomplished. Moreover, the ARMS method for the trnK gene was also valuable for authentication of *Curcuma* drugs (Sasaki et al. 2002).

Another PCR based technique utilized by scientists is **MASA-PCR**. This technique is mainly applied for studying and analyzing mutations present in any particular gene (Shibata et al. 1998, Hasegawa et al. 1995).

This technique was used for differentiating species of medicinal herbs by analyzing conserved gene fragments.

**MASA-PCR for differentiating species of medicinal herbs**

Researchers developed a method for identification of ginseng drugs at the DNA level using PCR-RFLP and mutant allele specific amplification (MASA) analysis, based on differences of the 18S rRNA gene sequence among three Panax species. In this study they extracted DNA of each species and amplified by PCR and extention was established (MASA analysis). Their results suggested that PCR-RFLP and MASA analysis under the established conditions are convenient for identifying three ginseng drugs (Fushimi et al. 1997).

**Amplified fragment length polymorphism (AFLP) and Restriction fragment length polymorphism (RFLP)**

Other useful techniques, which are used for authentication of medicinal herbs, are AFLP and RFLP. These are most common procedures for DNA fingerprinting. In these techniques, restriction enzymes are used to cut DNA strands into small fragments and these fragments are separated by electrophoresis. These techniques are also used for the same purpose as RAPD analysis but all these techniques differ in reproducibility according to the type of medicinal plants (Cheng et al. 1997, Vos et al. 1995).
Korea are homogenous genetically whereas samples of
P. quinquefolius from different sources are much
heterogenous. For authentication of the two ginsengs,
the minisatellite DNA was used (Ha et al. 2002).

AFLP analysis was performed in Echinacea purpurea
germplasm and related wild species. On the other hand
tetraene and cichoric acid contents were assessed by
HPLC and observation suggested that AFLP data was
statistically significant predictor of phytochemical
marker in E. purpurea germplasm and related wild
species (Baum et al. 2001).

Withania somnifera, which is an important medicinal
plant and their withanolide compounds have promising
anti-cancer activity. The genetic variability among 35
individuals of W. somnifera and 5 individuals of W.
Coagulans were studied using AFLP marker technique
and it was found that AFLP technique was useful tool
for detecting variation at genomic level in inter and
intra specific level and thus it is an efficient tool for
detecting genetic variation in plant species (Negi et al.
2000).

ALFP technique was also utilized to assess genetic
relationship between Papaya and related species.
Vasconcella was considered as a section within the
genus Carica but ALFP technique helped in
identifying genetic variation between C. papaya
accessions and Vasconcella group (Van Droogenbroeck et al. 2002).

RFLP analysis for differentiating species of
medicinal plants and their adulterants and for their
authentication
For molecular authentication of Panax species, RFLP
was utilized and thus difference was observed between
P. ginseng and P. quinquefolius. Through this
technique, ginsengs were discriminated from its
common poisonous adulterants (Ngan et al. 1999).

Utilizing PCR and PCR-RFLP based on Nuclear
Ribosomal DNA Internal Transcribed Spacer
Sequences identified Fritillaria pallidiflora, which is
commonly used antitussive herb. Utilizing these
techniques, they differentiated and authenticated F.
pallidiflora from other species of Fritillaria and thus
concluded that these methods were effective and
accurate for identification of F. pallidiflora (Wang et al.
2005).

Medicinally used Rheum species were studied and
identification of Rhei rhizoma was done by utilizing
PCR-RFLP and Amplification Refractory Mutation
System (ARMS) analysis and thus concluded that
these methods reliable to classify the botanic origins of
22 drug samples of Rhei Rhizoma (Yang et al. 2004).
PCR-RFLP was also used in this year for the
authentication of Atractylodes-derived crude drugs
(Mizukami et al. 2000).

DNA microarray
Gene profiling has been facilitated by microarray
analysis. With the help of this technique, hundreds or
thousands of genes can be detected simultaneously and
thus the technique provides better analyzing tool for
screening target genes whose expression is different
from genes in other samples. With the help of DNA
microarray, one can study the whole genome picture
(Nuwaysir et al. 1999 and Ekins et al. 1999).

Unlike conventional method such as Northern blotting
in which few genes are studied, in microarray analysis
full genome can be analyzed. Through this technique,
new genes and their level of expression in different plant tissues and in different conditions are identified.

This technique is very useful in identifying particular standard herbal medicines from its processed components for example:

DNA microarray technique was utilized for detecting processed medicinal plants like *Dendrobium* species (*Herba dendrobii*) and florescently-labeled ITS 2 sequences were used as a probe and via this technique the particular herb can be identified from medicinal formulations and different components (Zhang *et al.*, 2003).

**Sequencing of conserved DNA regions**

Some scientists utilized direct sequencing of conserved DNA regions for accurate identification of standard medicinal plant.

**Sequencing of conserved DNA regions for authentication of medicinal herbs**

For the identification of *Panax notoginseng* and its adulterants, DNA sequencing was utilized and thus concluded that this method is an accurate and reliable in origin identification of the genuine *notoginseng* (Cao *et al.*, 2001).

PCR method was used for identification of Bulb from *Fritillaria cirrhosa*, which is an important traditional Chinese herbal medicine. They cloned the SS-rRNA spacer region sequences of *F. thunbergii*, *F. pallidiflora*, *F. ussuriensis*, *F. delavayi*, *F. cirrhosa*, *F. anhuiensis*, *F. puqensis* by PCR. They designed the pair of specific primers for differentiation of bulb of *F. cirrhosa* from each other by using PCR and observed that this method is rapid and more accurate for identification of the bulb of *F. cirrhosa* at the DNA level (Li *et al.*, 2003).

*Acorus gramineus* and three types of *Acorus calamus* were also analyzed for their phylogenetic relationship by comparing specific sequence of a 5 S-rRNA gene spacer region. It was observed that *Acorus gramineus* was distinguishable from chemotypes of *Acorus calamus* and this spacer region data correlated well with essential oil chemotype of *Acorus calamus* (Sugimoto *et al.*, 1999).

**Examination of Herbal Drugs for their Effectiveness, Usefulness and Mechanism of Action**

The effectiveness, usefulness and mechanism of action of herbal medicines can also be evaluated using molecular biological techniques like Reverse transcription-PCR (RT-PCR), In-situ hybridization, Real time PCR, Northern blotting etc.

**Reverse transcription-PCR (RT-PCR)**

This is the technique used for detecting RNA expression of any particular gene by reverse transcribing RNA into cDNA and followed by PCR. This technique is used to analyze the effect of herbal drugs on specific genes in human or animal cells.

![Image](image_url)

**Figure 6:** Analysis of IFN-Gamma in PBMC after 6 hours of treatment. 1. DNA ladder, 2. Control, 3. PHA Positive control, 4. *Beriberis aristata*.

![Image](image_url)

**Figure 7:** Agarose gel electrophoresis of PCR reactions on U. dioica genomic DNA. Biochemical and molecular data on *urtica dioica* plant peroxidase. Douroupi, T, and Margaritis I.H.

**In-situ hybridization**

This technique allows the examination of mRNA expression or specific DNA sequences in particular tissue or cells. In this technique, labeled probes are used that can hybridize to the nucleic acid sequence of interesting fixed tissues or cells and this tissue or cells are then examined microscopically (Wilcox 1993).

**RT-PCR and In-situ hybridization for evaluation of effectiveness, usefulness and mechanism of action of herbal drugs**

It was reported that in chronic pancreatitis acinar cells of pancreas of WBN/kob rats were deteriorated by
apoptosis. To observe the effects of herbal medicine TJ-10 (Chaihu-Guizhi-Tang) on these mice which were kept on pellet diet (MB-3) for 20 weeks. Pancreatic cells were examined every 4 weekly on histopathological examination and then expression of apoptosis related factors such as Fas and Fas ligand (Fas L) mRNA and their proteins were analyzed with the help of RT-PCR, In-situ hybridization and immunohistochemistry. Apoptosis was observed using TUNEL method. Using all these techniques, it was observed that control group rats (intubated with TJ-10) developed chronic pancreatitis at 12 weeks and progressively acinar cell damage occurred at 16 weeks. Fas and Fas L expression level increased at 12-20 weeks. Expression level of Fas and Fas L mRNA correlated to the apoptotic index in acinar cells and on the other hand in treatment group rats, pancreatic acinar cells apoptosis and Fas and Fas L expression at 12-20 weeks decreased significantly. Thus, it was concluded that TJ-10 can inhibit apoptosis in pancreatic acinar cells with chronic pancreatitis by suppressing Fas and Fas L expression (Shibing et al. 2001).

The effectiveness of Chinese Jianpi herbs like Sijunzi decoction and another Chinese recipe (SRRS) was examined in the treatment of Gastric Cancer. Human gastric adenocarcinoma cell line SGC-7901 was grafted onto nude mouse was used as an animal model. The effect of therapy was assessed by tumor size, tumor weight, TUNEL test, electron microscopy, S-P immunohistochemistry and Reverse transcriptase-PCR (RT-PCR). The low expression level was p53 mRNA and bcl-2 mRNA was observed in treatment group and thus it suggests cell apoptosis as a cause of inhibition of gastric cancer cell growth in vivo (Zhao et al. 2002).

The differentiation of rat bone marrow stromal cells into neuron in vitro was induced by baikalin, a kind of flavanoid isolated from an important medicinal plant Scutellariae Radix. The expression of neuronal or glia specific markers and gene was observed by RT-PCR, western blot and immunofluorescence cytochemistry staining and thus these observations suggested that baikalin is a useful vehicle for autotransplantaion in both cell and gene therapy for a variety of diseases of the central nervous system (Jia et al. 2002).

The mechanism of action of Chinese herbal extract of Paeoniae radix (PRE), which is the root of traditional Chinese herb, was studied on human hepatoma cell lines. They used human hepatoma cell lines HepG2 and Hep3B. They observed that the inhibitory effect of water-extract of PRE on human hepatoma cell growth was induced by apoptosis in a p53 independent pathway. They utilized microarray technique and RT-PCR analysis for differential gene expression of Paeoniae radix treated HepG2 and found up regulation of BNIP3 and down regulation of ZK1, RAD23B and HSPD1 during early apoptosis of the hepatoma cell mediated by Paeoniae radix (Lee et al. 2002).

Real time PCR
In this method, data is provided and one can quantitate the DNA or RNA during whole PCR process or we can say that this is a sort of quantitative assay (Schena et al. 1995).

Northern blotting
This is the technique used to analyze the RNA of interest. In this technique, the RNA molecules that bind with the specific probe is analyzed by matching with the reference bands (Sambrook et al. 1989).

Real time PCR and Northern blotting for evaluation of effectiveness, usefulness and mechanism of action of herbal drugs
Investigation was conducted on Gum resin of C. mukul plant, which contains Guggulsterones (plant sterols). These sterols are used in the complex mixture of compounds in herbal remedies termed gugulipid. These sterols are used for their lipid lowering, thyroid stimulating, platelet aggregation inhibiting properties etc. it was observed that Guggulsterones function as agonists for about three nuclear receptor proteins (estrogen receptor isoform ER; NR3A1, the progesterone receptor PR; NR 3C3 and the pregnane X receptor PXR; NR112 in cell based assays and in cultured hepatocytes (derived from rodents and humans) by PXR activation. Guggulsterones and Gugulipid also induce CYP3A gene expression, which encodes the most abundant drug-metabolizing cytochrome P450 in human liver and is responsible for the biotransformation of about 60% of of drugs and these results suggest that as on gugulipid therapy, CYP3A drug metabolizing pathway may be induced thus one must be careful in co-administrating medicines that are metabolized by way of this pathway because this remedy activates PXR and promotes drug metabolism and thus can induce herb-drug interaction. Expression of CYP3A4 (human) and CYP3A11
PCR analysis was performed for examining gene expression levels (Staudinger et al. 2004).

CONCLUSION

For chemoprofiling and for quality control analysis of herbal drugs, mainly routine pharmacognostic studies are made like evaluating microscopic and macroscopic features of herbal drugs along with HPLC, TLC, HPTLC, GC, Column chromatography, spectrophotometric techniques etc. with the help of these techniques, minor impurities in herbal drug material can be determined and thus ensures better quality of herbal material. Although these techniques are useful tools, they cannot determine the absolute chemical characteristics and genetic variation in chemical composition of same specie or genotype of medicinal plants. Therefore, there was need for better selection of useful compounds, standard plant specie and analyzing functionality of herbal medicine. Therefore, various molecular biological techniques have provided us better way to authenticate herbal medicines and to study their mechanism of action and effectiveness. In fact, with the help of molecular biological techniques and DNA based markers, significant advances have been made in understanding the mechanism of action, analyzing the usefulness, effectiveness, standard specie detection and confirmation of genetic make up of standard herbal medicine in purified as well as crude form. This advancement is much helpful in quality control analysis and ensures better authentication of herbal drugs.

REFERENCES


Manuscript received 03 - 05 - 2005
Accepted for publication 02 - 07 - 2005