6. SUMMARY AND CONCLUSION

10 indigenous drugs of high therapeutic values were selected for the current study. The names of the selected indigenous drugs are: Tukhm-e-kasoos (seeds of Cuscuta reflexa), Barg-e-sudab (leaves of Ruta graveolens), Filfil siyah (fruits of Piper nigrum), Zarishk (bark of Berberis aristata), Kurkum (floral parts of Crocus sativus), Amla (fruits of Emblica officinalis), Mulethi (Root of Glycyrrhiza glabra), Rewand chini (Root of Rheum emodi), Unnab (berries of Zizyphus jujube) and Senna (leaves of Cassia angustifolia and Cassia acutifolia). The market samples of the selected indigenous drugs were procured from Khari Baoli, New Delhi and authenticated by Dr. H.B Singh at NISCAIR, Pusa road, New Delhi. The samples of these drugs were also supplied by CCRUM, Ministry of Health and Family Welfare, Government of India. These samples were also sent to NISCAIR for identification and found to be genuine. The drugs sold in the market were, however, found to be adulterated or substituted by some other herbs of same or different genera and families. The basis of adulteration was the remarkable morphological similarities among the genuine drugs and their adulterants. They were compared on various features including the organoleptic characteristics like colour, taste, odour, texture, etc. and were found to be significantly similar. After an acute observation, it was concluded that it was almost impossible to differentiate between the genuine drugs and their adulterants in their intact or powdered forms. The most surprising aspect of the study was that when the adulterants were studied for their therapeutic value, they were found to have entirely different medicinal properties. The reasons for adulteration include sourcing of raw material by the traders from illiterate plant collectors, mixing weeds and other undesirable herbs from the sight of collection and deliberate adulteration by traders to get more economic benefits in case of costly medicinal herbs, but the consequences are indeed hazardous and sometimes life threatening (Khan et al., 2009). The mixing of two or more herbs not only decreases the therapeutic efficacy of the indigenous drugs but can also have adverse effects on the body due to antagonistic effects of the secondary metabolites present in these herbs. In some drugs like Filfil siyah (fruits of Piper nigrum) and Barg-e-sudab (leaves of Ruta graveolens), the adulterants are even known to have adverse effects on the human body. The observations made earlier and during this study are alarming, and, therefore, it is highly desirable to develop easy,
simple, sensitive and cost effective techniques for authentication of herbal drugs and discrimination of adulterants from genuine medicinal herbs. These techniques are essential for both pharmaceutical companies as well as government’s quality control agencies to maintain public health. There are many molecular markers available in the literature for the identification of plants at the level of lowest taxon including herbal drugs. Limitations of chemical and morphological approaches for authentication have, however, generated a need for newer, more specific and sensitive methods in quality control of herbal formulations.

SCAR, a PCR-based marker, represents single, genetically defined locus identified by amplification of genomic DNA with a pair of specific oligonucleotide primers. SCAR markers may contain high-copy number and dispersed genomic sequences within the amplified region. Thus, they are valuable in large-scale and locus-specific applications such as marker-assisted screening and map-based gene cloning. A SCAR marker, generated from polymorphic regions that differ in size among species, permits sample authentication based on SCAR size shifts. Different DNA-based markers viz. AFLP, SSR, ISSR and RAPD can be used to generate these markers. However, high reliability of SCAR markers could lead to the displacement of RAPD and other DNA-based markers which are costly, time consuming and tedious. Also high detection sensitivity and avoidable electrophoresis step makes it an economical molecular tool. It can be carried out in any laboratory, in a very short period using unknown genomic DNA from any developmental stage, tissue and organ of the herbs (Kethidi et al., 2006). The quantity of the sample required is very small as compared to other conventional pharmacognostic techniques and no prior information about the genomic DNA is required. SCAR markers can enable screening of numerous samples, accurately at one time thus, adding to the cost efficiency of the experiment (Kasai et al., 2000). Also SCAR markers are not affected by the presence of introns that could eliminate the priming sites (Harmandez et al., 1999). Thus, so far SCAR marker seems to be the best suitable technology for authentication of traditional medicinal herbs, both at the stage of formulation, development and also in the finished products. The developed SCAR markers were also found to be highly sensitive.

The first step in SCAR marker development was genomic DNA isolation. DNA of high quality and purity was isolated from each selected drug as well as the adulterants using the modified CTAB method (Khan et al., 2007). The DNA was further
quantified for RAPD. RAPD was performed in all the drugs and their adulterants using 20 operon primers obtained from Operon Life technologies, India. There was a high degree of polymorphism obtained in each RAPD profile and almost an equal number of similar and unique bands were obtained in each drug. The most prominent and intense unique band was, however, selected in each drug and its respective adulterant. These unique RAPD amplicons were cloned in to pGEMT Easy vector and the inserts were further sequenced using SP6 and T7 promoters. Homology searches were performed using non-redundant database at Genebank using BLAST at NCBI with the sequenced RAPD amplicons obtained from all the drugs and their adulterants. BLAST results revealed that these sequences did not have significant similarity with the sequences in the database and also were not contaminated with the vector sequence. Based on the characterized sequences, specific primers for all the herbs, both genuine and adulterant were designed and their thermodynamic efficiency was confirmed. Each specific pair of primers also known as the SCAR primers was used to amplify the genomic DNAs of the genuine drug as well as the adulterants. When amplification was performed using the SCAR primers of the genuine drug, a band of desired size was obtained with the genomic DNA of the genuine drug and no amplification was observed in the adulterant. Similarly, with the SCAR primers of the adulterant, amplification was observed with the genomic DNA of the adulterant and no band could be seen with the genomic DNA of the genuine drug. This confirmed the specificity of SCAR markers which were developed for each drug as well as the adulterant. SCAR markers of 589bp in *Cuscuta reflexa*, 464bp in *Cuscuta chinensis*, 600bp in *Ruta graveolens*, 750bp in *Euphorbia dracunculoides*, 350bp in *Piper nigrum*, 589bp in *Carica papaya*, 500bp in *Emblica officinalis*, 800bp in *Ipomoea batatas*, 400bp in *Crocus sativus*, 800bp in *Carthamus tinctorious*, 652bp in *Berberis aristata*, 220bp in *Coscinium fenestratum*, 613bp in *Glycyrrhiza glabra*, 488bp in *Abras precatorious*, 553bp in *Rheum emodi*, 707bp in *Rheum palmatum*, 351bp in *Zizyphus jujube*, 370bp in *Zizyphus nummularia*, 829bp in *Cassia angustifolia*, 589bp in *Cassia acutifolia*, 398bp in *Cassia tora* and 589bp in *Cassia sophera* were successfully developed. The reproducibility of the developed SCAR markers was tested by repeating each experiment twice or even thrice in some cases. Similar results were, however, obtained each time which confirmed the high reproducibility of SCAR markers. The sensitivity of these SCAR markers was also tested by using the
mixtures of genuine drugs and the adulterants in the aforementioned ratios (Table 11) for PCR amplification using the SCAR primers of both the genuine drug as well as the adulterant. It was inferred that the developed SCAR markers were highly sensitive and capable of detecting even 10% of the genuine material present in the mixture. Since we have developed SCAR markers for the adulterant also, these can, therefore, be utilized to detect as low as 10% of adulterant present in the given dry samples. The SCAR markers were also validated by using them on various samples collected from the market of different states of the country. All the major markets of medicinal herbs were surveyed and the selected indigenous drugs were collected from 12 different places in India. Each drug was examined using the SCAR primers of both the genuine drug as well as the respective adulterant. A high degree of adulteration was detected in each drug procured from almost all the markets. Some drugs were even found to be completely replaced by some other herbs. The drug Barg-e-sudab (leaves of Ruta graveolens) was not found in any of the market samples. Instead, it was replaced by Euphorbia dracunculoides. The drugs Rewand chini (roots of Rheum emodi), Zarishk (barks of Berberis aristata) and Mulethi (roots of Glycyrrhiza glabra) were also replaced by their adulterants in most of the market samples. The drugs Kurkum (floral parts of Crocus sativus), Filfil siyah (fruits of Piper nigrum), Unnab (berries of Zizyphus jujube), Tukhm-e-kasoos (seeds of Cuscuta reflexa) and Senna (leaves of Cassia angustifolia) were found mixed with varying quantities of their respective adulterants in most of the market samples. The results of the survey of market samples were indeed appalling.

Since consequences of such high levels of adulteration have an adverse impact on public health, the use of this technique by pharmaceutical industries and government monitoring agencies is highly desirable for quality assurance of indigenous drugs. To make this technology more simple and amenable to the users, we modified the existing protocol by eliminating some of the steps involved in the use of SCAR markers for authentication of medicinal herbs. Thus, we have been able to develop a rapid, cost effective and even simpler protocol with minimum labour requirement, expertise and instruments. The modified protocol was optimized to enable screening of numerous samples in a single experiment, making it suitable as a fool proof method for correct identification of genuine medicinal herbs and their adulterants in the market samples and processed drugs. This will be a major step towards controlling
adulteration in the market samples. This in turn will increase the therapeutic efficacy of indigenous drugs and will prove to be a boon to the traditional systems of medicine.