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Chemical marker compounds and their essential role in quality control of herbal medicines

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Abstract

Many countries are registering their standardized herbal drugs with proven clinical efficacy, safety and as dietary supplements. But India is unable to exploit the World market due to unsatisfactory system of quality control which necessitates the establishment of standards for herbal single drugs and compound formulations. In order to ensure international community to use AYUSH drugs with confidence and without compunction, developing acceptable quality control standards of herbal medicines aims to ensure their consistency, safety and efficacy with good manufacturing practices is very much essential. Study of chemical markers is applicable to many research areas, including authentication of genuine species, search for new resources or substitutes of raw materials, optimization of extraction and purification methods, structure elucidation and purity determination. Systematic investigations using chemical markers may lead to discoveries and development of new drugs.

Key words: Marker, Standardization, Formulation, Quality control, Herbal drugs

Introduction

Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health (Sane, 2002). In India, around 20,000 medicinal plant species have been recorded recently but more than 500 traditional communities use about 800 plant species for curing different diseases (Kamboj, 2000; Verma and Singh, 2008).

In other developing countries too, plants are the main source of medicine. Two of the largest users of medicinal plants are China and India. Traditional Chinese Medicine uses over 5000 plant species; India uses about 7000. According to Export Import Bank, the international market for medicinal plant related trade having a growth rate of 7% per annum. China's share in world herbal market is US$ 6 billion while India's share is only US$1 billion.

Herbal drug technology includes all the steps that are involved in converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge will remain important. Herbal medicinal products may vary in composition and properties, unlike conventional pharmaceutical products, which are usually prepared from synthetic, chemically pure materials by means of reproducible manufacturing techniques and procedures. Correct identification and quality assurance of the starting material is, therefore, an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy (Straus, 2002; De Smet PAGM, 2002).

Current regulations for standardization of crude drugs

In recent years, there is a spurt in the interest regarding survival of AYUSH forms of medication. In the global perspective, there is a shift towards the use of medicine of herbal origin.

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An innovative research effort to define the advantage of traditional system of medicine with respect to their safety and efficacy could result in a better utilization of these complementary systems of medicine (Verma and Singh, 2008).

India keen to explore the medicinally important plants which can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization such as UV-Visible, TLC, HPLC, HPTLC, GC-MS, Spectrofluorimetric and other sophisticated techniques.

Evolve systematic approaches and to develop well-designed methodologies for the standardization of herbal drugs such as of drug preliminary phytochemical screening, fingerprint profiling, standardization, and quantification of marker compound(s) with reference to herbal raw materials and polyherbal formulations need to be developed (Rajini and Kanaki, 2008).

Importance of standardization

To assess the quality of the raw material, estimate the amount of active principle present in it and to achieve batch-to-batch consistency of the finished product such as Physical/ Chemical/Organoleptic Characters, Efficacy and Safety, Shelf-life is very much important. Standardization of drug means confirmation of its identity and determination of its quality, purity and detection of nature of adulterant by various parameters.

Parameters required for quality evaluation of herbal drugs

Authentication of raw material, foreign matter, organoleptic evaluation, macroscopic and microscopic properties, ash values, extractive values, chromatographic fingerprinting, marker component isolation, pesticide, heavy metals, aflatoxins contamination estimations, shelflife which are to be carried out according to WHO guidelines for herbal drugs.

Techniques for standardization of metals, minerals and other form of compounds

Analytical methods such as Atomic Absorption Spectroscopy, x-ray Diffraction Studies, x-ray Fluorescence Spectroscopy, ICP - MS, x-ray Photoelectron spectroscopic studies, Transmission Electron Microscopy, NIR, NMR, IR, HRMS, HPLC, UV *etc*.

Development of extraction conditions, starting extract, and finished products, markers chemicals for standardization, chemical fingerprint to the extent allowed by modern analytical techniques and methods, safety evaluation like heavy metal, microbial load, bacterial load, fungal count, pesticide residue *etc.* Optimization the method of preparation of manufacturing of product and controls to assure consistency of product; stability, disintegration, dissolution, bioavailability (if known), assurance of batch-to-batch reproducibility, analytical guidelines for testing during course of study and information on matching placebo, if applicable; availability of retention samples for future testing, if desired.

Standardization of herbal drugs for Global competitiveness such as raw materials needs to be authentic, physico-chemical standards, storage conditions, size and shape. Processing of raw material include material, energy inputs, operational uniformity, safety and occupational health, intermediate quality whereas finished product include physicochemical properties, biological assay, storage stability, user safety *etc*.

Markers in herbal drug technology

Markers are chemically defined constituents of a herbal drug which are of interest for quality control purposes independent of whether they have any therapeutic activity or not.

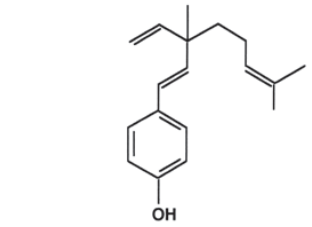
Markers may serve to calculate the amount of active component of herbal drug or preparation in the finished product if that marker has been quantitatively determined in the herbal drug or preparation when the starting materials are tested.

The bioactive extract should be standardized on the basis of active principles or major compounds along with the chromatographic fingerprints (TLC, HPTLC, HPLC and GC). In the standardization of crude drug materials, chemical and instrumental analyses are routinely used for analyzing synthetic drugs to confirm its authenticity.

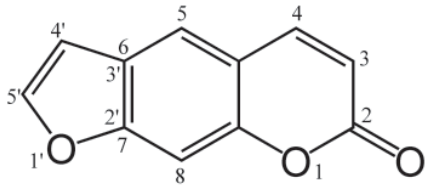
India can emerge as the major country and play the lead role in production of standardized, therapeutically effective herbal formulation. Proper utilization of rural resources to generate new herbal formulations not only can make India as one of the leading country in world in the field of medicine but also helps to generate lot of revenue, which will improve financial status and make India as financially independent.

Chemical fingerprinting has been demonstrated to be a powerful technique for the quality control of herbal medicines. A chemical fingerprint is a unique pattern that indicates the presence of multiple chemical markers within a sample.

Psoralea corylifolia Linn. is one of the most important medicinal plants used in the formulation as an ingredient for the treatment of Bars /Leucoderma/Vitiligo (Cardosa *et al*., 2002; Huang *et al*., 2000; Anonymous, Ayurvedic Pharmacopoeia of India; Maisch, 1889 and Wilson, 1999). Besides this, there are many formulation existing in the market for Vitiligo which required to contain the active principle in it such as pure psoralen and bakuchiol which were isolated from *Psoralea corylifolia* and then characterized by all the analytical and spectroscopic data their structures are as shown in Figure 1 and the Mass spectrum and HPLC chromatograms are as shown in the Figures 2(a-b). These can be quantified by using by HPLC and compounds identified by LCMS in the formulations.



Bakuchiol



Psoralen

Figure1. Strucures of bakuchiol and psoralen

Psoralen Mass Spectra shows M^+ peak at m/z of 187.0 with good intense peak whereas Bakuchiol mass spectra shows M+ Peak at m/z of 257.4 as shown in the Figure2b. HPLC chromatogram of pure Psoralen shows a peak at retention time of 20.288 minutes and pure Bakuchiol shows a peak at retention time of 34.176 minutes as shown in the Figure 2(a-d). Now with the help of psoralen and bakuchiol as marker compound, one can use these for quality control check for the various marketed formulations such as Purim, Deraflex, Cutis, Pigmento, Neemwin and Tolenorm formulations in which Purim, Deraflex contain both the compounds whereas Pigmento contain only psoralen and no bakuchiol indicating the spurious/adulteration of formulation and also in Cutis, Neemwin and Tolenorm formulations only Bakuchiol was present and Psoralen was absent, thus, adulterated formulation and not the genuine as shown in the Figures 3(a-f). Therefore, it is very much essential to know the content of the active compound species in the required limits and their presence in the finished product as to attain therapeutic property of the formulation. Thus, with the help of the quality control check up, this type of adulterated formulations can be known and make us aware of spurious drugs.

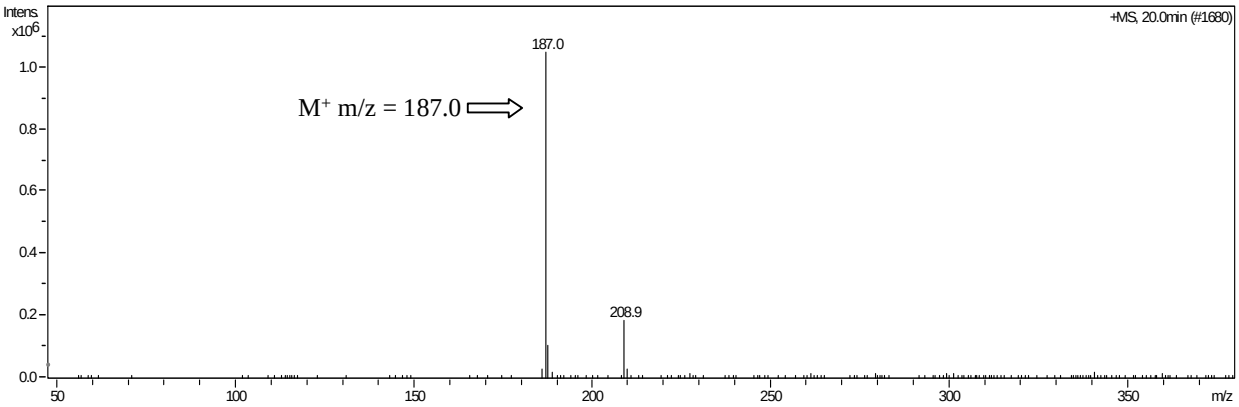


Figure 2a. Mass spectra of psoralen compound

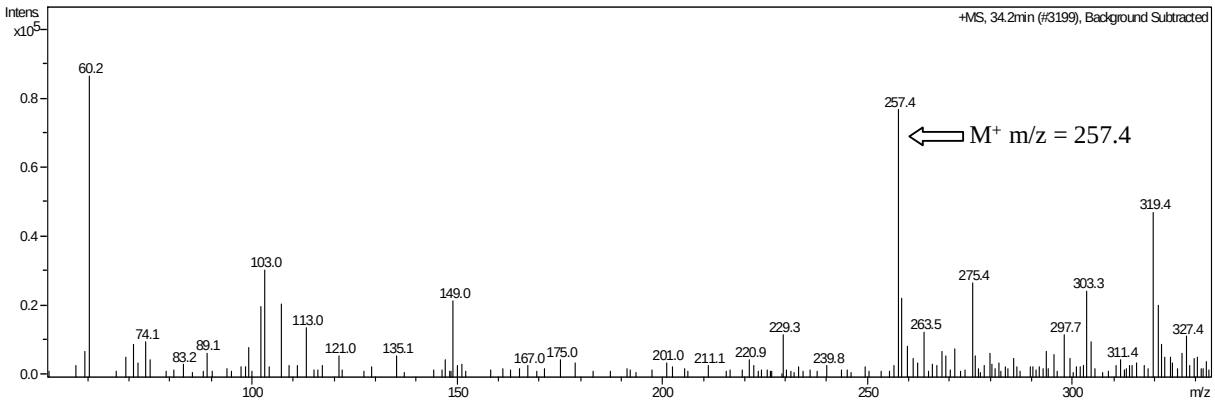


Figure 2b. Mass spectra of bakuchiol compound

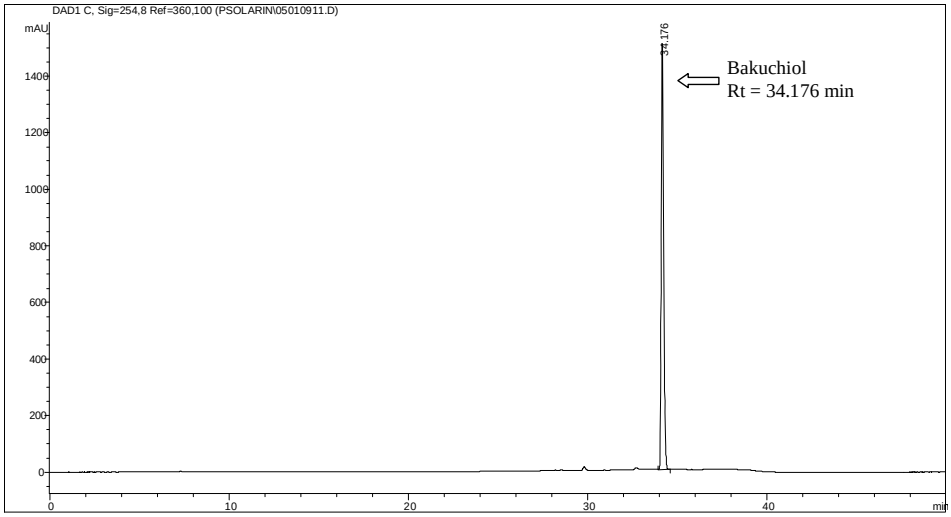


Figure 2c. HPLC chromatograms of pure bakuchiol

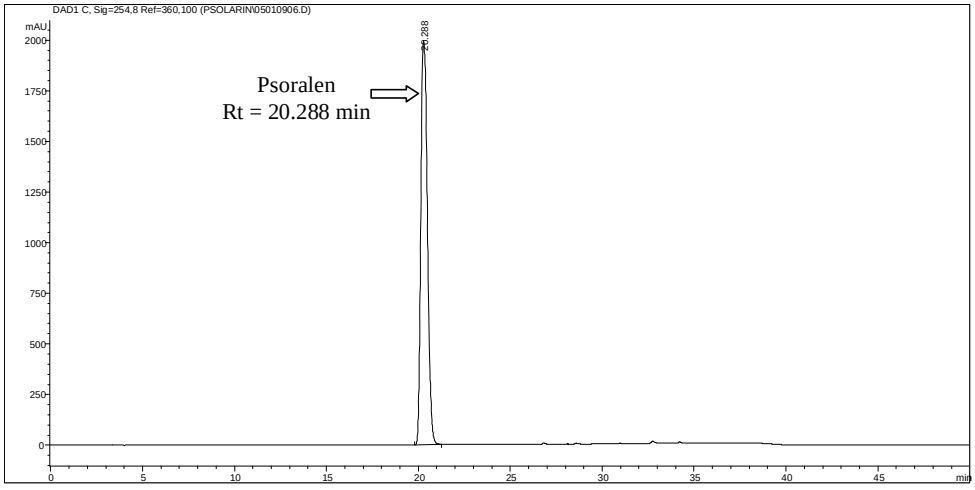


Figure 2d. HPLC chromatograms of pure psoralen

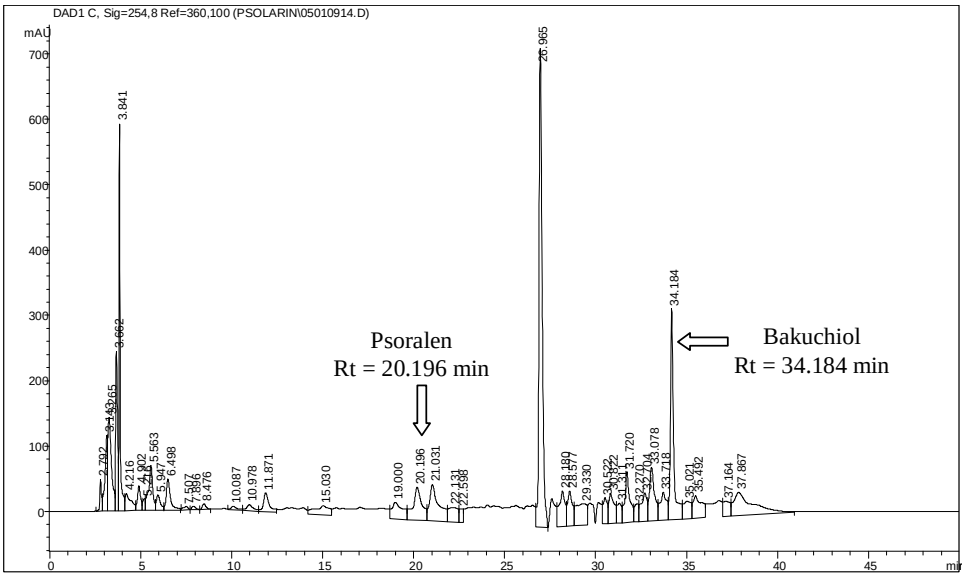


Figure 3a. HPLC chromatograms of purim formulation

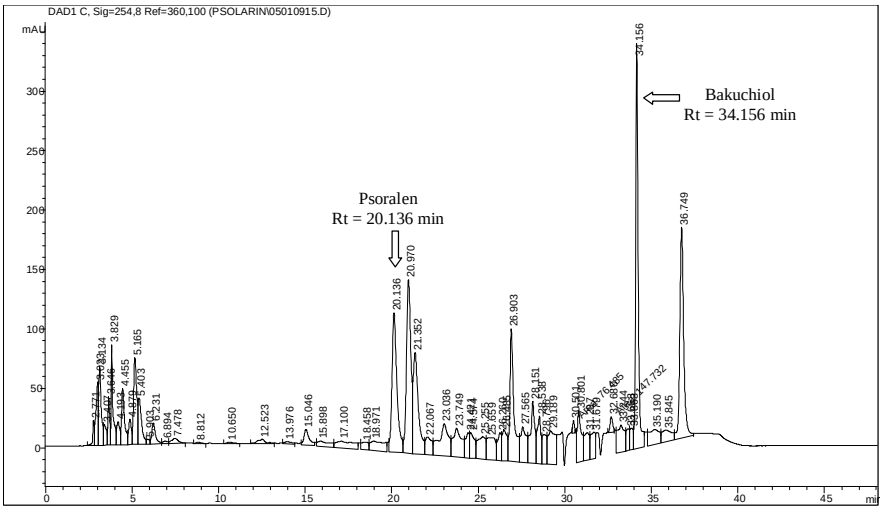


Figure 3b. HPLC chromatograms of deraflex formulation

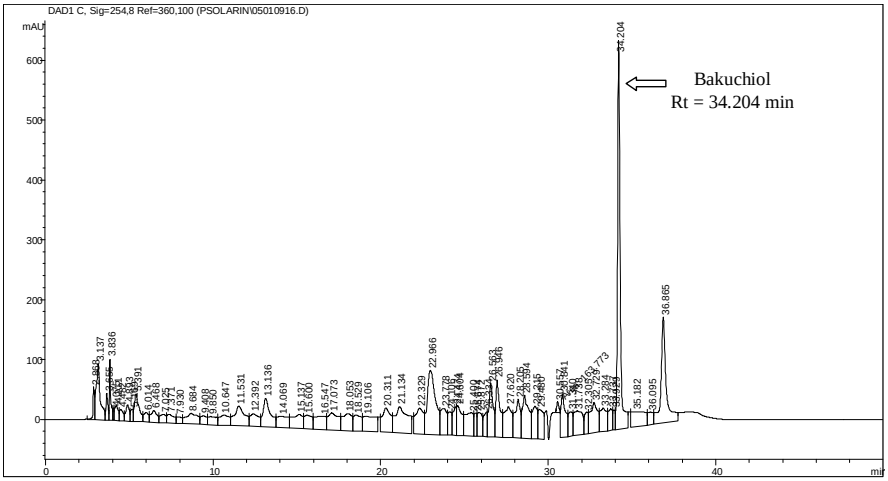


Figure 3c. HPLC chromatograms of cutis formulation

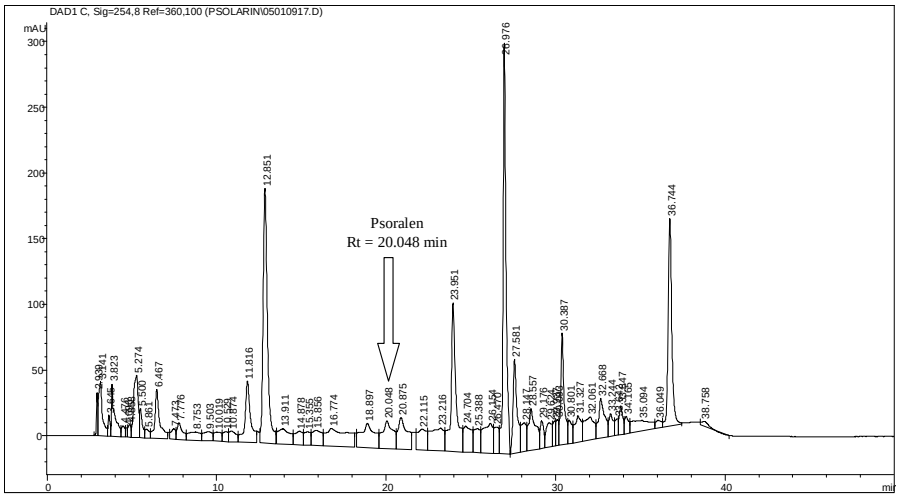


Figure 3d. HPLC chromatograms of pigmento formulation

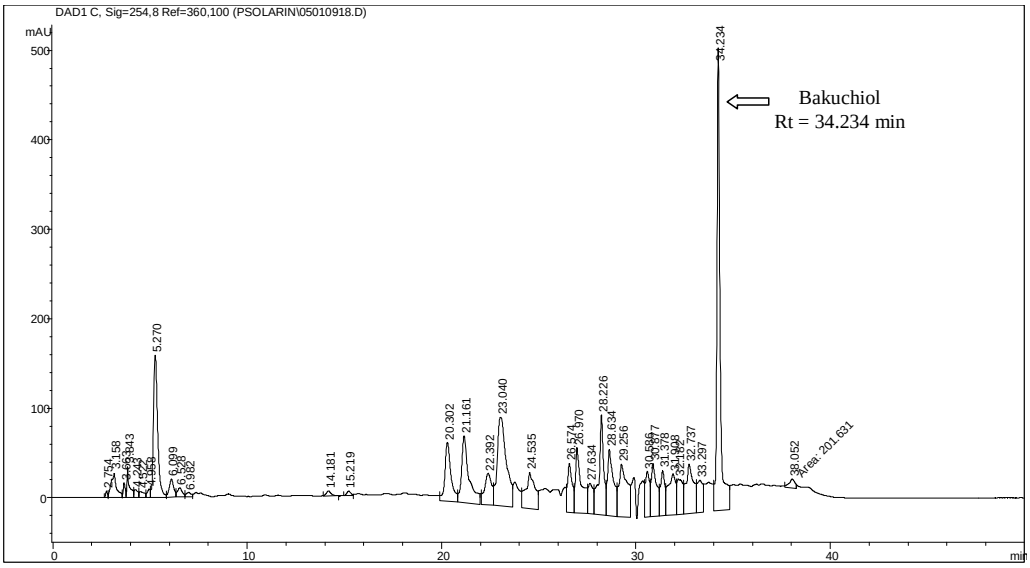


Figure 3e. HPLC chromatograms of neemwin formulation

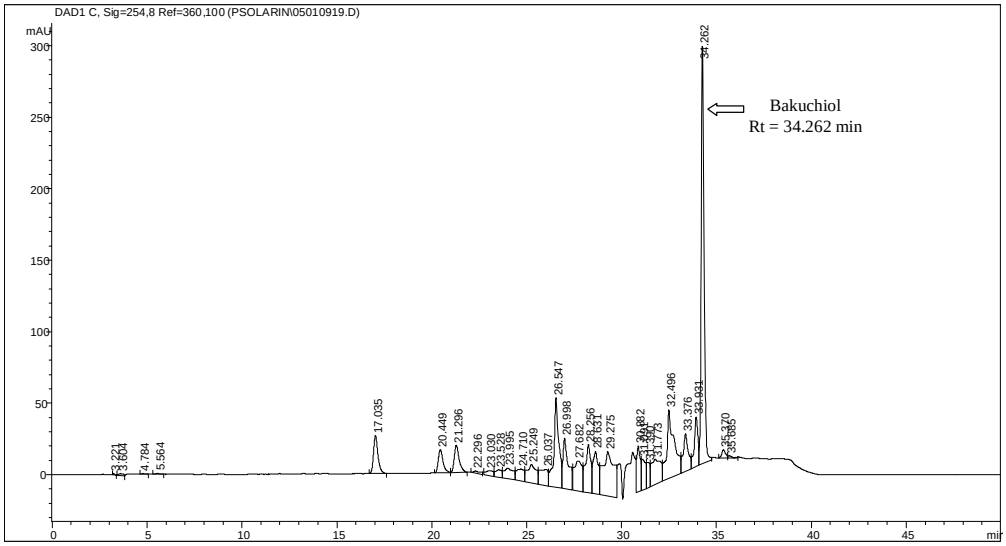


Figure 3f. HPLC chromatograms of tolenorm formulation

Methods of identification, limitations and emerging techniques

Many factors may affect the ultimate chemical profile of any herb, intrinsic factors such as genetics, and extrinsic factors such as cultivation, harvesting, drying and storage conditions. Routine chemotaxonomic studies provide only a qualitative account of secondary metabolites. For quantitative studies, use of specific markers that can be easily analyzed to distinguish between varieties, remains a preferred option. Such metabolites being used as markers may or may not be therapeutically active, but should ideally be neutral to environmental effects and management practices.

A standardized extract means that the manufacturer has verified that the active ingredient believed to be present in the herb are present in the preparation and that the potency and the amount of the active ingredient is assured in the preparation.

Furthermore, there are many technical challenges in the production of chemical markers. For example, temperature, light and solvents often cause degradation and/or transformation of purified components; isomers and conformations may also cause confusions of chemical markers. Marker compounds are pure, single isolated compounds, secondary metabolites mostly with terpenes, steroid, alkaloid, flavonoid aromatic hetero aromatic frameworks and glycosides having alcoholic, carbonyl, olefinic, acid, ester and amide functionalities highly useful for single / crude drugs: may or may not survive in multiherbal formulations

Chemical markers are pivotal in the current practice of quality control. Chemical markers should be used at various stages of the development and manufacturing of an herbal medicine. Authentication and differentiation of species, collecting and harvesting, quality evaluation and stability assessment, diagnosis of intoxication and discovery of lead compounds. Lack of chemical markers remains a major problem for the quality control of herbal medicines. In many cases, we do not have sufficient chemical and pharmacological data of chemical markers.

Development of markers for herbal drugs

The term "marker compounds can be defined as standard reference compounds used for the purpose of comparison, quality control purposes".

Development of marker provides a suitable and an important parameter for quality control of plants and herbal formulations. To help the research laboratories, drug dealers, manufacturers and pharmacies, policy makers of herbal drug to identify the genuine drugs to society in improving the health care system.

Types of chemical markers uses in herbal plant analysis

Marker assisted selection of desirable chemotypes along with authentication of species identity; prediction of the concentration of active phytochemicals may be required for quality control in the use of plant materials for pharmaceutical purposes. Identification of DNA markers that can correlate DNA fingerprinting data with quantity of selected phytochemical markers associated with that particular plant would have wide applications in quality control of raw materials.

Applications of chemical markers in different fields and areas

Markers can have a vital role in various applications such as applications of molecular markers in herbal drug technology for authentication, detection of adulteration/substitution of medicinal plants, marker assisted selection of desirable chemotypes, DNA markers as new pharmacognostic tool, markers applications in foods and nutraceuticals, purpose of safety and efficacy of the drugs.

Recent years, with the advance in analytical techniques considerable emphasis is being laid on marker compounds based identification of single drugs as well as compound formulation. The marker compounds can be defined as standard reference compounds used for the purpose of comparison.

Marker compounds are characteristic phytochemicals found in a plant. They are often chosen to represent the standard for a standardized extract. For example, in the case of say, *Boswellia serrata* also known as Shallaki or Salai Guggul. It is extensively used in Ayurveda for joint support and provides an overall sense of well being. It offers broad health and immunomodulating benefits, anti-inflammatory, anti-atherosclerotic and anti-arthritic activities. It also improves circulation of blood, it is used in cosmetic products. The gum resin of *Boswellia serrata* is known to contain 4 major Pentacyclic Triterpenic acids which are as Beta- Boswellic acid, acetyl beta- boswellic acid, 11-keto-beta-boswellic acid and acetyl-11-keto-beta-boswellic acid. The non-phenolic fraction of gum resin had marked sedative and analgesic action. It has expectorant effect and can reduce body weight. In clinical trials, promising results were observed for patients suffering with rheumatoid arthritis, chronic colitis, ulcerative colitis, bronchial asthma and edema.

In gum resin of *Boswellia serrata* Linn., the marker compound is Boswellic acid and the standardized extract of *Boswellia serrata* is fixed to contain a consistent level of this compound (usually a few percent). However, marker compounds are need not necessarily be active compounds.

Multiherbal formulations marker compounds

Marker compounds of ingredients not necessarily the markers for formulations. Some markers undergo transformations and decompose, vanish or generate new markers. Marker compounds specific to multi-herbal formulations need to be isolated. Formation of new markers depends on the various manufacturing processes.

Role of chemical markers mainly in the stability test of proprietary products to evaluate the product quality over time and determine recommended shelf life. Diagnosis of herbal intoxication in which 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 and other hyphenated techniques. The components responsible for the therapeutic effects lead compounds for new drug discovery may be investigated as lead compounds for new drug discovery.

Conclusion

Traditional medicine will contribute to human health care in the 21st century. There are many challenges to the safety and effective use of traditional medicine; efficacy, quality and standardization. Marker compounds are highly useful for quality control; markers of ingredients may not be the markers of formulations. Therefore, markers specific to formulation need to be isolated. The WHO Strategy will meet the gaps and challenges; active principles in the plants responsible for the medicinal properties of plant drugs must be identified and quantified. They may be used as chemical markers for both qualitative and quantitative assessments. Fingerprinting

profile of the marker compounds in plant drugs which shows the presence/percentage of the active principle along with the closely related bioactive principles is necessary for all herbal formulations.

Eventually standardization of herbal drugs essential to be based on scientific evidence and profile of maker compounds. At this moment, there is a vital need to carry out work in this direction.

Acknowledgement

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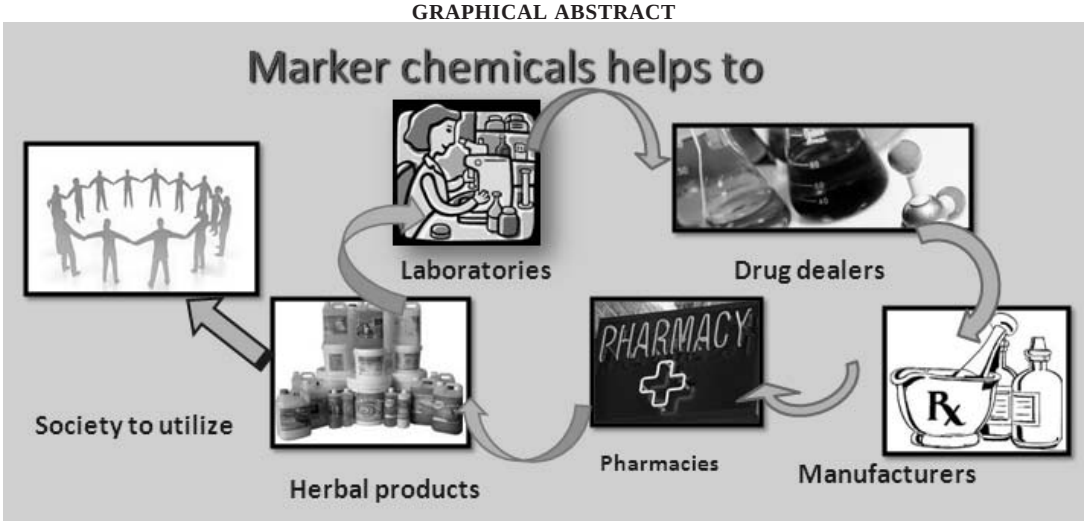
GRAPHICAL ABSTRACT



- Isolate marker chemicals from the standard herbal drugs or plant.
- Supply the need for the marker species for checking and comparing marketed drugs.
- Identify the product with the required content of the active chemical constituent.
- Quality Assurance of markers species help pharmacies to analyze their drugs with the standard one and obtain a genuine drug.
- Active Marker species content can be compared within the market products.
- Finally the product can be utilized to serve the society for welfare and promoting good health.

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