Herbal drug standardization and quality assurance of raw materials: a rational approach

R.B. JadHAV*, C.R. Patil
Department of Pharmacognosy & Phytochemistry, College of Pharmacy, Malegaon, Baramati-413115

ShrINIVAS BhPoe
Sanjivani Remedies Ltd., Pune

and

C.V. Murumkar
Department of Botany, Tuljaram Chaturchand College, Baramati- 413102

* Correspondent author, E-mail: rambhadav123@yahoo.co.in

Abstract

Most of the herbal raw material used in production of herbal medicines is procured from wild sources. Such chemically inconsistent material causes considerable problems in achieving quality herbal products. A systematic cultivation can help not only in getting expected quality herbs but it also has an impact on healthcare system, national economy and conservation status of biodiversity. Herbal raw material producers and herbal product manufacturers always underestimate medicinal plant cultivation technologies. This ultimately leads to difficulties in developing the products of desired quality. In this paper, need for application of 'quality assurance approach' in herbal product standardization at agro-climatic and processing level has been discussed.

Introduction

Even in the era of combinatorial chemistry and biotechnology products like herbal medicines, cosmetics and nutraceuticals are having huge demand in the marketplace. A need has been generated for enforcing more stringent quality control on such products and for this, it is very essential that, the plant material grown, harvested and processed for manufacturing should be as per the standardized protocols to ensure product uniformity, efficacy and safety. In addition, systematic cultivation efforts help in preserving biodiversity by restricting the present practice of destructive harvesting of wild source (Ved et al. 1998). If our country wants to exploit its rich biodiversity, diverse agro-climatic conditions and wealth of traditional knowledge, the only way is to generate products, which will comply with the quality specifications at international market.

Specifying the chemical composition related quality standards in case of herbals is more challenging compared to the standardization of synthetic drugs. Herbal products contain number of constituents with complex chemical nature and are inconsistent in composition. Also, standard monographic documentation about herbs is limited. Following discussion highlights certain flaws in the present method of herbal standardization and suggests a quality assurance approach for optimizing the quality of herbal raw material that may minimize the complications in standardization process.

Major difficulties in standardization of herbals

1. Uncertainty about active constituents

Herbal material is chemically complex in nature as it consists of several constituents. In most of the cases biological activity is not exclusively dependent upon the so-called active constituents, but is due to overall chemical constitution of the plant. Eventhough biologically inert, many constituents affect the pharmacokinetics and stability of the active constituents (Handa, 1994). Because of the involvement of several chemical constituents in biological activity, it is not possible to optimize all of them.
during quality control. Also, many of these constituents still remain to be chemically characterized, which further complicates the process of quality control.

2. Inconsistency of chemical composition

Consistent chemical composition is the critical parameter in quality assurance. Herbal material is affected by number of factors mentioned below, which impart considerable changes in their chemical make up (Sane, 1997). Following are known to alter the composition of the herbal material.

a) Agro-climatic factors
b) Geographical variations
c) Natural association with other plants
d) Harvesting time
e) Post-harvest handling
f) Storage of raw material
g) Unit operations (size reduction, drying and extraction)
h) Manufacturing process and equipment’s used
i) Storage of the finished product.

3. Factors concerning the safety aspects in use of herbal material

It is also critical to establish the safety of the herbal material considering possible toxic constituents, changes in chemical composition during storage or harmful contamination’s arising from plant protection measures. Following are the sources of such harmful substances.

- Substitutes and adulterants
- Toxic plant constituents
- Contaminations due to pesticides, heavy metals fumigation agents, radioactivity, microorganisms and toxins.

Current approaches in chemical standardization of herbals

Above stated factors reveal difficulties in standardization of the herbals. Presently the herbal products are evaluated using chemo-profiling of the plants by using chromatographic techniques like Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC) and Gas Chromatography (GC), etc. Chemoprofiling of the herbal extract and its correlation with the biological activity is presently attempted by following ways.

1. In some cases, individual herb is tested to establish correlation between active constituents and recommended therapeutic activity. These active constituents are referred as ‘marker compounds’ and are used in standardization of test herb, e.g. in Ashwagandha (Withania somnifera Dunal), concentrations of the active constituents, Withanolides, are used as markers for the standardization. In other cases where the active constituents are not known or have not been characterized, the compounds abundantly present are used as marker compounds, e.g. in Bilva (Aegle marmelos Correa ex Roxb.), Aegelin is used as marker. Aegelin is not linked with the recommended therapeutic activity of Bilva, but since its presence in this herb has been well established it can be used as a marker compound for standardization (Dobriyal et al. 1998).

2. The chemical constituents of plant can be categorized as follows:
   a) Constituents related to efficacy into constituents with known therapeutic activity and constituents without known therapeutic activity (marker compounds).
   b) Constituents related to safety are grouped as toxic constituents, potential impurities and potential contaminants. Only the constituents known to be relevant to efficacy and safety are used to characterize the quality and where active constituents are not known, optimum quality cannot be indicated on the basis of abundantly present constituents (marker compounds) that are therapeutically irrelevant. The quality standards can only be derived from the spectrum of constituents that are evaluated by using pharmacological, clinical and toxicological trials to establish characteristics for each constituent. Specific identity, purity and assay tests are determined for such constituents. Further, tolerance limits for these constituents is
established. The test samples are assayed for such constituents and are compared with standards to determine the quality of the material (Ralf et al., 2000).

3. In another approach, repetitive TLC studies of the herbal extracts of same species are carried out. Four to five compounds, which are predominantly present in the TLC pattern, are selected as marker compounds. Such compounds are isolated, purified, and if possible, chemically characterized. Studies carried out by using different compositions of such isolated and purified marker compounds are then correlated with the biological activity of that herb. In actual standardization, TLC a pattern of specified biological markers in the test extract is compared with that of standards derived from earlier studies (Bhutani, 2000).

All above approaches mainly rely on the chemo-profiling of the test material and its comparison with the specified standards (as marker compounds or complete TLC spectrum of ingredients), but there are certain drawbacks (Narayana et al., 1997) in these approaches as:

- Lack of validated markers
- Unavailability of the specific assay methods for the detection or estimation of the markers
- Lack of standards (qualitative and quantitative) for establishing tolerance limits of the constituents.

**Quality herbal product through 'quality by assurance approach'**

Reproducing the desired chemoprofile is main necessity of quality assurance and so, quality of raw material used is the key element in producing desired chemo-profile. Hence, herbal material cultivated, collected and stored as per the standardized protocol will ensure the desired uniformity and thus efficacy of the products.

Above approaches of chemo-profiling by using chromatographic techniques may satisfy the needs of herbal standardization to certain extent, but the question of 'reproducible quality' still persists due to use of herbal material with inconsistent composition. Thus herbal drug standardization should cover overall process to assure a quality in final product. To assure such availability of good quality raw material, 'Land to Laboratory' approach of quality assurance, although difficult, is more rational and economical than existing approach. Quality assurance of herbal raw material is a part of 'Total Quality Management', in which uniformity, efficacy and safety of the herbal material is established through judicious and scientific interventions at each process of the herbal raw material production. Thus, 'quality by assurance' means, identification of the variants affecting the quality by applying analytical techniques and monitoring the process throughout (Narayana et al., 1997).

Authentication of the plant species as well as part of the plant is a crucial step in the quality assurance. In addition to the assistance of botanists and pharmacognostics, 'HPTLC fingerprint' can prove to be of great help in identification of plant species. Here, thought should be given on various chemomones (chemical races) and polyploidy forms of plant, which differ in chemical makeup and such study also helps in selecting high yielding strains. Parallel HPTLC run of possible adulterants along with different parts of the authentic plant can eliminate doubtful adulterations and other unwanted parts of plants. Plants collected from different geographical zones also show variations in their chemical constitution. Screening of these variations will guide not only in selecting appropriate climatic zone for cultivation (or material selection) but also in studying economic factors associated with the production of that plant in a particular area.

Seasonal variation in the chemical constituents of interest can also be identified and guidelines can be prepared for defining appropriate season for collection. There are several reports on the seasonal variations of chemical constitution. Seasonal variation of lignans in Phyllanthus amarus Schum. & Thonn. have been reported (Sane et al. 1997). If collected during monsoon, P. amarus possesses highest percentage of phyllanthin and hypophyllanthin than the samples collected in winter and summer. Natural association of certain plants may also affect the biological efficacy of the herb, therefore, care should be exerted during plant collection e.g., Tinospora cordifolia (Willd.) Miers. ex Hook.f. & Thoms. (Guduchi/Gulvel), a hepatoprotective herb, grows on different host plants. HPTLC analysis of Gulvel growing on five different host plants has been reported (Sane, 2002). It was
observed that Gulvel growing on Neem tree (*Azadirachta indica* A. Juss.) is more efficacious than those growing on other host plants.

Other factors like altitude, temperature, rainfall, diurnal variation, radiation characteristics, soil type, fertilizers and growth regulators, etc. influence the secondary metabolite production of plant (Trease, 1992). Therefore, agricultural protocol should be prepared taking into account all these factors. Table 1 summarizes data regarding the effect of these factors on secondary metabolite production (medicinal compounds).

Factors like specific period, season and ontogenetic stage of plant is essential in deciding harvesting time. Table 2 depicts some examples.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Plant species</th>
<th>Effect on secondary metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Altitude</td>
<td><em>Chrysanthemum cinerarifolium</em> (Trev.) Bocc. <em>(Pyrethrum)</em> <em>Cinchona succirubra</em> <em>Gentiana lutea</em> Linn.</td>
<td>At higher altitude (1900-2700 m) gives best yield of flowers and pyrethrin (Trease <em>et al.</em>, 1992). At lower altitude plant grows well but produces no alkaloids (Trease <em>et al.</em>, 1992). Bitter constituents increase with altitude (Trease <em>et al.</em>, 1992).</td>
</tr>
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<td>2</td>
<td>Temperature</td>
<td><em>Nicotiana rustica</em> Linn.</td>
<td>The mean optimum temperature for nicotine production is 28°C (Trease <em>et al.</em>, 1992).</td>
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<td>3</td>
<td>Diurnal variation and type of radiation</td>
<td><em>Bryophyllum pinnatum</em> <em>(Lam.) Kurz</em> <em>Datura stramonium</em> var. <em>tafida</em></td>
<td>A dark-adapted plant contains more polyphenols (Flavonoids) in leaves (Yogeeswaran, 2000). Long exposure to intense light results in sharp increase in hyoscine content at the time of flowering (Trease <em>et al.</em>, 1992).</td>
</tr>
<tr>
<td>4</td>
<td>Fertilizers</td>
<td><em>Dioscorea</em> spp. <em>Rosmarinus officinalis</em> Linn.</td>
<td>Apart from N, P and K, application of S, Ca and Mg increases the productivity as well as the diosgenin content (Mishra, 1992). Iron application did not produce any significant increase in oil yield but appeared to cause a marked rise in verbenone concentration in the oil of irrigated plants (Moretti <em>et al.</em>, 1998). The geraniol and citronellol content of the Citronella Java oil is affected by NPK application. Geraniol: Citronellal ratio is highly affected by the K application (Mishra, 1992). Spraying foliar nutrients like Fe, Cu, Zn and B, either individually or in combination at pre-flowering stage increases the total alkaloid content of leaves, stems and roots (Mishra, 1992).</td>
</tr>
<tr>
<td>5</td>
<td>Soil</td>
<td><em>Atropa belladonna</em> Linn.</td>
<td>Soil pH 6 and higher causes better accumulation of alkaloids in leaves (Mishra, 1992).</td>
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Table 2. Ontogenetic variations of some secondary metabolites in plants

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plant species</th>
<th>Ontogenetic variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Artemisia annua</em> Linn.</td>
<td>Artemisinin content is maximum at the 16 week harvest (early flowering stage) and declines if harvested later (Farooqi <em>et al.</em>, 1996).</td>
</tr>
<tr>
<td>2</td>
<td><em>Salvia officinalis</em> Linn.</td>
<td>Oil obtained at flowering stage greatly differs in composition than at vegetative stage (Piccaglia <em>et al.</em>, 1997).</td>
</tr>
<tr>
<td>3</td>
<td><em>Alpinia galanga</em> Willd.</td>
<td>Maximum amount (27.1%) of cineole is found in oil at 42 months after planting (Joy <em>et al.</em>, 2000).</td>
</tr>
<tr>
<td>4</td>
<td><em>Digitalis purpurea</em> Linn.</td>
<td>Glycoside content varies with age. Purpurea glycoside A is formed terminally and its content increases up to 50% of total glycosides (Trease <em>et al.</em>, 1992).</td>
</tr>
<tr>
<td>5</td>
<td><em>Anni visnaga</em> (Linn.) Lam.</td>
<td>Khellin and visnagin contents are maximum in unripe fruits (Trease <em>et al.</em>, 1992).</td>
</tr>
<tr>
<td>6</td>
<td><em>Mentha piperita</em></td>
<td>Menthone: Menthol ratio is minimum at the end of flowering (Chalchat <em>et al.</em>, 1997).</td>
</tr>
<tr>
<td>7</td>
<td><em>Ipomoea violacea</em></td>
<td>Lysergic acid amide: Chanoclavine ratio increases as the seed matures (Trease <em>et al.</em>, 1992).</td>
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Table 3. Effects of drying on chemical composition of herbal raw material

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plant species</th>
<th>Drying conditions</th>
<th>Effects on chemical constitution</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td><em>Bacopa monnieri</em> (Linn.) Penn. Shoots</td>
<td>Pre-treatment of shoots at 80°C for 30 min.</td>
<td>Retains higher amount of bacoside-A in dried material (Gupta <em>et al.</em>, 1998).</td>
</tr>
<tr>
<td>2</td>
<td>American Ginseng roots</td>
<td>Drying temperature of 32-44°C</td>
<td>Reduces concentration of malonyl-ginsenosides and increases concentration of gypenoside. While ginsenosides are not affected. Optimum temperature for drying of Ginseng is 38°C (Reynolds, 1998).</td>
</tr>
</tbody>
</table>
| 3       | *Taxus media* Intact clippings | a) Different drying conditions like Tobacco Drying barn, greenhouse, oven and freeze drying  
b) Duration of drying extended to 10 to 15 days, as in Shade house and laboratory conditions. | Gives nearly total recovery of taxol and cephalomannine                                             |
                                                                                                                 | Recovery of all taxanes is adversely affected (El-Sohly *et al.*, 1997).                          |
Post-harvest handling and storage of crude drugs also have adverse effect on chemical composition; therefore, unit operations like drying, size reduction and extraction should be optimized to reduce chemical changes in crude drugs.

The main objective of drying is to reduce the water content to the acceptable levels. Excessive water content causes enzyme activation (which subsequently leads to undesirable biotransformation), water aided chemical reactions, growth of microorganisms and insects/mites infestation. In addition to the increase in stability, drying also reduces the bulk of the material and thus transportation cost. Various factors like method of drying, drying temperature, duration and drying conditions should be optimized to minimize chemical changes. Table 3 illustrates the effects of drying conditions on chemical constituents of some plant drugs.

Size reduction is another important unit operation performed to convert crude material into suitable form for manufacturing dosage forms and for storage. It also increases the surface area of material, which can offer greater mass transfer rate during extraction process. The choice of method depends upon factors like form of material, stability of constituent and degree of fineness. Chopping of roots and rhizomes of Echinacea purpurea (Linn.) Moench alter the level of some alkaloids, however, drying process does not affect their levels significantly (Perry et al., 1997). Extraction of active constituents by using solvents of different polarities is very important in getting the desired constituents in concentrated form. Extraction protocols thus need to be designed and optimized to maximize the recovery of chemical entities and minimize chemical modifications. Method of extraction, contact time of solvent, solvent to plant material ratio, nature and concentration of extracting solvent are also other important parameters to be optimized. Pilot plant to large-scale extraction should also be monitored for any chemical changes. There was significant difference in the volatile constituents (major and minor) of Palmarosa oil obtained in laboratory (Clevenger’s apparatus) and in field distillation unit (Kaul et al., 1998).

Chemical constitution of the plant material should remain unchanged during storage. Factors like temperature, moisture content of crude drug, humidity, light, oxygen, and form of crude drugs influence preservation status of crude drugs. Hence, these need to be optimized. Based on HPTLC analysis of the Phyllanthus amarus samples collected in four successive years, it was proved that P. amarus should be used within one year of its collection in order to retain its original characteristics (Sane et al., 1997).

In addition to the above factors responsible for variation in uniformity of plant material, also its safety should be established. Contaminations due to pesticides, heavy metals, fumigation agents, radioactivity, microorganisms and toxins are concerned with safety status plant material. Also, toxic phytoconstituents that may be present in given plant or arise from adulteration or as product of degradation should also be considered. Since herbal therapy is required to be administered chronically, due consideration should be given to the maximum allowable limits for the content of pesticide, heavy metals and toxin residue.

Q.A. Protocol for herbal raw material production

- Characterization of geographical variations.
- Selection of right species (as well as high yielding strain) of the plant.
- Identification of the possible substitutes and adulterants.
- Optimization of factors affecting the production of secondary metabolite of plant.
- Minimization of parameters causing variation during collection, post-harvest treatment and storage of raw material.

Conclusion

The main barrier in the way of wider acceptance of the herbal drugs is the non-availability or inadequacy of standards necessary for assessment of their quality. Present methods of herbal drug standardization concentrate on few segments of quality assurance protocol,
which obviously finds difficulties in obtaining reproducible quality. The major factor that determines the actual quality of finalized product is the raw material being used. Hence, instead of only testing the quality of end product, efforts should be made to achieve the desired quality of raw material through minimization of the variants affecting the quality. By this approach, we can eliminate maximum possibilities of contamination’s and deviations from expected standards. In addition, detailed profiling of the variants can enable the manufactures to utilize their local resources properly. Thus, effort to produce a quality raw material can considerably support the production of semi-finished and finished herbal products of international quality standards. The stress on only technical know-how is not enough but need to evaluate the agro-climatic conditions is also essential for standardization of herbal products. On the eve, when the whole world is showing interest in Indian herbal products in healthcare system, a rational approach presented here will justify the face value of herbal usage at global level in coming future.

References


