Analysis of phytochemical variability in Neem formulations

S Gunasekaran1 and B Anita2*

1Periyar Univeristy, Salem- 636 011, Tamil Nadu, India
2Department of Physics, D.G.Vaishnav College, Chennai- 600 106, Tamil Nadu

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Herbal drug development includes various steps, starting from a passport data on raw materials, correct identification, pharmacognistic and chemical quality, standardization, safety and randomized clinical trials. Addressing standardization is vital and needs broader consideration. Adulteration in market samples and availability of sub-standard products is one of the greatest drawbacks in promotion of herbal products from India, therefore, present study was undertaken to evaluate the phytochemical variability in neem formulations available in the market. Five Ayurvedic formulations, containing neem as the main ingredient was chosen for the study. The samples were tested for the amount of limonoids like 2', 3' dehydrosalannol, salannin, nimbolide and azadiradione using HPLC (High-performance liquid chromatography) technique. The wide variations in the amount of the active principles in the samples highlight the need for stringent quality control measures in herbal industry.

**Keywords:** Azadirachta indica, Neem, Active principles, Limonoids, Phytochemical variability, HPLC, Quality control.

**IPC code; Int. cl.**——A61K 36/00

**Introduction**

Herbal medicines, particularly Asian herbal medicine have gained immense popularity in recent times. Currently, they are not regulated as medicines and can be purchased from outlets ranging from health food stores to internet sites. A critical evaluation of their safety is therefore important. Adulteration in market samples is one of the greatest drawbacks in promotion of herbal products from India. Plant samples in the market are stored under undesirable conditions over the years and often contain a mixture of other plant species, thus adversely affecting their bio-efficacy.

Botanical medicines produced in our country are seldom evaluated for their chemical efficacy and the composition of complex mixtures of herbs are only crudely analysed. Recently, many international authorities and agencies, including the World Health Organisation (WHO), European Agency for the Evaluation of Medicinal Products and European Scientific Cooperation of Phytomedicine have started creating new mechanisms to induce and regulate quality control and standardization of botanical medicine. Global definitions of botanical products are being developed with international cooperation and a new perspective of standardization, validation, safety and efficacy of botanical medicines is evolving. In view of the wide concern over the quality of herbal medicines, the present study was undertaken to make an assessment of the phytochemical composition of commercial formulations containing neem (Azadirachta indica A. Juss.) The medicinal properties of A. indica are recognized historically and almost every part of the tree has been used in folklore and traditional system of medicine for the treatment of a variety of human ailments, particularly against diseases of bacterial and fungal origin.

The amount of four bioactive principles in neem, namely, 2', 3'-dehydrosalannol, nimbolide, salannin and azadiradione (Plate 1 gives the structure of these compounds) was compared in five commercial products containing neem as the main ingredient. All the compounds chosen for the investigation are limonoids (triterpenoids). The limonoids exhibit antifungal, bactericidal and antimalarial activity against several strains of Plasmodium falciparum. It also shown antibacterial activity against Staphylococcus aureus and S. coagulase. The bio-efficacy and mode-of-action of some limonoids of salannin group has been studied by Doul et al. In the current work, the analyses are based on the estimation of the above mentioned bioactive principles using HPLC technique. Column chromatography and thin
layer chromatography has been used by Rao et al. to investigate the variation in the chemical composition of Indian samples of *Centella asiatica* (Linn.) Urban. Variation of bioactive components in *Curcuma longa* Linn. in Thailand has been studied by earlier workers. Bioassays were carried out by Kumar et al. in order to compare the biological efficacy of eight commercial neem formulations. Sangwan et al. have used HPLC technique to compare the amount of Withaferin A and six other unidentified molecules in commercial products containing *Ashwagandha*. A rapid concentration method for the determination of azadirachtin-A and B, nimbin and salannin in neem oil samples by using graphitized carbon solid phase extraction has been developed by Ramesh and Balasubramaniam, while, LC/MS was used by Caboni et al. to determine the amount of azadirachtoids in methanolic seed extracts of the neem tree. In the current work the amount of individual constituent is expressed as peak area units of the HPLC peak in a unit amount of neem formulation. The study was undertaken only to display a general scenario of phytochemical variability in herbal medicinal products, rather than their ranking as good or bad products. Further, the aim of this study is to emphasize the need for stringent quality control measures in herbal preparations.

**Materials and Methods**

The HPLC analysis was carried out at Asthagiri Herbal Research Foundation, Chennai, using a Shimadzu LC 10 ATVP High Performance Liquid Chromatograph fitted with a Luna 5 µ reverse phase column and a variable wavelength programmable UV-Visible detector set at 215 nm. Working reference standards of the bioactive principles were obtained from Asthagiri Herbal Research Foundation, Chennai. All chemicals used were of analytical or HPLC grade. On-line detectors (usually a UV monitor set at 220 nm or 280 nm) allow automated detection and quantification of eluting bands. The technique has been used by researchers to analyse the quantitative variations in Azadirachtin-A contents in neem trees grown in different regions of Andhra Pradesh, India.

**Chromatographic conditions**

The Phenomenex C-18 110A column of dimension 250 × 4.6 mm with particle size 5 µm was used. An isocratic high performance liquid chromatography was performed using a mixture of acetonitrile and water in the ratio 60:40 as mobile phase. The flow rate was maintained at 0.5 ml/min. The column was maintained at room temperature and the detection was carried out by UV detector at 215 nm for all the
samples. 1mg of the neem extract was dissolved in 1ml of the mobile phase and 20 µl of this aliquot was injected into the column.

Quantification of bioactive principles in commercial neem formulations

The neem formulations were procured from the local market and are coded as A, B, C, D and E. The analysis is based on the estimation of 2', 3'-dehydrosalannol (Rt = 21.81 min.), salannin (Rt = 30.99 min), nimbolide (Rt = 21.68 min.) and azadiradione (Rt = 24.27 min.) using HPLC technique. The chromatograms of the working reference standards of the phytochemicals are given in Fig. 1. In order to extract the active principles from neem formulations, the following method has been adopted: 5 g of the drug sample was soaked in 30 ml of methanol and kept at room temperature for a day and the methanolic extract was filtered out. This alcoholic extraction was repeated thrice for complete extraction of the active principles. The methanol extract thus collected was evaporated and the solvent was removed using rotary evaporator. The drug extract obtained per 5 g of the drug was 1, 0.75, 0.56, 0.86 and 0.64 g for the five samples. This extract was used for the quantification of various bioactive principles by HPLC method. The stock solution for the analysis of sample A was prepared by dissolving 24 mg of the extract in 1ml of mobile phase. The stock solution for the samples B, C, D and E were prepared by dissolving 21.9, 29.41, 41.0 and 20 mg, respectively of the extract in 1 ml of mobile phase and the analysis was carried out for each sample. The chromatograms of the five samples analysed is shown in Fig. 2.

Fig. 1 — Chromatogram of the phytochemicals

Fig. 2 — Chromatograms of commercial neem formulations
Results and Discussion

The results of the analysis, projected in Table 1 suggest that the products had widely varying amounts of each of the four active principles. While sample B and E contain varying amounts of all the four compounds, samples C and D contain small amount of 2', 3'-dehydrosalannol only. Sample A contains some amount of 2',3'-dehydrosalannol and salannin. The graphical representation of the amount of phytochemicals in the samples is projected in Fig. 3. The recommended daily dosage of the formulation is 1 g for sample A, 500 mg for sample B, 7.5 g for sample C, 700 mg for sample D and 5 g for sample E. The amount of active principle intake in terms of the recommended daily dosage, as indicated by the manufacturer, by a person consuming any of the commercial samples studied is given in Table 2.

The results clearly suggest that the commercial products vary by several orders of magnitude with respect to their phytochemical composition. Since phytochemicals, particularly unique ones, like salannin, nimboide in neem are the main reasons for the health benefits of the herbs, inordinate variations in their daily dose may result in blotchy health benefits.

Some factors that cause such variations in phytochemical in commercial products are: (i) diversified bioresources of heterogenous nature from the wild or under cultivation, (ii) physiological and ecological variations in plantations, (iii) harvest and post-harvest operations, (iv) processing of

<table>
<thead>
<tr>
<th>Active principle</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample C</th>
<th>Sample D</th>
<th>Sample E</th>
</tr>
</thead>
<tbody>
<tr>
<td>2',3'-dehydrosalannol</td>
<td>0.946</td>
<td>1.344</td>
<td>0.2378</td>
<td>0.4219</td>
<td>3.02</td>
</tr>
<tr>
<td>Salannin</td>
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<td>-</td>
<td>-</td>
<td>1.6391</td>
</tr>
<tr>
<td>Nimboide</td>
<td>-</td>
<td>3.599</td>
<td>-</td>
<td>-</td>
<td>1.905</td>
</tr>
<tr>
<td>Azadiradione</td>
<td>-</td>
<td>0.637</td>
<td>-</td>
<td>-</td>
<td>0.0978</td>
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<table>
<thead>
<tr>
<th>Active principle</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample C</th>
<th>Sample D</th>
<th>Sample E</th>
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</thead>
<tbody>
<tr>
<td>2',3'-dehydrosalannol</td>
<td>0.946</td>
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<tr>
<td>Salannin</td>
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<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>9.525</td>
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<tr>
<td>Azadiradione</td>
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<td>0.3185</td>
<td>-</td>
<td>-</td>
<td>0.489</td>
</tr>
</tbody>
</table>

Table 1 — Amount of active principles in commercial Neem formulations (mg/g) by HPLC method
Table 2 — Amount of active principle per suggested daily dosage of Neem formulation (mg)

(As indicated by the manufacturer)

Fig. 3 — Amount of phytochemicals in the market samples of neem formulations
biomass, and (v) manufacture process of product. The current study emphasizes the necessity of taking efforts towards narrowing down the phytochemical variations and maintenance of compositional uniformity of herbal products.

**Conclusion**

The results indicate the wide variations in the amount of phytochemicals in the formulations, therefore, there is need to enforce strict quality control measures in the pharmaceutical sector and also there is need to adopt better manufacturing processes where the loss of active ingredients is minimum.

**References**