Chapter 7

Summary and Conclusions
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7.1. Summary

7.1.1. Preamble: Integration of Ayurveda with modern medicine
Ayurveda is a treasure of wisdom gained by Indian Rishis and Vaidyas about the therapeutic power of several herbal formulations for curing several human ailments. It is time tested experiences of over 5000 years. Unlike the modern medicine, Ayurveda lacks proper standardization and validation; seems arbitrary, and therefore does not find universal acceptance. On the other hand, modern medicines are tailor-made synthetic proprietary drugs, designed with fair knowledge of the disease at molecular level; seems precise, well targeted to the disease in particular and accepted all over the world being experimentally validated standardized therapy. The modern drugs go through rigorous, stringent regulatory norms, before being introduced for human use. All the same, these modern drugs being synthetic are more likely to have side effects than herbal medicines, which are natural. In order to reap the full benefits of ancient wisdom and bring them in line with modern medicine, it is necessary to put these herbal formulations also through validation, standardization, and stability criteria to make it more acceptable in the world market. Therefore this study was undertaken to pave a path for validation standardization and stability of herbal formulations.

7.1.2. Ashwagandha: A chosen herb for validation standardization and stability studies
Ashwagandha root is one of the most popular herbal parts used in many Ayurvedic formulations. Therefore, root of Ashwagandha was selected as the best suited herbal part to develop methods of validation standardization and stability studies.

It is generally considered that plants and plant parts are inherently richer in antioxidants and Ayurveda wisely exploits the specific secondary antioxidant metabolites for a given particular
disease in specific organs. Ashwagandha roots have a class of steroidal lactones called withanolides that have documented medicinal properties useful in the treatment of several diseases.

For Ayurvedic medicine to be effective and to be accepted in the world market, it has to be consistent in quality with an assurance of curative potential over a period of time that it stands on the shelf before it reaches the consumers.

7.1.3. Pertinent issues to address the problem

There are several pertinent issues that need to be tackled to address this problem. Some of the important issues are;

- Firstly, the best plant variety, with proven medicinal property has to be selected.
- Secondly, the plant has to be grown under ideal condition, nutritional support and proper agronomical inputs, so as to get maximum medicinal value.
- Thirdly, the method of formulation by all the manufacturers should be standard, well defined, uniform, to get the assured curative potential in the final product.
- Fourthly, the active principles, if known, be quantified and stated on the label.
- Fifthly, the antioxidant (anti-inflammatory) value be physically, chemically measured and stated.
- Sixthly, when the herbal formulation is being orally administered, it is necessary to ascertain how much active principle components of the herbal formulation is being absorbed and assimilated in human system to get the desired curative effects on the disease of the patient.
- Seventhly, the actual medicinal value to be checked in vivo, in animal model before being introduced into the market.
- Eighthly, the shelf-life of formulation should be noted on the label of final product.
It is in view of the above, in the present studies physical, chemical and biological approach were used to validate, standardize and conduct shelf-life stability studies by taking Ashwagandha as an example. Highlights and conclusions based on the above theoretical surmise of validation, standardization and stability was given below:

7.1.3.1. Quality control in the farmer’s field
- It is observed that macronutrients N, P, Ca and K deficiencies in the soil do affect the withanolide contents in the roots.
- It was also observed that Mo contents in roots vary under different soil macronutrients deficiency conditions.
- Further, it conclude that Mo in roots correlate with withanolide contents in roots.
- Mo is a micronutrient to be regulated in the soil for high quality, withanolide rich root products.

7.1.3.2. Efficient extraction of roots
- An efficient method of getting withanolide rich fraction (WRF) has been developed.

7.1.3.3. Fractionation and quantification of withanolides by HPTLC
- Solvent system has been developed for fractionation and quantization of withanolides in WRF.
- WRF has higher content of withaferine A followed withanolide A and withanolide B

7.1.3.4. Simple quick antioxidant assay of withanolides
- The fractionated withanolides on HPTLC plates can individually assessed for their antioxidant activities and thus assist in quantitative and qualitative standardizations of Ashwagandha formulations.
7.1.3.5. **Quicker physical technique (Cyclic Voltammeter) for quality assessment at different stages in the production of formulation and for shelf life**

- The technique identified two components in WRF, one fast decaying and the other slow decaying.
- Among the major withanolides 1, 2 deoxywithastramonolide is most stable followed by withanolide A.

7.1.3.6. **Biological assessment of Anti-inflammatory and hepatoprotective activities**

- Assessment of anti-inflammatory activity provides a tool for biological assessment.
- As it has been found anti-inflammatory activity has a shorter life, it may be attributed to slow decaying components.
- Hepatoprotective activity may be attributed to the antioxidant potential of WRF.
- Anti-inflammatory of WRF possibly mediates through the suppression of TNFα and IL-1β.

7.1.3.7. **Bioavailability**

- Sino-Veda – Canada MDCK cell culture is a suitable model for quickly in vitro assessing bioavailability of herbal formulations.
- It was observed that non polar aglycone withanolides has higher penetrability to pass through biological membrane.
- Polar glycosylated withanoside V showed low permeability.
- However, withaferine A, regarded as most potent withanolide, surprisingly showed no permeability.
- This observation needs to be confirmed with more appropriate cell line CACO₂.
7.1.4. Conclusions

Ayurveda, a treasure of therapeutic knowledge of medicinal herbs, can be made popular, on par with modern medicine, only by putting these formulations through strict rigors, of standardization and validation.

The standardizations have to begin at the framers field, continue for method of extraction / fractionation / concentration, of active principles, upto formulation. Validation involves the determination of potency by quick antioxidant assay, anti-inflammatory or by specific biological assays. Efficacy must also be ensured through the bioavailability and shelf-life of the active principles in the product.

In conclusion, it may be stated that although validation, standardization, stability information of Ayurvedic formulations are very complex. However, the problem is not insurmountable, with the proper holistic approach; one can achieve this as demonstrated in this study, with Ashwagandha root as an example.
7.2. Accomplishments

Taking Ashwagandha as example,

- The importance of quality control at the farmer’s field has been demonstrated,
- The precise extraction for the concentration of active principles increases efficacy,
- Antioxidant assay by cyclic voltammeter gives a quick handle for evaluation of the potency,
- Anti-inflammatory and hepatoprotective activities have been employed to demonstrate therapeutic value,
- A new approach for the investigation of bioavailability in vitro absorption technology has been employed,
- Although the method has been employed with Ashwagandha as an example, the same can also be applied to most of the Ayurvedic products
7.3. Limitations of the Work

- Although, Mo deficiency has been shown to affect withanolide concentration in roots, other nutrients may also be involved in determining the concentrations of withanolides in Ashwagandha roots.
- HPTLC used in the study, is quick and cheap, convenient, however resolution power of HPTLC is limited, and therefore HPLC although costly, may have been a better choice.
- The cyclic voltammetric method for validation has a limitation that the active principles, insoluble in water may be difficult to be evaluated by this method.
- Bioavailability studies could have been carried out by using Caco-2 cells rather than MDCK cells.
7.4. Future Prospects

Life is a play of redox. It is the ability of living system to neutralise oxidative stress that keeps the life going. Therefore antioxidant in food supplementations and herbal medicines are supportive to life by protection form diseases. Therefore it is our considered opinion that therapeutic power in herbal formulations may reside, particularly in its antioxidant activity.

A simple inexpensive, reliable method of assessing antioxidant power in any herbal formulations can be achieved by cyclic voltammeter. A cyclic voltammeter can quickly validate the most of Ayurvedic formulations and keep track on its stability, efficacy during formulation process and storage.

The application of such a simple technique as first fairly reliable measure of efficacy can be a valuable method of validation of Indian Traditional Herbal Medicine. This method can be further explored for general application, of all herbal formulations.