CHAPTER 9

Conclusions

This chapter provides the overall conclusions and future directions for research into APDs
9. Conclusions

Limited natural distribution and yet a high demand for medicinal plant species have pushed several of them into the endangered species list, demanding immediate conservation strategies. Cultivation of medicinal plant species has been promoted by the National Medicinal Plants Board (NMPB), but only for 116 of the 2,400 medicinal plant species used by the codified systems of medicine in India (http://3846065317Modified-Minor-Ammendments-Operational Guidelines Centrally Sponsored Scheme). Agro technology for the cultivation of several rare species like *Aconitum heterophyllum* is still at its infancy. Short supply of medicinal plants has led to extensive substitution and adulteration in the raw drug market.

Ayurveda approaches the problem of unavailability of drugs by recommending the use of functionally similar substitutes through the concept of APD. Exploring APDs can help (i) validate substitutes for endangered species and (ii) identify new plants/ bio-activities for plants based on the logic of APD. References to ADs and APDs lie scattered in classical Ayurvedic texts from c. 14th Century CE onwards. The logic behind selection of an APD is not explained in the classical texts of Ayurveda, nor researched. Through this thesis, the concept of APD has been systematically studied.

The overall aim of this Ph.D study was to understand the Ayurvedic logic behind selection of APDs for rare ADs. The objectives were

- to compile a list of ADs and APDs from Ayurvedic texts and living tradition
- to study the relationship between a selected 20 pairs of AD and APDs based on Ayurvedic principles
- to evaluate the legitimacy of substitution in two pairs of AD and APDs using phytochemistry and pharmacology
Conclusions

- to enhance the awareness on possibility of substitution among Ayurveda practitioners.

A trans-disciplinary research (TDR) strategy involving Ayurveda, phytochemistry and pharmacology studies was adopted to explore the possible logical relationship between ADs and APDs. The TDR strategy was adopted because of the limitations of a ‘single disciplinary research’ in solving the "real world" problems, which do not fit into any single system of scientific discipline.

Compilation of a list of AD-APD pairs: A systematic literature review of 20 Ayurvedic books and documentation through specially designed formats has provided a fully referenced list of APDs for 156 drugs, including 123 plant drugs, 19 metals and minerals, 12 animal products and 2 processed drugs. Many APDs are found to be relevant to contemporary practice, but rarely practiced, maybe because of unawareness about APDs.

Analysis of selected 20 AD-APDs based on Ayurvedic principles: From the 156 AD-APD pairs, 20 were prioritized for Ayurvedic analysis based on factors including the current status of availability of ADs, abundance of APDs, non-controversial botanical identity of AD and APDs, utility of AD in current practice and scientific curiosity due to obvious botanical/chemical dissimilarities between AD and APDs. (Fig. 9.1). The analysis showed that the rasapanchaka, doṣa karma and panchamahābhūta were required to be similar between ADs and APDs. Among the 20 analysed pairs, 85% had similar panchamahābhūta composition, 80% similar in rasa and guṇa, 95 % in veerya and 85 % in terms of vipāka. 90 % of AD-APDs had similar action at the doṣa level (doṣakarma). However, there was a lot of difference in the actual clinical applications between the pairs maybe because there are several indications for each of the AD/ APD.
Conclusions

Figure 9.1: Compiled and analyzed AD-APD pairs

Since the pharmacological activities of a herbal drug is linked to the presence of particular chemical constituents in plant drugs, the physico-chemical, phyto-chemical and chromatography of 2 pairs of AD-APDs (i) Ativiṣā (Aconitum heterophyllum) - Mustā (Cyperus rotundus) and (ii) Dāḍima (Punica granatum) - Vṛkṣāmla (Garcinia indica) were compared using established phytochemistry and pharmacology methods.

Substitution of Ativiṣā (A. heterophyllum) by Mustā (C. rotundus):

The panchamahābhūta composition of Ativiṣā and Mustā appear to be similar with the dominance of agni, vāyu and ākāśa mahabhutas. The rasapanchaka factors like kaṭu and tikta rasa, laghu and rūkṣa guṇa and kaṭu vipāka are also similar between them. Both pacify kapha and pitta doṣas. Dīpana, pāchana, grāhi, and lekhana are prominent karmas of both drugs. They are efficiently used in the treatment of atisāra, jwara, kṛmiroga, medoroga, ajīrṇa and śotha. Both A. heterophyllum and C. rotundus contained alkaloids, glycosides, saponins and phytosterols. C. rotundus additionally contained phenolic compounds, tannins
and flavonoids. *Ativiṣā* had 1.57% (w/w) of alkaloids, but negligible in *Mustā*. HPTLC of successive extracts of *Ativiṣā* and *Mustā* showed several bands at common Rf values. HPLC fingerprints of aqueous extracts of *A. heterophyllum* and *C. rotundus* also showed that both plants had common peaks at retention times 1.5, 4.25, 5.2, 7.6 and 8.4 mins, indicating similar phytochemical profiles, however this needs further study.

The AD-APD pair, *Ativiṣā* (*A. heterophyllum*)-*Mustā* (*C. rotundus*) were comparable in their *jwarahara* (antipyretic), *atisāraghna* (antidiarrhoeal) and *medohara* (antihyperlipidemic) activities, when studied using established pharmacological models in Wistar albino rats. Both drugs were proven to be safe as seen from the acute oral toxicity studies in mice at a dose of 2000 mg/kg body weight. They were studied for anti-hyperlipidemic activity in high fat diet fed obese rat models. Powders of both *A. heterophyllum* (at 400 mg/kg) and *Cyperus rotundus* (800 mg/kg) significantly lowered total cholesterol, triglycerides, LDL, VLDL, blood sugar and body weight, compared to the control, proving the *medohara* activity. The antidiarrhoeal activity of both drugs was evident from the significant delay in the onset of diarrhea and reduction in the number of episodes of watery diarrhoea as compared to the control (P<0.05), in castor oil induced diarrhoea in Wistar albino rat model. The AD-APD pair significantly controlled pyrexia in rats (>100.4°F) induced by yeast two hours after administration with *A. heterophyllum* (400 mg/kg body weight) and *C. rotundus* (800 mg/kg body weight) as compared to the control (P<0.01). However, the difference observed in the studied pharmacological activities between the AD-APD pair, was not statistically different, supporting the substitution of *Ativiṣā* by *Mustā* to manage *medoroga* (hyperlipidemia), *atisāra* (diarrhoea) and *jwara* (pyrexia). It is to be noted that, *Must* was required to be administered in double the quantity (800 mg/kg body weight) as opposed to *Ativiṣā* (400 mg/kg body weight). This is interesting since, living traditions advise consumption of double the quantity of *Mustā*, if substituted for *Ativiṣā*. 
Substitution of Dāḍima (P. granatum) by Vrkshamlā (G. indica):

Both Dāḍima and Vrkšamlā have a similar panchamahābhūta composition of jala and agni. They are amla and kaśāya in taste and change to sour after the digestion. Both of them reduce vitiated pitta and kapha doṣas and mainly have hṛdyā and grāhi karmas. This AD-APD pair is used to treat dāha and trṣnā. Glycosides, phytosterols, phenolics and flavonoids were detected in the juices of both P. granatum and G. indica. Only P. granatum had fixed oils, whereas saponins were detected in G. indica. Both of them had phenolic substances in comparable proportions (1476.6±86 mg/L and 1112±42mg/L respectively), but the total acids in G. indica was much higher than in P. granatum. Hydroxycitric acid (HCA), was detected only in G. indica and not in P. granatum. This was confirmed by comparing the IR spectrum with that of standard HCA. Comparison of the HPLC profiles of both drugs showed some common peaks at RT 6.27, 12.12, 12.94, 21.49, 30.28 and 32.22 mins, which may represent the presence of common phytoconstituents in both drugs. However, this requires further studies. One of the possible dīpana actions of both Dāḍima and Vrkshamlā was demonstrated by the increase in iron dialysability by 2.5 and 4 folds respectively in in vitro cell free model compared to the control. They have enhanced bioavailability of iron in in vitro cell based (Caco2 and HepG2) models as well. In Caco2 cells, on treating with the lysates of P. granatum and G. indica the iron storage protein, ferritin was increased up to 900±10.16 ng/ml and in 715.5±8.38 ng/ml respectively, with a statistical significance compared to FeSo4 control (41.5±2.42 ng/ml) (P<0.001). There was an increase of ferritin in HepG2 cells up to 601.02±5.5 ng/mL and 305.24±3.98 ng/mL respectively, which are again significantly high compared to the control (187±3.64 ng/mL). This validates the substitution of Dāḍima by Vrkshamlā for dīpana action with particular reference to iron bioavailability enhancement. This supports the Ayurvedic recommendation of use of these plants in the management of
pāṇḍu (Iron deficiency anemia). Further experiments in *in vivo* and clinical systems can confirm this action.

The studied AD-APD pairs possessed similar *rasapanchakas* as well as bioactivities. This supports the concept that *dravyas* with similar *rasapanchaka* have similar bioactivities (*karma*) and demonstrates that the APD can substitute the ADs at least for the studied bioactivities. No striking similarities were observed from the chemical studies conducted except the fact that most of the phytoconstituent groups were similar in the AD-APD pairs.

*Chikitsa* (treatment) as per Ayurveda is ‘*dhātu samyata,*’ which means bringing (increased or decreased) *dhātus* and *doṣas* to normal levels, which is ultimately achieved by *panchamahābhūtas.* Increase or decrease of a particular *dhātu* can be achieved by different ‘*dravyas,*’ having similar *panchamahābhūta* composition. Similar *panchamahābhūta* composition is necessary, because Ayurveda states that, ‘qualities of similar nature have an increasing effect on the system and opposing qualities pacify the effects’ (*Sarvada sarvAbhāvanaam saamaanyam vrddhi kaaranam, hraasa hetuh visheshashca* – Cha. Su. 1/44). Figure 9.2 depicts the probable logical relationship between ADs and APDs.

From the current study, it appears that an APD needs to have biological action(s) similar to that of AD. The AD-APD need to be similar actions at the *doṣa* and *dhātu* levels through similar *panchamahābhūta,* which in turn manifests as similar *rasapanchakas.* Similar *rasapanchakas* shall have a comparable effect on every tissue of the body, which is otherwise called as *dravyakarmukata* (therapeutic efficacy). However, an APD may also achieve the necessary *karma* in spite of dissimilarity in *rasapanchaka,* E.g., *dīpana* can be achieved either through *ushna veerya* or *kaṭu/tikta/kaṣāya rasa* or *kaṭu vipāka* or *tikshna/sookshma guṇa.* The observed differences in the *rasapanchakas* of AD and APDs, yet
their ability to achieve similar *doṣa* and *dhātu karmas* could be because of this. These are internally consistent with the principles of Ayurveda. Ayurvedic scholars may argue that there is nothing new in what is being said here and that this was already known, this is the first time this has been demonstrated through a systematic analysis.

**Figure 9.2:** Logical relationships between ADs and APDs
In this Ph.D study, traditional healers were engaged to enable the process of collaborative research work. CAPTURED (Capacity and Theory building for Universities and Research Centers in Endogenous Development) program was helpful in capacity building the researcher including the team. Benefit sharing approach included sharing of research findings with the stakeholder community about the possibility of using APDs and finding other substitutes as advised by APD concept.

The concept of APD has some limitations, which are prompted by the Ayurvedic seers themselves. BhaiṣajyaRatnavālī says that, use of APD is permitted in a compound formulation, if the AD is not the main ingredient.

**Limitations of the study:** This work documents APDs from selected 20 Ayurvedic books. Unpublished and several regional language books also may contain information on APDs, which can certainly enhance the list of APDs. There is a need to also document the living practices further about APD concept. This work has attempted selected scientific studies of only 2 APD pairs, there are several to be explored to obtain a comprehensive idea.

**Future Directions**

**(A) Research approach towards the APD concept**

(i) Other indications of Ativiśā and Dāḍima (ADs) need to be studied and compared with Mustā and Vṛksāmla (APDs) respectively

(ii) Scientific studies, including clinical trials of prioritized AD-APDs to prove their legitimacy

(iii) Documentation of AD-APDs from regional texts as well as living practices and creating a computerized, referenced database
(iv) Prioritization of AD-APDs based on interest (conservation or scientific) for further scientific research

(v) Research to quantify and understand *panchamahābhūta, rasapanchaka* and their action on *doṣas* and *dhātus* is critical to understand the potential of Ayurveda, not just APD. So far the Ayurveda practitioners operate only from a theoretical basis

(vi) Phytochemical studies including isolation and characterization of molecules/groups which cause similar actions in AD and APD may provide interesting insights into chemistry and bioactivity

(B) **Collaboration of regulatory authorities, Ayurveda industry and R&D institutions**

(i) A national level road map for researching AD-APDs can contribute to developing coordinated strategies identify new substitutes for endangered species as well as provide tool to identify new plant medicines with identified pharmacological actions

(ii) Regulatory authorities and pharmacopoeia need to approve the use of scientifically proven APDs. This can ease the stress on critically endangered medicinal plant species