Chapter 7

Summary and Future directions

The process of scientific discovery is, in effect, a continual flight from wonder
7.1 SUMMARY AND CONCLUSION:

Today, Aloe is used as an ingredient in a myriad of health and cosmetic products. It is included in large range of skin moisturizers, face and hand creams, cleansers, soaps, suntan lotions, shampoos and hair tonics, shaving preparations, bath aids, makeup and fragrance preparations, and baby lotions and wipes. Topical preparations of Aloe particularly Aloe barbadensis are used to treat frostbite. The cosmetic industry has made claims of its rejuvenating, moisturizing, and healing properties.

The aloe plant, being a cactus plant, contains 99 and 99.5 per cent water, with an average pH of 4.5. The remaining solid material contains over 246 different ingredients including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid.

To utilize the available resources of genus Aloe in Uttaranchal, the present study “Conservation and Assessment of Physico-chemical and Molecular diversity of the genetic resources of the genus Aloe occurring in Uttaranchal, India (Kumaon and Garhwal region)” was undertaken with the following objectives

1. To screen Kumaon and Garhwal region of Uttaranchal for available resource of Aloe.
2. To authenticate, conserve and multiply with development of agro-technology.
3. To evaluate them for their biochemical potentials.
4. To identify them by using molecular marker technique like ISSR.

In the present study Uttaranchal region was screened for the genus Aloe where 5 species of Aloe were collected from 8 different locations at different altitudes ranging in between 1082’ to 7983’ MSL in Kumaon and Garhwal regions. Aloe barbadensis ecotypes were found in Kumaon region only. While other species were found in Garhwal region. Aloe humilis was found at the highest altitude at Joshimath (7983’ MSL) from Garhwal region.

The present study indicates that even though Uttaranchal is not the natural habitat for the occurrence of the genus Aloe, it was found distributed all over the Kumaon and Garhwal
region. This means these species may have been introduced by humans in this region in the past and in the course of time they have adopted to the climate.

Agro-technology developed in accordance with the norms of WHO for cultivation of medicinal plants proved to be effective as all the species could be grown and maintained successfully in Uttarakhand at Pithoragarh (5397’ MSL) and at high altitude (Joshimath, 9000’ MSL). The cultivation in glass houses showed improvement in morphological parameters like length, width and number of leaves etc. These parameters can be correlated to proper nourishment of the plants. Rather higher yield was observed at these sites than the yield at the temperate region, which are the natural habitat of the Aloe. Covered conditions i.e. glass and polyhouses gave more yield (2400 q/ha) as compared to open fields i.e. 1750 q/ha. In ideal conditions i.e. in natural habitat of the Aloe barbadensis, 525 quintals /hectare yield is reported. These cultivation practices can open up the new avenues for commercial cultivation of Aloe in this region.

In the nutraceutical assessment of all the cultivated plants, it was found that the variations in the biochemical parameters were influenced by agro-climate, soil type and its nutrients, altitude and species specific differences. It was observed that all the plants showed increase or decrease in the biochemical constituents relative to their controls which were collected from wild. Ecotype 2 collected from the slopes and water deficient soil showed high moisture content due to its retention. Moisture was found relatively high in its control as compared to other ecotypes. Other species of Aloe collected from the plains i.e. Aloe saponaria and Aloe perryi showed the maximum moisture content.

All the biochemical parameters like total carbohydrates, proteins, fats, total minerals were found to be increased in the cultivated species as compared to their respective controls. Chlorophyll a, b and total chlorophyll were estimated from fresh leaf sample while other estimations were carried out by using dried leaf powder. This increase was found in all the ecotypes and species under cultivation. In case of phenolics, tannins and proline the concentration was found to be decreased as the cultivated plants were maintained and provided with better irrigation and fertilizers. Ecotype 1 and Aloe perryi were found to be the best in biochemical composition among the ecotypes and species respectively.
11 minerals were quantitatively determined from *Aloe barbadensis* ecotypes and other species. It was observed that Ecotypes 4 contains most of the minerals like Na, K, P, Cu and Li in higher concentration than other ecotypes. *Aloe perryi* was found to be the best source of minerals as its content of these minerals were maximum of all the species. In all the plants Co was absent or beyond the detectable limit. Thus it can be concluded that low biochemical composition estimated in control plants which were collected from wild can be improved by these practices, fertilizers and irrigation.

Aloin content was tested for all the species under cultivation from whole leaf powder, fresh latex and lyophilized latex. It was found that aloin content in all the three samples was maximum in *Aloe perryi* as compared to all the species. Among the *A. barbadensis* ecotypes, Ecotypes 1 was found to be the best source of aloin. Thus the plants rich in particular component can be used and cultivated for trading, cottage industry set ups.

High altitude cultivation studies were carried out Auli (9000’ MSL) where morphological parameters, biochemicals, minerals and aloin content were studied. Ecotype 1 was cultivated at this location and its wild source was used as a control. It was observed during the study that moisture content was reduced even though the standard agro technology was followed. All the biochemical showed decrease or least significant increase in these plants. Proline was found to be increased as survival molecule. All the secondary metabolites used for the survival in harsh climate were found to be increased. Ecotype 1 at this site can be cultivated for the production of secondary metabolites which constitutes the major portion of the herbal drug and other synthetic products. Cultivation at the high altitude (Auli) of Ecotype 1 was found to be satisfactory but due to high acidic and very low nutrient content of soil along with high degree of slopes, heavy rains and frost conditions the nutraceutical constituents showed reduction.

Prebiotic and symbiotic potential of the *Aloe barbadensis* ecotypes were evaluated by using the gut microorganisms i.e. probiotics. Other species of *Aloe* were not used for the study because they are not recommended for the edible purposes. Normal growth pattern was observed for all the probiotics by using gel of ecotypes, as a source of carbon in media. Ecotype 1 showed best symbiotic potential for all the probiotics - *Lactobacillus plantarum* (2084), *Lactobacillus lactis* (2589), *Lactobacillus acidophilus* (2285).
Lactobacillus delbrueckii (2292) Bifidobacterium bifidum. Hence, Ecotypes 1 can be used as a rich source of prebiotic for the industrial cultivation of above mentioned microorganisms for various food preparations and medicines.

In antimicrobial activity assessment, water, methanol and hexane extracts of whole leaf powder were used and it was found that methanol extracts were best as it showed minimum MIC and maximum zone of inhibition against all the food pathogens viz E. coli, S. typhii, S. aureus and B. subtilis. Here also Ecotype 1 showed the maximum zone of inhibition as it may contain higher amount of anti-microbial compounds extracted by methanol. The antimicrobial compounds can be isolated and produced on commercial scale by using these plants and can be used in food preparations also.

DNA extraction protocol yielded good quality and quantities of DNAs from the leaves of all the Aloe barbadensis ecotypes and other species. ISSR markers successfully identified the variations among A. barbadensis ecotypes and with the species. It was found that the primers amplified by the ecotypes of Aloe barbadensis and Aloe perryi were different from the primers amplified by other species due to variation in the genetic make up and the microsatellite regions. Clustering in dendrogram based on ISSR variation grouped 10 plants in three groups and indicated correlation between geographical position and genetic differentiation.

**Future Directions:**

In recent times, the use of Aloe has reached a level of concern, since some herbalists and organizations are promoting oral consumption of it as a prophylaxis and treatment to alleviate a variety of unrelated systemic conditions. Promoters offer a number of whole leaf formulations that are widely available for consumption at various concentrations in liquid, powder, and tablet form.

Aloe is to be uniquely valued for its content of active biochemicals. These are substances which interact with living cells in very small amounts, producing significant changes to cell metabolism and cell behaviour. These substances interact with specialized receptors on the cell surface to produce these changes, in a way which might be described as “pharmacological.” Yet the substances within Aloe which are doing this are entirely non-toxic natural substances and they leave no residues in the tissues. Aloe itself is not a
food, but pharmacologically active substances of the same general type are well
distributed among unprocessed whole foods. None of our foods contain the same range of
active cell-stimulating constituents as Aloe in the same proportions, but the principles
involved in using Aloe are much the same as when one uses some foods as medicines.

During the present study at DARL, Pithoragarh some of the products having Aloe
gel as one of the major ingredients were prepared. Those are given in Fig. 7/36 and 7/37.

There is a vast scope for the development of the new products and food
supplements. Nutraceutical evaluation can lead to development of these products using
rich source of particular Aloe ecotypes for specific constituents. Aloin like compounds
can be extracted for the bulk and crude drug manufacturing. New prebiotics can be
isolated, synthesized or tailored from these rich prebiotic source and important
antimicrobial compounds can be isolated for the food purposes. By evaluating the elite
sources they can be conserved by genetic information.

The present work indicates that the Aloe genus can be fully utilized for its
medicinal importance and development of the new products like anti-frost bite which was
the concern of DRDO for this study so that troops can be benefited.

Cultivation practices, nutritional evaluation, prebiotic, antimicrobial and genetic
diversity studies can lead understanding to mass and commercial scale cultivation, small
scale processing and production industries in the Kumaon and Garhwal region. These
activities will improve the socioeconomic and nutritional status of the people in
Uttaranchal.
Figure 7/36 Antileucoderma and Anti toothache medicine developed at DARL

Figure 7/37 Anti eczema medicine and honey developed at DARL