18. **Altitudinal variation induces variation in secondary metabolites contents**

The data obtained from study of quantitative variation confirm that altitudinal variation of the growing site have profound and reproducible effects on the quantitative composition of profiles of certain secondary metabolites in the rhizome of *Bergenia ciliata*. This quantitative variation in phenolic contents of *Bergenia ciliata* is probably a combined response to altitudinal variation. Due to widespread occurrence on specific soils from variable altitude, *Bergenia ciliata* was investigated for the altitudinal alteration phenolics contents. The mountains, which are masses of tangled peaks and valleys, are known to be associated with several sacred beliefs and represent one of the most rugged ranges in the region. In the ruggedness of feature they are not surpassed by any inhabited tract in the world. Along altitudinal variation several environmental forces are likely to elicit either positive or negative responses in the biosynthesis and accumulation of phenolic compounds. However, the effect of altitudinal variation is not same for the all phenolic compounds and their derivatives. In the study of impact of altitudinal variation on secondary metabolites contents of *Bergenia ciliata*, the results have shown variations at the phytochemical level, that is, compound (GA), (PCA), (BG), (EPCT), (CT), (VA), (SYA) and (GC) contents.

The finding of the study determined the best region of altitude of 5700 feet of *Bergenia ciliata* for commercial production of the targeted compounds (GA), (PCA), (BG), (EPCT), (CT), (VA), (SYA) and (GC) due to its average compound contents in the sample field no. 254027.

On the basis of result it can be concluded that biosynthesis of compound Bergenin in natural condition was highly affected at various altitudes. Except few populations, the biosynthesis
of all targeted compounds was positively correlated on increasing altitudinal variation and
after reaching on particular height slight negative correlation was observed except in case of
(CT) and (VA). Besides altitude, random variation in other phenolic compounds may be by
other environmental factors, because plant was growing under open environment. The
present study also sent a word of caution to the entire drug collectors and manufacturers that
the raw material collected from any area is not acceptable in terms of quality. This may
directly be associated with the fact that certain batches of medicines from manufacturers are
less potent than others or vice versa.

18.1. Development of analytical HPTLC/HPLC method and validation

The developed analytical method in the present study could constitute an excellent approach
for determining the real content of the phenolic compounds in complex extracts of *Bergenia
ciliata*.

The proposed analytical HPTLC and HPLC method was validated according to ICH
guidelines in terms of accuracy, linearity, specificity, intraday and inter-day precision, and
repeatability. Statistical analysis proves that the developed analytical HPTLC and HPLC
method is reproducible and selective for the analysis of compounds (GA), (PCA), (BG),
(EPCT), (CT), (VA), (SYA) and (GC). Since the proposed mobile phase effectively resolves
compounds (GA), (PCA), (BG), (EPCT), (CT), (VA), (SYA) and (GC) simultaneously, the
method can be used for qualitative as well as quantitative analysis of compounds (GA),
(PCA), (BG), (EPCT), (CT), (VA), (SYA) and (GC) in extracts. This developed method
provides more chemical information that can be used for the identification of the crude drug
as well as for the quantitation of eight pharmacologically active marker compounds that are
directly associated with the quality with the quality of the herbal medicine. The HPTLC
fingerprinting method has the advantages of rapidity, simplicity and visuality, whereas the HPLC fingerprinting method bears the advantage of specificity, more separation ability and ability to derive detailed chemical information. Both fingerprinting methods can improve the reliability of identification of herbal drugs. The better understanding of content variations assists in identifying environment, soil type, and superior genotype for crop improvement, as well as to develop strategies for the effective in situ and ex situ conservation program.
**19. Biosynthesis of andrographolide in *Andrographis paniculata***

The biosynthesis of andrographolide (1) in *A. paniculata* seems to precede by both MVA and MEP/DXP pathways. The lower enrichment of carbon positions [using (\(^{13}\)C)acetate] through the MVA pathway in comparison to very high enrichment of carbon positions [using 1,6-(\(^{13}\)C)]glucose] through the MEP/DXP pathway indicates the existence of MVA pathway also. However, the very high enrichment of specific carbons from 1,6-(\(^{13}\)C)]glucose confirm that the major biosynthetic pathway to this diterpenoid (1) operates through DXP. This also implies that some biosynthetic steps proceed in different compartments and specific intermediates or precursors cross over through the chloroplast boundary. In case of andrographolide the diterpenoid skeleton has been biosynthesised from IPP units derived from each pathway. Initially, the first C\(_{10}\) unit is derived from prenylation with DMAPP derived from either MVA or MEP pathways. This is followed by second and third prenylations with IPP. A possible biosynthetic pathway of isoprene units and andrographolide (24) from there onwards is shown in Figure 8-9 & 39 (Chapter-1 section-3.2. and Chapter-4 section-15.2.).
20. Biosynthesis of artemisinin in *Artemisia annua*

The biosynthesis of artemisinin (21) has been reinvestigated using $^{13}$C-labeled precursors and the enrichment of carbon signals in $^{13}$C-enriched artemisinin points out that MVA and DXP pathways participate in the biosynthetic process. MVA pathway is predominantly existent during the biosynthesis of artemisinin but it also involves geranyl (C10) moiety biosynthesized in plastids via DXP pathway and once again proof of cross talk over between the plastid and cytosol constituents is indicated.
21. Derivatives: act as inhibitors of bacterial cell-to-cell communication

Recent studies have shown that various eukaryotic specimens, including plant, fungi, and even animals produce compounds that interfere with bacterial QS system (Singh et al., 2015). Unluckily, most of the QS blockers identified so far are too reactive and toxic, which increases the concern of using these blockers in medicine, agriculture, and industry. However, QS inhibition as a target to control bacterial diseases of human, plant, and animal seems to hold much promise. In this investigation, we therefore decided to screen various derivatives of gallic acids for their anti-QS property by using different assays including disk diffusion, violacean production, and biofilm formation spectrophotometrically. For disk diffusion assays, *C. violaceum* 12472, a bioindicator strain was used. Results revealed that the (1a), (2a), (4), (5) and (7) showed anti-Qs activity by inhibiting violacein pigment production and biofilm formation without interfering with its growth. We therefore assumed that the negative effect of the derivatives on violacein production is not caused by inhibition of growth but rather by disruption of QS signaling system. This approach is highly encouraging because when growth is not affected there is no selective pressure for development of resistance in bacteria. Therefore, derivatives of gallic acid have a great advantage for human use than toxic anti-QS compounds such as halogenated furanone and convectional antibiotics.