Chapter 6: Concluding remark and future scope

The present study deals with phytochemical evaluation, molecular characterization and in vitro studies of selected species of Alpinia and Kaempferia such as Alpinia galanga, Alpinia malaccensis, Alpinia nigra, Alpinia calcarata, Kaempferia galanga, Kaempferia rotunda and Kaempferia parishii. Phytochemical evaluation was carried out through GC-MS based phytoconstituent analysis of essential oils and extracts, phytochemical screening, evaluation of total phenolic and flavonoid contents as well as their bioactivity studies.

GC-MS analysis of leaf oil revealed that eucalyptol, α-phellandrene and β-pinene comprised maximum peak area in A. galanga, A. malaccensis and A. nigra respectively. However leaves of Alpinia calcarata and Kaempferia species found to contain no oil. Rhizome oil contained highest percentage of eucalyptol, α-phellandrene, β-pinene, ethyl p-methoxy cinnamate and benzoic acid in species of A. galanga, A. malaccensis, A. nigra, K. galanga and K. rotunda respectively. Rhizomes of Alpinia calcarata and Kaempferia parishii did not contain essential oil. Leaf extract comprised highest percentage of benzenepropanal, acetylcyclopentanone, α-caryophyllene, olealdehyde, 2-(3,4-dimethoxyphenyl)-7-hydroxy-3-methoxy-4H-chromen-4-one, phytol and phytol in species of A. galanga, A. malaccensis, A. nigra, K. galanga, K. calcarata, K. galanga, K. rotunda and K. parishii respectively whereas rhizome extract of these species contained maximum percentage of carotol, 5-hydroxymethylfurfural, pyranone, hydroquinone, ethyl p-methoxycinnamate, vinylacetic acid and totarol respectively.

Phytochemical screening carried out revealed presence of alkaloids, flavonoids, steroids, triterpenoids in all the seven species indicating possible bioactive potential of these plants. Evaluation of total phenolic content (TPC) of leaf and rhizome extracts of Alpinia and Kaempferia species revealed that among the seven species screened, leaves of Alpinia galanga had the highest TPC value. Likewise total flavonoid content (TFC) was found highest in Alpinia nigra leaf extract.

Evaluation of antioxidant activity of the essential oils and extracts by DPPH assay revealed that leaf oil of Alpinia nigra had highest activity with lowest IC₅₀ value (4μg/ml). Antimicrobial activity of essential oil and extract samples of Alpinia and Kaempferia species was evaluated by the inhibition zone diameters, MIC and MBC...
values. Among all samples the IZD value was found highest (against *C. albicans*) in *A. nigra* leaf extract and Gentamycin was used as positive control. MIC was highest in *A. malaccensis* leaf extract against *S. aureus* while MBC was highest in *A. galanga* rhizome extract against *S. aureus*. Essential oils of *Alpinia* and *Kaempferia* species was screened for anticancerous activity against two cell lines i.e. HeLa and MCF7 and it was found that *K. galanga* and *A. nigra* possessed considerably high activity.

The results revealed that *Alpinia* and *Kaempferia* species have numerous phytochemical constituents which may be liable for a range of pharmacological activities like antioxidant, antimicrobial and anticancer activities. Thus the identified important constituents of different species may be used as biomarkers for development of chemical fingerprint of respective plant species for authentic identification and quality control of herbal drug. The oils and extracts exhibited strong antioxidant and antimicrobial activity thus explaining its potential in giving shield against various oxidative diseases and its utilize as natural antioxidants and antimicrobial molecules in food and pharmaceutical industries. Advance work on separation and characterization of compounds of these classes will offer additional data on the dynamic principle accountable for their pharmacological properties. The essential oils, extracts and the chemical constituents of *Alpinia* and *Kaempferia* species with high bioactive potential can be used for formulation of novel drugs.

Molecular characterization carried out with RAPD, ISSR, SSR and other sequence based markers revealed species-specific and reproducible DNA banding pattern in all seven species. Further, the presence of the signature sequences obtained in *A. nigra* and *A. calcarata* from highly conserved sequence based markers (rbcL, rpoB and trnH-psbA) could lead to develop suitable barcode markers. These findings would be of enough significance for authentic identification and further improvement of the taxa.

In the present study, *in vitro* culture methods have been standardised for selected taxa such as *Alpinia galanga*, *Alpinia malaccensis*, *Kaempferia galanga* and *Kaempferia rotunda*. Molecular marker (RAPD and ISSR) based assessment of field grown micropropagated plantlets revealed genetic stability up to 3 years. The protocol of micropropagation established for 4 species has got significance for large scale commercial production of authentic plants for stable supply of drug to the market.