CHAPTER VII

SUMMARY AND CONCLUSION

*Kaempferia galanga* L., an endangered medicinal plant of Zingiberaceae is mainly cultivated for its aromatic rhizome. As an economically important medicinal species, it is used in several ayurvedic preparations. The plant exhibits dormancy during drought and sprouts only in spring. Conventional propagation of the species is through rhizomes and there is no seed setting under natural condition. Hence, in vitro methods are desirable for its conservation and multiplication.

The present study was undertaken to develop a suitable protocol for its direct and indirect organogenesis and somatic embryogenesis, as adequate investigations have not been attempted earlier.

Studies on genetic diversity and phytochemical analyses also formed an integral part of this study. Besides, antimicrobial screening and antioxidant evaluation also have been done to validate the traditional medicinal significance.

The results are summarized herewith

- Evolved distinct and reproducible protocol for the *in vitro* regeneration from rhizome bud, rhizome disc, shoot tip and leaf-sheath explants.
- Standardized callus mediated organogenesis from rhizome bud, rhizome disc, leaf-sheath and leaf explants.
- Achieved a direct pathway for somatic embryogenesis from leaf-sheath explants and indirect pathway for somatic embryogenesis from callus tissue of rhizome bud explants.
- Studied genetic diversity by RAPD to diagnose the variation among the populations.
- Through GC-MS analysis of ethanol extract, about 72 phytochemical compounds have been identified.
- Established antimicrobial activity of the plant extract by using Gram-positive and Gram-negative bacteria and fungi which were sensitive to the plant extract.
- Confirmed by antioxidant studies the presence of flavonoids and polyphenols.
In vitro propagation

7.1. Direct Organogenesis

The excised in vivo explants were surface sterilized with fungicide, with 70% ethanol and 0.1% HgCl$_2$ for 2 minutes for controlling the fungal and bacterial contamination. Multiple shoots were induced from rhizome bud, rhizome disc, shoot tip, and leaf-sheath explants. Explants of 2 months old rhizomes of about 6-7 mm size exhibited maximum response with a survival of 80%.

- BAP showed the highest frequency with optimal number of shoot bud regeneration than KN.
- BAP 1.0 mg/l and KN at 3.0 mg/l induced maximum number of shoots in rhizome bud explants.
- BAP 2 mg/l and KN 1.0 mg/l induced maximum multiple shoots from rhizome disc explants.
- BAP 2.0 mg/l and NAA 1.0 mg/l induced maximum number of shoots in shoot tip and leaf-sheath explants.
- Simultaneous root formation was achieved in shoot induction medium in the case of rhizome bud and rhizome disc explants.
- Half MS medium fortified with NAA 2.0 mg/l induced maximum number of shoots from the shoot tip and leaf-sheath explants.
- Rooted plantlets were successfully transferred to the field.

7.2. Indirect Organogenesis

The induction of callus growth and subsequent differentiation and organogenesis were accomplished by the differential application of growth regulators and the control of conditions in the culture medium.

- Full strength MS medium was used for callus induction and their regeneration.
- Of the three auxins and their different concentrations tested for their callusing ability 2,4-D (2.0 mg/l) induced maximum callus.
- Morphology, texture and colour of the callus were dependent on the nature of the hormone used.
BAP 2.0 mg/l and NAA 1.0 mg/l showed maximum frequency of shoot bud differentiation in all the explants derived calluses.

BAP (1.5 mg/l) in combination with GA₃ (1.0 mg/l) had maximum induction of shoot elongation.

Of the three auxins used, NAA (1.5 mg/l) showed the highest frequency of root induction within 15 days of culture.

Rooted plantlets were successfully transferred to the field.

7.3. Somatic Embryogenesis

In the present study, a protocol was developed for induction of somatic embryogenesis directly from leaf-sheath and indirectly using rhizome bud explants. Successful regeneration of plants from leaf-sheath, via direct somatic embryogenesis has been reported for the first time.

Direct somatic embryogenesis avoids the passage through callus and thus avoids the genetic instability often associated with somatic embryos obtained indirectly from callus.

NAA 2.0 mg/l and BAP 0.5 mg/l induced higher percentage of somatic embryos directly from leaf-sheath.

Higher percentage of somatic embryos was initiated indirectly when the callus subcultured on MS medium with NAA (1.0 mg/l) and BAP (0.5 mg/l).

BAP (0.5 mg/l) and NAA (1.0 mg/l) produced the highest frequency of somatic cells in half MS liquid medium.

More advanced stages of somatic embryos normally globular, scutellar, club, and banana shaped were observed in liquid medium containing BAP (0.5 mg/l) and NAA (1.0 mg/l).

Half strength MS medium was found to be optimum for induction, maturation and germination of somatic embryos.

The combination of BAP (1.0 mg/l) and NAA (0.4 mg/l) was found to be effective for somatic embryo germination.

Rooted plantlets were successfully transferred to the field.
7.4. Genetic diversity

- A total of 109 DNA fragments were generated by the 12 primers with an average of about 6.2 bands per primer. Primers yielded bands ranging from 1 to 9.

- Approximately 86.9% polymorphism estimated from 93 of 109 fragments were polymorphic with 12 primers used among the 16 *K. galanga* accessions.

- The value of genetic distance ranging from 0.1 to 0.8 was observed among the 16 accessions and the overall genetic diversity was observed to be 0.2747 which indicates fairly high polymorphism.

- The cluster analysis based on genetic distance values classified all the *K. galanga* accessions into two major groups/clusters (I & II). Group I constituted the wild varieties and group II constituted the commercially cultivated varieties. This shows that wild varieties are still undergoing variations in their genetic makeup. The group II was further divided into three subgroups showing that variations have occurred within the commercially cultivated varieties in different areas of Kerala. Thus the RAPD work suggests to screen the elite clones from the divided groups by phytochemical screening.

7.5. Phytochemical Analysis

- *Kaempferia galanga* yielded 72compounds in GC-MS analysis.

- Rhizome extract with 22 compounds, leaf extract with 39 compounds and root extract with 17 compounds.

- Two compounds namely n-Hexadecanoic acid and 2-Propenoic acid, 3-(4-methoxyphenyl), ethyl ester were common for all extracts.

- Some compounds are known for activities like anticancer, antitumour, antioxidant, hypocholesterolemic, anti-inflammatory, antiachene, antiseptic, antiandrogenenic, anti- microbial, sedative, anesthetic and analgesic etc.

- Some other compounds have herbicidal, nematicidal, fungicidal, insecticidal, pesticidal and termiticidal properties.

- Thirty three compounds do not have reports on pharmaceutical or medicinal usage.
7.6 Antimicrobial Analysis

- The activity of different solvent extracts of *Kaempferia galanga* on Gram-negative and Gram-positive bacteria confirmed that the plant contains compounds that exhibit measurable *in vitro* antimicrobial activity against some bacteria and fungi used in this study.

- All plant parts exhibited antimicrobial activity and the highest activity is noted in the rhizome.

- Among the solvents used, ethanol extracts showed the highest activity with regard to the inhibition of both bacterial and fungal growth.

- Rhizome extracts were found to be highly active against the growth of bacterial strains such as *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus cereus*, *B. subtilis* and *Escherichia coli*.

- Fungal strains such as *Aspergillus niger* and *A. flavus* which usually parasitize man and animal and cause pulmonary aspergillosis, *Candida albicans* which causes serious systemic infections viz. candidiasis and vulva vaginitis were also found to be susceptible to the plant extract.

7.7. Antioxidant Studies

- *In vitro* antioxidant assays were carried out in rhizome and leaf ethanol extracts.

- Both extracts showed significant antioxidant scavenging activities against superoxide radical, hydroxyl radical, and lipid peroxidation and showed little antioxidant scavenging activities against DPPH radical.

- Leaf extracts showed higher antioxidant activity than rhizome. IC₅₀ values for leaf-extract were 14 µg/ml, 47µg/ml and 85 µg/ml against, hydroxyl radical, superoxide radical and lipid peroxidation respectively.

- IC₅₀ values were recorded for rhizomes extracts were 22 µg/ml against hydroxyl radical, 87µg/ml against superoxide radical and 96 µg/ml, against lipid peroxidation.