Summary

6. SUMMARY

***In vitro* propagation**

- Asymbiotic seed germination

KC medium was found to be most effective in inducing 95 % seed germination in *P.pallida.*

100 % seed germination was observed in *A.graminifolia* on BM medium.

- Callus induction

2,4-D at lower concentration (2.26 µM) induced callus in both the orchids.

Hormone-free basal medium supported the conversion of callus into complete plantlets.

- Multiple shoots development

P. pallida: KC medium with individual concentrations of BA and Kinetin induced multiple shoots.

A.graminifolia : BM medium with Kinetin induced multiple shoots.

- Pseudobulb culture

P.pallida : IAA (11.4 µM) induced shoot and root.

A.graminifolia : Kinetin (2.32 µM) induced shoot and root.

- Rooting and Hardening

P.pallida : NAA (10.76 µM) induced rooting.

A.graminifolia : IAA (11.4 µM) induced rooting.

Well rooted orchids were acclimatized successfully with 60 % survival rate.

Phytochemical analysis and biological evaluation

- Preliminary phytochemical analysis

P.pallida : alkaloids, phenolic compounds, terpenoids in Chloroform extract.

A.graminifolia : alkaloids, terpenoids, steroids, flavonoids, phenolic compounds, fats and oils in methanol extract.

- Estimation of total phenol and flavonoid content

Chloroform extract of *P.pallida* have highest phenol and flavonoid content (86.55 μg GAE/mg DW and 58.60 μg QE/mg DW).

Methanol extract of *A.graminifolia* having highest phenol and flavonoid content (75.85 μg GAE/mg DW and 59.86 μg QE/mg DW).

FT-IR and GC-MS analysis

The FTIR spectrum of chloroform and methanol extracts of *P.pallida* and *A.graminifolia* reveals the presence of carboxyl, amine and alcohol groups.

GC-MS analysis of *P.pallida* : 1-Heptanol, 3-methyl, Naphthalene, Phenol 4-Methoxymethyl-, 1- Heptanol, 6- methyl-, trans-Cinnamic acid, Phosphonic acid, Diotadecyl, n-Hexadecanoic acid, Ergost-5- EN-3-ol.

GC-MS analysis of *A.graminifolia* : Butane-1,2,3,4-Tetraol, Pentadecanoic acid, Benezene, 1,1'- (Thiobis Methylene), 4H-1- Benzopyran-4-one,2,3-dihydro-5,7-, 4H-1-Benzopyran-4-one,5,7-Dihyd

Antibacterial activity

The chloroform extract of *P.pallida* showed the maximum zone of inhibition against the bacteria *Pseudomonas aeruginosa* (15 mm) followed by *Enterococcus facealis* (14.3 mm) and *Salmonella enteria* (12 mm).

The highest activity and zone of inhibition were recorded when methanol extract of *A.graminifolia* used against *Enterococcus faecalis* (13 mm), *Bacillus subtilis* (13 mm), and *Pseudomonas aeruginosa* (12 mm).

Antioxidant activity

Water extract shows maximum activity in both the orchids.

Anticancer activity

P.pallida : water extract was cytotoxic towards the MCF-7 cell line with IC_{50} value 292.8 µg/ml. *A.graminifolia* : water extract was cytotoxic towards the MCF-7 cell line with IC_{50} value 390.7µg/ml.

Nanoparticle synthesis

Aqueous extracts of both *Pholidota* and *Arundina* was used for the silver nanoparticle synthesis. The synthesized nanoparticles was characterized by using FTIR, UV Spectroscopy, SEM and XRD and exhibits good at aqueous extracts

Isolation, identification and bioactivity evaluation of endophytes

P.pallida : The isolated endophyte S1 shows high homology towards *Guignardia vaccinii. A.graminifolia* : The isolated endophyte S2 shows high homology towards *Colletotrichum karstii.* The isolated endophytes (S1 & S2) do not possess antimicrobial and antioxidant activities.

Conclusion

Orchids are considered as the high source of herbal medicine. Unfortunately, they are among the most threatened of all flowering plants due to over collection by increasing orchid lovers, over-exploitation as herbal healthcare, destruction of habitats by reclamation, shifting cultivation and deforestation, loss of pollinator, fragmentation of populations, genetic drift, anthropogenic pressures and unauthorized trade. There is no doubt some of the orchid species have already been extinct from the wild.

Modern propagation and production technology has made orchids accessible to a much broader section of the society. The fact that all major commercial tissue culture laboratories in the world are involved in orchid micropropagation emphasizes how popular these flowers have become. Development of new hybrids and their commercial

cultivation have now become a lucrative industry in many countries of the world. The rising popularity of orchids has created a demand for high quality plant materials for the development of orchid industry.

This study reports a simple and effective protocol for the successful *in vitro* asymbiotic seed germination, callus induction and regeneration, multiple shoots induction, pseudobulb culture, rooting and hardening of *P.pallida* (endangered) and *A.graminifolia* (vulnerable) orchids. Besides, the study also describes the antibacterial and antioxidant activities of orchid leaves. The endophye isolated from the leaves of *P.pallida* (S1) and *A.graminifolia* (S2) are not responsible for the antibacterial and antioxidant activities. This study further expands the possibilities for the conservation and utilization of the threatened orchid species inorder to meet the demands of the horticultural trade, hobbyists and plant based pharmaceutical industries.