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REINTRODUCTION OF ENDAMIC AND ENDANGERED ORCHID

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Abstract : Orchids are widely used either directly as folk remedies or indirectly in the preparation of modern pharmaceuticals. The distribution of orchids in peninsular India are about 200 species of 60 genera Kanyakumari District harbors about 80 species in 29 genera and 22 species are found to be endemic, i.e. only confined to peninsular India. The peninsular India contains 33 species that are reported from Himalayas and 35 species commonly distributed in Malaya or Thailand or Java. The increased exploration and critical taxonomic analysis of orchids in different regions with comparable climate and elevation bound to throw more light on inter and intra-specific variations of orchids and a better knowledge of their distribution patterns (Joseph, 1987).

A significant number of modern pharmaceutical drugs derived from these plants serve as a potential source of therapeutic aids in health system all over the world for humans and animals. Our ancestors have made selfless efforts to explore nature health problems for the benefit of mankind associated with mind and body. Pharmacologists, microbiologists, biochemist, botanists and natural-products chemists all over the world are currently investigating medicinal plants for phytochemical and lead compounds that could be developed for the treatment of various diseases (Acharya et al., 2008).

INDEX TERMS:

Alkaloids, Flavonoids, Coumarins, Glycosides, Polysaccharides, Phenols, Tannins, Terpenes and Terpenoids

1. INTRODUCTION:

In today's fast-changing world, conversations around climate change, sustainability, and eco-friendly livingare no longer confined to environmentalists alone. Businesses, too, are realizing that their survival depends on how responsibly they treat the planet. In recent years, organizations across the globe have been increasingly focusing on sustainable practices to address environmental challenges. One emerging concept that aligns with this movement is Green Human Resource Management (Green HRM). Green HRM integrates environmental management into human resource practices, with the goal of promoting sustainability, reducing waste, and developing a workforce that is environmentally conscious. Nature has been bestowed with large number of diverse types of plants that possess therapeutic properties. Orchids the nature's extravagant group of plants distributed throughout the world from the tropics to high alpine. The plant kingdom of the family Orchidaceae comprises 25,000-35,000 species (Dressler, 1993). Incredible range of the orchid exhibits diversity in shape, size and colour of their flowers. The most

pampered plants occupy the top position among all the flowering plants valued for cut flower production and as potted plants. Orchids are known for their long lasting beautiful flowers fetch a very high price in the international market (Joshi et al., 2009).

1.1 IMPORTANCE OF GREEN HRM:

1.2 OBJECTIVES OF GREEN HRM

Collection and identification of orchids from Western Ghats of Kanyakumari District
Conservation of selected orchids through micropropagation by establishing the standard protocol
Reintroduction of hardened orchid
In-vitro phytochemical analysis of selected orchids

In-vitro antioxidant activity of selected orchids

II .LITERATURE REVIEW

The compounds which act as radical scavengers prevent the radical chain reaction of oxidation delay or inhibit the oxidation process are known as antioxidant. Oxidative stress is the major causative factor in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, aging, diabetes, cancer, immune suppression, neurodegenerative diseases and others. Oxidative stress plays an important role in manifesting of diabetes and vascular complications. The antioxidant potential of in vitro estimation of aqueous extract of aseptically regenerated Dendrobium aqueum showed a dose dependent DPPH free- radical scavenging potential. Free radicals have attracted a great deal of attention in excessive production of reactive oxygen species and oxidative stress leads to derangements of normal physiological phenomenon. To counteract the harmful effects of free radicals, antioxidant defense mechanism operates to detoxify or scavenge these free radicals. Vanda tessellata (Roxb.) have been used in the indigenous medicine such as Ayurveda and local traditional medical practices. Petroleum ether extract of Vanda tessellata Roxb by using established in-vitro antioxidant methods 1, 1-Diphenyl-2-Picryl hydroxyl (DPPH) and NO scavenging activity showed that petroleum ether extract had significant NO inhibition.

Antioxidants prevent oxidative stress caused by free radicals, which damages cells free radicals have attracted a great deal of attention in recent years. Excessive production of reactive oxygen species and oxidative stress leads to derangement of normal physiological phenomenon. To counteract the harmful effects of free radicals, antioxidant defense mechanism operates to detoxify or scavenge free radicals. Antioxidants are compounds which act as radical scavengers, prevent the radical chain reaction of oxidation delay or inhibit the oxidation process. It has been established that oxidative stress is the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, aging, diabetes, cancer, immune suppression, neurodegenerative diseases and others (Sudha et al., 2001).

Traditional medicine is widespread and plants still presents a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, anti-necrotic, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant or antiradical scavenging mechanism as part of their activity. Oxidation processes are intrinsic in the energy management of all living organisms are therefore reserved under strict control by several cellular mechanisms. The biological screening of Vanda coerulea (Griff. ex. Lindl) crude hydro-alcoholic stem extract displayed the best DPPH /NOH radical scavenging activity and in vitro inhibition of type 2 prostaglandin (PGE-2) release from UVB (60 mJ/cm2) irradiated HaCaT keratinocytes. Bio-guided fractionation and phytochemical analysis led to the isolation of five stilbenoids: imbricatin methoxycoelonin, gigantol, flavidin and coelonin. Stilbenoids most concentrated in the crude hydroalcoholic stem extract of Vanda coerulea is considered as biomarkers. Dihydro-phenanthropyran and dihydrophenanthrene displayed the best DPPH/NOH radical scavenging activities as well as HaCaT intracellular antioxidant properties using DCFH-DA probe: IC50 8.8 mM and 9.4 mM compared to bibenzyle (IC50 20.6 mM) (Chellaram et al., 2013).

III. MATERIALS AND METHODS

3.1 Collection of Epiphytic and Terrestrial Orchids

The orchids grown in the diverse areas of the wild sanctuaries of extinct and endangered orchids of epiphytic and terrestrial habitat gathered during the field visit are micropropagated.

3.2 Micropropagation of Orchids

Micropropagation has been demonstrated as an excellent method of large-scale production of true-to-type. Several factors were responsible for the success of micropropagation which includes explants types, nutrients, growth regulators, other additives and culture conditions (temperature, light intensity and duration) are essential for the development of micropropagation protocols. Plant tissue culture medium usually consists of major inorganic salts, trace elements, a carbon source, vitamins, growth regulators and gelling agent. The systematic study of the nutrient requirements of plant tissues under in vitro conditions has led to the development of a range of nutrient formulations.

3.3 Sterilization of Pod

Pod obtained from young and actively growing shoots of mature Orchid species were placed in enamel trays containing tap water with two to three drops of detergent and a drop Tween-20 (surfactant). The explants were stirred gently and then washed with running tap water until all the traces of soap were completely removed. Further explants were washed in double distilled water. These explants were then treated with 0.1% (w/v) mercuric chloride solution under sterile conditions for one or two minutes and transferred to the inoculation chamber, where they were washed with double distilled sterile water for at least seven times to remove traces of surface sterilizing chemicals present on the surface of the explants. The exposed cut end was trimmed off to eliminate toxic effect of sterilant which may have moved into cells during sterilization.

3.4 Nutrient media

Murashige and Skoog's (1962) are used as a medium for the propagation of orchid and all trials were later carried out on MS medium.

3.5 Preparation of stock solutions

Weighing of all constituents of a nutrient medium individually and their mixing were made to the highest level of accuracy. Concentrated stock solutions of major salts, minor salts, myoinositol, iron source, vitamins and phytohormones were prepared on need basis which not only saved time but also it was more accurate. The stock solutions were kept in dark glass bottles and stored in refrigerators.

Table : Components of Murashige and Skoog (MS) medium

Components	Constituents	Con.(mg/L)	Stock con.(50x) g
A	Ammonium Nitrate Potassium Nitrate	1650 1900	82.5 95.0
B	Magnesium Sulphate Manganese Sulphate Zinc Sulphate Copper Sulphate	370 22.3 8.6 0.025	18.5 1.115 0.43 0.00125
C	Calcium Chloride Potassium Iodide Cobalt Chloride	440 0.83 0.025	22 0.0415 0.00125

D	Potassium Dihydrogen Orthophosphate Boric Acid Sodium Molybdate	170 6.2 0.23	8.5 0.31 0.0125
E	Ferrous Sulphate Sodium Ethylene Diamine Tetra Acetic Acid	27.8 37.3	1.39 1.865
F	F1-Thiamine HCL F2-Nicotinic Acid F3-Pyridoxine HCL F4-Glycine	0.1 0.5 0.5 2.0	0.005 0.025 0.025 0.1
G	Myo-Inositol	100	5.0

3.6 Preparation of Nutrient Medium

Required quantities from (pre-prepared) stock solutions of MS major and minor salts, vitamins and myo-inositol were mixed together for one litre basal medium. The plant growth regulators were added at required concentration either individually or in combinations. In general, use of 3% sucrose is better than lower or higher concentration. However, there are several accounts of the usage of higher concentrations of sucrose (5%) for root initiation and proliferation. Prior to the addition of agar the pH of the medium was adjusted to 5.8 by using 0.1 N NaOH or 0.1N HCL.

In general, agar is the most frequently used gelling agents in tissue culture, after the addition of agar the medium was warmed up till the agar melted. The liquid medium was poured equally in the culture vials (10ml in each vial). The medium was autoclaved at 121º C for 15 psi (1.06 kg/cm2) pressure for 15 minutes. The mouth of the culture vials were sealed tightly after autoclaved, undisturbed to cool. The culture vials were labeled and kept under sterile condition.

3.7 Inoculation

The platform of the laminar flow cabinet, hands of the operator and others were sterilized by wiping them thoroughly with 70% alcohol and the alcohol was allowed to dry. Prior to inoculation all the sterilized implements, glass wares and vials were kept inside the laminar air flow chamber in the presence of UV light for about 30 minutes. The washed explants were first kept on sterile tissue paper in order to remove the excess water from the surface. The explants were transferred to the cutting board and trimmed to 0.5-1 cm and planted / transferred on the culture medium, the mouth of the culture vials was held close to the flame and sealed immediately after inoculation and labeled. Then, the inoculated culture vials were incubated in dark and light conditions at 24±1ºC. Light conditions provided by cool – white fluorescent tubes (polylux XL) were of 4000 lux. The explants were inoculated in 100 ml sterilized culture bottles. One or two nodes and shoots per culture bottle were used and five to seven replicates per treatment. The response was observed daily.

3.8 Sub-Culture

After three weeks of incubation, the microshoots were sub-cultured for further multiplication to culture bottles containing 30 ml culture media. Whenever the culture medium gets depleted, the cultures were sub-cultured onto the same medium fortified with the same hormonal strength in which they were raised initially. Culturing of shoots in culture bottles makes the multiplication process cost effective and provides required space for the growth. After one or two sub-cultures, multiple shoot buds and shoots were transferred to MS medium supplemented with different concentration of plant growth regulators.

Table: 1 Inoculation of *Geodorum densiflorum* (Lam) Schltr. seed using different concentration of MS Media supplemented with 15% Coconut Water

S. No	PGR Treatment mgL^{-1})	Number of replicates	% of Callus Induction	Morphogenesis of immature seed
1	Control	10	-	-
2	BAP (1)	10	3.37±0.74	++
3	BAP (2)	10	3.45±0.56	+
4	BAP (5)	10	3.78±0.37	++
5	KIN (1)	10	3.17±0.12	+
6	KIN (2)	10	2.92±0.48	++
7	KIN (5)	10	4.38±0.21	-
8	NAA (1)	10	3.91±0.96	++
9	NAA (2)	10	3.45±0.24	+
10	NAA (5)	10	4.68±1.48	++

Data showing the mean of 10 replicates ± standard error (SE) No response; + Small quantity; ++ Moderate quantity; +++ Large quantity of calli

Table: 2 Influence of sub-culture on Growth Regulators (PGRs) on development of protocorm like bodies (PLBs) from immature seed callus of *Geodorum densiflorum* (Lam) Schltr. on MS Media supplemented with 15% Coconut Water

S. No	PGR Treatment (mgL^{-1})	Number of replicates	Number of PLBs from each Callus	Response of seed explant
1	Control	10	-	-
2	BAP (5)+NAA(5)	10	-	-
3	BAP (5)+NAA(2)	10	5.37±0.74	Healthy and Green PLBs formed
4	BAP (2)+NAA(2)	10	4.53±0.65	Few Green PLBs formed
5	BAP (2)+NAA(1)	10	5.33±0.96	Few Green PLBs formed
6	BAP (1)+NAA(1)	10	4.92±0.48	Few Green PLBs formed
7	BAP (5)+KIN(5)	10	-	-
8	BAP (5)+KIN(2)	10	5.17±0.31	Few Green PLBs formed
9	BAP (2)+KIN(2)	10	4.68±1.48	Few Green PLBs formed
10	BAP (2)+KIN(1)	10	-	-
11	BAP (1)+KIN(1)	10	13.93±0.64	Healthy and Green PLBs formed

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Table: 3 Effect of Activated Charcoal (AC), Polyvinylpyrrolidone (PVP) and Gibberlic acid (GA3) provided individually or in combinations in MS medium on the elongated shoots of *Geodorum densiflorum* (Lam) Schltr. compared after 60 days of transfer

S.No	Treatment	Average Initial height (cm) (X±SE)	Mean length of shoots (cm) (X±SE)	Percentage of increase in shoot length (%)
1	Control	0.72±0.01	1.40±0.05^{efg}	106.02^{fghi}
2	GA (0.5 µM)	0.61±0.01	0.75±0.03^{g}	41.30^{i}
3	GA (1.0 µM)	1.06±0.05	2.80±0.11^{bc}	227.36^{de}
4	GA (1.5 µM)	0.87±0.03	3.20±0.10^{b}	328.46^{bc}
5	GA (2.0 µM)	0.75±0.01	2.00±0.08^{de}	261.06^{cd}
6	AC (0.5%)	0.63±0.01	2.20±0.09^{cd}	368.09^{b}
7	AC (0.1%) + GA (0.5µM)	0.61±0.02	0.90±0.05^{g}	82.61^{ghi}
8	AC (0.1%) + GA (1.0 µM)	0.64±0.02	4.00±0.08^{a}	565.63^{a}
9	AC (0.1%) + GA (1.5 µM)	0.60±0.02	1.60±0.06^{def}	174.74^{defg}
10	AC (0.1%) + GA (2.0µM)	0.64±0.02	1.20±0.09^{fg}	183.85^{defg}
11	PVP (0.5 µM)	0.69±0.03	0.78±0.05^{g}	89.42^{ghi}
12	PVP(0.1 µM) + GA (0.5µM)	0.67±0.02	1.20±0.08^{fg}	165.50^{defg}
13	PVP (0.1 µM) + GA (1.0µM)	0.79±0.03	1.10±0.05^{fg}	67.80^{hi}
14	PVP (0.1 µM) + GA (1.5µM)	0.52±0.02	1.00±0.06^{fg}	144.87^{efgh}
15	PVP (0.1 µM) + GA (2.0µM)	0.69±0.03	0.79±0.05^{g}	37.98^{i}

Values followed by same superscripts in a column are not significantly different at P=0.05, N=45, ± Standard Error

. **Table: 4 Effect of Cytokinins supplemented with BAP and KN individually to MS and MS+TDZ at varying concentration on the differentiation and development of shoots from *in-vitro* developed PLBs of *Geodorum densiflorum* (Lam) Schltr. compared after 60 days of culture**

S.No	Cytokinin (µM)	% of Cultures Responding	Average number of shoot/culture	Average length of shoots (cm) X±SE
1	Control	73.33^{ab}	0.80±0.16^{f}	3.30±0.25^{a}
2	BAP (0.5)	53.33^{b}	1.22±0.41^{def}	3.50±0.38^{a}
3	BAP (1.0)	60.00^{ab}	2.56±0.71^{abcd}	3.00±0.29^{ab}
4	BAP (1.5)	60.00^{ab}	2.24±0.65^{abcde}	3.30±0.35^{a}
5	BAP (2.0)	73.33^{ab}	3.18±0.59^{ab}	1.50±0.12^{bcde}
6	KN (0.5)	60.00^{ab}	1.26±0.31^{def}	2.50±0.24^{abcd}
7	KN (1.0)	73.33^{ab}	2.87±0.56^{abc}	1.20±0.12^{cde}
8	KN (1.5)	66.67^{ab}	2.43±0.58^{abcde}	2.30±0.20^{abcde}
9	KN (2.0)	60.00^{ab}	1.20±0.30^{ef}	2.90±0.22^{abc}
10	MS+TDZ (0.5)	93.33^{a}	1.93±0.26^{bcdef}	1.80±0.13^{abcde}
11	MS+TDZ (1.0)	73.33^{ab}	1.53±0.34^{cdef}	2.00±0.17^{abcde}
12	MS+TDZ (1.5)	86.67^{ab}	3.58±0.52^{a}	1.00±0.10^{de}
13	MS+TDZ (2.0)	86.67^{ab}	1.87±0.29^{bcdef}	0.60±0.05^{e}

Each culture contained three PLBs. Values followed by same superscripts in a column are not significantly different at P=0.05, N=39, ± Standard Error

Table: 5 Effect of Auxin supplementation in Half-strength MS Medium of *in-vitro* regenerated roots/responding shoots of *Geodorum densiflorum* (Lam) Schltr.

S.No	% of responding culture	Concentration of sucrose	Average number of roots/responding shoot	Average length of roots (cm) (X±SE)
1	Control	5%	2.32±0.08^{cd}	1.06±0.13^{c}
2	IBA (0.4)	5%	2.00±0.06^{cde}	0.85±0.18^{c}
3	IBA (0.8)	5%	1.50±0.07^{e}	0.60±0.11^{c}
4	IBA (1.2)	5%	1.75±0.07^{de}	0.72±0.09^{c}
5	IBA (1.5)	5%	1.83±0.08^{cde}	4.32±0.53^{a}
6	IAA (0.4)	5%	4.29±0.15^{a}	1.96±0.29^{b}
7	IAA (0.8)	5%	2.45±0.09^{c}	0.65±0.10^{c}
8	IAA (1.2)	5%	2.07±0.07^{cde}	1.60±0.24^{b}
9	IAA (1.5)	5%	3.09±0.126^{b}	1.05±0.16^{c}

Each culture contained two shoots. Values followed by same superscript(s) in a column are not significantly different at p=0.05, N=27, ±Standard Error

Table: 6 Evaluation of *in-vitro* raised plantlets of *Geodorum densiflorum* (Lam) Schltr. in different hardened planting substrates

Planting substrate (1:1:1)	No. of plant transferred	No. of plant survived
Sterilized forest leaves, charcoal and brick pieces	32	19
Autoclaved charcoal, dried cow dung and brick pieces	25	15

Table: 7 Effect of Coconut Water on callus induction of immature seeds of *Acampe praemorsa* on MS Media

S.No	Supplemented medium with Coconut Water	Response of immature seeds in culture		Number of Days Taken
		Morphogenesis	% Callus Induction	
1	Control	-	-	-
2	MS media + CW 10%	++	59±0.81	33
3	MS media + CW 15%	+	61.4±0.43	35
4	MS media + CW 20%	++	60.6±1.32	37

Data showing the mean of 10 replicates ± standard error (SE) No response; + Small quantity; ++ Moderate quantity; +++ Large quantity of calli

Table: 8 Influence of Growth Regulators (PGRs) on development of protocorm like bodies (PLBs) from immature seed callus of *Acampe praemorsa* on MS Media supplemented with 15% Coconut Water

PGR Treatment (mgL^{-1})	No of PLBs from each callus	Type of response	Development of shoot buds from PLBs
MS (Control)	-	-	-
BAP (5) + NAA (5)	-	-	-
BAP (5) + NAA (2)	1.3±0.32	Greenish PLBs formed	---
BAP (2) + NAA (2)	4.7±0.36	Greenish PLBs formed	---
BAP (2) + NAA (1)	7.33±0.96	Greenish PLBs formed	-
BAP (1) + NAA (1)	15.93±0.64	Healthy green PLBs formed	Shoot buds and plantlet formation
BAP (5) + KIN (5)	3.1±0.31	Greenish PLBs formed	--
BAP (5) + KIN (2)	5.17±0.31	Green PLBs formed	-
BAP (2) + KIN (2)	9.3±2.72	Green PLBs formed	---
BAP (2) + KIN (1)	10.77±0.92	Healthy green PLBs formed	---
BAP (1) + KIN (1)	11.17±1.32	Healthy green PLBs formed	Shoot buds and plantlet formation

Table: 9 Effect of PGR treatments in the influence of number of shoot/ explant and shoot length

S.No	PGR	Number of Shoot/Explant	Shoot Length (cm)
1	0.5KIN+0.5 BAP	2.11 ± 0.04^{ef}	3.73 ± 0.13^{e}
2	0.5KIN+1.0 BAP	1.82 ± 0.11^{ef}	2.26 ± 0.23^{h}
3	0.5KIN+2.0 BAP	1.50 ± 0.04^{h}	2.06 ± 0.08^{i}
4	0.5KIN+5.0 BAP	1.11 ± 0.10^{i}	1.5 ± 0.24^{j}
5	1.0KIN+0.5 BAP	3.71 ± 0.24^{d}	3.25 ± 0.21^{fg}
6	1.0KIN+1.0BAP	1.99 ± 0.15^{fg}	2.84 ± 0.28^{gh}
7	1.0KIN+2.0BAP	1.59 ± 0.21^{h}	1.27 ± 0.06^{j}
8	1.0KIN+5.0BAP	1.00 ± 0.14^{i}	1.22 ± 0.12^{j}
9	2.0KIN+0.5BAP	2.34 ± 0.12^{e}	5.66 ± 0.07^{b}
10	2.0KIN+1.0BAP	1.75 ± 0.15^{gh}	3.4 ± 0.34^{ef}
11	2.0KIN+2.0BAP	1.51 ± 0.25^{h}	2.58 ± 0.29^{h}
12	2.0KIN+5.0BAP	1.0 ± 0.16^{i}	1.22 ± 0.10^{j}

Table: 10 Rooting of *in-vitro* regenerated shoots of *Acampe praemorsa* and type of Auxin supplementation in MS Medium

S.No	% of responding culture #	Average number of roots/responding shoot	Average length of roots (cm) (X±SE)
1	Control	2.14±0.53^{ab}	374±0.14^{ab}
2	IBA (0.1)	1.50±0.07^{e}	0.60±0.11^{c}
3	IBA (0.5)	1.75±0.07^{de}	0.72±0.09^{c}
4	IBA (1)	1.83±0.08^{cde}	4.32±0.53^{a}
5	IBA (2)	NR	NR
6	IAA (0.1)	1.42± 0.53^{a}	1.71± 0.48^{ab}
7	IAA (0.5)	1.85± 0.69	1.85± 0.69
8	IAA (1)	NR	NR
9	IAA (2)	NR	NR

Table: 11 Evaluation of *in-vitro* raised plantlets of *Acampe praemorsa* in different hardened planting substrates

Planting substrate (1:1:1)	No. of plant transferred	No. of plant survived
Sterilized forest leaves, charcoal and brick pieces	32	19
Autoclaved charcoal, forest tree barks and brick pieces	25	15

Table. 12 *In-vitro* Qualitative Analysis of *Geodorum densiflorum* (Lam) Schltr. Leaf extracts

Phytoconstituents	Control	Ethanol	Methanol	Chloroform	Acetone	Aqueous
Terpenoid	+	-	-	+	-	+
Flavonoid	+	+	-	+	+	-
Reducing Sugar	-	-	+	-	-	-
Phenol	-	+	+	-	+	+
Alkaloid	-	+	+	-	+	+
Saponin	-	+	+	+	+	+
Tannin	+	+	+	+	+	+
Steroid	+	+	-	+	+	+
Aminoacid	+	-	-	+	-	-
Glycosides	+	-	-	-	-	-

+ Presence - Absence

Table.13 *In-vitro* Qualitative Analysis of *Geodorum densiflorum* (Lam) Schltr. Root extracts

Phytoconstituents	Control	Ethanol	Methanol	Chloroform	Acetone	Aqueous
Terpenoid	+	+	+	-	+	+
Flavonoid	+	+	-	-	-	-
Reducing Sugar	+	-	-	+	-	-
Phenol	-	-	-	-	+	+
Alkaloid	+	+	-	+	+	-
Saponin	-	-	-	-	-	+
Tannin	-	+	+	+	+	-
Steroid	+	+	+	+	+	-
Aminoacid	+	+	-	-	-	+
Glycosides	-	-	-	+	-	-

+ Presence - Absence

Table.14 *In-vitro* Qualitative Analysis of *Acampe praemorsa* Leaf extracts

Phytoconstituents	Control	Ethanol	Methanol	Chloroform	Acetone	Aqueous
Terpenoid	+	-	+	+	+	+
Flavonoid	+	+	+	+	-	-
Reducing Sugar	-	-	-	+	-	+
Phenol	-	+	-	-	-	+
Alkaloid	-	+	-	-	-	+
Saponin	-	+	-	-	+	+
Tannin	-	+	-	+	+	-
Steroid	+	-	+	+	+	+
Aminoacid	+	-	-	-	+	+
Glycosides	+	-	-	-	+	-

+ Presence - Absence

Table.15 *In-vitro* Qualitative Analysis of *Acampe praemorsa* Root extracts

Phytoconstituents	Control	Ethanol	Methanol	Chloroform	Acetone	Aqueous
Terpenoid	+	+	+	+	+	+
Flavonoid	+	+	-	-	+	-
Reducing Sugar	+	+	-	-	-	-
Phenol	+	-	-	+	+	-
Alkaloid	+	+	-	+	-	-
Saponin	+	-	-	+	+	-
Tannin	-	-	+	+	-	-
Steroid	-	-	+	+	+	+
Aminoacid	-	+	-	-	-	-
Glycosides	-	-	-	+	+	-

+ Presence - Absence

IV. RESULT AND DISCUSSION

The orchid's grownup in the diverse areas of the wild sanctuaries in epiphytic and terrestrial forms collected during the field visits are grown in greenhouse as well it was micropropagated and reintroduced. The orchids of endemic and endangered situations of both epiphytic and terrestrial forms in Kanyakumari

District was collected from the hill locks, rock cervices and grasslands their morphological characteristics, flowering and fruiting seasons are also noted.

The terrestrial orchid *Geodorum densiflorum* (Lam) Schltr. seed inoculated in the supplemented MS media and 15% of coconut water showed callus induction percentage in different concentration of BAP (1), BAP (2) and BAP (5) supplemented media and 15% of coconut water inoculated in *Geodorum densiflorum* (Lam) Schltr. The seed showed minimum percentage of callus induction in BAP (1) of 3.37±0.74% to BAP (5) of 3.78±0.37%; KIN (1), KIN (2) and KIN (5) in the supplemented media and 15% of coconut water inoculated in *Geodorum densiflorum* (Lam) Schltr. seed varied from the minimum percentage of callus induction in KIN (2) of 2.92±0.48% to KIN (5) of 4.38±0.21% and NAA (1), NAA (2) and NAA (5) in the supplemented media and 15% of coconut water in inoculated *Geodorum densiflorum* (Lam) Schltr. seed varied from the minimum percentage of callus induction in NAA (2) of 3.45±0.24% to NAA (5) of 4.68±1.48%.

The evaluation of morphogenetic effect of inoculated *Geodorum densiflorum* (Lam) Schltr. seed supplemented with MS media and 15% of coconut water showed morphogenesis of seed in different concentration of BAP (1), BAP (2) and BAP (5), KIN (1), KIN (2) and KIN (5) and NAA (1), NAA (2) and NAA (5) showed no response in control and KIN (5); small quantity in BAP (2), KIN (1) and NAA (2); moderate quantity in BAP (1), BAP (5), KIN (2), NAA (1) and NAA (5).

The effect of sub-culture on growth regulators (PGRs) showed the development of protocorm like bodies (PLBs) from immature seed callus of *Geodorum densiflorum* (Lam) Schltr. on MS media supplemented with 15% coconut water showed no response in the formation of protocorm like bodies (PLBs) in the media supplemented with Control, BAP (5) +NAA (5), BAP (5)+KIN(5) and BAP (2) +KIN (1). Among the tested BAP +NAA the number of PLBs varied from BAP (2) +NAA (2) 4.53±0.65 to BAP (5) +NAA (2) 5.37±0.74 and in BAP+KIN the number of PLBs varied from 4.68±1.48 to 13.93±0.64 PLBs.

The response of seed influence of sub-culture on growth regulators (PGRs) showed the development of protocorm like bodies (PLBs) from immature seed callus of *Geodorum densiflorum* (Lam) Schltr. on MS media supplemented with 15% coconut water and their response of seed explant showed Healthy and Green PLBs formed in BAP (5)+NAA(2) and BAP (1)+KIN(1); Few Green PLBs formed in the media supplemented with BAP (2)+NAA(2), BAP (2)+NAA(1), BAP (1)+NAA(1), BAP (5)+KIN(2) and BAP (2)+KIN(2).

The effect of Activated Charcoal (AC), Polyvinylpyrrolidone (PVP) and Gibberlic acid (GA3) provided individually or in combinations in MS on the elongated shoots of *Geodorum densiflorum* (Lam) Schltr. compared after 60 days of transfer of control showed an average initial height 0.72±0.01 cm; AC (0.1%) + GA (0.5µM), AC (0.1%) + GA (1.0 µM), AC (0.1%) + GA (1.5 µM) showed a minimum average initial height of GA (0.5 µM) of 0.61±0.01 cm and maximum height of 1.06±0.05 cm. GA (0.5, 1.0, 1.5 and 2.0 µM) and AC (0.1%) + GA (2.0µM) showed minimum average initial height of AC (0.1%) + GA (1.5 µM) of 0.60±0.02cm and maximum height of AC (0.1%) + GA (0.5µM) & AC (0.1%) + GA (2.0µM) of 0.64±0.02cm. The minimum average initial height of GA (0.5 µM) of 0.61±0.01 cm and PVP (0.1 µM) + GA (0.5µM), PVP (0.1 µM) + GA (1.0µM), PVP (0.1 µM) + GA (1.5µM) and PVP (0.1 µM) + GA (2.0µM) showed minimum average initial height of PVP (0.1 µM) + GA (1.5µM) of 0.52±0.02cm and maximum height of PVP (0.1 µM) + GA (2.0µM) of 0.79±0.03cm.

The evaluation on the effect of Activated Charcoal (AC), Polyvinylpyrrolidone (PVP) and Gibberlic acid (GA3) provided individually or in combinations in MS on the elongated shoots of *Geodorum densiflorum* (Lam) Schltr. of control showed 1.40±0.05cm. GA (0.5 µM), GA (1.0 µM), GA (1.5 µM) and GA (2.0 µM) showed minimum response of GA (0.5 µM) of 0.75±0.03cm to GA (1.5 µM) 3.20±0.10 cm, AC (0.5%), AC (0.1%) + GA (0.5µM), AC (0.1%) + GA (1.0 µM), AC (0.1%) + GA (1.5 µM) and AC (0.1%) + GA (2.0µM) showed minimum response of AC (0.1%) + GA (0.5µM) 0.90±0.05 cm to maximum response of AC (0.1%) + GA (1.0 µM) 4.00±0.08cm; PVP (0.5 µM); PVP(0.1 µM) + GA (0.5µM); PVP (0.1 µM) + GA (1.0µM); PVP (0.1 µM) + GA (1.5µM) and PVP (0.1 µM) + GA (2.0µM) showed minimum response of PVP (0.5 µM) 0.78±0.05cm to maximum response of PVP (0.1 µM) + GA (0.5µM) 1.20±0.08cm.

The assessment of Activated Charcoal (AC), Polyvinylpyrrolidone (PVP) and Gibberlic acid (GA3) provided individually or in combinations in MS media on the elongated shoots of *Geodorum densiflorum* (Lam) Schltr. compared after 60 days of transfer of control showed 106.02cm. GA (0.5 µM), GA (1.0 µM), GA (1.5 µM) and GA (2.0 µM) showed minimum response of GA (0.5 µM) of 41.30cm and maximum response of GA (1.5 µM) of 328.46cm; AC (0.5%), AC (0.1%) + GA (0.5µM), AC (0.1%) + GA (1.0 µM), AC (0.1%) + GA (1.5 µM) and AC (0.1%) + GA (2.0µM) showed minimum response of AC (0.1%) + GA (0.5µM) of 82.61cm and maximum response of AC (0.1%) + GA (2.0µM) of 183.85cm; PVP (0.5 µM), PVP(0.1 µM) + GA (0.5µM), PVP (0.1 µM) + GA (1.0µM), PVP (0.1 µM) + GA (1.5µM) and PVP (0.1 µM) + GA (2.0µM) showed minimum response of PVP (0.1 µM) + GA (2.0µM) of 37.98 cm and maximum response of PVP (0.1 µM) + GA (0.5µM) of 165.50cm.

The evaluation of Control, BAP (0.5), BAP (1.0), BAP (1.5) and BAP (2.0) showed minimum culture responding of BAP (0.5) of 53.33% and maximum response of BAP (2.0) of 73.33%; KN (0.5), KN (1.0), KN (1.5) and KN (2.0) showed minimum response of KN (0.5) & KN (2.0) of 60.00% and maximum response of KN (1.0) 73.33% and BM+TDZ (0.5), BM+TDZ (1.0), BM+TDZ (1.5) and BM+TDZ (2.0) showed minimum response of BM+TDZ (1.5) & BM+TDZ (2.0) of 86.67% and maximum response of BM+TDZ (0.5) of 93.33%.

The average number of shoot/culture in control showed the response of 0.80±0.16, BAP (0.5), BAP (1.0), BAP (1.5) and BAP (2.0) showed minimum response of BAP (0.5) of 1.22±0.41 and maximum response of BAP (2.0) of 3.18±0.59; KN (0.5), KN (1.0), KN (1.5) and KN (2.0) showed minimum response of KN (2.0) of 1.20±0.30 and maximum response of KN (1.0) of 2.87±0.56; MS+TDZ (0.5), MS+TDZ (1.0), MS+TDZ (1.5) and MS+TDZ (2.0) showed minimum response of MS+TDZ (1.0) of 1.53±0.34 and maximum response of MS+TDZ (1.5) of 3.58±0.52.

The average length of shoots (cm) X±SE showed the effect of cytokinins supplemented with BAP and KN individually to MS and MS+TDZ at varying concentration on the differentiation and development of shoots from *in-vitro* developed PLBs of *Geodorum densiflorum* (Lam) Schltr. compared after 60 days of culture of control showed 3.30±0.25cm, BAP (0.5), BAP (1.0), BAP (1.5) and BAP (2.0) showed minimum response of BAP (2.0) 1.50±0.12cm and maximum response of BAP (0.5) 3.50±0.38cm; KN (0.5), KN (1.0), KN (1.5) and KN (2.0) showed minimum response of KN (1.0) 1.20±0.12cm and maximum response of KN (2.0) of 2.90±0.22cm; MS+TDZ (0.5), MS+TDZ (1.0), MS+TDZ (1.5) and MS+TDZ (2.0) showed minimum response of MS+TDZ (2.0) of 0.60±0.05cm and maximum response of MS+TDZ (1.0) 2.00±0.17cm.

The assessment on the effect of auxin supplementation in half-strength MS medium of *in-vitro* regenerated roots/responding shoots of *Geodorum densiflorum* (Lam) Schltr. supplemented with IBA (0.4), (0.8), (1.2) and (1.5) varied from IBA (0.8) of 1.50±0.07cm to 2.00±0.06cm; IAA(0.4), (0.8), (1.2) and (1.5) varied from IAA of 2.07±0.07cm to 4.29±0.15cm. The effect of auxin supplementation in half-strength MS medium of *in-vitro* regenerated roots/responding shoots of *Geodorum densiflorum* (Lam) Schltr. showed the average length of roots in IBA (0.4), (0.8), (1.2) and (1.5) showed minimum average length of roots of IBA (0.8) of 0.60±0.1cm to maximum of 4.32±0.53cm; IAA (0.4), (0.8), (1.2) and (1.5) showed minimum average length of roots IAA (0.8) of 0.65±0.10cm and maximum average length of roots IAA (0.4) of 1.96±0.29cm.

The survival of *in-vitro* plantlets grown in the substrate provided with sterilized forest leaves, charcoal and brick pieces was noticed. The number of plant transferred is 32 and the number of plant survived is 19. The hardened plantlets grown in autoclaved charcoal, forest tree barks and brick pieces of the transferred *in-vitro* plantlets of 25 and the number of plant survived was noticed as 15. The survived plants were reintroduced in the natural habitat.

The morphogenetic response of immature *Acampe praemorsa* seeds showed no response of control, small quantity of morphogenetic response in MS media + CW 15% and the response of moderate quantity in MS media + CW 10% and MS media + CW 20% and the percentage of callus induction showed no response in control. MS media + CW showed minimum response of 59±0.81% in MS media + CW 10% and maximum response of 61.4±0.43% in MS media + CW 15%.

The number of days taken for morphogenesis of callus formation in control showed no response and the supplemented MS media + CW showed growth response in a short duration of 33 days in MS media + CW 10% and long duration of 37 days in MS media + CW 20%. The PGR Treatment (mgL^{-1}) of the control and BAP (5) + NAA (5) showed no response of PLBs formation on the other hand, minimum PLBs formation of BAP (5) + NAA (2) of 1.3±0.32 PLBs and maximum PLBs of BAP (1) + NAA (1) of 15.93±0.64. The plant growth regulators showed minimum response of 3.1±0.31 in BAP (5) + KIN (5) and maximum response of 9.3±2.72 in BAP (2) + KIN (2).

The plant growth regulators supplemented with KIN+BAP showed the minimum response of 1.00±0.14 in 1.0KIN+5.0BAP and maximum response of 3.71±0.24 in 1.0KIN+0.5 BAP and the shoot length of treated plants showed minimum response in 1.0KIN+5.0BAP of 1.22±0.12cm and maximum response in 2.0KIN+0.5BAP of 5.66±0.07cm. The responding root treated with IBA showed minimum number of roots in IBA (0.1) of 1.50±0.07% and maximum roots of IBA (0.1) of 1.83±0.08%. Average length of roots (cm) showed a minimum response of IAA (0.1) of 1.42± 0.53cm and maximum response of IAA (0.1) of 1.85± 0.69cm. The plantlets were acclimatized in growth room after two weeks of hardening, their response was recorded and the rooted plants were successfully established in their habitat.

The *in- vitro* qualitative analysis of *Geodorum densiflorum* (Lam) Schltr. **leaf extracts** revealed the presence of Terpenoid, Flavonoid, Tannin, Aminoacid, Glycosides, Steroid in **Control** and absence of Reducing Sugar, Phenol, Alkaloid and Saponin. **Ethanol extracts** showed the presence of Flavonoid, Phenol, Alkaloid, Tannin, Saponin, Steroid and absence of Terpenoid, Reducing Sugar, Aminoacid and Glycosides; **Methanol extracts** showed the presence of Reducing Sugar, Phenol, Saponin, Alkaloid, Tannin and absence of Terpenoid, Flavonoid, Steroid, Aminoacid and Glycosides; **Chloroform extracts** showed the presence of Terpenoid, Flavonoid Saponin, Tannin, Aminoacid, Steroid and absence of Reducing Sugar, Phenol and Glycosides; **Acetone extracts** showed the presence of Flavonoid, Saponin, Phenol, Alkaloid, Tannin, Steroid and absence of Terpenoid, Reducing Sugar, Aminoacid and Glycosides and **Aqueous extracts** showed the presence of Terpenoid, Phenol Alkaloid, Saponin, Steroid, Tannin and absence of Flavonoid, Reducing Sugar, Aminoacid and Glycosides.

Qualitative Analysis of *Geodorum densiflorum* (Lam) Schltr. **Root extracts** showed the presence of Terpenoid, Flavonoid, Reducing Sugar, Alkaloid, Steroid, Aminoacid in **Control** and absence of Phenol Saponin, Tannin, and Glycosides. **Ethanol extracts** showed the presence of Terpenoid, Flavonoid, Tannin, Alkaloid, Aminoacid, Steroid and absence of Phenol, Reducing Sugar, Saponin and Glycosides; **Methanol extracts** showed the presence of Terpenoid, Tannin, Steroid and absence of Phenol, Alkaloid, Reducing Sugar, Flavonoid, Saponin Aminoacid and Glycosides. **Chloroform extracts** showed the presence of Reducing Sugar, Tannin, Steroid, Alkaloid and Glycoside and absence of Terpenoid, Flavonoid, Phenol, Saponin and Aminoacid; **Acetone extracts** showed the presence of Terpenoid, Alkaloid, Phenol, Tannin, Steroid and absence of Flavonoid, Reducing Sugar, Saponin, Aminoacid and Glycosides **and Aqueous extracts** showed the presence of Terpenoid, Phenol, Saponin, Aminoacid and absence of Flavonoid, Reducing Sugar, Alkaloid, Steroid, Tannin and Glycosides.

In-vitro analysis of *Acampe praemorsa* **leaf** extract showed the presence of terpenoid, flavonoid, steroid aminoacid and glycoside and absence of reducing sugar, phenol, saponin, alkaloid, tannin. **Ethanol** extract of *Acampe praemorsa* leaf showed the presence of flavonoid, alkaloid, saponin, phenol, tannin and absence of terpenoid, steroid reducing sugar, aminoacid and glycoside. **Methanol** extract of *Acampe praemorsa* leaf showed the presence of terpenoid, flavonoid, steroid and absence of reducing sugar, phenol, alkaloid, tannin, saponin, aminoacid and glycoside. **Chloroform** extract of *Acampe praemorsa* leaf showed the presence of terpenoid, flavonoid, tannin, steroid reducing sugar and absence of saponin, phenol, alkaloid aminoacid and glycoside. **Acetone** extract of *Acampe praemorsa* leaf showed the presence of terpenoid, tannin, steroid, saponin, glycoside, aminoacid and absence of flavonoid, alkaloid, reducing sugar, phenol and **aqueous** extract of *Acampe praemorsa* leaf showed the presence of terpenoid, alkaloid reducing sugar, phenol, saponin, steroid, aminoacid and absence of flavonoid, tannin and glycoside.

Phytochemical analysis of *in-vitro Acampe praemorsa* (Roxb.) **root** extract showed the presence of terpenoid, reducing sugar, flavonoid, phenol, alkaloid, saponin and absence of tannin, steroid aminoacid and glycoside. **Ethanolic** extract of *Acampe praemorsa* (Roxb.) root showed the presence of terpenoid, flavonoid, reducing sugar, alkaloid, aminoacid and absence of phenol, saponin, tannin, steroid and glycoside. **Methanolic** extract of *in-vitro Acampe praemorsa* (Roxb.) root showed the presence of

terpenoid, tannin, steroid and absence of phenol, alkaloid flavonoid, reducing sugar, saponin, aminoacid and glycoside. **Chloroform** extract of *Acampe praemorsa* (Roxb.) root showed the presence of terpenoid, phenol, saponin, alkaloid, tannin, steroid, glycoside and absence of flavonoid, reducing sugar and aminoacid. **Acetone** extract of *Acampe praemorsa* (Roxb.) root showed the presence of terpenoid, flavonoid, phenol, saponin, steroid, glycoside and absence of reducing sugar, tannin, alkaloid and aminoacid. **Aqueous** extract of *Acampe praemorsa* (Roxb.) root showed the presence of terpenoid, steroid and absence of alkaloid, tannin, flavonoid, reducing sugar, phenol, saponin, glycoside and aminoacid.

The hydroxyl radical scavenging of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf extract varied from the minimum inhibition of 64.21 % ±0.021 (25µl) to 71.48 % ±0.028 (100µl). **Aqueous extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf varied from the minimum inhibition of 55.27 % ±0.016 (25µl) to 53.21 % ±0.008 (100µl). **Ethanol extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf varied from the minimum inhibition of 42.26 % ±0.008 (25µl) to 47.28 % ±0.016 (100µl). **Methanol extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf varied from the minimum inhibition of 63.51 % ±0.021 (25µl) to 67.73 % ±0.020 (100µl). **Acetone extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf varied from the minimum inhibition of 57.14 % ±0.012 (25µl) to 65.51 % ±0.004 (100µl). **Chloroform extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf varied from the minimum inhibition of 68.64 % ±0.012 (25µl) to 73.68 % ±0.012 (100µl). Antioxidant potential of the standard antioxidant **Gallic acid** varied from the minimum inhibition of 81.08 % ±0.009 (25µl) to 89.96 % ±0.008 (100µl).

Hydroxyl radical scavenging of *in-vitro Geodorum densiflorum* (Lam) Schltr. root extract varied from the minimum inhibition of 71.27 % ±0.024 (25µl) to 77.09%±0.021 (100µl). **Aqueous extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr. root extract varied from the minimum inhibition of 62.48% ±0.008 (25µl) to 67.48% ±0.035 (100µl). **Ethanol extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr. root varied from the minimum inhibition of 59.26% ±0.020 (25µl) to 64.68 %±0.032 (100µl). **Methanol extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr. root varied from the minimum inhibition of 51.10% ±0.020 (25µl) to 56.95% ±0.004 (100µl). **Acetone extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr. root varied from the minimum inhibition of 55.24% ±0.012 (25µl) to 57.73% ±0.004 (100µl). **Chloroform extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr. root varied from the minimum inhibition of 37.51 % ±0.026 % (25µl) to 41.81% ±0.008 (100µl). Antioxidant potential of the standard antioxidant **Gallic acid** varied from the minimum inhibition of 79.91% ±0.004 (25µl) to 83.96% ±0.030 (100µl).

Hydroxyl radical scavenging of *in-vitro Acampe praemorsa* (Roxb.) leaf extract varied from the minimum inhibition of 69.81% ±0.118 (25µl) to 75.35% ±0.009 (100µl). **Aqueous extract** of *in-vitro Acampe praemorsa* (Roxb.) leaf varied from the minimum inhibition of 51.03% ±0.016 (25µl) to 56.63% ±0.012 (100µl). **Ethanol extract** of *in-vitro Acampe praemorsa* (Roxb.) leaf varied from the minimum inhibition of 47.02% ±0.004 (25µl) to 49.81% ±0.065 (100µl). **Methanol extract** of *in-vitro Acampe praemorsa* (Roxb.) leaf varied from the minimum inhibition of 51.17% ±0.052 (25µl) to 57.03% ±0.052 (100µl). **Acetone extract** of *in-vitro Acampe praemorsa* (Roxb.) leaf varied from the minimum inhibition of 65.50% ±0.004 (25µl) to 69.11% ±0.004 (100µl). **Chloroform extract** of *in-vitro Acampe praemorsa* (Roxb.) leaf varied from the minimum inhibition of 57.68 % ±0.049 (25µl) to 61.71% ±0.024 (100µl). Antioxidant potential of the standard antioxidant **Gallic acid** varied from the minimum inhibition of 83.21% ±0.024 (25µl) to 89.35% ±0.043 (100µl).

Hydroxyl radical scavenging of *in-vitro Acampe praemorsa* (Roxb.) root extract varied from the minimum inhibition of 72.39 % ±0.065 (25µl) to 78.98±0.055 % (100µl). **Aqueous extract** of *in-vitro Acampe praemorsa* (Roxb.) root varied from the minimum inhibition of 56.23 % ±0.016 (25µl) to 61.26 % ±0.021 (100µl). **Ethanol extract** of *in-vitro Acampe praemorsa* (Roxb.) root varied from the minimum inhibition of 61.53% ±0.032 (25µl) to 66.75 % ±0.030 (100µl). **Methanol extract** of *in-vitro Acampe praemorsa* (Roxb.) root varied from the minimum inhibition of 55.26 % ±0.014 (25µl) to 59.42% ±0.029 (100µl). **Acetone extract** of *in-vitro Acampe praemorsa* (Roxb.) root varied from the minimum inhibition of 69.53% ±0.082 (25µl) to 73.15% ±0.012 (100µl). **Chloroform extract** of *in-vitro Acampe praemorsa* (Roxb.) root varied from the minimum inhibition of 58.87% ±0.033 (25µl) to 63.91% ±0.041 (100µl). Antioxidant potential of the standard antioxidant **Gallic acid** varied from the minimum inhibition of 79.84 % ±0.004 (25µl) to 83.33 % ±0.016 (100µl).

DPPH scavenging activity of *in-vitro Geodorum densiflorum* (Lam) Schltr. leaf extract varied from the minimum inhibition of 71.89%±0.021 (25µl) to 76.08%±0.035 (100µl). **Aqueous extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf varied from the minimum inhibition of 63.25%±0.096 (25µl) to 67.92%±0.231 (100µl). **Ethanol extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf varied from 78.96%±0.021 (25µl) to 82.82%±0.041 (100µl). **Methanol extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf varied from 68.26% ±0.049 (25µl) to 74.81% ±0.028 (100µl). **Acetone extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf varied from 52.98%±0.028 (25µl) to 58.98% ±0.028 (100µl). **Chloroform extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr leaf varied from 59.41%±0.024 (25µl) to 63.62%±0.016 (100µl). Antioxidant potential of the standard antioxidant **Gallic acid** varied from 77.89%±0.061 (25µl) to 85.19%±0.021 (100µl).

DPPH scavenging activity of *in-vitro Geodorum densiflorum* (Lam) Schltr root extract varied from the minimum inhibition of 29.51% ±0.020 (25µl) to 34.43%±0.024 (100µl). **Aqueous extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr root varied from the minimum inhibition of 55.77%±0.136 (25µl) to 58.82%±0.028 (100µl). **Ethanol extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr root varied from the minimum inhibition of 58.40% ±0.291 (25µl) to 61.51% ±0.020 (100µl). **Methanol** extract of *in-vitro Geodorum densiflorum* (Lam) Schltr root varied from the minimum inhibition of 63.57%±0.043 (25µl) to 68.82%±0.016 (100µl). **Acetone extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr root varied from the minimum inhibition of 78.48% ±0.035 (25µl) to 81.61% ±0.035 (100µl). **Chloroform extract** of *in-vitro Geodorum densiflorum* (Lam) Schltr root varied from the minimum inhibition of 78.73%±0.148 (25µl) to 82.43% ±0.134 (100µl). Antioxidant potential of the standard antioxidant **Gallic acid** varied from the minimum inhibition of 87.98% ±0.038 (25µl) to 95.96% ±0.029 (100µl).

The evaluation of DPPH scavenging activity of *in-vitro Acampe praemorsa* (Roxb.) leaf extract varied from the minimum inhibition of 74.36%±0.016 (25µl) to 79.87%±0.016 (100µl). **Aqueous extract** of *in-vitro Acampe praemorsa* (Roxb.) leaf varied from the minimum inhibition of 64.96%±0.018 (25µl) to 69.89%±0.016 (100µl). **Ethanol extract** of *in-vitro Acampe praemorsa* (Roxb.) leaf varied from 78.45%±0.008 (25µl) to 83.50%±0.036 (100µl). **Methanol extract** of *in-vitro Acampe praemorsa* (Roxb.) leaf varied from 72.17%±0.008 (25µl) to 75.54%±0.020 (100µl). **Acetone extract** of *in-vitro Acampe praemorsa* (Roxb.) leaf varied from the minimum inhibition of 61.48%±0.012 (25µl) to 65.68%±0.004 (100µl). **Chloroform extract** of *in-vitro Acampe praemorsa* (Roxb.) leaf varied from 55.49%±0.012 (25µl) to 62.96% ±0.051 (100µl). Antioxidant potential of the standard antioxidant **Gallic acid** varied from 72.44%±0.016 (25µl) to 78.68%±0.016 (100µl).

The assessment of DPPH scavenging activity of *in-vitro Acampe praemorsa* (Roxb.) root extract varied from the minimum inhibition of 74.62%±0.016 (25µl) to 79.19%±0.020 (100µl). **Aqueous extract** of *in-vitro Acampe praemorsa* (Roxb.) root varied from the minimum inhibition of 66.01% ±0.029 (25µl) to 68.83%±0.028 (100µl). **Ethanol extract** of *in-vitro Acampe praemorsa* (Roxb.) root varied from the minimum inhibition of 65.51%±0.053 (25µl) to 69.53% ±0.029 (100µl). **Methanol extract** of *in-vitro Acampe praemorsa* (Roxb.) root varied from the minimum inhibition of 50.61% ±0.136 (25µl) to 54.68% ±0.103 (100µl). **Acetone extract** of *in-vitro Acampe praemorsa* (Roxb.) root varied from the minimum inhibition of 64.31% ±0.016 (25µl) to 71.28% ±0.035 (100µl). **Chloroform extract** of *in-vitro Acampe praemorsa* (Roxb.) root varied from the minimum inhibition of 57.68%±0.020 (25µl) to 61.79% ±0.035 (100µl). Antioxidant potential of the standard antioxidant **Gallic acid** varied from the minimum inhibition of 86.14% ±0.021 (25µl) to 91.45%±0.132 (100µl)

Extensive research is still necessary to be able to fully recommend the orchid species for their medicinal uses. Micropropagation of rare and endangered orchids in large scale has to be developed further because of their commercial and medicinal values. A proper ratio of auxins and cytokinins is required for optimal protocorm multiplication though the efficiency of type and concentration of plant growth regulators varied with the varieties. The present study describes an efficient protocol for mass multiplication of a medicinal orchid *Geodorum densiflorum* (Lam) Schltr. and *Acampe praemorsa* (Roxb.). The application of this protocol could minimize the stress on its population in the wild by meeting the demand of pharmaceutical industries through micropropagation. The regenerated plants could be relocated to the sites where the population of the micropropagated species has dwindled.