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MICROPROPAGATION, PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF A WILD EPIPHYTIC ORCHID *ACAMPE PRAEMORSA (ROXB)* OF KANYAKUMARI DISTRICT, INDIA

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ABSTRACT

Orchids are most abundant in the forest of Western Ghats of India have been threatened in their natural habitat due to habit dilapidation and anthropogenic activities. The scarcity of pollinators and poor seed setting are the major constraints in the natural propagation, leading to a continuous depletion of its natural population. Propagation from seeds is held back by low germination and survival rates due to the inept environmental conditions as a result their wild populations are diminishing at an alarming rate. The large-scale production of *Acampe praemorsa* (Roxb) requires efficient *invitro* propagation techniques to avoid overexploitation of natural populations. The immature embryos were inoculated on MS media, along with and devoid of different growth additives. The culture seeds showed positive germination response in the nutrient media but the frequency and onset of germination response and associated morphogenetic changes leading to seedling development varied with the nature of growth stimulus. In the basal MS medium 43.75±0.75% seeds were germinated (control). MS media supplemented with coconut water (CW) (15%), supported highest germination (70.75±0.75%) induced protocorm multiplication and complete seedlings were obtained in 131.50±1.73 days. Additional presence of activated charcoal (AC) (0.2%) in the MS medium inhibited the seed germination, while use of coconut water (15%) or (2 g/L) in the medium, favoured enhance early germination response and differentiation of protocorms.

KEYWORDS: *Acampe praemorsa*, phytochemicals, antioxidant, micropropagation.

INTRODUCTION

Nature has been bestowed with large number of diverse types of plants that posses therapeutic properties. Orchidaceae is one of the largest flowering plant families with cosmopolitan in distribution. Orchids are profuse particularly in the humid tropics and sub-tropics so far 17,000 species have been known in the world and about 1,500 species in India. In peninsular India there are about 200 species in 60 genera and about 80 species in 29 genera in Kanyakumari District among these 22 species are found to be endemic, i.e. only confined to peninsular India. Orchid seeds are unique in being exceedingly small, dust like in appearance, and more or less fusiform in shape; these lack endosperm and have undifferentiated embryos enclosed within transparent seed coats. Orchidaceae are widely used either directly as folk remedies or indirectly in the preparation of modern pharmaceuticals. 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).

MATERIALS AND METHODS

The epiphytic orchids *Acampe praemorsa* (Roxb) was observed from the teak (*Tectona grandis*) plantation, at an altitude of about 500 to 1500 feet of Kanyakumari District, the southernmost end of the peninsular India lies between 8º-20º north of the equator and between 70º-85º in longitude**.** Photographs of the vegetative and reproductive (inflorescence) parts were compared with the description published in orchids of Nilgiris (Joseph, 1987).

Description

Acampe praemorsa (Roxb) is an epiphytic wild orchid **(Plate-1)**. Robust plant with stout stem, 16 cm long, covered by sheathing bases of leaves, with persisting old inflorescence axis and long stout aerial roots among the leaves. Leaves alternate distichous, large and coriaceous, 8-17cm oblong, unequally deeply cleft at apex. Single

branch possess 4 leaves, first leaf 22-2.5cm, second leaf 28-2.5cm, third leaf 18-2.5cm and fourth leaf 18-2.2cm. Inflorescence short, erect, corymbose panicles, 6-10 cm long, leaf opposed, peduncle stout with several copular sheathing bracts. Flowers dense, not wide opening, yellow, midly sweet scented. Bracts 3.0 x 3.5 mm, broadly ovate, obtuse, persistent ovary with pedicel 13 mm long, perianths fleshy with horizontal dark purplish streaks. Dorsal sepals 13.5x7.5 mm, obvovate-oblong and obtuse with a mucro. Lateral petals 7.5x3.0 mm, oblanceolate-spathulate and obtuse. Lip 8.7 mm long, fleshy, trilobed, saccate at base; sidelobes small, narrow, erect, thick; midlobe 6.5x4 mm, ligulate, ovate-oblong, more or less reflexed, obtuse, fleshy, irregularly crenulate at margins, tuberculate on the upper surface; base saccate, long slender, papillose within. Column short, stout, 2 mm long with two small terminal horns, on each side. Fruits sub-sessile, erect, more or less in cluster, sub-cylindric, longitudinally ribbed; young fruits 7 cm long (**Plate-2**).

Plate-1 *Acampe praemorsa* (Roxb) - Habit

Plate-2 *Acampe praemorsa* (Roxb) – Flower

Phytochemical Analysis and antioxidation assay

The phytochemical analysis of *A. praemorsa* (Roxb), aqueous, silver nitrate and ethanol extracts were carried out to analyse the presence of alkaloid, flavanoid, phenol, terpenoid, saponin, reducing sugar, tannin, steroid and glycosides (Harbone, 1976). Antioxidation assay of the *A. praemorsa* (Roxb) and extracts were done for hydroxyl, DPPH and reducing power activity (Nabavi *et al*., 2008 & Olabinri *et al*., 2010).

Micropropagation

The green and undehisced capsules, harvested from subsist plants served as a source for young seeds with immature embryos were thoroughly washed under running tap water for 15 to 20 min and surface sterilized for 7 min with $HgCl_2$ solution (0.1%), again with 1 to 2 drops of 'teepol' as a wetting agent prior to washing with sterilized distilled water were also treated with streptomycin (0.03%) for 5 min and repeatedly washed with sterilized double distilled water to remove the traces of sterilizing agents. Subsequently, these capsules were dipped in 70% ethyl alcohol for 30 s, flame sterilized were split open longitudinally with a sterilized blade to scuff out the immature embryos, under aseptic conditions. The effect of MS media tested on *in vitro* seed germination and subsequent seedling development in *A. praemorsa* (Roxb) and effect of different growth additives (activated charcoal; 0.2%) and CW (coconut water; 15%) was also assessed during the experimentation. The seeds were inoculated on MS media in cultures vessels incubated at 25±2°C under 12 h photoperiod provided by cool white fluorescent tubes (40 µmol m^{-2} s^{-1}). Eight replicates were used for each treatment. The cultures were examined regularly observations such as germination frequency and number of days taken for the onset of germination, protocorm formation, emergence of leaf as well as root primordia and seedling development were recorded. Sub-culturing was done at four week intervals.

RESULTS AND DISCUSSIONS

Phytochemical Analysis

Phytochemical analysis of *Acampe praemorsa* (Roxb) showed the presence of alkaloid, flavanoid, phenol, terpenoid and steroid constituents. On the other hand, aqueous extract revealed the presence of saponin, phenol, terpenoid, tannin and glycoside. Meanwhile, ethanol extract revealed the presence of flavanoid, phenol, tannin and steroid. However, silver nitrate assorted in the plant extract revealed the presence of terpenoid, tannin and steroid constituents (Table: 1).

Table: 1 Qualitative Analysis of *Acampe praemorsa* (Roxb) and extracts

S.No	Phytochemicals	*Acampe praemorsa*	Aqueous	Ethanol	Silver nitrate
1	Alkaloid	+	-	-	-
2	Flavanoid	+	-	+	-
3	Saponin	-	+	-	-
4	Phenol	+	+	+	-
5	Terpenoid	+	+	-	+
6	Reducing Sugar	-	-	-	-
7	Tannin	-	+	+	+
8	Steroid	+	-	+	+
9	Glycoside	-	+	-	-

+ Presence - Absence

Antioxidation Assay

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of *Acampe praemorsa* (Roxb) varied from the minimum inhibition of 59.27 ± 0.010 % (25µl) to the maximum inhibition of 61.93 ± 0.010 % (100µl). On the other hand, aqueous extract varied from the minimum inhibition of 57.00 ± 0.010 % (25µl) to the maximum inhibition of 69.99 ± 0.010 % (100µl). Meanwhile, ethanol extract varied from the minimum inhibition of 45.95 ± 0.010 % (25µl) to the maximum inhibition of 55.55 ± 0.010 % (100µl). However, silver nitrate extract varied from the minimum inhibition of 54.21 ± 0.011 % (25µl) to the maximum inhibition of 56.01 ± 0.011 % (100µl). Antioxidant potential of the standard antioxidant Gallic acid varied from the minimum inhibition of 57.39 ± 0.020 % (25µl) to the maximum inhibition of 64.73 ± 0.020 % (100µl) (Table: 2).

Table: 2 Hydroxyl Radical Scavenging activity of *Acampe praemorsa* (Roxb) and Extracts

Concentration of medicine and extracts	*Acampe praemorsa*	Aqueous Extract	Ethanol Extract	Silver nitrate (Nanoparticle)	Gallic acid (Standard)
25µl	59.27 ± 0.010	57.00 ±0.010	45.95 ±0.010	54.21 ±0.011	57.39 ±0.020
50µl	59.36 ±0.020	59.21 ±0.005	47.29 ±0.020	55.00 ±0.010	59.25 ±0.000
75µl	61.27 ±0.020	63.61 ±0.005	49.98 ±0.005	55.06 ±0.010	61.42 ±0.005
100µl	61.93 ±0.010	69.99 ±0.010	55.55 ±0.010	56.01 ±0.011	64.73 ±0.020

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of *Acampe praemorsa* (Roxb) varied from the minimum inhibition of 60.37 ± 0.011 % (25µl) to the maximum inhibition of 69.74 ± 0.010 % (100µl). On the other hand, aqueous extract varied from the minimum inhibition of 56.00 ± 0.005 % (25µl) to the maximum inhibition of 58.83 ± 0.011 % (100µl). Meanwhile, ethanol extract varied from the minimum inhibition of 51.01 ± 0.015 % (25µl) to the maximum inhibition of 54.93 ± 0.010 % (100µl). However, silver nitrate extract varied from the minimum inhibition of 55.55 ± 0.011 % (25µl) to the maximum inhibition of 55.93 ± 0.020 % (100µl). Antioxidant potential of the standard antioxidant Gallic acid varied from the minimum inhibition of 52.07 ± 0.011 % (25µl) to the maximum inhibition of 69.71 ± 0.000 % (100µl) (Table: 3).

Table: 3 DPPH Radical Scavenging activity of *Acampe praemorsa* (Roxb) and Extracts

Concentration of medicine and extracts	*Acampe praemorsa*	Aqueous Extract	Ethanol Extract	Silver nitrate (Nanoparticle)	Gallic acid (Standard)
25µl	60.37 ± 0.011	56.00 ±0.005	51.01 ±0.015	55.55 ±0.011	52.07 ±0.011
50µl	63.96 ±0.010	57.21 ±0.010	52.51 ±0.011	55.61 ±0.020	57.10 ±0.010
75µl	67.21 ±0.011	58.37 ±0.000	54.62 ±0.000	55.79 ±0.020	64.63 ±0.020
100µl	69.74 ±0.010	58.83 ±0.011	54.93 ±0.010	55.93 ±0.020	69.71 ±0.000

Micropropagation of *Acampe praemorsa*

The germination entities developed into protocorms which differentiated first leaf and root primordia in 91.50±1.73 and 121.25±2.50 days of inoculation respectively and complete seedlings were obtained in 156.25±2.50 days. Incorporation of Coconut water (15%), supported early and highest germination (70.75±0.75%) and induced protocorm multiplication. The morphogenetic stages leading to seedling development however, advanced in medium containing additives. In the activated charcoal enriched medium, protocorms differentiated first leaf and root primordia

and complete seedlings with 2 to 3 leaves and 1 to 2 roots were obtained within 136.50±1.73 days. Coconut water however, pronounced effect and complete seedlings were obtained in 131.50±1.73 days. MS medium supplemented with coconut water proved to be an optimal combination for seed germination (70.75±0.75%) and seedling development (131.50±1.73 days) in *A. praemorsa* without significant differences from activated charcoal added to MS medium cultures (Plate - 1).

Plate: 1 Micropropagation of *Acampe praemorsa* (Roxb)

Hardening of *Acampe praemorsa* (Roxb)

Tissue culture raised plantlets have to become photoautotrophic from their earlier photo heterotrophic state, following their transfer to *ex vitro*. In addition, they have to become adapted to a lower humidity level and higher irradiance, which impose on them transpiration induced water stress. Therefore, these plantlets show poor survival when they are transferred *ex vitro*. In view of this, tissue culture-raised plantlets are subjected to specific culture regimes aimed at making them capable of surviving the uncontrolled and harsher *ex vitro* environments; this is called *in vitro* hardening or *in vitro* acclimatization. The well-developed seedlings with 2 to 3 leaves and 1 to 2 roots were gradually sub-cultured on hormone free and subsequently on one half and one fourth strength nutrient medium, respectively for 3 months as a part of hardening procedure. These plantlets were then removed from culture vessels and thoroughly washed with lukewarm water to remove adhering medium completely without causing damage to the roots. Subsequently, the seedlings were treated with a mild fungicide (Bavistine; 0.01%) solution and streptomycin (0.03%) for 5 min. These plantlets were then transferred to green house and potted in clay pots with charcoal pieces and brick pieces (1:1) these showed 85% survival rate (Plate: 2).

Plate 2 Hardening of *Acampe praemorsa* (Roxb)

Reintroduction of Hardened Orchid on a wild tree

CONCLUSION

The presence of large number of orchid species in Indian forests are now at the verge of extinction and some of them have become so rare that a large number of botanical teams were unable to trace them. The preliminary phytochemical screening and antioxidant potential of *Acampe praemorsa* insist us to conserve the medicinal orchid from destruction.

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