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CERTIFICATE

This is to certify that the plant materials brought by Ms. Sharone Gladies., Ph. D. Research Scholar, Department of Botany, PSGR Krishnammal College for Women, Avinashi Road, Coimbatore, are identified as *Pholidota imbricata* L. and *Arundina graminifolia* (D. Don) Lindl. (Orchidaceae).

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Publications

DOI : 10.5958/2349-2104.2019.00003.2 Indianjournals.com

In vitro regeneration of *Pholidota pallida* Lindl. (Orchidaceae)

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ABSTRACT

Orchids exhibit a wide range of diversity in forms, size, colour and texture of flowers beyond the imagination of human mind. Orchids grow in nature through seeds but in the absence of appropriate hosts, they do not germinate in adequate number. This obstacle was overcome by adapting tissue culture technique for appropriate germination of orchids. *In vitro* techniques have been successfully carried out for the mass propagation of orchid plantlets. Hence, a preliminary study was carried out to develop a suitable protocol for mass multiplication of *Pholidota pallida.* MS (Murashige and Skoog) medium was found to be suitable for the asymbiotic seed germination of *Pholidota pallida*. MS medium supplemented with IAA (Indole-3-acetic acid), IBA (Indole-3-butyric acid), BAP (6-Benzylaminopurine) and KIN (Kinetin) individually and in combinations induced direct protocorm like bodies. Hormone-free MS basal medium was found suitable for the conversion of PLBs (protocorm-like bodies) into complete plantlets **(Keywords:** *Pholidota pallida,* asymbiotic seed germination, *in vitro* regeneration).

INTRODUCTION

The Orchidaceae is the largest monocotyledonous family with an estimated 25,000 species placed in about 600-800 genera, comprising about 10% of the Angiosperms and with 80,000 hybrids (Dressler, 1981). The orchids are present both in normal and in extreme cold environments and habitats. The flowers of the plants vary in size, shape and colour, due to which they are highly valued ornamentally (Kasulo *et al.*, 2009). India is home to a large number of tropical and temperate orchids. In India, orchids are employed for a variety of therapeutic uses in different systems of traditional medicines. The tubers of orchids are even used as a food source. Orchids are in a symbiotic relationship with a mycorrhizal fungus in order to germinate under natural conditions (Rasmussen *et al.*, 1990). This fungal association provides the seeds with carbohydrates, nutrients, minerals and water (Rasmussen, 1992). Symbiotic germination methods are being replaced by non-symbiotic germination procedures.

The orchid of interest for the present study, *Pholidota pallida* Lindl. occur in northeast India (The Plant List, 2013). It exists both as an epiphyte and as terrestrial orchids under suitable conditions. It has a high medicinal value and is used in Chinese traditional medicine along with other species of *Pholidota* (Teoh, 2016). It is also used in the treatment of headache, fever, abdominal pain, cuts, ulcer, skin rashes and skin diseases (Mohanty *et al.*, 2015; Quattrocchi, 2012). *P. pallida* plant extracts were found to possess antimicrobial activity (Hoque *et al.*, 2016). The plant extracts exhibited antioxidant activity (Nagananda *et al.*, 2014). Prasathkumar and Ramesh (2016) developed a protocol for micropropagation of *P. pallida* using axillary buds from asymbiotically germinated seedlings. In view of the immense medicinal importance, the present study was undertaken to develop a protocol for mass multiplication of *P. pallida.*

MATERIAL AND METHODS

Plant material

The un-dehisced capsules of *Pholidota pallida* Lindl. (Figure 1a), were collected during July-August from Western Ghats, Tamil Nadu. The seeds from the capsules were used for asymbiotic seed germination studies. The freshly collected capsules were washed with the detergent Teepol (0.1%), rinsed in distilled water, surface sterilized with mercuric chloride solution (0.1%) for 3 min and rinsed thrice in distilled water. The capsules were dipped in 80% ethyl alcohol for 1 min, flamed, cut longitudinally and the seeds were inoculated

Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.255

IN VITRO REGENERATION OF *ARUNDINA GRAMINIFOLIA* (D. DON) HOCHR

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(Date of Receiving-12-01-2021; Date of Acceptance-19-03-2021)

ABSTRACT

We can see Orchids come in a wide variety of shapes, sizes, colours, and textures far beyond the human mind's imagination. They emerge from seeds in nature, but in the absence of suitable hosts, they do not germinate in sufficient numbers. This problem was solved by using the tissue culture technique for its germination. One of the successful method used for mass propogation of orchid plantlets is *in vitro* techniques. Therefore, an initial analysis was conducted in order to establish an appropriate procedure for mass multiplication of *Arundina graminifolia*. MS (Murashige and Skoog) medium was found to be suitable for the asymbiotic seed germination of *Arundina graminifolia*. Direct protocorm like bodies were induced by using combinations and individual supplement of MS medium with IAA (Indole-3-acetic acid), IBA (Indole-3- butyric acid), BAP (6-Benzylaminopurine) and KIN (Kinetin). Hormone-free MS basal medium was found suitable for the conversion of PLBs (protocorm-like bodies) into complete plantlets

Keywords: Contaminate, spoilage, fungi, bacteria and surface sterilized

INTRODUCTION

Among angiosperms orchidaceae is one of the largest families. Orchidaceae family includes 800 genera and 25000 species. Orchids are well known for their economic importance and ornamental beauty. *Arundina* comes from Latin word arundo which means reed and graminifolia means grass like leaves. *Arundina graminifolia* is commonly known as bamboo orchid. Bamboo orchid is a terrestrial perennial orchid with erect stem, forming into large clumps growing to a height between 70cm to 2m (Supriya das *et al*; 2013).

Orchids are the most fascinatingly beautiful flowers and unique group of plants of nature. With their exotic shapes, hues and the added advantage of longevity, these flowers of rare beauty have become increasingly popular in the 21st century. They belong to the Orchidaceae family and consisting 600-800 genera and 25,000-35,000 species. Today growing orchid is more than just a hobby; it is an international business covering around 10% of the world floriculture trade. Especially, some of the exquisitely rare hybrids of orchid are among the top ten cut flowers. It has become possible by adopting *in vitro* tissue culture techniques for their rapid multiplication. Since, orchids are strictly out breeders, seed propagation results in unwanted heterozygous types. So, vegetative propagation techniques require accurate regeneration protocol for obtaining *in vitro* cultured true to type plants. In fact, the technical aspects of micro propagated orchids have improvised significantly in past few years. But the loop holes in micro propagation stems from the somaclonal variation, phenolic compound exudation explants, hardening and so on (Dipika Sarmah; 2017)

A. graminifolia a reedly terrestrial tropical orchid species generally grows in clumps. It is available in newly developed habitats of anthropogenic origin, such as road cuts and abandoned farm fields [1, 2] and mostly occur in limited areas, its natural habitat being steep, rocky sites or open grassy areas [1]. The rhizome of the plant is used as antibacterial agent and its root decoction is commonly used for the ailments of diabetes, tumour, hyperliposis and hepatitis. The phenolic compound of this orchid has antihepatitic and antiHIV activity (Bimal Debnath *et al*., 2016)

High frequency micropropagation of *Arundina graminifolia* (D. Don.) Hochr. through protocorm-like bodies (PLBs) using node explants was established. Node explants derived from three-year-old field grown plants were cultured on half-strength MS medium with either 6.97 µM kinetin (Kn), or 15% coconut water (CW) or 13.3 µM BA favoured sprouting of the axillary bud. Subsequent culture of these emerged buds on medium having 44.4 µM BA facilitated formation of PLBs (a mean of 5.4) from the base. Transfer of PLBs to medium with the same level of BA (44.4 µM) PLBs favoured enhanced proliferation. The 6th subculture of PLBs on medium with 44.4 µM BA yielded > 100 PLBs. The PLB proliferation did not exhibit a decline up to 10th subculture. Conversion of PLBs to shoots or plantlets occurred at high rate (89%) upon transfer to half-strength MS medium containing 6.97 µM Kn. Half-strength MS medium with 1 g l-1 activated charcoal was effective for rooting, and the rooted plants exhibited 91% survival in field condition (Kottackal Poulose Martin; 2007)

Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.161

BIOACTIVITY OF ENDEMIC ORCHIDS OF WESTERN GHATS; *PHOLIDOTA PALLID* LINDL AND *ARUNDINA GRAMINIFOLIA* (D. DON) HOCHR.

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(Date of Receiving-07-01-2021; Date of Acceptance-21-03-2021)

ABSTRACT

Pholidota pallida And *Arundina graminifolia* is an Endemic orchid species belongs to orchidaceae family. It is mainly found in Western Ghats of India. It is used to treat various diseases such as liver affection, wound healing, rheumatism, abdominal pain, ear pain, diabetes etc. The present study is to identify the phytochemicals, Anticancer, Antioxidant, Antimicrobial and nanoparticle synthesis from leaves extract of *Pholidota pallid* and *Arundina graminifolia*. The phytochemical analysis revealed the presence of Alkaloids, Terpenoids, Phenols, Sugar, Saponins, Flavonoids, Quinin's and Steroids. While comparing *Pholidota pallida* possess more bioactivity than *Arundina graminifolia*.

Keywords: *Pholidota pallida, Arundina graminifolia*, Endemic, Nanoparticle synthesis

INTRODUCTION

Phytochemically some orchids have been reported to contain alkaloids, terpenoids, flavonoids and stilbenoids (Singh *et al*., 2009) and more than 44 orchid species of 34 genera have medicinal value (Ghanaksha *et al*., 1993). Orchids are also commercially important for its glycosidal value where four kinds of glycosides have been reported to be present in some orchids (Bose *et al*., 1989). Loroglosin from Loroglossum, coumarin from Angraceum fragrance and saponin from *Paphiopedilum javanium* (Bose *et al.,* 1989) are commercially important glycosides. *Vanilla planifolia* is the main source of commercial vanilla flavor (A. Marjoka *et al.,* 2016)

Pholidota pallida belongs to family Orchidaceae, one of the native epiphytic orchids from the Western Ghats forests of Karnataka. The pseudo bulb of *P. pallida* is used in controlling intestinal worms and abdominal pain and root is used in. Based on its importance the present work was designed to evaluate its phytochemical constituents and free radical scavenging activity of pseudo bulb cold and hot successive extracts. The phytochemical secondary metabolite screening of extracts revealed the presence of alkaloids, flavonoid, phenols, phytosterols and total antioxidant components. Based on the quantitative estimation studies it revealed that extracts have a good amount of secondary metabolites. The cold and hot successive extracts were subjected to free radical scavenging activity on DPPH and ABTS radical cation decolorization assay the result revealed that the highest DPPH scavenging activity was seen in the hot methanolic extract and highest ABTS scavenging activity was seen in the cold methanolic extract. The present investigations prove that the *Pholidota pallida* plant is a reservoir of the phytochemicals that can be utilized for the development of Phyto-therapeutics (Nagananda G S, *et al.,* 2014).

A. graminifolia a reedly terrestrial tropical orchid species generally grows in clumps. It is available in newly developed habitats of anthropogenic origin, such as road cuts and abandoned farm fields and mostly occurs in limited areas, its natural habitat being steep, rocky sites or open grassy areas. The rhizome of the plant is used as antibacterial agent and its root decoction is commonly used for the ailments of diabetes, tumour, hyperliposis and hepatitis. The phenolic compound of this orchid has antihepatitic and anti HIV activity (Bimal Debnath *et al.,* 2016).

MATERIALS AND METHODS

Pholidota pallida and *Arundina graminifolia* leaves were collected from TBGRI, Palode, Trivandrum. Leaves were washed thoroughly using distilled water and shade dried. Then it was pulverized through mechanical grinder. The powdered plant material was extracted using solvents petroleum ether, chloroform, ethanol, methanol, Aqueous solution respectively. Qualitative analysis of phytochemicals mainly alkaloids, terpenoids, phenols, sugar, saponins, flavonoids, quinines, steroids were screened out on *Pholidota pallid* and *Arundina graminifolia*. Antioxidant, Anti-cancerous, Antimicrobial analysis of leaves of both *Pholidota* and *Arundina* were screened out. Nanoparticle synthesis was also analyzed in aqueous extract. Based on the results got it is concluded that *Pholidota* has the highest bioactivity than *Arundina graminifolia*. Current study gives innovative and new findings on the capacity of these orchids and supports the continued research of medicinal orchids of Western Ghats.

DOI : 10.5958/2349-2104.2021.00007.3 Indianjournals.com

Isolation and characterization of fungal endophytes from *Dendrobium crumenatum* Sw. and *Dendrobium nodosum* Dalzell (Orchidaceae)

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ABSTRACT

Orchids rely on specific fungal association for seed germination and early growth. Apart from mycorrhizal fungi within orchid roots, many of the nonmycorrhizal endophytic fungi have also been reported. Although orchids host many non-mycorrhizal fungi in leaves, studies on such fungi especially in tropical and subtropical orchids have been few. The present study established the presence of non-mycorrhizal fungi in leaves of two orchids *Dendrobium crumenatum* Sw. and *Dendrobium nodosum* Dalzell. The endophytic colonies were separated from leaf segments and cultured under laboratory conditions for extraction of the DNA. The DNA samples were sequenced using 18S rRNA ITS universal primers and the resultant data was analysed using NCBI BLAST tool. The sequence of the fungus isolated from *Dendrobium crumenatum* (513bp) showed 95% similarity to *Aspergillus flavus* and that of *Dendrobium nodosum* (556bp) had 96% similarity to *Rhizopus oryzae* (**Keywords:** *Dendrobium crumenatum, D. nodosum, Aspergillus flavus, Rhizopus oryzae*).

INTRODUCTION

The term 'endophyte' has been coined to all organisms, including bacteria (Kobayashi and Palumbo, 2000), fungi (Stone *et al.*, 2000), algae (Peters, 1991) and insects (Feller, 1995), that grow inside living plant tissues without causing disease symptoms (Tao *et al.*, 2008). Endophytic fungal associations increase abiotic stress resistance of host plant (Redman *et al.*, 2002) and improve plant adaptability to various environmental conditions (Swarts *et al.*, 2010). In the case of orchids, the fully photosynthetic orchid species appear to rely on fungi for seed germination and early growth (Warcup, 1981). The fungi that colonize the non-photosynthetic orchids supply carbon from living tree roots (Hamada and Nakamura, 1963). Orchid mycorrhizae have often been characterized as belonging to several anamorphic genera: *Epulorhiza*, *Ceratorhiza* and *Moniliopsis* (Ma *et al.*, 2003; Pereira *et al.*, 2003), other studies have revealed teleomorph genera (*Ceratobasidium*, *Oliveonia*, *Sebacina*, *Thana-tephorus* and *Tulasnella*) as well as several genera of Basidiomycota (Zettler *et al.*, 2004). Apart from mycorrhizal fungi within orchid roots, many of the nonmycorrhizal endophytic fungi have also been reported (Kasmir *et al.*, 2011). Although orchids host many non-mycorrhizal fungi in leaves, studies on such fungi especially in tropical and subtropical orchids have been lacking (McCormick *et al.*, 2004).

Dendrobium crumenatum Sw. commonly called pigeon orchid and *D. nodosum* Dalzell (epiphytic on branches of tall trees in evergreen forests of Western Ghats and the Servarayan Hills in the Eastern Ghats). These two orchids were selected to study the diversity of the fungal symbiont. *Dendrobium,* the second largest genus in Orchidaceae, has a vast diversity in vegetative and floral characteristics and has been of considerable interest due to its broad geographic distribution and high value of hybrids as a floricultural commodity. *Dendrobium* hybrids that range over 30,000 in number, have been preferred commercially due to the number of flowers per inflorescence, recurrent flowering, and variety of flower colours, colour patterns and relatively short production cycle from seeding to a full bloom.

Traditional approaches for revealing fungal endophytes involved isolation procedures, sterilization techniques, cultural conditions and sporulation of isolates (Ganley and Newcombe, 2006). Endophyte isolations commonly resulted in a considerable number of sterile mycelia (Lacap *et al.*, 2003), and these fungi