Plant Archives Vol. 18 No. 2, 2018 pp. 2448-2452 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EXTRACTION AND ESTIMATION OF CHLOROPHYLLS FROM EPIPHYTIC ORCHIDS AND THEIR ANTIOXIDANTS SCAVENGING ACTIVITY ANALYSIS

Deepak Kumar Soni, Sushil Kumar Shahi*, Pramila Khandel, Deepika Mahobiya, Ravishankar Singh, Ravi Kumar Yadaw and Leeladhar Kanwar

Bio-resource Tech Laboratory, Department of Botany, Guru Ghasidas Viswavidyalaya, Bilaspur (Chhattisgarh), India.

Abstract

Epiphytic orchids are the Angiospermic plants having tremendous medicinal properties and charming, longer time sustaining fragrant flowers are facing high risk of endangerment in the wild due to several anthropogenic activities and climate changes. They are becoming rare, endangered and threatened every year as reported in Red data list of International Union for the Conservation of Nature and Natural resources (IUCN). To evaluate chlorophylls and carotenoids contents and their antioxidants activity in some epiphytic orchids using different solvents, present investigation was carried out. Di-ethyl ether solvent was found to be more effective among other solvents to extract chlorophyll and carotenoids in all tested orchid. However, Di-ethyl ether (DEE) extraction showed that *Aerides multiflorum* Roxb.*„ Rhynchostylis retusa* (L.) Blume., *Dendrobium herbaceum* Lindl. and *Vanda tassellata* (Roxb.) Hook. contain chlorophylls in larger amount and carotenoids in trace less amount than other orchids studied. A thorough experiment on antioxidants activity of mentioned orchids showed variant result and found that *Bulbophyllum* has the most DPPH inhibition activity.

Key words : Chlorophylls, carotenoids, orchids, *Rhynchostylis*, *Dendrobium*, *Vanda*. *Bulbophyllum*. *Aerides*, antioxidants, DPPH inhibition.

Introduction

Chlorophyll is a photosynthetic vital green pigment having tetrapyrrole ring with a central magnesium ion found in plants and green algae. It has two types; chlorophyll, a and b with ratio approximately 3:1 in higher plants absorb light mainly in two different wavelength regions *viz*., 650 – 700 nm and 400 – 500 nm of the visible spectrum. (Aminot and Ray, 2000; Arnon, 1849).

Family orchidaceae is known for its well-developed plants and widely distributed occurrence around the world with over and above 30,000 species belonging to 750 genera approximately and native to all the continents in the world constituting one of the largest family among monocots (Kong *et al*., 2003; Dressler, 1981).

This family possess several pharmacognostical properties *viz*., antibacteria, antituberculosis and used in treatment of many diseases *viz*., ear in oitis, inflammation dyspepsia, bronchitis, rheumatism and fevers etc. (Murti and Panigrahi, 1999). Luning (1974) investigated phytochemicals present in family orchidaceae.

Although, almost orchid species possess autotrophy but some orchids show mixotrophy comprising photosynthesis and myco-heterotrophy having two metabolism *viz*., C3 pathway and facultative CAM (Leake, 1994; Gebauer and Meyer, 2003; Selosse *et al*., 2004; Julou *et al*., 2005; Serafini *et al*., 2007). Serafini and co-workers (2007) reported that photosynthesis was CO_2 Dependent in *Dactylorhiza*, *Cephalanthera*, *Platanthera* and *Limodorum*. They also measured chlorophyll contents in these species and stated that Photosynthesis was temperature-dependent in all orchid species, and the highest rates were recorded where temperature ranges from 25^0C to 31^0C. Caroteinoids are well reported in various researches from all around the world. Vechetel and Ruppel (1992) have reported that carotenoids save chlorophyll and thylakoid membrane

***Author for Correspondence :** E-mail: sushilkshahi@gmail.com

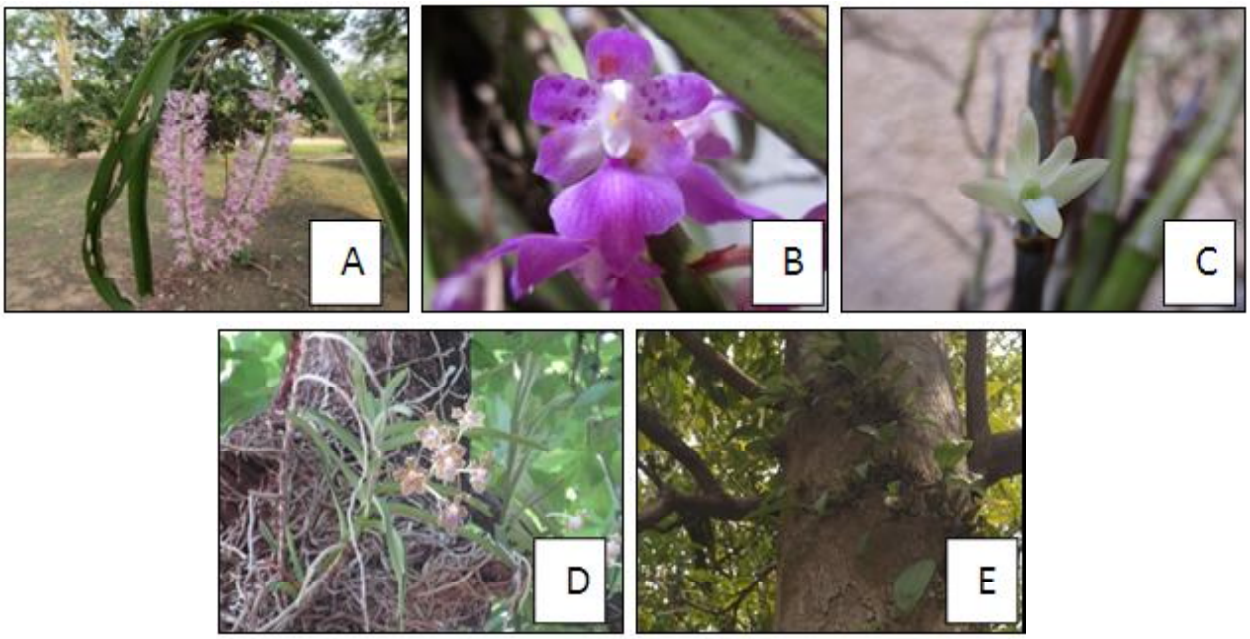

Plate 1 : Showing floral photographs of selected orchid species: A- *Rhynchostylis retusa*, B- *Aerides multiflorum*, C- *Dendrobium herbaceum*, D- *Vanda tessellata*, E- *Bulbophyllum* sp.

during peroxidation process. Furthermore, Dunn *et al.* (2004) have stated that aqueous extracts of some solvents *viz*., Methanol and acetone were less capable of extracting carotenoids from commonly grown fern species. Which further quantified by Sumanta *et al.* (2014) and also concluded that DEE is the best extractant for chlorophylls and DMSO for carotenoids.

Materials and Methods

Collection of orchid samples

In this study, five wild orchid species of Chhattisgarh (*viz. Vanda tessellata*, *Rhynchostylis retusa*, *Aerides multiflorum*, *Dendrobium herbaceum* and *Bulbophyllum* sp.) for phytochemical investigation. These all species are mostly epiphytes in nature and grow on tree trunks condition under varying temperature (15-35^{0}C) in plane land areas.

For test, healthy mature leaves were collected and thoroughly washed with tap water followed by double distilled water to avoid any contamination and kept in room temperature (25^{0}C). After desiccation of orchid leaf samples, they were crushed using mortar and pistle in addition with related solvent used for extraction. Finally, the chlorophyll a, chlorophyll b and carotenoids were analyzed using spectrophotometric tool.

Identification of orchids

All orchids were identified using literatures available in some books (Murti *et al.*, 1989 and Singh *et al.*, 2001) and internet sources.

Analysis methodology

0.5 gram orchid leaf samples were taken and carefully crushed by adding the solvent used in pestle mortar and transferred in test tubes (25 ml capacity) and covered using aluminium foil. Later all samples centrifuged for 10,000 rpm for 15 minutes at 4^{0}C. Further supernatants were separated and 0.5 ml supernatants from each sample were taken in fresh test tubes and 5 ml respective solvents were added. Later each sample solution was analyzed for their Chlorophyll-a, Chlorophyll-b and carotenoids content using spectrophotometer also DPPH (2,2-Diphenyl-1-picrylhydrazyl) inhibition activity was analyzed to see the scavenging property of selected orchids extracts.

The antioxidants activity (Percentage inhibition of DPPH) was calculated by following formula (Chinsamy *et al.*, 2014; Vijaykumar, 2013):

$$\% \text{ Inhibition} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100$$

Results and Discussion

In tropical orchids, two types of photosynthesis occur; C_3 in thin leafy orchids and C_4 in thick leafy orchids (Hew and Khoo, 1979). The main constituent of photosynthesis is chlorophyll a which converts light energy into chemical energy and chlorophyll b (an accessary pigment) indirectly transfers the light energy to chlorophyll a (Costache *et al.*, 2012).

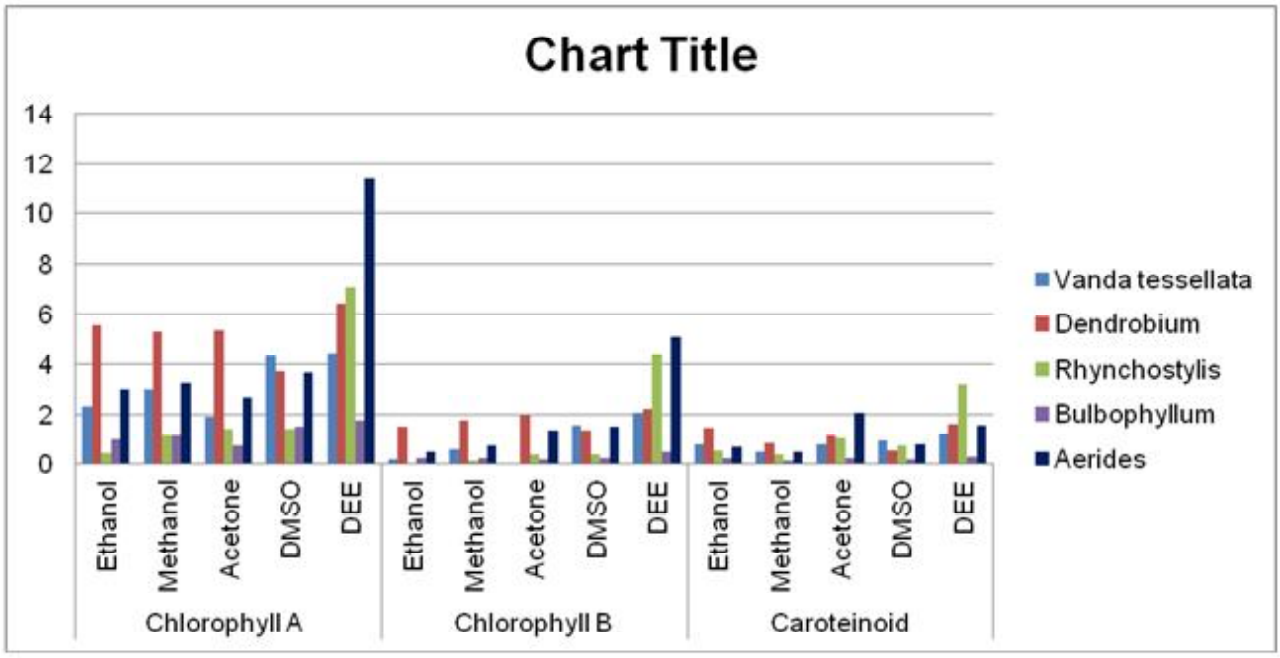

Fig. 1 : Showing data of Chlorophyll a, b and carotenoids contents in µg/ml obtained through data calculation using absorption (in nm).

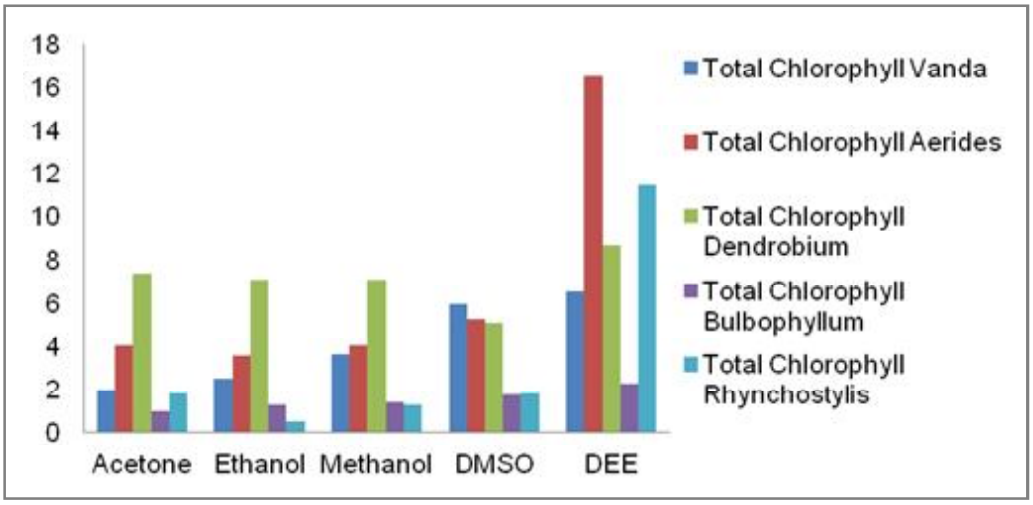

Fig. 2 : Total chlorophyll variation in five selected orchids.

Table 1 : Equations to determination of concentrations (µg/ml) chlorophyll a (Ch-a), chlorophyll b (Ch-b) and total carotenoids (Cx+c) by different solvents in spectrophotometer. (Porra, 1989; Lichtenthaler, 1983 & 1987).

S. no.	Solvent	Formula
1	95% Ethanol	Ch-a=13.36A_{664} – 5.19 A_{649}Ch-b=27.43A_{649} – 8.12 A_{664}C x+c=(1000A_{470} –2.13Ca – 97.63Cb)/209
2	Diethyl-ether (DEE)	Ch-a=10.05$A_{660.6}$ – 0.97$A_{642.2}$Ch-b=16.36$A_{642.2}$ – 2.43$A_{660.6}$C x+c=(1000A_{470} – 1.43Ca – 35.87Cb) /205
3	80% Acetone	Ch-a=12.25$A_{663.2}$ – 2.79$A_{646.8}$Ch-b=21.5$A_{646.8}$ – 5.1$A_{663.2}$C x+c=(1000A_{470} – 1.82Ca –85.02Cb) /198
4	Dimethyl-sulphoxide (DMSO)	Ch-a=12.47$A_{665.1}$ – 3.62$A_{649.1}$Ch-b=25.06$A_{649.1}$ – 6.5$A_{665.1}$C x+c=(1000A_{480} – 1.29Ca-53.78Cb) /220
5	Methanol	Ch-a=16.72$A_{665.2}$ – 9.16$A_{652.4}$Ch-b=34.09$A_{652.4}$ – 15.28$A_{665.2}$C x+c=(1000A_{470} – 1.63Ca – 104.96Cb)/221

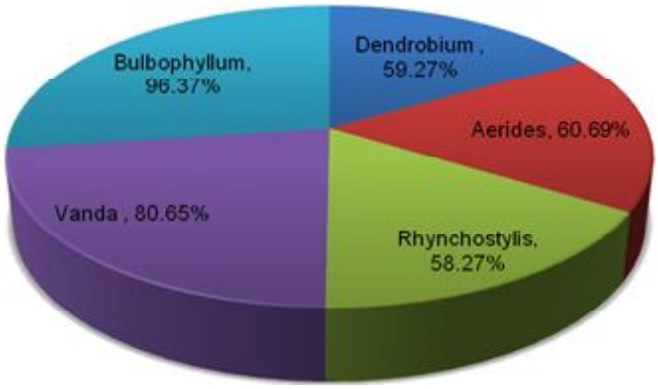

Fig. 3 : Pie Chart showing the antioxidants activity (Percentage inhibition of DPPH) of five selected orchid taxa of Chhattisgarh of DEE Extraction samples.

Extraction in high quantity of photosynthetic pigments was observed in DEE ((except chlorophyll b and Carotenoids in *Bulbophyllum* species). This reason of high extraction of photosynthetic pigments by DEE was supported and explained by Nayek and coworkers (2014) in their work on commonly grown fern species.

Lowest amount was found in *Bulbophyllum* sp. which may be due to its leaf amount and presence of CAM region beside the leaf.

The chlorophyll extraction in different solvents for examined orchids are in the sequence of :

Vanda and *Bulbophyllum* sp. : Acetone < Ethanol < Methanol < DMSO < DEE.

Aerides multiflorum : Ethanol < Acetone < Methanol < DMSO < DEE.

Dendrobium herbaceum : DMSO < Ethanol < Methanol < Acetone < DEE.

Rhynchostylis retusa : Ethanol < Methanol < DMSO < Acetone < DEE

S. no.	Name of Orchid	Percentage inhibition of DPPH
1	*Dendrobium herbaceum*	59.27%
2	*Aerides multiflorum*	60.69%
3	*Rhynchostylis retusa*	58.27%
4	*Vanda tessellata*	80.65%
5	*Bulbophyllum* sp.	96.37%

The antioxidants activity was analyzed in following sequence:

Rhynchostylis < Dendrobium < Aerides < Vanda < Bulbophyllum.

Vattakandy and Chaudhari (2013) worked on antioxidant activity of *Dendrobium herbaceum*. Vijaykumar (2013) studied in *Vanda tessellata* and

Table 2 : Spectrophotometry of orchid leaf samples for determination of absorbance (in nm according to formula used) for Chlorophyll a, Chlorophyll b and Carotenoids with various extraction solvents.

Extraction solvent used	*Vanda tessellata*			*Rhynchostylis retusa*			*Aerides multiflorum*			*Dendrobium herbaceum*			*Bulbolphyllum* sp.		
	Chl a	Chl b	Cx+c	Chl a	Chl b	Cx+c	Chl a	Chl b	Cx+c	Chl a	Chl b	Cx+c	Chl a	Chl b	Cx+c
95% Ethanol	0.199	0.660	0.195	0.039	0.013	0.117	0.266	0.970	0.253	0.495	0.201	0.464	0.089	0.016	0.077
Diethyl-ether (DEE)	0.462	0.196	0.333	0.741	0.378	0.827	1.185	0.487	0.899	0.659	0.234	0.417	0.181	0.058	0.078
80% Acetone	0.165	0.041	0.166	0.125	0.049	0.241	0.245	0.122	0.499	0.486	0.208	0.367	0.069	0.003	0.004
Dimethyl-sulphoxide (DMSO)	0.398	0.166	0.301	0.127	0.048	0.189	0.341	0.149	0.265	0.340	0.143	0.200	0.134	0.044	0.054
Methanol	0.252	0.131	0.201	0.093	0.046	0.105	0.275	0.146	0.202	0.455	0.256	0.381	0.096	0.050	0.064

Chinsamy *et al*. (2014) have previously reported antioxidants activities in *Bulbophyllum* sp. In 2016, Chand *et al*. have assessed antioxidants activity in leaves of some wild orchids of Nepal including *Rhynchostylis retusa*.

Conclusion

Spectrophotometric analysis of chlorophylls and carotenoids showed variation in data obtained using 5 different polar solvents. This work not only supports the ability of DEE as the best photosynthetic pigments extractant, but also reports the presence of those pigments in variety of epiphytic orchids. Based on the extraction of DEE solvents after all numerical calculations, it can be said that high amounts of chlorophyll are found in the *Aerides* and the lowest chlorophyll is found in the *Bulbophyllum*. Therefore, it would be fair to say that the amount of chlorophyll in the orchids with flashy leaves (except the *Aerides* sp.) and pseudobulb is less than flattened leaves. Also found that extraction of chlorophyll from *Dendrobium herbaceum* leaves using DEE followed by *Rhynchostylis retusa* is comparatively more than other solvents. In *Bulbophyllum* sp. the chlorophyll content was lower than other orchids which indicates that C_3 pathway is occur but due to crassulasion acid metabolism in pseudobulb may be the reason of less chlorophyll content. The plant *Bulbophyllum* sp. has the most antioxidants activity based on DPPH scavenging activity analysis among other orchid plants examined. Further more research is needed to explore the reason behind these differences.

References

Aminot, A. and F. Rey (2000). Standard procedure for the determination of chlorophyll a by spectroscopic methods. International Council for the Exploration of the Sea. ISSN 0903-2606.

Aron, D. (1949). Copper enzymes isolated chloroplasts, polyphenoloxidase in Beta vulgaris. *Plant Physiology*, **24**: 1-15.

Chinsamy, M., J. F. Finnie and J. V. Staden (2014). Anti-inflammatory, antioxidant, anti-cholinesterase activity and mutagenicity of South African medicinal orchids. *South African Journal of Botany*, **91**: 88–98.

Costache, M. A., G. Campeanu and G. Neata (2012). Studies concerning the extraction of chlorophyll and total carotenoids from vegetables, *Romanian Biotechnolo. Letters*, **17(5)** : 7702–7708.

Dressler, R. L. (1981). *The orchids*. London, UK: Harvard University Press.

Gebauer, G. and M. Meyer (2003). 15N and 13C natural abundance of autotrophic and myco-heterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. *New Phytol*., **160** : 209-223.

Hew, C. S. and S. I. Khoo (1979). Photosynthesis Of Young Orchid Seedlings. *New Phytol.* (1980), **86** : 349-357.

Kong, J. M. N., G. Khang, C. L. Sai and C. T. Fatt (2003). Recent advances in traditional plant drugs and orchids. *Acta Pharmacology Sinca*, **24(1)** : 7-21.

Leake, J. R. (1994). The biology of myco-heterotrophic (saprophytic') plants. *New Phytol*., **127** : 171-216.

Lichtenthaler, H. K. and A. R. Wellburn (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.*, **11** : 591–592.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic membranes. *Method Enzymol*., **148** : 350–382.

Murti, S. K. and G. Panigrahi (1989). Flora of Bilaspur District, *M. P. Botanical survey of India*, Series 3, **3** : 1-906

Luning, B. (1974). *The Orchid*. (Editor C.L. Withner), John Wiley, New York.

Nayek, S., I. H. Choudhury, N. Jaishee and S. Roy (2014). Spectrophotometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents. *Research Journal of Chemical Sciences*, **4(9)** : 63-69.

Porra, R. J., W. A. Thompson and P. E. Kreidemann (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectrometry. *Biochim. Biophys. Acta*, **975** : 384–394.

Serafini, D., F. Brilli, P. Pinelli, S. Delfine and S. Loreto (2007). Photosynthetic properties of an orchid community in central Italy. *Journal of Plant Interactions*, **2(4)** : 253-261.

Vattakandy, L. S. and G. S. Chaudhari (2013). Estimation of total phenolic content and antioxidant activity of *Dendrobium herbaceum* Lindl. *International Journal of Pharmacy. Photon*., **104**: 302-305.

Vechetel, B. W. and H. G. Ruppel (1992). Lipid Bodies in Eremosphaeraviridis De Bary (Chlorophyceae). *Plant Cell Phys*., **31** : 41-48.

Vijaykumar, K. (2013). *In Vitro* Anti-Oxidant Activity of PET-Ether Extract of *Vanda Tessellata* Roxb. *International Ayurvedic Medical Journal*, **1(5)** : 1-4.