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ANTIOXIDANT POTENTIAL OF *CYMBIDIUM ALOIFOLIUM (L.)* LEAF

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ABSTRACT
Cymbidium aloifolium is a potent medicinal epiphytic orchid plant in the Indian systems of medicine. Traditionally this plant was used for the treatment of Anti-inflammatory, Paralysis, Joining fractured bones, Fever, Weakness of eyes, chronic illness, Burns, Sores etc. Nitric oxide radical scavenging of *Cymbidium aloifolium* leaf extract showed highest scavenging activity of aqueous extract 49.21 % ±0.008 (100µl) and lowest scavenging activity of ethanol extract 32.26 % ±0.008 (25µl) and reducing power activity of *Cymbidium aloifolium* leaf extract showed highest scavenging activity of aqueous extract 38.82%±0.028 (100µl) and lowest scavenging activity of 13.57%±0.043 (25µl) methanol extract.

KEY WORDS: Orchid, *Cymbidium aloifolium*, epiphytic, antioxidant.

INTRODUCTION
Orchids belong to family Orchidaceae, one of the largest families of flowering plants with both terrestrial and epiphytic members. Orchids are grown primarily as ornamentals and are valued as cut flowers because of their exotic beauty and their long lasting blooming period (Hew *et al*., 1997). Plants have been used for medical purposes since the beginning of human history is the basis of modern medicine. Most chemotherapeutic drugs for cancer treatment are molecules identified and isolated from plants or their synthetic derivatives**.** 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 of 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 (Suja and Williams. 2016).

Cymbidium aloifolium is an epiphytic herbaceous orchid belongs to Orchidaceae family (Nongdam and Chongtham. 2011). *Cymbidium aloifolium* occupies a significant position in the everyday life of tribal people of North-Eastern India due to its medicinal and ornamental values. The indigenous people especially in hilly regions take immense pride in treasuring this plant because of its high utility in traditional healing and cure floriculture trade. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) including peroxides, super-oxides, hydroxyl radicals and nitrous oxide, generated in the living organisms by cellular metabolism are known to play a vital role in oxidative cellular damage (Halliwell, 1997). Bio prospecting of plants with anti-proliferative activity has been a major focus on the search of plants based cure (Binish and Suja, 2015). Oxidative stress, resulting from these free-radicals play an important role in manifesting various disorders including ageing and diseases like diabetes, cancer, cardiovascular diseases, Parkinson's and so on. Thus to alleviate such condition in the body, a defense mechanism becomes mandatory. Several types of natural and artificial antioxidants are in regular use worldwide for such oxidative stress (Mukherjee *et al*., 2011).

MATERIALS AND METHODS
Collection of Plant Material
The epiphytic orchid *Cymbidium aloifolium* (L.) was collected from Pechipari 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.

Processing of Plant samples

The freshly collected ***Cymbidium aloifolium*** (L.) leaves were harvested and properly washed in tap water and then rinsed in sterile distilled water. The harvested leaves were dried in the hot air oven at 40º C for 3 days and the dried leaves were pulverized using sterile laboratory mortar and pestle to obtain a powered form. The powdered samples were stored in airtight glass containers for further analysis.

Preparation of extracts

Cymbidium aloifolium (L.) dried leaves powder was extracted with ethanol, methanol, chloroform acetone and aqueous in soxhlet extractor for 72 hours and after exhaustive extraction the extracts were filtered with the help of rotary evaporator.

Antioxidant assays

Nitric oxide radical scavenging assay

Nitric oxide radical scavenging activity was determined as per the standard procedure. 3ml of reaction mixture containing sodium nitroprusside (10Mm in phosphate buffered saline) and various concentrations (25, 50, 75 & 100µg/ml) of the extract were incubated at 37º C for 4 hours. To the incubation solution, 0.5ml of Griess reagent was added and the absorbance was read at 546nm. The percentage of inhibition was calculated using the formula

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Reducing power assay

Reducing power activity was determined as per the standard procedure (Berker *et al*., 2010). The control and solvent extracts of the sampling plants (2.5ml) was mixed with 2.5ml of 200mM sodium phosphate buffer and 2.5ml of 1% potassium ferricyanide and the mixture was incubated at 50ºC for 20 minutes. After the addition of 2.5ml of 10% trichloroacetic acid the reaction mixture was centrifuged at 3000 rpm for 10min. About 5ml of the upper layer was mixed with 5ml of deionised water and 1 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power. IC_{50} value (µg extract/ml) is the effective concentration at which the 0.5 absorbance for reducing power and ascorbic acid was used as a standard.

RESULTS AND DISCUSSION

Antioxidation Activity

An antioxidant is a chemical compound that inhibits the oxidation of other molecules. Antioxidation assay of nitric oxide radical scavenging and reducing power activity of *Cymbidium aloifolium* (L.) leaf extracts after the addition of chemical ingredients were measured at 523 nm using ELICO Spectrophotometer were analyzed and tabulated.

Nitric Oxide scavenging activity

Nitric oxide (NO) radical involved in a variety of biological functions including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. Nitric oxide radical scavenging of *Cymbidium aloifolium* leaf extract varied from 34.21 % ±0.021 (25µl) to 39.48 % ±0.028 (100µl); aqueous extract of *Cymbidium aloifolium* leaf varied from 45.27 % ±0.016 (25µl) to 49.21 % ±0.008 (100µl); ethanol extract of *Cymbidium aloifolium* leaf varied from 32.26 % ±0.008 (25µl) to 37.28 % ±0.016 (100µl); methanol extract of *Cymbidium aloifolium* leaf varied from 35.51 % ±0.021 (25µl) to 37.73 % ±0.020 (100µl); acetone extract of *Cymbidium aloifolium* leaf varied from 39.14 % ±0.012 (25µl) to 40.51 % ±0.004 (100µl); chloroform extract of *Cymbidium aloifolium* leaf varied from 38.64 % ±0.012 (25µl) to 39.68 % ±0.012 (100µl) and antioxidant potential of the standard antioxidant Gallic acid varied from 99.08 % ±0.009 (25µl) to 99.96 % ±0.008 (100µl) (Table: 1).

Table: 1 Nitric Oxide Scavenging activity of *Cymbidium aloifolium* Leaf Extracts.

Conc. of extracts	Control	Aqueous Extract	Ethanol Extract	Methanol Extract	Acetone Extract	Chloroform Extract	(Standard)
25µl	34.21±0.021	45.27±0.016	32.26±0.008	35.51±0.021	39.14±0.012	38.64±0.012	99.08±0.009
50µl	34.28±0.004	45.99±0.004	33.77±0.012	35.69±0.035	39.59±0.032	38.95±0.008	99.64±0.021
75µl	36.52±0.120	46.27±0.009	34.51±0.020	36.61±0.016	40.29±0.028	39.19±0.026	99.75±0.026
100µl	39.48±0.028	49.21±0.008	37.28±0.016	37.73±0.020	40.51±0.004	39.68±0.012	99.96±0.008

Reducing power activity

Reducing the capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Antioxidant scavenging of *Vanda roxburghii* R.Br. leaf extracts varied from 17.98 % ± 0.570 (20µl) of chloroform extract to 61.91% ± 1.196 (100µl) of ethanol extract and hydroxyl radical scavenging activity of *Vanda roxburghii* R.Br. leaf extracts varied from 11.98 % ± 0.789 (100µl) of chloroform extract to 58.5 % ± 0.475 (500µl) of ethanol extract highlight the medicinal importance of the sampling plant (Subin *et al*., 2018). Reducing power activity of *Cymbidium aloifolium* leaf extract varied from 29.51% ±0.020 (25µl) to 34.43%±0.024 (100µl); aqueous extract of *Cymbidium aloifolium* leaf varied from 35.77%±0.136 (25µl) to 38.82%±0.028 (100µl); ethanol extract of *Cymbidium*

aloifolium leaf varied from 18.40% ±0.291 (25µl) to 19.51% ±0.020 (100µl); methanol extract of *Cymbidium aloifolium* leaf varied from 13.57%±0.043 (25µl) to 18.82%±0.016 (100µl); acetone extract of *Cymbidium aloifolium* leaf varied from 28.48% ±0.035 (25µl) to 36.61% ±0.035 (100µl); chloroform extract of *Cymbidium aloifolium* leaf varied from 18.73%±0.148 (25µl) to 34.43% ±0.134 (100µl) and antioxidant potential of the standard antioxidant Gallic acid varied from 71.98% ±0.038 (25µl) to 73.96% ±0.029 (100µl) (Table: 2).

Table: 2 Reducing power activity *Cymbidium aloifolium* Leaf Extracts

Conc. of extracts	Control	Aqueous Extract	Ethanol Extract	Methanol Extract	Acetone Extract	Chloroform Extract	(Standard)
25µl	29.51±0.020	35.77±0.136	18.40±0.291	13.57±0.043	28.48±0.035	18.73±0.148	71.98±0.038
50µl	31.98±0.050	36.43±0.033	18.73±0.008	14.90±0.020	35.77±0.020	23.78±0.012	72.06±0.054
75µl	32.90±0.021	37.78±0.030	19.02±0.066	16.40±0.021	36.53±0.030	25.16±0.021	73.53±0.026
100µl	34.43±0.024	38.82±0.028	19.51±0.020	18.82±0.016	36.61±0.035	34.43±0.134	73.96±0.029

CONCLUSION

Antioxidant potential of the sampling plants justified the traditional use of the plants further experiments are required to elucidate their mechanism of action at cellular and molecular levels may lead as a potential source of natural antioxidants at a low cost.

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