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Free radical scavenging activity versus flavonoid content in twelve *Dendrobium* orchids collected from Darjeeling Hills of Eastern Himalaya

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ABSTRACT

Orchids are praised not only among nature lovers but scientific community as well. Darjeeling Himalayas is natural home for above 500 orchid species of which *Dendrobium* is second most dominant genus. Orchids contain flavonoid and show free radical scavenging activity. But, there is hardly any research that explains a correlation between free radical scavenging activity and flavonoid content in orchids. So, twelve *Dendrobium* species collected from Darjeeling Hills of Eastern Himalayas were used to fill this scientific gap. Except *Dendrobium candidum* all other species- *D. chrysotoxum, D. chysanthum, D. densiflorum, D. nobile, D.*

bicameratum, D. moschatum, D. fimbriatum, D. aphyllum, D. anceps, D. jenkinsii and *D. denudans* exhibited very low to low DPPH scavenging activity. Maximum quantity of flavonoid was present in *D. candidum* while the lowest amount of flavonoid in *D. fimbriatum*. Though an overall positive correlation was observed between flavonoid content and free radical scavenging activity, but in all the species it was not a perfect match. So, flavonoid molecules are not the only candidates for free radical scavenging property. Moreover, presence of prooxidant molecules cannot be ruled out.

Keywords: Orchid, *Dendrobium*, free radical, flavonoid, correlation

1. INTRODUCTION

Orchid flowers exhibit incredible diversity in shape, size, structure, colour and fragrance [1]. The family Orchidaceae is considered to be the most highly evolved among the monocotyledons [2]. Orchids are characterized by three different habitats forms- epiphytic, terrestrial and saprophytic with distinct floral morphology, pollination mechanism, association with unique fungal partners and miniscule seeds.

It is estimated that over 22,500 species with 779 genera are distributed throughout the world [3] of which Indian orchids represent 10% of the world Orchid flora with Himalayas as their natural home [4]. There are 1331 species belonging to 186 genera widely distributed throughout the country [5&6] with 545 orchid species reported from the Eastern Himalayan region [6]. Darjeeling Himalayan region with 85 genera with 311 species [7] is a rich repository of orchids. *Bulbophyllum* with 25 species is the most dominating genus in the region followed by *Dendrobium* having 22 species distributed in natural habitat [8].

42 species of orchids of this region have reported medicinal properties. The medicinal importance of orchids is indicated by Susruta and Vagbhata of ancient Sanskrit literature as early as 250-300 BC [6]. Orchids are reported to contain alkaloids, triterpenoids, flavonoids, stilbenoids, anthocyanins, arundinan, bibenzyl, cypripedin, dendrobine, gigantol, glucoside, glycoside, gymopusin, hircinol, jibantine, kinsenoside, loroglossin, nidemin and orchinol, phenanthrene, phenanthropyran, rotundatin, moscatin etc [9]. So, they are widely used in herbal system of medicine. But, most research on orchids is concentrated on its ecology, conservation, regeneration, availability etc with little attention to its biochemistry and medicinal property. So, the present research is aimed on exploration of comparative free radical scavenging activity and flavonoid content of twelve species of *Dendrobium* collected from Darjeeling hills.

2. MATERIALS AND METHODS

Collection of plant materials

Twelve Orchid species- *D. chrysotoxum, D. chysanthum, D. densiflorum, D. nobile, D. bicameratum, D. moschatum, D. fimbriatum, D. aphyllum, D. anceps, D. jenkinsii, D. denudans* and *D. candidum* were considered for the present study. Plant materials were collected either from wild or from reputed orchid nursery of Kalimpong. Orchids are rare or very restricted in availability. So, outmost care was taken to collect samples causing very little or no injury the plant body. Few leaves were detached from twigs, filled in zipper bags and preserved in insulated ice packed boxes for transportation to Laboratory for all downstream experiments.

Preparation of leaf extracts

The leaf samples were washed several times in running tap water followed by distilled water to remove the dirt and dust. They were crushed in mortar-pestle with a little methanol and finally dipped in methanol for about 48 hours with occasional shaking. After incubation, the extracts were centrifuged and filtered to remove the cellular debris. The methanol was evaporate at low temperature and finally dissolved and stored in methanol at refrigerated condition.

In vitro DPPH scavenging activity

DPPH scavenging activity was measured by a decrease in absorbance at 517 nm of methanolic solution of coloured DPPH brought about by the sample [10-12]. A stock solution of DPPH (100µM) in methanol was prepared. 200µl of methanol extract from *Dendrobium* leaf samples at a concentration of 100 and 200 mg/ml were added to 2800 µl of DPPH solution. Decreases in the absorbance in presence of the different fractions were noted after 30 min. Percentages of DPPH scavenging activity were calculated as {(Absorbance of control - Absorbance of sample/Absorbance of control) x 100}. DPPH activity was expressed as ascorbic acid equivalent.

Quantification of flavonoids

Quantification of flavonoid content was conducted by adding 250µl of each leaf extracts, 1.25ml double distilled water and 75µl of 5% $NaNO_2$ in a glass vessel. The vessel was kept undisturbed at room temperature for 5minutes. Following this, 150µl of 10% $AlCl_3$ was added to it and kept for 6 minutes at room temperature. After that 500µl of 1Mm NaOH and 275µl of double distilled water was added and the solution incubated for 30 minutes. Finally absorbance was measured at 510nm by UV-Vis spectrophotometer [13].

Correlation studies

Correlation studies between free radical scavenging activity and flavonoid content of twelve *Dendrobium* species under study were conducted using SPSS and Microsoft Office Excel Worksheet.

3. RESULTS AND DISCUSSION

***In vitro* DPPH scavenging activity**

Reactive oxygen species and antioxidants have diverse roles to play in the life of organisms. A majority of the disease and disorders are mainly due to the imbalance between pro-oxidation and anti-oxidation homeostatic phenomenon in the body [14]. DPPH scavenging activities of twelve species of *Dendrobium* at a concentration of 100 and 200 mg/ml concentration are depicted in Fig 1. Except *Dendrobium candidum* all other eleven species (*D. chrysotoxum, D. chysanthum, D. densiflorum, D. nobile, D. bicameratum, D. moschatum, D. fimbriatum, D. aphyllum, D. anceps, D. jenkinsii* and *D. denudans*) exhibited very low to low DPPH scavenging activity. In all these plant leaf extracts the free radical scavenging activities were less than 50%. Maximum and minimum free radical scavenging activity was observed in *D. candidum* (91.77%) and *D. chysanthum* (1.94%) respectively at a concentration of 200mg/ml.

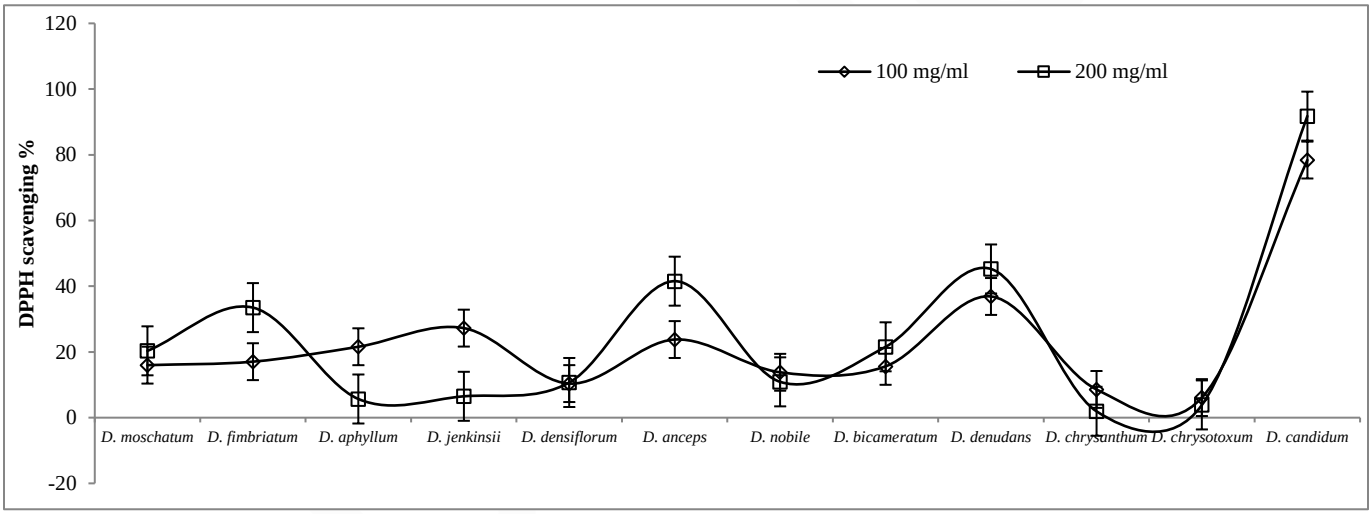

Fig.1. Free radical scavenging activity of *Dendrobium* spp

DPPH scavenging activity is directly related to free radical scavenging potential of an organism. Free radical is responsible for several degenerative disorders and aggravates pathogen related infections. Some of our studied *Dendrobium* (*D. anceps*, *D. bicameratum*, *D. candidum* and *D.* aphyllum) have no reports on medicinal use, despite this very high DPPH scavenging activity was reported in *D. candidum*. So, biochemistry of *D. candidum* needs to be explored. The other species of our study have reported medicinal property. Fresh leaf juice of *D. moschatum* is used in earache and dry seeds dried as haemostatic [4&15-17]. Decoction of flowers and leaves of *D. fimbriatum* are used for liver upsets, cholera and nerves debility while poultice of fresh leaves used to cure boils and pimples [4&18]. Whole plant parts of *D. jenkinsii* used to treat nerves, cholera, pimples and boils [15&17]. Fresh leaf poultice of *D. densiflorum* is recommended for bone fracture [15] while dry seed powder is used as haemostatic [16]. Whole plant parts of *D. nobile* is used in the treatment of pulmonary tuberculosis, flatulence, general debility, cut and wounds, dyspepsia, night sweats, fever and anorexia. It is also stomachic and tonic [4,9,15&19] while powdery seeds and root-powder used to heal wounds and nervous disorder [16&18] but pseudobulb extract used to cure eye infections and to soothe burns [20]. Raw stem of *D. denudans* relieves high fever and body aches [21]. Dry deeds of *D. chrysanthum* are used as haemostatic [16&18]. Whole plant extract of *D. chrysotoxum* is antitumorous and anticancerous [15] while its dry seeds are used as haemostatic [16]. But, all these

species (*D. moschatum* , *D. fimbriatum* , *D. jenkinsii* , *D. densiflorum* , *D. nobile* , *D. denudans* , *D. chrysanthum* and *D. chrysotoxum*) showed low or very low DPPH scavenging activity.

Highly variable degree of concentration dependent DPPH scavenging activities was recorded. Six out of twelve species of *Dendrobium* (*D. bicameratum, D. moschatum, D. fimbriatum, D. anceps, D. denudans* and *D. candidum*) showed increase in DPPH scavenging activity with increase in concentration of methanol extract, where as in one species (*D. densiflorum*) the level of activity was quite similar. Species like *D. chrysotoxum*, *D. chysanthum*, *D. nobile*, *D. aphyllum* and *D. jenkinsii* showed less scavenging activity at higher concentration (200mg/ml). The ascorbic acid equivalent of DPPH scavenging activities of all the *Dendrobium* orchids are depicted in table I. In a similar work on ethanol extract of *D. moniliforme* stem [22] and methanol extract of *D. sonia* [23] flower were conducted.

Table 1: DPPH as ascorbic acid and flavonoid as quercetin equivalent in *Dendrobium* spp

Orchid species	DPPH scavenging activity equivalent Ascorbic acid (µg AE/g)		Flavonoids equivalent to Quercetin (mg QE/g)
	100mg/ml (50% concentration)	200mg/ml (100% concentration)	
D. moschatum	107	156.7	3.252
D. fimbriatum	119.1	306.7	2.976
D. aphyllum	170.9	>10	3.004
D. jenkinsii	235.5	10	3.176
D. densiflorum	43	46.8	2.980
D. anceps	195.9	398.5	3.261
D. nobile	82.2	50	3.121
D. bicameratum	102.8	170.6	5.229
D. denudans	345.2	440.8	3.439
D. chrysanthum	23	>10	3.367
D. chrysotoxum	>10	>10	3.012
D. candidum	818.8	971.3	5.297

Quantification of flavonoid

Flavonoid molecules possess a variety of mechanisms of action including free radical scavenging [14], anti-allergic, anti-inflammatory, antimicrobial and anti-cancer [24]. The presences of flavonoids in the extracts are tested for their antioxidant activity. The quantity of flavonoids detected in twelve *Dendrobium* species collected from eastern Himalayas showed variable amount of flavonoids. Maximum quantity of flavonoid was present in *D. candidum* (5.297mg QE/g) followed by *D. bicameratum* (5.229 mg QE/g) while the lowest amount of flavonoid in *D. fimbriatum* (2.976mg QE/g).

Correlation studies

Correlation study between flavonoid content and free radical scavenging activity was conducted to find out contribution of flavonoid molecules in free radical scavenging activity. A positive correlation was detected between free radical scavenging activity and flavonoid content for both 100mg/ml (0.599977) and 200mg/ml (0.588477) concentrations. But in some species proportional relation between flavonoid content and free radical scavenging activity was not observed. We tried to draw a logical conclusion to this paradox. It was interesting to observe that the magnitude of free radical scavenging activity were very much different in *D. candidum* (91.77% for 200mg/ml concentration) and *D. bicameratum* (21.56% for 200mg/ml concentration) but they had almost similar quantity of flavonoids in their extract. Similarly *D. chrysotoxum* (3.012mg QE/g) and *D. chrysanthum* (3.367mg QE/g) exhibited moderate amount of flavonoids but their free radical scavenging activity was very less (6.11% at 100mg/ml and 3.88% at 200mg/ml concentration for *D. chrysotoxum* and 8.59% at 100mg/ml and 1.94% at 200mg/ml concentration for *D. chrysanthum*), thus there is a possibility for the presence of pro-oxidants. Presence of prooxidant in these plant extracts can further justified by the fact that with increase in concentration of extracts the free radical scavenging activity decreases in these two species along with other species like *D. nobile*, *D. aphyllum* and *D. jenkinsii*. The amount of flavonoid in *D. fimbriatum* (0.2976mg/ml) was the lowest but its free radical scavenging activity was comparatively higher (17.04% for 100mg/ml and 33.49% for 200mg/ml concentration) than other species with higher flavonoid content. Thus a logical conclusion can be drawn that some molecules other than flavonoids have much role to play in free radical scavenging activity.

4. CONCLUSION

It is well known that incredible orchids are facing threat in its habitat for climate change and habitat destruction. But, to our knowledge, molecules exhibiting free radical scavenging activity take a prime protective role during stress period arising out of adverse situation in its habitat and metabolic pathways. The quantity of free radical scavenging activity in all the *Dendrobium* except one showed a very low level of free radical scavenging activity. Even in some of the orchid involvement of pro-oxidants cannot be ignored. So metabolic profiling of Himalayan orchids can show a direction in finding out other probable reasons behind their stress.

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