

Antioxidant Properties of Medicinal Orchid in Indian Vegetation Flora

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Abstract: Modern generation recently interest in plant-derived food. Mainly many- plants are used as for medicine. It's used as a plant is food of medicine. Plant extracts have been shown to possess health-care promoting properties. In the present recently investigation *Bulbophyllum kaitense* plant different extracts from petroleum ether, chloroform, ethanol and aqueous were assessed for their FE-EDTA scavenging, DPPH radical scavenging, H₂O₂ radical scavenging, Inhibition of Lipid peroxidation and total Flavonoids activities. The extracts from aqueous and ethanol extract possessed the highest antioxidant activities except for petroleum ether and chloroform. Although ethanolic extracts showed the best performance in the total phenolic content assay it was little effective at retarding the oxidation of FE-EDTA scavenging assay 1.5mg of the ehtanolic extracts were as effective as 265.1 for 1.5mg respectively. Thus both extracts are rich source of alternatives to synthetic substances as food of medicine with antioxidant properties.

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Keywords: *Bulbophyllum kaitense*, FE-EDTA, DPPH, H₂O₂, Lipid peroxidation and Flavonoids.

1. Introduction:

India is one of the mega diversities hot spots of the world contributing to the world's biological resource from the long stretches of Eastern Ghats on the east. The grater Himalayan range on the northern plains and Western Ghats on the west. The Eastern Ghats range is unique in its own way to host many valuable floras from time immemorial. The Eastern Ghats are spread over four states from the northeast to southwest from the northeast to southeast to southwest along the east coast covering an area of about 75,000 km with average width of 200 km in the north and 100km in the south (Bhairavamurthy 1982). The forest types that predominantly persist in the eastern ghats range are the tropical semi- evergreen forests moist deciduous and dry deciduous types inhibited by a number of terrestrial and epiphytic orchids. Orchids are the most beautiful flowers and comprise a unique group of plants. They represent the most highly evolved family growing abundantly in association with established trees. The family orchidaceae to which orchids belong is the largest family among cotyledons, containing 600-800 genera, orchids include terrestrial, epiphytic and saprophytic forms. Epiphytic orchids are largely tropical and subtropical in distribution however, there have been no investigation on orchids in the world for their pharmacological medicinal and food industries. Orchids are highly prized ornamental plants but also have importance in medicinal and food industries vanilla genus is import ants as the natural vanilla flavoring. The fresh dried stem of *Dentrobium nobile*

is used in the preparation of a drug that works as aphrodisiac, analgesic and longevity. Some orchid species antidote for scorpion bite and curing ailments. Tuber paste of *Habenaria fusifera* is used for cuts, wounds and poisonous bits. Tuber extract of *Habenaria plantaginea* and *H. roxburghii* is used for scorpion and snack bites. Paste obtained from *Acampe pracemorsa*, *Luisia zeylanica* and aerial roots of *Cymbidium aloifolium* are used for fixing human bone fractures. Tubers of *Bulbophyllum neilgherrense* as consumed for good health. Pseudo-bulb extract of *Malaxis acuminata* is used in tonic preparations and of *Pholidata imbricate* for rheumatic swelling, *Velamin* root extract of *Vanda tessellate* is used for treating dysentery. Some other orchid species such as *Malaxis rheedii*, *Liparis prazeri* and *Vanilla wightiana* have ornamental value in addition to botanical values (Seshagiri rao 1998). In ancient time India, orchids used for medicinal purposes were described as medicinal and ornamental plant. Indigenous people of eastern Himalayas believe that *Dentrobium nobile* flowers can cure eye ailments (Mandal and data 2003). It has been reported that antitumore and antimicrobial activities and inhibition of in-vitro lipid peroxidation by *Dentrobium nobile* (Puma devi 2009). Lipid peroxidation has been identified as one of the basic reactions involved in the free radical induced cellular damages in cell (Hallwell and Gutteidge 1992).

2. Material and Methods

2.1. Plant Material

The *Bulbophyllum kaitense* (Tamil vernacular name: Oru ethal elai) belongs to the family orchidaceae was first identified at Sethurpatti nadu urachi kolli hills of Namakkal District, Tamil Nadu, India. Herbarium specimens were prepared and taxonomic identification of the plant *Bulbophyllum kaitense* was confirmed at the Rapinat Herbarium and Centre for Molecular Systematic, Tiruchirappalli, with the voucher number: RHT. 872. A voucher specimen of plant was deposited to that the Rabinat Herbarium for future reference.

2.2. *Bulbophyllum kaitense* Reichb

Kingdom - Plantae
Unranked - Angiosperms
Unranked - Monocots
Order - Aspergales
Family - Orchedaceae
Genus - Bulbophyllum
Species - kaitense

2.3. Preparation of stem extract

250 g of stem were powder and extracted separately in soxhlet apparatus using petroleum ether, chloroform, ethanol and aqueous solvent systems. The extract were filtered through a cotton plug followed by what man filter paper No. 1 and then concentrated by using a rotary evaporator at 40 – 50°C and reduced pressure to get 5.20 g, 4.65 g, 3.50 g, and 4.80 g, yield from petroleum ether, chloroform, ethanol and aqueous fractions respectively. The extracts were preserved in airtight containers and kept at 4°C until further use. All the extracts were tested for antioxidant activity.

2.4. DPPH radical scavenging activity (Gyamfi et al., 2002)

Different aliquots of 0.5 ml, 1.0 ml and 1.5 ml of sample extract Solutions were taken in different test tubes. To these entire tubes methanol was added and made up to 1 ml. to this 4 ml of methanolic DPPH was added and shaken well. The mixture was allowed to stand at room temperature for 20 minutes. The control contains only methanol and DPPH. The readings were noted at 517 nm against methanolic blank. The change in absorbance of the samples was measured free radical scavenging activity was expressed as the inhibition percentage calculated using the formula.

Calculation

$$\text{Percentage of antioxidant-radical activity} = \left[A - \frac{B}{A} \right] \times 100$$

where,

'A' is absorbance of control and 'B' is absorbance of sample.

2.5. Fe – EDTA scavenging activity (Koracevic et al., 2001)

Standardized solution of Fe- EDTA complex (Fenton's reagent) reacts with Hydrogen peroxide by

Fenton type of reaction leading to the formation of hydroxyl radicals (OH). In the presence of 100 μM EDTA, FeCl₃, 6H₂O and ascorbic acid were prepared degassed H₂O prior to use. The reaction tube contained final concentration 0.5 ml O-Toluidine, 0.5 μM EDTA, 0.5 mM H₂O₂, 100 μMl – ascorbic acid. 100 μM FeCl₃, 6H₂O in 100 mM phosphate buffer. PH 7.4 in 0.5 ml total volume following incubation 37°C at 30 minutes after 0.5 Buffer solution and 0.5 ml O-Toluidine were added to produce purple colour complex incubate for 30 minutes at 100 ° C. Antioxidants in the sample suppress the degradation of O-Toluidine. This reaction is measured calorimetrically at 620 nm. This inhibition of colour development is measured as antioxidant properties. The present inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Abs.620 nm control} - \text{Abs.620 nm sample}}{\text{Abs 620 nm control}} \times 100$$

Where,

Abs = Absorbance,

2.6. Hydrogen peroxide scavenging activity (Badami et al., 2005)

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of 0.5 ml, 1.0 ml and 1.5 ml the plant extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The inhibition was calculated. Ascorbic acid was used as standard.

Calculation

$$\text{Percentage of H}_2\text{O}_2 \text{ radical scavenging activity} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Where,

A control is the absorbance of the control. A test is the absorbance in the presence of the sample.

2.6. Antioxidant activity of lipid peroxidation (Vadivelan et al., 2009)

Varying volume of 0.5 ml, 1.0 ml and 1.5 ml of plant samples and control were taken in a test tube. The inhibition of lipid peroxidation induced by FeSO₄ plant extract in homogenate was assayed by measuring the amount of lipid peroxidation product. The antioxidant activity of the compounds was often described by their delay the onset of auto-oxidation by scavenging ROS, or their ability to act as chain – breaking antioxidant by inhibiting the propagation phase of lipid autoxidation. The OD values were taken at 532 nm.

2.8. Determination of total phenolic content (Rohman et al., 2010)

The plant extracts were diluted to 50 times with deionized water prior to analysis. The different concentrated 0.5 ml, 1.0 ml and 1.5 ml of diluted

extract was mixed with 1 ml of diluted folin-ciocalteu reagent (10 times diluted with deionized water). After incubating the mixture at room temperature for 4 minutes, 0.8 ml of 7.5% (W/V) sodium carbonate anhydrous solution was added into the mixture. The mixture was immediately vortexed for 10 s and incubated in dark environment at room temperature for 2 hour. Blank was prepared by replacing 1 ml of plant extract with 1 ml of deionized water. The absorbance of mixture was measured against blank at 765 nm by using UV light spectrophotometer.

2.9. Determination of total Flavonoid content (Sakanaka et al., 2005)

Total flavonoids content was determined by using the stem extract. Varying volume of 0.5 ml, 1.0 ml and 1.5 ml of plant samples and rutin standard solution was mixed with 1.25 ml of distilled water in a test tube, followed by addition of 75 μ l of a 5 % (w/v) sodium nitrite solution. After 6 min, 150 μ l of 10 % (w/v) aluminum chloride solution was added, and the mixture was allowed to stand for a further 5 min before 0.5 ml of 1 M NaOH was added. The mixture was made up to 2.5 ml with distilled water and mixed well. The absorbance was measured immediately at 510 nm.

3. Result

3.1. Evaluation Of *In-Vitro* Free Radical Scavenging Potential

The different methods were used for free radical scavenging activity.

3.2. DPPH scavenging assay

The free radical scavenging activity of *B. kaitense* stem extracts. Different concentration of DPPH free radical scavenging activity (%) of total aqueous extract comparing other extracts in petroleum ether, chloroform, and ethanol are presented in Table 1. The plant stem aqueous extract was the highest having scavenging activity. Aqueous extract showed significantly higher express activity. The scavenging activity of was observed in ethanolic fraction in petroleum ether fraction and chloroform extract fraction respectively. The plant stem ethanolic, chloroform, and petroleum ether extract fraction is more or less activity respectively.

3.2. Fe – EDTA free radical scavenging potential

The Fe – EDTA free radical scavenging potential of *B. kaitense* stem extract. Different solvent of various solvent were petroleum ether, chloroform, ethanol and aqueous were used for the extraction.

Showed the data on free radical scavenging activities as assessed by Fe – EDTA assays. The results of Fe – EDTA scavenging ability were presented shows ethanolic extract to be the most potential scavenger. The suggest that ethanolic extract was highly performance activity. The significantly higher activity respectively. The methanolic extracts were compared to petroleum ether chloroform and aqueous extracts respectively. The results are summarized in Table 2.

3.4. Hydrogen peroxide free radical scavenging assays

The Hydrogen peroxide free radical scavenging potential *B. kaitense* stem extract. Showed the data on free radical scavenging activities as assessed by hydrogen peroxide assays. The results shows petroleum ether extract to be the most potential scavenger. This suggest that petroleum ether extract was highly exert potential activity. The significantly highest than activity. The petroleum ether extracts were compared to chloroform, ethanolic and aqueous extracts respectively. The presented in Table 3.

3.5. Lipid peroxidation scavenging

The lipid peroxidation free radical scavenging potential *B. kaitense* stem extract. The results showed data on chloroform extract to be the expressed potential scavenger. This suggests that chloroform extract had highly potential activity. The significantly higher than activity and the chloroform extracts were compared to petroleum ether extracts, ethanolic and aqueous extracts respectively. The results are summarized in Table 4.

3.6. Total phenolic content scavenging

The total phenolic free radical scavenging potential *B. kaitense* stem extract. The results showed on aqueous extract to be the as well as content of total phenolics a good correlation with most of the petroleum ether, chloroform and methanol extracts. The significantly higher activity. The aqueous extracts were compared to petroleum ether, chloroform and ethanolic extracts respectively. The results are given in Table 5.

3.7. Total flavonoids radical scavenging

The total flavonoids radical scavenging of *B. kaitense* stem extract. Different solvent petroleum ether, chloroform, ethanol and aqueous extracts. The results showed on aqueous extract to be the most no superior activity of flavonoids. The significant highest activity. The aqueous extracts were correlation of petroleum ether, chloroform and ethanolic extracts respectively. The results were summarized in Table 6.

Table1. In-vitro antioxidant activity of stem extract in *Bullbophyllum kaitense* by DPPH Method

Sample	Pet ether extract			Chloroform extract			Ethanol extract			Aqueous extract		
				1ml	1.5ml	0.5ml	1ml	1.5ml	0.5ml	1ml	1.5ml	
Mean value	45.52	77.65	91.20	33.40	42.41	53.0	53.68	83.46	96.38	46.18	99.26	113.2

Table 2. In-vitro antioxidant activity of stem extract in *Bullbophyllum kaitense* by Fe-EDTA method

Sample	Pet ether extract			Chloroform extract			Ethanol extract			Aqueous extract		
	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml
Standard Mean value	0.225	0.086	0.159	0.225	0.086	0.159	0.225	0.086	0.159	0.225	0.086	0.159
Toluidine mean value	0.055	0.088	0.090	0.055	0.088	0.090	0.055	0.088	0.090	0.055	0.088	0.090
Stem extract mean value	0.129	0.1202	0.2084	0.2128	0.2144	0.1660	1.2526	1.2902	1.1548	0.3328	0.4034	0.4422

Table 3. In Vitro Antioxidant Activity of stem extract of *Bullbophyllum Kaitense* by Hydrogen Peroxide Assay

Sample	Pet ether extract			Chloroform Extract			Ethanol extract			Aqueous extract		
	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml
Stem	67.2	93.60	74.6	86.26	74.0	31.6	97.6	94.2	57.2	44.8	31.4	28.6
Mean value	67.2	93.60	74.6	86.26	74.0	31.6	97.6	94.2	57.2	44.8	31.4	28.6

Table 4. In-vitro antioxidant activity of stem extract in *Bullbophyllum kaitense* by Lipid Peroxidation method

Sample	Pet ether extract			Chloroform Extract			Ethanol extract			Aqueous extract		
	0.5ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml
Stem	0.3700	0.4142	0.5050	0.2616	0.2852	0.5676	0.2524	0.2732	0.3118	0.2228	0.2184	0.2414
Mean value	0.3700	0.4142	0.5050	0.2616	0.2852	0.5676	0.2524	0.2732	0.3118	0.2228	0.2184	0.2414

Table 5. In-vitro antioxidant activity of stem extract in *Bullbophyllum kaitense* by Total phenolic method

Sample	Pet ether extract			Chloroform Extract			Ethanol extract			Aqueous extract		
	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml
Stem	54.56	115.32	144.80	87.44	151.12	195.40	96.85	215.26	265.28	189.18	230.46	276.20
Mean value	54.56	115.32	144.80	87.44	151.12	195.40	96.85	215.26	265.28	189.18	230.46	276.20

Table 6. In-vitro antioxidant activity of stem extract in *Bullbophyllum kaitense* by Total flavonoids method

Sample	Pet ether extract			Chloroform Extract			Ethanol extract			Aqueous extract		
	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml	0.5 ml	1 ml	1.5 ml
Stem	0.287	0.787	1.5	0.456	1.0456	1.214	0.5442	0.8936	1.770	0.3314	0.976	1.90
Mean value	0.287	0.787	1.5	0.456	1.0456	1.214	0.5442	0.8936	1.770	0.3314	0.976	1.90

4. Discussion

4.1. In-Vitro analysis for antioxidant activity

In DPPH assay, the free radical scavenging activity of *B. kaitense* stem extracts. The free radical scavenging activity (%) of total aqueous extract and fractions of comparing other extracts in petroleum ether, chloroform, and ethanol. In the present investigation the stem aqueous extract have the highest scavenging activity. Aqueous extract showed significantly higher activity. Scavenging activity of % was observed in ethanolic fraction in petroleum ether fraction % and chloroform extract fraction % respectively. The stem ethanolic, chloroform, petroleum ether extract fraction is more or less activity respectively. Kumar Chandini 2008 reported that DPPH radical scavenging activity (%) of total methanolic extract and fractions of three brown seaweeds were repeated. Methanolic extract of *Turbinaria conoides* showed significantly higher activity ($P < 0.05$) of 17.35% followed by *P. tetra stomatica* (14.78%) and *S. marginatum* (11.00%). The scavenging activity of 23.16% was observed in ethyl acetate fraction of *S. marginatum*, 19.55 and 17.79% in PE fraction of *T. conoides* and *P. tetra stomatica*, respectively. The DPPH radical scavenging activity of enzymatic extract has been attributed to the ability of

these extracts in pairing with the odd electron of DPPH radical (Park et al., 2004). Earlier study is a useful reagent for investigating the free radical scavenging activities of compounds (Duan, 2006). The DPPH, free radical is a stable at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation (Hemant, 2011). In several studies conducted on *Anoectochilus roxburghii* (Wall) Lindl. The following phenol constituents were isolated. 5-hydroxy- 3',4',7-trimethoxyflavonol-3- β -D-rutinoside (Ja et al., 2002) and quercetin-7-O- β -D-[6''-O-(trans-feruloyl)]-glycopyranoside which possess scavenging activity of DPPH radicals (He et al., 2006). On the other hand from stems of *D. aurantiacum* were isolated three 2-glucosyloxycinnamic acid derivatives, namely, cis-melilotoside, dihydromelilotoside, and trans-melilotoside which exhibited potent antioxidant activities (Yang et al., 2007). Zhang (2007) noticed that the another research, ethanol extract of stems of *D. nobile* was found to exhibit significant anti oxidant activity us in the DPPH assay led to the isolation of

bibenzyl derivatives, with significant antioxidant activity higher than or equivalent to vitamin C. Several phenanthrenes phoyunnanins A – C, 9,10-dihydrophenanthrene 4,4',7,7'-tetrahydro-xy-2, 2'-dimethoxy-9,9',10,10'-tetrahydro-1, 1'-biphenanthrene, lusianthridin, eulophiol, 2,4,7-trihydroxy-9, 10-dihydrophenanthrene and imbricatin, have been isolated from 60% ethanol extract of air-dried whole plant of *Pholidota yunnanesis* Rolfe. All compounds were found to show the DPPH free radical scavenging activity with EC50 from 8.8– 55.9 μ M (Guo, 2007).

The FE-EDTA free radical scavenging potential of *B. kaitense* stem extract. Different solvents petroleum ether, chloroform, ethanol and aqueous were used for the extraction. The results of FE-EDTA scavenging ability of ethanolic extract were found to be the most potential scavenger. It is confirmed that ethanolic extract has high antioxidants activity. The significantly higher activity respectively. The methanolic extracts were compared to petroleum ether, chloroform and aqueous extracts respectively. Living cells, including those of man, animals, and plants, are continuously exposed to a variety of challenges that exert oxidative stress. Oxidative stress arises in a biological system after an increased exposure to oxidants, a decrease in the antioxidant capacity of the system, or both. It is often associated with or leads to the generation of reactive oxygen species (ROS), including free radicals, such as cancer, rheumatoid arthritides, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing. Reactive free radicals may come from endogenous sources through normal physiological and metabolic processes such as mitochondrial respiration. Alternatively, they could result from exogenous sources such as exposure to pollutants and ionizing irradiation, and particularly oxygen derived radicals are capable of oxidizing biomolecules, resulting in cell death and tissue damage (Halliwell and Gutteridge, 2003). The antioxidant supplements or antioxidant – containing may be used to help the human body to reduce oxidative damage or to protect food quality by preventing oxidative deterioration (Elmastasa, 2007). The recent reported several protocols to determine their antioxidant activity based on spectrophotometric techniques (Barros, 2007). Progressively, electrochemical techniques have been tested and developed as an alternative tool, for the evaluation of different food extracts, expressed in terms of “antioxidant power”, due to their quickness, simplicity and low cost (Blasco, 2004; and Korotkova, 2002). Elmastasa, 2007 Tabulated in the natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress. The latter being considered a cause

of ageing and degenerative diseases. The antioxidants contained in foods, especially vegetables, are phenolic compounds (Phenolic acid and flavonoids), Carotenoids, tocopherol and ascorbic acid.

In another study investigating effectiveness of FE-EDTA free radical scavenging activity of cymbopogon citratus (DC) staff (Koracevic, 2001).

The hydrogen peroxide free radical scavenging potential *B. kaitense* stem extract. Different solvents petroleum ether, chloroform, Ethanol and aqueous extracts were used. The results showed petroleum ether extract was found to be potential scavenger. The petroleum ether extract was highly exert potential activity. The significantly higher than activity. The petroleum ether extracts were compared to chloroform, ethanolic and aqueous extracts respectively. In recent years, interest in plant derived food additives has grown. Plant extracts might substitute synthetic food antioxidants, which may influence human health when consumed chronically (Martinez, 2001).

The hydrogen peroxide free radical scavenging potential *B. kaitense* stem extract. Different solvents petroleum ether, chloroform, Ethanol and aqueous extracts were used. The results showed petroleum ether extract was found to be potential scavenger. The petroleum ether extract was highly exert potential activity. The significantly higher than activity. The petroleum ether extracts were compared to chloroform, ethanolic and aqueous extracts respectively. Hemant, 2011 analyzed that the hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (–SH) groups. Hydrogen peroxide can cross cell membrane rapidly, once inside the cell, hydrogen peroxide can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects.

In the similar study lipid peroxidation free radical scavenging potential *B. kaitens* stem extract. The results showed chloroform extract was found to be potential scavenger. This suggested that chloroform extract was highly potential activity. The significantly higher than activity and the chloroform extracts were compared to petroleum ether extracts, ethanolic and aqueous extracts respectively. Oxidative stress, involves a series of free radical chain reaction processes, is associated with several types of biological damage, DNA damage, diabetes, respiratory tract disorders, carcinogenesis and cellular degeneration related to aging (Anderson, 2000), Habib (2011) in the present investigation of different extracts of *Geodorum densiflorum* (LAM). Schltr root was found to exhibit significant antioxidant activity. Alcohol extract of rhizomes of *Gymnademina conopsea* showed effect on the collagen synthesis in rat lungs

exposed to silica under the influence on antioxidant activities. The extract can ameliorate silica – induced pulmonary fibrosis by increasing activities of antioxidant and alleviating damage of lipid peroxidation to the lungs (Wang, 2007).

The total phenolic free radical scavenging potential *B. kaitense* stem extract. Different solvent petroleum ether, chloroform, ethanol and aqueous extract the results showed on aqueous extract to be the as well as content of total phenolics a good correlation with most of the petroleum ether, chloroform and ethanol extracts. The significantly highest activity. The aqueous extracts were compared to petroleum ether, chloroform and ethanolic extracts respectively. Reported that the total phenolic content in callus of *Habenaria edgeworthii* found to be higher ranged between 10.33 and 14.30 mg GAE / g dry weight (DW) respectively (Moin, 2012).

Our findings the total flavonoids radical scavenging of *B. kaitense* stem extract results showed on aqueous extract to be the most no superior activity of flavonoids. The significant highest activity, the aqueous extracts were correlation of petroleum ether, chloroform and ethanolic extracts respectively. Moreover, from structure activity studies (Miranda, 2000). Miranda, 2000 noticed that the flavonoids are characterized as phenolic compounds with functional groups associated with the chelation of metal ions, there are numerous flavonoids, such as the prenylated and non-prenylated chalcones and flavanones found in beer and hops, which do not chelate copper ions *in vitro*. The total flavonoid content of ethyl acetate was found to be higher (37.25 µg Quercetin equivalents/mg DW) than methanol and water with the values of 14.00 µg and 11.24 µg Quercetin equivalents/mg DW respectively (Moin, 2012). Improved antioxidant status helps to minimize the oxidative damage and thus can delay or decrease the risk for developing many chronic age related, free radical induced diseases (Karuna, 2009). The interest in natural antioxidants, especially of plant origin, has greatly increased in recent years as the possibility of toxicity of synthetic antioxidants has been criticized (Jayaprakash, 2000). Svilaas, 2004 reported that antioxidants, found naturally in many plants, foods and beverages offer health benefits in preventing various diseases by fighting cellular damage caused by free radicals in the body. More recent reports revealed seaweeds to be a rich source of antioxidant compounds (Duan, 2006 and Kuda, 2005).

Conclusion

The stem aqueous and ethanol extract has shown high exact activity than free radical scavenging activity than petroleum ether and chloroform extracts. This indicated that *B. kaitense* could be a good source

of antioxidants. By using such plant extracts to develop drug industry.

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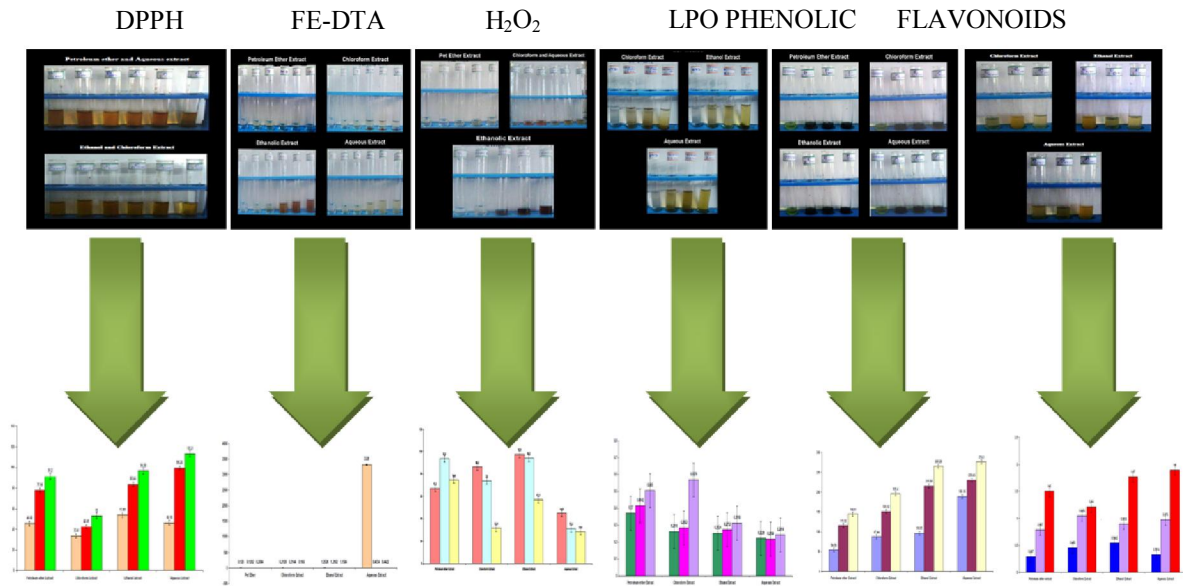
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Pictorial Abstract
Bulbophyllum Kaitense Orchid



Antioxidant Models





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