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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTI OXIDANT ACTIVITIES OF DIFFERENT PARTS OF *DENDROBIUM OCHREATUM*.

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ABSTRACT

This study was performed to evaluate the presence of phytoconstituents, antimicrobial and antioxidant activity of *Dendrobium ochreatum*. (Orchidaceae) stem, root leaf extracts. In this study nine different solvent extracts were subjected to examination. Preliminary phytochemical analysis revealed that the ethanolic extract of root, stem and hexane: ethanol extract of stem showed the maximum phytochemical constituents followed by ethanol extract of leaves. Ethyl acetate extract of stem possess moderate 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (IC50 172.27µg/ml), ABTS assay (IC50 140.06µg/ml) This research findings suggest that *Dendrobium ochreatum* exhibits potential antioxidant properties.

KEYWORDS: *Dendrobium ochreatum*, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS.

INTRODUCTION

Orchids are one of the largest and most diverse groups of angiosperms consisting of nearly 25,000 species with more than 850 genera. They are generally cultivated for beautiful flowers and are widely known for their economic importance and very less for their medicinal use *(Siddhartha Singh et al., 2012)*. The genus Dendrobium is represented by more than 1100 species widely distributed throughout Asia, Europe and Australia, thus being the richest

genus of the family Orchidaceae. There are 74 species and 2 variations of Dendrobium plants found in China, in Thailand, approximately 177 genera and 1,135 species are found in a variety of habitats*(Atichart et al., 2007), D. denneanum* has been archived in the Pharmacopoeia of the People's Republic of China. To elucidate the pharmacological mechanism of Dendrobium species, much research has been carried out on the low molecular compounds, such as bibenzyls *(Majumder et al., 1999; Zhang et al., 2007)*, coumarins *(Zheng et al., 2009),* alkaloids *(Wang et al., 2010)* and phenanthrenes *(Yang et al., 2006).* Polysaccharides is some of its main bioactive compounds that possesses immunoregulatory *(Shao et al., 2004; Chen et al., 2007; Feng et al., 2010),* anti-inflammatory (*Hitoshi et al., 1974; Popov et al., 2005),* anti-viral *(Wang et al., 2009)* and antioxidant *(Wu et al., 2007; Luo et al., 2009, 201.)* In vitro and in vivo studies of water-soluble crude polysaccharide (DDP) obtained from the aqueous extracts of the stem of *Dendrobium denneanum*. showed potent antioxidant activity.*(Aoxue Luo ,et al 2009*).

Antioxidant potential of methanolic extract of *D. normale.* was proved and the reason proposed for the activity is due to the presence of flavonoids, alkaloids, triterpenoids, steroids and carbohydrates.*(Srinivasa Rao Vandavasi et al., 2014)* Aqueous extract of aseptically regenerated Dendrobium aqueum was used for in vitro estimation of antioxidant activity, extract showed a dose dependent DPPH free-radical scavenging potential *(Sourav Mukherjee et al., 2012).*

Several works were carried out on either isolated polysaccharides, *(Jeong-Chae Lee et al., 2007)* or solvent extracts of *Dendrobium* species and result revealed that they possess potent antioxidant activity, here in this study an attempt has been made to carry out antioxidant activities of nine different solvent extract of each stem, leaves and roots of *Dendrobium ochreatum.* As no antioxidant activity has been reported on this particular species yet.

MATERIAL AND METHODS

The flowers of *Dendrobium ochreatum* from Janak nursery,Siliguri, Darjeeling district of West Bengal, India. Ascorbic acid ,DPPH,ABTS and all other solvents and chemicals used were of analytical grade purchased from local source.

Preparation of extract

Leaves, stem and root was separated from plants and shadow dried for 7 days. Then crushed into fine powder using mill grinder. Stem powder was filled in the thimble and extracted

successively with Hexane, chloroform, Ethylacetae and ethanol using a Soxhlet extractor for 72 h. The leaves and root powder was extracted with Hexane and ethanol. The solution of the extract was filtered through Whatman filter paper no. 1 and concentrated using rotary flash evaporator and stored in the refrigerator. Likewise nine solvent extracts were prepared by same procedure.

Table no -01: Total weight of plant parts.

Plant parts	Total weight (grams)
Leave powder	14
Stem powder	158
Root powder	25

Table -02: Yield value of different extracts.

Plant Parts	Solvents	Extracts weight(grams)
Leaves	Hexane	0.46
	Ethanol	0.86
Stem	Hexane	1.23
	Chloroform	8.38
	Ethyl acetate	2.228
	Ethanol	11.99
	Hexane-Ethanol	3.248
Root	Hexane	0.19
	Ethanol	4.2

Table -03: Code no of different extracts.

Sl. no	Code.no	Extracts/fractions
1.	LHDO	Leaves/Hexane extract
2.	LEDO	Leaves/Ethanol extract
3.	SHDO	Stem/ Hexane extract
4.	SCDO	Stem/Chloroform extract
5.	SEADO	Stem/Ethyl acetate extract
6.	SEDO	Stem/Ethanol extract
7.	SHEDO	Stem/ Hexane→ethanol extract
8.	RHDO	Root/Hexane extract
9.	REDO	Root/ Ethanol extract

Table -04 Qualitative preliminary phytochemical analysis of different solvent extracts of *Dendrobium ochreatum*

Sl.No	Compounds	Steroids	Alkaloids	Flavonoids	Glycosides	Saponins	Terpenoids	Tannins
1.	Leaves/Hexane extract	+	-	-	-	-	-	-
2.	Leaves/Ethanol extract	+	+	+	-	+	+	+
3.	Stem/ Hexane extract	+	+	-	-	-	-	-
4.	Stem/Chloroform extract	+	+	+	-	-	+	+
5.	Stem/Ethyl acetate extract	-	+	+	+	-	+	+
6.	Stem/Ethanol extract	-	+	+	+	+	+	+
7.	Stem/ Hexane→ethanol extract	+	+	+	+	+	+	+
8.	Root/Hexane extract	+	-	-	-	-	-	-
9.	Root/ Ethanol extract	+	+	+	+	+	+	+

\+ = PRESENT

\- = NOT DETECTED

ANTIOXIDANT ACTIVITY

DPPH radical scavenging assay

Free radical scavenging activity of plant extracts were measured by the 1,1-diphenyl picryl hydrazyl (DPPH) assay method. *(Aoxue Luo et al.,2010),* (*Thirunavukkarasu sappanimuthu et .al)* with slight modification. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 1.8mL of this solution was added to sample solutions in Methanol (0.2 mL) at different concentrations (5,50,100,500,1000,2000) µg/ml. The mixture was vortexed and allowed to stand in dark at room temperature for 30 min. A DPPH blank was prepared without compound and methanol was used for the baseline correction. Ascorbic acid was used as a reference standard. Decrease in the absorbance at 517 nm was measured using UV-Visible spectrophotometer and the remaining DPPH was calculated. The radical scavenging activity was expressed as the percentage inhibition and was calculated using the formula.

% of Inhibition = [(Ao - A1)/Ao] X 100.

Where Ao is the absorbance of the control (without compound) and A1 is the absorbance of the compound. The IC_{50} (concentration causing 50% inhibition) values of each compound was determined graphically.

Table 05- Percentage inhibition of different fractions of *Dendrobium ochreatum* (DPPH assay)

Sl no	Abs Of blank	Concentration µg/ml	% Inhibition								
			LHDO	LEDO	SHDO	SCDO	SEADO	SEDO	SHEDO	RHDO	REDO
1	1.215	5	11.76	12.51	12.83	12.83	14.07	12.18	13.41	12.26	14.15
2		50	15.65	15.55	15.96	18.27	23.45	19.75	20.24	13.74	25.34
3		100	17.94	18.02	19.67	22.22	30.78	25.43	26.09	15.31	37.36
4		500	32.42	39.51	39.58	50.61	62.71	44.77	56.29	24.19	71.93
5		1000	48.64	60.16	57.86	66.74	82.31	79.09	77.36	28.72	84.93
6		2000	62.46	74.65	77.44	73.91	86.09	89.87	86.58	35.63	87.57

Table 06- Percentage inhibition of standard (ascorbic acid) in various dilutions in DPPH assay

Sl .no	Concentration (µg/ml) of standard (ascorbic acid)	% Inhibition
1.	1	5.11
2.	5	19.01
3.	10	27.81
4.	20	46.91
5.	30	65.67
6.	50	96.54

Table no-07: IC50 values of standard and different partitions of *Dendrobiumochreatum* (DPPH assay)

Sl. No	Test samples	(IC_{50})
1.	Ascorbic acid (standard)	22.89
2.	LHDO	1334.98
3.	LEDO	601.93
4.	SHDO	573.78
5.	SCDO	391.52
6.	SEADO	172.27
7.	SEDO	731.71
8.	SHEDO	220.37
9.	RHDO	3054.48
10.	REDO	132.43

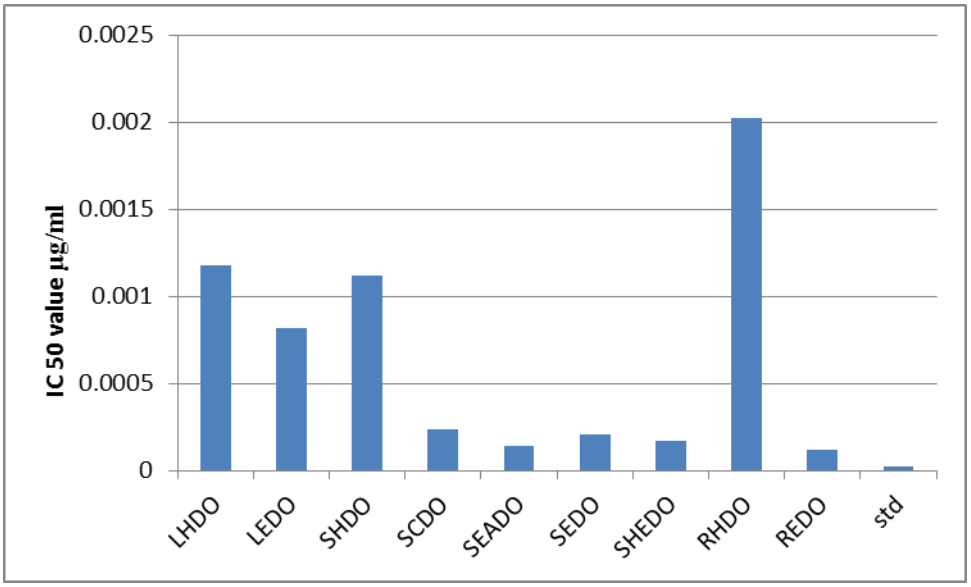

Fig-01- Bar diagram of IC50 values of the standard and different partitions of *dendrobium ochreatum* (DPPHassay)

ABTS radicals scavenging assay

The antioxidant activity of plant extracts were measured using 2,2′-azino-bis[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS) assay*(Wu et al., 2006), (Thirunavukkarasu sappanimuthu et .al)* with slight modification. The ABTS•+ was produced by the reaction between 7 mM ABTS in deionized water and 2.45 mM potassium persulfate, left to stand in the dark at room temperature for 16 h. Then, ABTS•+ solution was diluted with phosphate buffer (0.1M, pH 7.4) to give an absorbance value of ~0.700 at 734 nm. To the reaction mixture containing 0.2 ml of different concentration (5,50,100,500,1000,2000)µg/ml of compounds in ethanol was added to 1.8mL of ABTS•+ solution. After 30 min, the decrease in absorbance was measured at 734 nm. Ascorbic acid was used as standard (positive control). The % inhibition and the IC_{50} values were calculated as mentioned in the DPPH assay.

Table 08- Percentage inhibition of different fractions of *Dendrobium ochreatum* (ABTS assay)

Sl no	Abs Of blank	Concentration µg/ml	% Inhibition								
			LHDO	LEDO	SHDO	SCDO	SEADO	SEDO	SHEDO	RHDO	REDO
1	1.018	5	2.16	2.06	2.35	3.43	3.33	3.53	3.14	1.86	4.32
2		50	3.24	8.25	6.18	12.18	21.81	14.93	15.17	4.02	21.7
3		100	7.36	12.47	10.01	20.23	34.47	17.28	26.22	5.69	36.14
4		500	26.22	32.91	29.17	56.87	73.08	59.03	69.15	19.54	79.46
5		1000	47.15	92.73	48.42	82.8	91.74	86.54	90.66	30.35	94.59
6		2000	79.56	97.83	82.22	92.23	95.97	97.44	96.36	46.85	98.42

Table 09- Percentage inhibition of standard (ascorbic acid) by ABTS assay

Sl. No	Concentration (µg/ml)	% Inhibition
1.	1	1.27
2.	5	4.32
3.	10	13.45
4.	20	45.48
5.	30	75.73
6.	50	99.51

Table10-IC50 values of standard and different partitions of *Dendrobium ochreatum* (ABTS assay)

Sl no	Test samples	(IC_{50})
1.	Ascorbic acid (standard)	23.92
2.	LHDO	1178.96
3.	LEDO	815.54
4.	SHDO	1120.63
5.	SCDO	238.78
6.	SEADO	140.06
7.	SEDO	210.88
8.	SHEDO	169.84
9.	RHDO	2022.86
10.	REDO	123.21

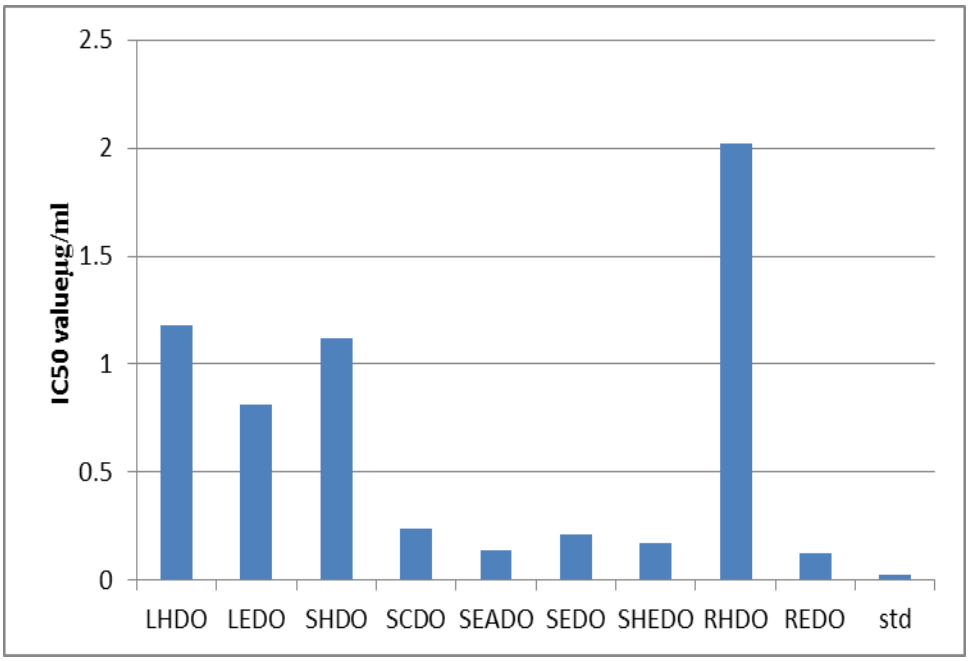

Fig 02: Bar diagram of IC50 values of the standard and different partitions of *dendrobium ochreatum* (ABTSassay)

DISCUSSION

Preliminary phytochemical screening of nine different solvent extracts of *Dendrobium ochreatum* showed a wide range of chemical constituents like steroids, alkaloids, flavanoids, glycosides,saponins,terpinoids, tannins. Whereas maximum phytoconstituents are present in ethanolic extracts of each root , stem and leaf. The DPPH radicals are widely used to investigate the scavenging activity. In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to the yellow colored diphenyl-picryl hydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. The method helps to determine the antiradical power of an antioxidant by measuring of a decrease in the absorbance of DPPH at 517 nm *(Srinivasa Rao et.al., 2014).* The result of scavenging activity assay suggests that the plant is potently active which indicates that the plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity.*(Narender Prasad D,et.al., 2012*).It has been reported previously that various polysaccharides isolated from *Dendrobium ochreatum* possess antioxidant properties *(AoXue Luo et.al., 2010).* Here in this study nine different fractions were subjected to antioxidant study, From the DPPH assay, it was observed that "SEADO"(ethyl acetate extract of stem) and "REDO" (ethanolic extract of root) showed moderate free radical scavenging activity (IC50172.27 µg/ml) and (IC50 132.43) respectively compared to standard ascorbic acid (22.89 µg/ml) in comparison to other fractions. LHDO and RHDO showed very poor free radical scavenging activity. From ABTS assay same fractions (SEADO and REDO) showed moderate free radical scavenging activity (IC50140.06µg/ml) and (IC50123.21 µg/ml) respectively compared to standard ascorbic acid(IC5023.92 µg/ml).

CONCLUSION

From the above study it reveled that ethanol is the best solvent for extracting maximum phytoconstituents. The antioxidant potential could be due to the presence of flavonoids, alkaloids, triterpenoids, steroids. The presence of the above said constituents in selected plant extract alone or in combination might be responsible for the observed antioxidant potential, based on this study further works are to be carried out in future.

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