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ORIGINAL ARTICLE

Phytochemical Evaluation and *in vitro* Free Radical Scavenging Activity of Cold and Hot Successive Pseudobulb Extracts of Medicinally Important Orchid *Pholidota pallida* Lindl.

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ABSTRACT
Pholidota pallida belongs to family Orchidaceae, one of the native epiphytic orchids from the Western Ghats forests of Karnataka. The pseudobulb of P.pallida is used in controlling intestinal worms and abdominal pain and root is used in rheumatism. Based on its importance the present work was designed to evaluate its phytochemical constituents and free radical scavenging activity of pseudobulb cold and hot successive extracts. The phytochemical secondary metabolite screening of extracts revealed the presence of alkaloids, flavonoid, phenols, phytosterols and total antioxidant components. Based on the quantitative estimation studies it revealed that extracts have a good amount of secondary metabolites. The cold and hot successive extracts were subjected to free radical scavenging activity on DPPH and ABTS radical cation decolorization assay The result revealed that the highest DPPH scavenging activity was seen in the hot methanolic extract and highest ABTS scavenging activity was seen in the cold methanolic extract. The present investigations proves that the Phalidota pallida plant is a reservoir of the phytochemicals that can be utilized for the development of phyto-therapeutics.
KEY WORDS: *Pholidota pallida, pseudobulb, extracts, phytochemical, free radical scavenging activity.*

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INTRODUCTION

Plants are known to be the source of effective and valuable therapeutic agents against various diseases [1]. Traditional usage of orchids in Indian medicine dates back to the vedic period [2]. The use of orchids as medicine is been reported by Charaka, Sushruta and Vagbhata in different system of medicines like Ayurveda, Siddha and Unani [3,4]. *Pholidota pallida* is an epiphytic orchids with the pseudobulbs and is one of the commonest species of orchid occurring in south India in the plains and as well as in elevation upto 900m [5]. Recent literature tells that pseudobulb of *P.pallida* is used in controlling intestinal worms and abdominal pain and root is used in rheumatism [6].

Antioxidants are molecules that inhibit oxidation of other molecules, which is a chain of chemical reaction transferring electrons or hydrogen to an oxidizing agent, producing free radicles. The characterestic feature of an antioxidant is its ability to trap free radicals. Excess of free radicles, which causes oxidative stress and death of cells, by oxidizing proteins, lipids, nucleic acids which initiates degenerative diseases. Oxidative stress is the term that refers to the imbalance between the generation of ROS and the activity of the antioxidant defences. Severe oxidative stress can cause cell damage and death. Oxidative damage is caused by the presence of free radical or radical-blocking or radical scavenging mechanism causing majority of disease conditions like atherosclerosis, hypertension,inflamatory response syndrome, aging, resperatory syndrome, liver and cancer [7,8]. Antioxidants like flavanoids, polyphenols, and phenolic acids scavange free radicles like peroxides, hydroxyl or lipid peroxyl radicals thus inhibiting oxidative stresss, are widely used to cure different diseases [7]. Based on the recent literature few orchids like *Flickingeria nodosa* (Dalz.) Seidenf. [9] and *Dendrobium aqueum* Lindl [10] have shown the presence of

good antioxidant activity; these are mainly due to presence of secondary metabolite like phenols, flavonoids, alkaloids and others which plays an important role in neutralising free radicals activity [2].So the present investigation was started with an aim to evaluate the phytochemical components and in vitro free radical scavenging activity of cold and hot successive pseudobulb extracts of medicinally important orchid *Pholidota pallida* Lindl.

MATERIALS AND METHODS

Preparation of the plant material: The pseudo bulb of the plant was collected from natural habitat and rinsed with distilled water to remove the dust particles. The water was removed by blotting over a filter paper. Then the plant materials were shed dried and powdered. Ten gram of powdered plant material was weighed and taken in muslin cloth and made into packets. The packets were used for successive extraction by using 3 different solvents namely petroleum ether, chloroform and methanol respectively. The cold and hot extraction

Qualitative phytochemical screening:

The different qualitative chemical tests were performed for establishing phytochemical profile of hydro methanolic extracts obtained from cold and hot extractions. The tests for alkaloids, Saponins, Phytosterols, phenols, Tannins, glycosides, flavanoids were performed on all the extracts to detect various phytoconstituents present in them [11, 2].

Estimation of alkaloids: The extracts were taken and the pH was adjusted to 1.2-2 with dilute HCl and were estimated for alkaloids by the method followed earlier [12] with absorbance measured at 435 nm.

Estimation of phytosterols: The extracts were dissolved in chloroform and were estimated for phytosterols by libermann-burchard method [13] with absorbance measured at 640 nm with cholesterol (1mg mL^{-1}) as the standard.

Estimation of total flavonoid : The extracts were dissolved in DMSO and were estimated for total flavonoid content by aluminium chloride method [14] with absorbance measured at 510 nm with quercetin (100µg mL^{-1}) as the standard.

Estimation of total phenols: The extracts were dissolved in 5mLof distilled water and were estimated for total phenols by Folin-Ciocalteau reagent method [15] with absorbance measured at 650 nm with catechol (50 µg mL-1) as the standard.

Total Antioxidant Capacity: The extracts (1mg mL^{-1}) were dissolved in DMSO and were checked for total antioxidant capacity by phosphomolybdenum method [16] with absorbance measured at 695nm and ascorbic acid (1mg mL^{-1}) serves as standard.

DPPH (1,1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging assay [17]: Standard ascorbic acid and extracts (1 mg mL^{-1}) at various concentrations (10-50 µg) were taken and the volume were adjusted to 100 µL with methanol. Five millilitre of a 0.1mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Methanol serves as a blank and the experiment was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

% DPPH radical scavenging activity = [(control OD – Sample OD) / Control OD)] *100.

ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolourization assay [18]: ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS (7mM) with 2.45mM Ammonium persulfate and the mixture was allowed to stand in dark at room temperature for 12-16h before use. Standard ascorbic acid and sample extracts (1mg mL^{-1}) at various concentrations (10-50 µg) were taken and the volume was adjusted to 500µL with methanol and 500µL of methanol serves as blank. 300 µL of ABTS solution was added; the final volume was made up 1 mL with ethanol and incubated in dark for 30min at room temperature. The absorbance was read at 745nm and the experiment was performed in triplicate. Radial cation decolourization activity was expressed as the inhibition percentage of cation by the sample and was calculated using the formula:

% ABTS radical scavenging activity = [(control OD – Sample OD) / Control OD)] *100.

IC_{50} value: IC_{50} value (concentration of sample required to scavenge 50% of free radical) were calculated from the regression equation, prepared from the concentration of the samples and percentage inhibition of free radical formation. Ascorbic acid was used as positive control and all tests were carried out in triplicate.

Statistical analysis: The experiments were set up in a completely randomized design. All values obtained from the mean replicates to the variance and presented as mean± standard error (SE). Analysis of variance was conducted by two ways ANOVA and the mean were compared by Tukey HSD test. All statistical analysis was performed at 1% significance level using IBM SPSS Statistics (version 20) by IBM.

RESULTS

Qualitative phytochemical screening: The different qualitative chemical tests were performed for establishing phytochemical profile of six extracts. Phytochemical screening was performed for all the extracts which revealed the presence of alkaloids, flavonoids, phenols and phytosterols in different extracts (Table1).

Quantitative estimation of phytochemicals: The quantitative estimation of the phytochemicals, from cold and hot pseudobulb stem revealed the presence of high alkaloid content (492.63µg mL^{-1}) in cold petroleum ether extract (fig.1), high phenol content (1171.2 µg mL^{-1}) in hot methanol extracts (Fig.2), high content of phytosterols (199.68µg mL^{-1}) in cold methanol extract (Fig.3), high content of flavonoids (65.12µg mL^{-1}) in hot petroleum ether extract (Fig.4) and high total antioxidant content(80.5 µg mL^{-1}) in cold petroleum ether extract(Fig.5). (Table2).

DPPH free radical scavenging activity assay: Free radical scavenging potential of extracts at different concentrations was tested by DPPH method. The percent of scavenging activity and IC_{50} value for standard ascorbic acid were found to be 69.76% and 42.19µg mL^{-1} and hot methanolic extract had showed highest percent of scavenging activity and IC_{50} value of 35.46% and 69.44µg mL^{-1} (Table 3) (fig. 6)

ABTS radical scavenging assay: Radical cation decolourization activity of the plant extracts was tested by ABTS method. The cold methanolic extract has shown the highest percentage of scavenging activity and IC_{50} value of 59.35% and 48.69µg mL^{-1}. The standard ascorbic acid had showed percentage scavenging activity and IC_{50} value of 88.83% and 30.98µg mL^{-1} respectively (Table 3) (fig. 7).

Table 1: Phytochemical screening of six extract of *Pholidota pallida*

Phytochemical Screening	CPE	CCH	CM	SPE	SCH	SM
Alkaloids						
Mayer's	+	+	+	+	+	+
Wagners	+	+	+	+	+	+
Hager's	+	+	+	+	+	+
Dragendroff's	+	+	+	+	+	+
Saponins						
Foam Test	-	-	-	-	-	-
Phytosterols						
Liebemann-Burchards	+	+	+	+	+	+
Phenols						
Ferric chloride	+	+	+	+	+	+
FC reagent	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-

Note:- C: cold, S: hot, PE: petroleum ether, CH: chloroform, M: methanol

Table 2: Quantitative estimation of phytochemicals

Extracts	Alkaloids (µg/ml) X*± SE	Phenols (µg/ml) X*± SE	Phytosterols (µg/ml) X*± SE	Flavonoid (µg/ml)X*± SE	Total Antioxidant (µg/ml) X*± SE
CPE	492.63±0.11	187.2±0.030	23.04±0.011	50.00±0.030	80.50±0.011
CCH	382.63±0.02	171.6±0.015	41.28±0.034	16.96±0.041	31.71±0.010
CM	391.05±0.028	651.6±0.015	199.68±0.023	42.00±0.080	47.68±0.015
SPE	83.00±0.0200	690.0±0.100	172.8±0.020	65.12±0.020	67.96±0.020
SCH	124.20±0.026	643.2±0.011	126.85±0.02	29.60±0.046	10.71±0.011
SM	463.15±0.010	1171.2±0.020	83.65±0.017	16.24±0.000	13.56±0.000

Table 3: Percentage of scavenging activity and IC_{50} value of different extracts against different assays

Extracts	DPPH% of Scavenging	ABTS% of Scavenging	DPPH IC_{50} (µg mL^{-1})	ABTS IC_{50} (µg mL^{-1})
STD	69.76^{a}	88.83^{a}	42.19^{a}	30.98^{a}
CPE	7.72^{e}	16.33^{g}	604.03^{g}	200.32^{g}
CC	23.69^{d}	30.94^{e}	142.99^{e}	81.03^{e}
CM	31.52^{c}	59.35^{b}	73.77^{c}	48.69^{b}
SPE	8.57^{e}	19.35^{f}	428.79^{f}	154.08^{f}
SC	31.04^{c}	35.73^{d}	79.31^{d}	69.31^{d}
SM	35.46^{b}	47.62^{c}	69.44^{b}	53.51^{c}

Note: Mean of 15 replicate. Mean values with different superscripts ([a, b, c, d, e, f, g]) differ significantly at P<0.01 by Tukey (HSD) test

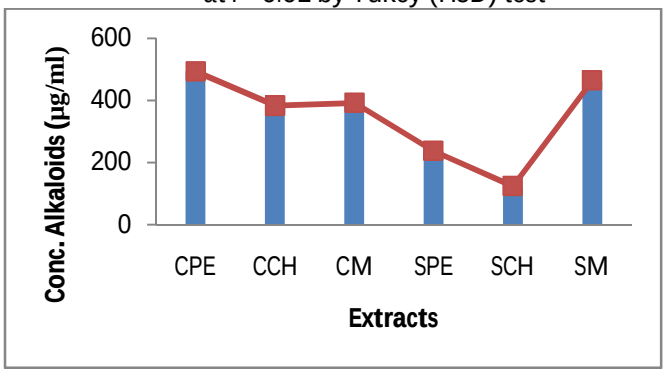

Fig 1: Concentration of Alkaloids (µg mL^{-1}) present in different extracts.

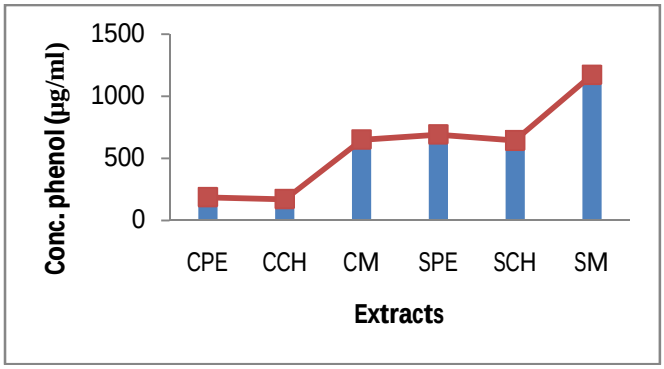

Fig 2: Concentration of Phenols (µg mL^{-1}) present in different extracts.

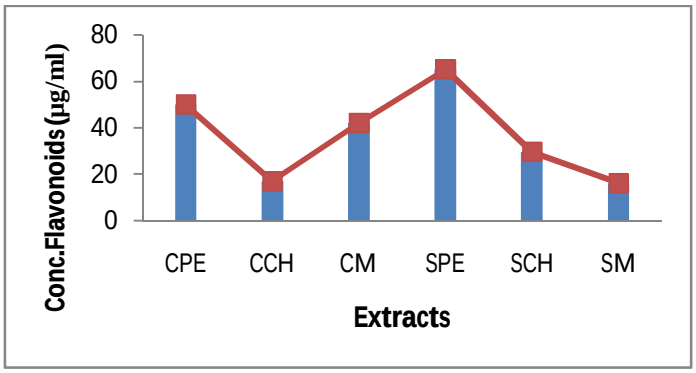

Fig 3: Concentration of Flavonoids (µg mL^{-1}) present in different extracts.

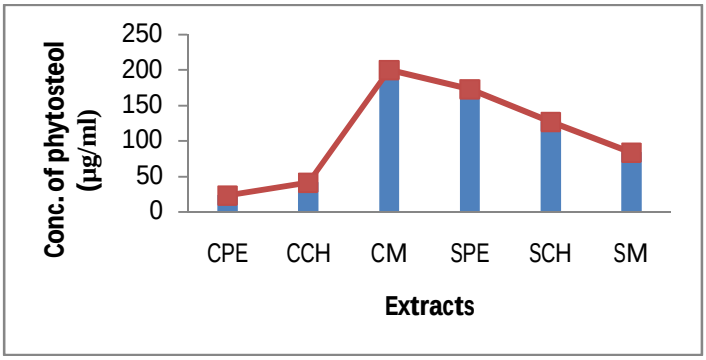

Fig 4: Concentration of Phytosterols (µg mL^{-1}) present in different extracts.

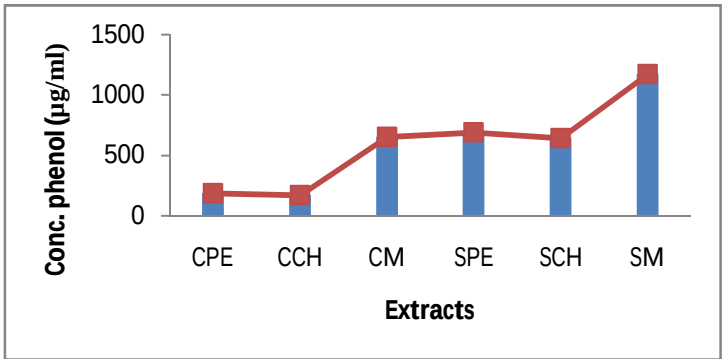

Fig 5: Concentration of Total Antioxidant content (µg mL^{-1}) present in different extracts.

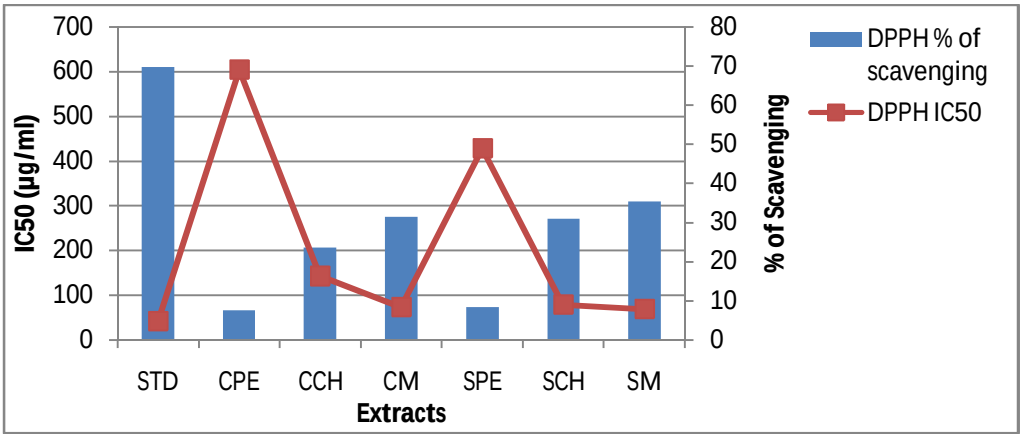

Fig. 6: DPPH scavenging activity of different extracts and its IC_{50} (µg mL^{-1})

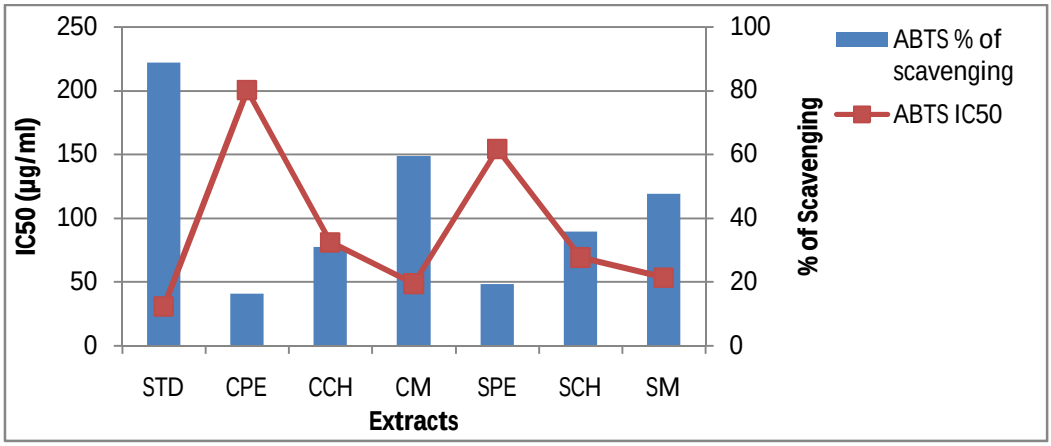

Fig. 7: ABTS scavenging activity of different extracts and its IC_{50} (µg mL^{-1})

DISCUSSION

The hot and cold successive extracts were screened for the presence of various phytochemicals like phenols, alkaloids, phytosterols, flavonoids and total antioxidant contents respectively. The hot extracts revealed the presence highest amount of phenols, and flavonoids. While cold extraction is more effective for extracting alkaloids. These phytochemicals may be responsible for showing scavenging effect on the DPPH radical and ABTS cation radical respectively [2]. The high value of DPPH scavenging activity and ABTS scavenging activity with an evident IC_{50} value was observed. The DPPH is involved in their hydrogen donating ability while ABTS does not involve formation of any intermediate radical but directly produces ABTS mono cation radical. Hence the more scavenging activity of DPPH and ABTS directly correlates with the antioxidant capacity [19,2]. The hot extract has more scavenging activity for DPPH while cold extract has shown better scavenging activity against ABTS. The hot methanolic extract has shown good scavenging activity against DPPH and cold methanolic extract has given better result against ABTS cation radical. It has been observed that the scavenging effect on the free radicals increases sharply

up to certain extent with the increase in the concentration of the samples. A strong correlation has been observed between the total phytochemical content and scavenging capacity [20, 19].

CONCLUSION

The results of the present investigation suggest that the extract of *Phalidota pallida* posse's significantly good antioxidant potential in both established extraction methods. This activity may be due to its various important phyto-constituents that are present in abundance in the plant. The plant can be a source to herbal drug industry. Since the plant is a reservoir of the phytochemicals that can be utilised for the development of phyto-therapeutics. Further research can be taken up to isolate the novel compounds present in the plant and biological evaluation of these novel compounds.

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