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Research Article

FOLKLORE MEDICINAL ORCHIDS FROM SOUTH INDIA: THE POTENTIAL SOURCE OF ANTIOXIDANTS

SAMEERA PARVEEN, RAMESH CK*, RIAZ MAHMOOD, PALLAVI M

[1]Postgraduate Department of Studies and Research in Biotechnology, Jnana Sahyadri Campus, Kuvempu University, Shimoga 577451, Karnataka, India. [2]Department of Studies and Research in Biotechnology, Sahyadri Science College, Kuvempu University, Shimoga, Karnataka, India. Email: ckramck@gmail.com

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ABSTRACT

Objective: Orchids are widely used the economically important ornamental plant. Conventionally, they were also used for the treatment of several diseases. In the present study, five species of medicinal Orchids from South India were selected to evaluate their antioxidant potential.

Methods: The selected species were extracted by Soxhlet method using 70% ethanol. The extracts obtained were analyzed for various quantitative and antioxidant assays followed by correlation analysis in between quantitative and antioxidant activity.

Results: Antioxidant data revealed that among the extracts of five orchids, *Coelogyne breviscapa* was proved to be superior in terms of antioxidant activities, followed by *Aerides maculosum, Dendrobium macrostachyum, Pholidota pallida,* and *Vanda testacea*. Correlation analysis was performed, and the results proved simple positive correlation and highest average value of "r" (correlation coefficient) for antioxidant activities with quantitative were the total antioxidants, total phenolics, total flavonoids, and ascorbic acid content. Among the qualitative antioxidant activities, the highest average value of "r" was shown by 2, 2-diphenyl-1-picrylhydrazyl, iron chelating, 2,2-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid, and superoxide radical.

Conclusion: The study documents that orchid plants have significant antioxidant potential which can contribute to human health.

Keywords: Antioxidant, Correlation, Folklore, Orchids, Phytochemicals.

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INTRODUCTION

Free radicals are reactive chemical species having a single unpaired electron in an outer orbit and are thus unstable [1]. This unstable configuration creates energy to pair with another electron which is released through reactions with adjacent molecules in the cytoplasm of the cell and hence damage it. Humans are constantly exposed to free radicals. Excess of free radicals in the cell prompts a state called "oxidative stress" a major factor in the development and progression of life-threatening diseases, including neurodegenerative and cardiovascular disease [2,3]. The protective effects against free radical damage are balanced by the supplementation of both endogenous and exogenous antioxidant systems combating the undesirable effects of reactive oxygen species (ROS)-induced oxidative damage in the body [4]. Plants are a potent source of useful antioxidant which plays a pivotal role to combat the oxidative stress. Several types of natural and artificial antioxidants are in regular use worldwide for such oxidative stress.

Orchids are belonging to the family Orchidaceae the most highly evolved among monocotyledon with 600–800 genera and 25,000–35,000 species in the world [5]. Orchids were used traditionally in the treatment of a number of diseases, namely, coughing, abdominal pains, heart attack, malaria, tuberculosis, asthma, wounds, bronchitis, ringworm, rheumatism, and kidney disorders [6]. In India, orchids are employed for a variety of therapeutic use in different systems of traditional medicines such as Ayurveda, Siddha, and Unani. Asthavarga is the important ingredient of various classical Ayurvedic formulations such as Chavyanprasa in which four of orchid constituent have been reported, namely, Riddhi, Vriddhi, Jivaka, and Rishbhaka [7]. Recently, there has been tremendous progress in medicinal plants research; however, orchids have not been exploited fully for their medicinal application. Pharmacological and phytochemical investigations may reveal bioactive compounds that could add value to medicinal and related orchid species. In this study, five orchid species with medicinal folk claims were selected, namely, *Aerides maculosum* (AM), *Coelogyne breviscapa* (CB), *Dendrobium macrostachyum* (DM), *Pholidota pallida* (PP), and *Vanda testaceae (VT)* of Karnataka, South India. Review of literature from ethanobotanical reports indicates that the above orchids were used from ancient times for the treatment of various diseases; for example, root and leaf infusion of AM was given for 2 months for tuberculosis [8], paste of pseudobulbs of CB are used for insects bite and swellings [9], tender shoot tips of DM were used as an ear drop for ear ache, pimples, and skin eruptions, and also the plant material was tied overnight to relieve pain [10,11]. Bulb of PP was used in intestinal worms, abdominal pain, and rheumatism [6]. The plant extract of VT called "Rasna" is useful in rheumatism, nervous disorders, and scorpion stings, and leaf is used for cuts and wounds, malarial fever, asthma, earache, antiviral, and anticancer agent [6,12]. Therefore, in the present study, five orchid species with various medicinal folk claims were evaluated for their antioxidant potential by performing various quantitative and antioxidant assays.

METHODS

Collection of plant material

The above selected five orchid species were collected from the forest area of Shimoga District, Karnataka, and were identified and authenticated by Dr. Prashantha K. M, Department of Botany, Sahyadri Science College, Kuvempu University, Shimoga.

Preparation of plant extracts

Different parts of the orchid species were used for the extraction. The selection of different plant parts of orchid species was with respect to medicinal folk claims in various ethnobotanical studies. The parts selected from different orchids for extraction were pseudobulbs in

CB and PP, leaves in AM and VT, and whole plant in DM. The above-selected parts of each orchid were cleaned thoroughly, shade-dried, and pulverized mechanically. Exactly 100 g of powder was subjected to Soxhlet extraction using 70% ethanol. Further, the extracts were concentrated at low temperature and reduced pressure. The yield of crude extracts obtained was noted, stored in desiccators for a maximum of 3 days, and later preserved in the deep freezer (−20°C) for the further use.

Qualitative phytochemical analysis

The preliminary qualitative studies of all the ethanolic extracts of orchids were examined for the presence of various secondary metabolites using standard protocols [13,14].

Determination of quantitative phytochemical analysis

Total phenolic content

The total phenolic content of orchid extracts was determined according to the standard protocol [15]. The total phenolic content of each sample was calculated and expressed as gallic acid equivalent in µg/mg of dry extract. All samples were analyzed in triplicates.

Total flavonoid content

The flavonoid content was determined by aluminum chloride method of Zhishen *et al.* [16]. Total flavonoid content of the extract was expressed as catechol equivalent in µg/mg of dry mass.

Ascorbic acid (AA) content

The AA content was determined by 2,4-dinitrophenyl hydrazine method as described by Sadasivam and Manickam [17]. AA equivalents in µg/mg of extract were calculated using the standard graph of AA.

Total antioxidant capacity

The total antioxidant capacity of all the extracts was performed by phosphomolybdenum method of Prieto *et al.* [18]. The total antioxidant capacity of each extract was expressed as equivalents of AA.

Evaluation for *in vitro* antioxidant activities

2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH free radical scavenging assay was measured by employing the method of Wong *et al.* [19]. The scavenging activity of extracts against DPPH radical was determined by measuring the absorbance at 517 nm. DPPH radical scavenging activity of standard butylated hydroxytoluene (BHT) was assayed for comparison. Radical scavenging capacity was expressed as effective concentration (EC_{50}) which is the amount of antioxidants necessary to decrease the initial concentration by 50%.

2,2-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) radical Scavenging activity

ABTS radical cation (ABTS• +) was produced by reacting ABTS solution 7 mM with 2.45 mM ammonium persulfate incubated in the dark for overnight at room temperature [20]. The scavenging activity of extracts against ABTS radical was determined by measuring the absorbance at 734 nm, and EC_{50} was calculated.

Ferrous ion (Fe^{2+}) chelating

The chelation of ferrous ions was estimated by the method of Dinis *et al.,* 1994 [21]. The effective percentage of ferrozine–Fe^{2+} complex formation was calculated, and the results were expressed as EC_{50}. EDTA was used as a standard metal chelating agent.

Superoxide anion radical scavenging

Superoxide anion radical scavenging activity of orchid extracts and standard BHT was assessed using the method of Nishikimi *et al.* 1972 [22]. The decreased absorbance of the reaction mixture indicated an increased superoxide anion radical scavenging activity. The percentage effect was calculated and expressed as EC_{50}.

Correlation analysis

Correlation analysis was performed to determine the relationship between qualitative antioxidant and quantitative activities [23]. For bivariate analysis, correlation coefficient (r) was analyzed by Pearson method using Graph Pad Prism 5. Regression lines were plotted for EC_{50} values of all the extracts from different qualitative activities against equivalence of respective extract from different quantitative estimations.

Statistical Analysis

All the experimental data were presented as mean±standard error of the three parallel measurements. Analysis of data was conducted using Graph Pad Prism 5-a statistical tool to measure the means and standard error.

RESULTS

Qualitative phytochemical analysis

The results of the preliminary qualitative phytochemical investigation of all the orchid extracts revealed the presence of several bioactive compounds, namely, polyphenols, flavonoids, terpenoids, steroids, glycosides, and alkaloids. Saponins were present only in CB and absent in all other extracts.

Determination of quantitative phytochemical analysis

Total phenolic content

The total phenolic content of five different orchid extracts was expressed in terms of gallic acid equivalent (µg/mg of extract) using

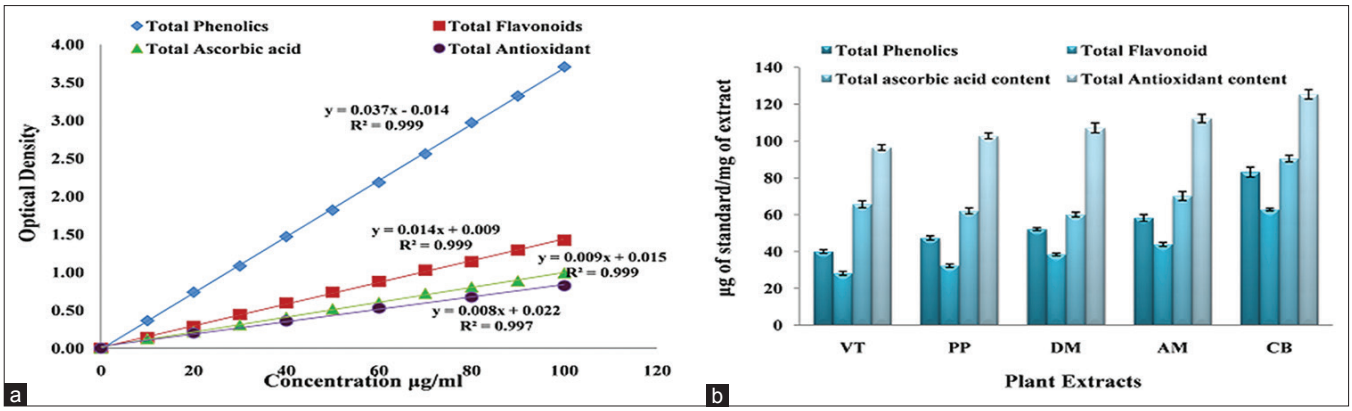

Fig. 1: Quantitative phytochemical evaluation of five Orchids extracts (a) graph of standard curves, (b) Equivalence graph of quantitative estimations

the following equation based on the calibration curve: y=0.037x−0.020, R^2=0.999 (Fig. 1a). Among the five extracts, highest phenolic content was recorded in extracts of CB with 83.13±2.7 µg/mg followed by AM (58.25198±1.82), DM (52.089±0.82), PP (47.321±1.16), and VT (40.027±1.04) (Fig. 1b).

Total flavonoid content

The total flavonoid content of five orchid extracts was expressed in terms of catechol equivalence (µg/mg of extract) using the following equation based on the calibration curve: (y=0.014x+0.009, R^2=0.999) (Fig. 1a). The highest flavonoid content was recorded in the extract of CB with 62.75±0.71, followed by AM (43.86±1.13), DM (38.39±0.79), PP (32.30±0.94), and VT (28.13±1.07) (Fig. 1b).

Total AA content

The AA content of five orchid extracts was compared with the standard curve of AA (y=0.009x+0.015, R^2=0.999) (Fig. 1a), and the results were expressed in terms of AA equivalence in µg/mg of extract. From the results, it was found that the extract of CB have maximum content of AA with 90.44±1.82 µg/mg. For the extracts AM, VT, PP, and DM, the AA content was 70.13±2.39, 65.67±1.31, 62.10±1.60, and 60.07±1.87 µg/mg, respectively (Fig. 1b).

Total antioxidant capacity

The standard curve of AA (y=0.008x+0.022, R^2 = 0.997) (Fig. 1a) was used to express the total antioxidant capacity of five orchid extracts in terms of AA equivalence (µg/mg of extract). The results revealed that the extract of CB consists of a potent antioxidant capacity of 125.35±2.55 µg/mg, followed by AM, DM, PP, and VT with the antioxidant content of 112.21±2.27, 107.12±2.69, 102.75±1.55, and 96.48±1.52 µg/mg, respectively (Fig. 1b).

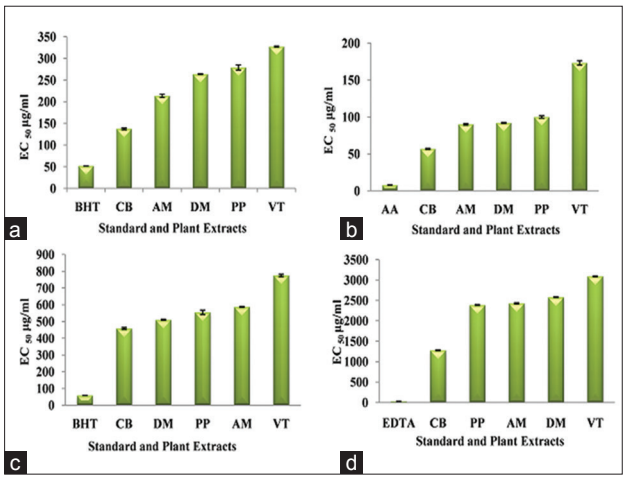

Fig. 2: *In vitro* antioxidant activities (EC_{50}) of five Orchid extracts, (a) *2, 2- diphenyl-1-picrylhydrazyl* radical scavenging activity, (b) 2,2-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid radical scavenging activity, (c) superoxide radical scavenging assay, (d) Fe^2+ chelating activity

Evaluation of *in vitro* antioxidant activities

DPPH radical scavenging

The five different extracts of orchids were screened for free radical scavenging ability by DPPH assay in percentage inhibition and expressed in terms of effective concentration (EC_{50}). The assay is based on the measurement of hydrogen-donating ability of antioxidant molecules present in the extracts to reduce purple color to colorless. The EC_{50} values of test extracts were found to be potent in CB (137.31±2.15 µg/ml), followed by AM (213.54±3.56 µg/ml), DM (263.59±1.13 µg/ml), PP (278.83±5.84 µg/ml), and VT (327.26±1.11 µg/ml). The results were compared with standard BHT (EC_{50}=51.34 µg/ml) (Fig. 2a). The results revealed that extracts act on the metal ion in a dose-dependent manner in terms of $EC_{50.}$

ABTS radical scavenging

ABTS is a protonated radical that has a characteristic maximum of 734 nm, which decreases with the scavenging of proton radicals. The scavenging effect of ABTS increased with concentration. Fig. 2b shows the ABTS scavenging ability of extracts and standard in dose-dependent manner, and the results were expressed in effective concentration (EC_{50}). The EC_{50} results obtained revealed that the extract CB is having highest scavenging activity with 56.69±0.71µg/ml, followed by AM, DM, PP, and VT with EC_{50} of 89.99±0.71, 91.86±1.11, 99.82±1.77, and 172.95±2.97 µg/ml, respectively. The EC_{50} of standard AA was 7.75±0.12 µg/ml.

Superoxide radical scavenging

The superoxide scavenging activity by PMS–NADH–NBT system where radicals generated from dissolved oxygen by PMS-NADH coupling has the ability to reduce NBT and measured the decrease in absorbance at 560 nm with the orchid extracts and standard BHT having the capacity to quench radicals in the reaction mixture. As shown in Fig. 2c, the EC_{50} value of standard BHT was 57.93±0.67 µg/ml and of extracts are in the order: CB, DM, AM, PP, and VT with values of 457.93±5.98, 510.02±3.45, 554.31±12.93, 586.59±3.03, and 774.68±7.34 µg/ml, respectively.

Ferrous Ion (Fe^{2+}) chelating

Ferrous ions (Fe^{2+}) chelating estimation in extracts may render important antioxidative effects by retarding metal-catalyzed oxidation which assesses the chelation capacity of the coexisting chelator. According to the results, the extract of CB possesses potent antioxidant activity with the EC_{50} value of 1276.56±12.95 µg/ml. The other extracts of PP, AM, DM, and VT have registered EC_{50} of 2385.69±9.84, 2427.64±14.73, 2574.96±8.40, and 3090.23±10.53 µg/ml, respectively (Fig. 2d). However, the EC_{50} value of standard EDTA was 25.73±0.23 µg/ml. Therefore, the decrease in concentration-dependent color formation in the presence of the extract indicates that it has iron chelating activity.

Correlation analysis

The correlations between quantitative estimation of plant extracts and antioxidant activity assays were also studied using the Pearson's correlation analysis, and the results are represented in Table 1. Among the quantitative and qualitative activities examined for five orchid extracts, a simple positive correlation was found between all the variables. From the results, it is noticeable that highest average

Table 1: Relationship between qualitative and quantitative antioxidant activities correlation coefficients (r) value

Quantitative estimation	Correlation coefficient (r)				
	DPPH	ABTS	Superoxide	Iron Chelating	Average
Total phenolics	0.982	0.825	0.742	0.960	0.877
Total flavonoids	0.983	0.816	0.726	0.937	0.865
Total antioxidant	0.996	0.874	0.782	0.940	0.898
Total AA	0.853	0.525	0.405	0.843	0.657
Average	0.954	0.760	0.664	0.920	

AA: Ascorbic acid, DPPH: 2, 2- diphenyl-1-picrylhydrazyl, ABTS: 2,2-Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid

value of "r" (correlation coefficient) between quantitative and antioxidant activities (DPPH, ABTs, superoxide, and iron chelating) was 0.898, 0.877, 0.865, and 0.657 for total antioxidant, total phenolics, total flavonoids, and AA content, respectively. Among the qualitative antioxidant activities with quantitative relation, the highest average value of "r" was shown by DPPH radical scavenging (0.954), followed by iron chelating (0.920), ABTS (0.760), and superoxide radical (0.664). It is clear from the results that there is a direct correlation between quantitative measures of antioxidant phytoconstituents and their antioxidant expression through various assays performed.

DISCUSSION

Nature and plants play a significant role in serving and maintaining human life and his health by providing a valuable source of novel natural products. The role of plants in medicine is expanding beyond their traditional uses and enduring in new drugs research and development. The exploration of traditional plant medicine conducted with modern theories and technique can enrich western medicine by absorbing new idea and concepts from traditional plant medicine from all over the world. Nowadays, numerous modern drugs have been isolated from traditional plants of medicinal uses [24].

The orchids are also known traditionally as the remedy for several diseases despite their ornamental importance. Some of the orchids are listed in the earliest known Chinese *Materia Medica* used in treating many diseases. Several orchids such as *Orchis latifolia*, *Orchis mascula*, *Cymbidium aloifolium*, and *Zeuxine strateumatica* and some species of *Dendrobium*, *Eulophia*, and *Habenaria* can treat many of diseases [25]. In India, orchids have been used in medicinal treatment since Vedic period, but the potential of most of the orchid species for therapeutic use is yet to be explored scientifically.

Plants with medicinal values are the wealthy resource of bioactive substances that produce a distinct physiological action on the human body [26]. Many studies on orchids have been conducted so far, and many phytochemicals and pharmaceutical properties were also reported. In the present investigation, selected five orchid species were subjected to qualitative and quantitative phytochemical estimation and its antioxidant ability. The phytochemical estimation reveals the presence of bioactive compounds such as alkaloids, flavonoids, tannins, phenols, terpenoids, and steroids. Based on the recent literature, the presence of bioactive compounds has been reported in few of the different parts of the orchid's species, namely, *Coelogyne stricta* [27], *Dendrobium pandurantum* [28], *Vanda tessellata* [29], *Rhynchostylis retusa* [30], *C. aloifolium* [31], *Phalaenopsis* [32], *Luisia zeylanica* [33], and *Geodorum densiflorum* [34]. Thus, the orchid plants have an abundant source of phytochemicals having important properties such as antioxidant activity. Hence, plants are being examined closely for quantitative phytochemical and antioxidants, owing to the beneficial health effects of phytochemical and antioxidants [35].

Several plants with potent antioxidant activities have been reported to possess significant free radical scavenging activity [36,37]. Aqueous extract of aseptically regenerated *Dendrobium aqueum* was used for *in vitro* estimation of antioxidant potential showed a dose-dependent DPPH free-radical scavenging potential [38]. According to Chand *et al.* [39], thirteen wild orchid species of Nepal showed total flavonoid and phenolic content and their antioxidant activity in a considerable manner. The ethanol extracts of the orchids in the present study, especially those orchids that are recorded as folklore medicinal value, showed the considerable amount of total flavonoids, total phenols, total AA, and total antioxidant content potentiating to their higher antioxidant activity. Thus, ethanolic extracts of five orchids exhibited potent antioxidant activities in all the assays, namely, DPPH, ABTS, superoxide, and iron chelating radical scavenging assays. Among the five orchids, extracts of CB was proved to be superior in terms of antioxidant activities, followed by AM and DM and then PP and VT. The data, therefore, suggest that the extracts of orchids are a potential source of natural antioxidants and may be due to the presence of phytoconstituents, namely, alkaloids, sterols, phenolic and flavonoids, and glycosides which are known for their antioxidant property [40]. The phenolics and flavonoids are well-known antioxidants which provide protection against the oxidative stress generated from ROS at the cellular level and damaging effects of free radicals [41]. The reduction activity of these compounds serves as a significant indicator of its antioxidant potential by donating hydrogen atoms to the radical molecules and terminating free radical chain reaction which would strengthen the steric hindrance [42,43].

The present work documents that antioxidant components of five orchid extracts are responsible for their lower percentage inhibition associated with high scavenging activity. The correlation study was performed using a bivariate analysis for ascertaining the strength of the relationship between the antioxidant activities and quantitative analysis. The degree of correlation in the study suggests a simple, positive, and high degree of correlation existing between the variables tested. It is evident from the results that the quantitative estimations performed in the study are positively correlated with the antioxidants studies wherein total antioxidant recorded a high "r" (correlation coefficient) value followed by total phenolics, total flavonoids, and AA content. Previous reports have indicated that there is a direct correlation between antioxidant activity and the presence of phenolics, flavonoids, and total antioxidants in the plants [23,43-46]. The average "r" value was found to be the highest in DPPH radical scavenging activity (0.954) followed by other assays under study irrespective of quantitative phytochemical estimations, indicating that it is a best suited and reliable radical scavenging activity [47,48]. The linear expression obtained from regression analysis is helpful in measuring the variables in terms of qualitative and quantitative parameters. Researchers have shown that the antioxidants of plant origin with free-radical scavenging properties could have enormous importance as therapeutic agents in diseases caused due to oxidative damage.

CONCLUSION

On the basis of the results obtained, it can be concluded that the ethanolic extracts of five orchids exhibit high antioxidant potential. The correlation study also proves that total antioxidant, total phenolic, flavonoids,and AA content shows a good correlation among antioxidant activities, thereby indicating the good source of antioxidants preventing various oxidative damages. Orchid plants as mentioned in folklore have the significant contribution to human health. These plants can be further assessed to rigorous experimentation expand beyond their traditional use and could be targeted for much needed therapeutic agents into viable bioactive compounds responsible for the therapy and treatments. Further research in this direction is underway.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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