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

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ABSTRACT

Objective: Orchids are widely used 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 brevisscapa was proved to be superior in terms of antioxidant activities, followed by Aerides maculosaum, 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.

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 protecive 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 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. Athavarga is the important ingredient of various classical Ayurvedic formulations such as Chaeyanprasa in which four of orchid constituent have been reported, namely, Riddhi, Vridhidi, Jwaka, 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 maculosaum (AM), Coelogyne brevisscapa (CB), Dendrobium macrostachyum (DM), Pholidota pallida (PP), and Vanda testacea (VT) of Karnataka, South India. Review of literature from ethnobotanical 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 leafis 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. Prashanthaa 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 GraphPad 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
the following equation based on the calibration curve: \( y = 0.037x - 0.20, R^2 = 0.999 \) (Fig. 1a). Among the five extracts, highest phenolic content was recorded in extracts of CB with 83.13±2.7 \( \mu g/mg \) 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 (\( \mu g/mg \) 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 (30.39±0.79), PP (32.30±0.94), and VT (28.13±1.07) (Fig. 1b).

**Total antioxidant capacity**

The standard curve of AA (eq. 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 (\( \mu g/mg \) of extract). The results revealed that the extract of CB consists of a potent antioxidant capacity of 125.35±2.55 \( \mu g/mg \), 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 \( \mu g/mg \), respectively (Fig. 1b).

**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 \( \mu g/ml \)) followed by AM (213.54±5.56 \( \mu g/ml \)), DM (263.59±1.13 \( \mu g/ml \)), PP (270.83±5.84 \( \mu g/ml \)), and VT (327.26±1.11 \( \mu g/ml \)). The results were compared with standard BHT (\( EC_{50} = 51.34 \mu g/ml \)) (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 has highest scavenging activity with 56.69±0.71 \( \mu g/ml \) 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 \( \mu g/ml \), respectively. The \( EC_{50} \) of standard AA was 7.75±0.12 \( \mu g/ml \).

**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 \( \mu g/ml \) 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 \( \mu g/ml \), 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 \( \mu g/ml \). The other extracts of PP, AM, DM, and VT have registered \( EC_{50} \) of 2385.69±9.84, 2427.64±14.73, 2574.96±18.40, and 3090.23±10.53 \( \mu g/ml \), respectively (Fig. 2d). However, the \( EC_{50} \) value of standard EDTA was 25.73±0.23 \( \mu g/ml \). 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 coefficient (r) |
|---|---|---|---|---|---|
| **Quantitative estimation** | **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 | 0.857 | 0.845 | 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 ideas and concepts from traditional plant medicine from all over the world. Nowadays, numerous modern drugs have been isolated from traditional plants of medical 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 medical 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 panduratum [28], Vanda tessellata [29], Rhynchostylis retusa [30], C. aloifolium [31], Phalaenopsis [32], Luaisa zeylanica [33], and Geodorum densiforum [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 phytochemicals 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 aequum 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, steroids, 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 its 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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