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## The antioxidant activity of selected wild orchids of Nepal

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### ABSTRACT

**Objective:** To assess the antioxidant activity as well as the total polyphenolics and flavonoids content of thirteen extracts from nine wild orchids of Nepal.

**Methods:** First, a preliminary phytochemical screening of thirteen extracts of nine orchids was carried out using established protocols. Then, the total polyphenolic and flavonoid contents of each extract were evaluated using the Folin-Ciocalteu's phenol reagent and aluminium chloride methods respectively. Finally, antioxidant activity was determined by 2, 2-diphenyl-1-picryl hydrazyl free radical scavenging assay and expressed in terms of half inhibition concentrations (IC<sub>50</sub> values).

**Results:** All the tested parameters showed significant variation at  $P = 0.05$ . The total flavonoids varied with the greatest amounts found in the leaves of *Rhynchostylis retusa* and the smallest amounts found in the roots of *Gastrochilus acutifolius* (*G. acutifolius*). The amount of total polyphenolics was highest in the stems of *Vanda cristata* and lowest in the leaves of *G. acutifolius*. Antioxidant activity was highest in the stems of *Vanda cristata* and lowest in the leaves of *G. acutifolius*.

**Conclusions:** Some of the orchid extracts studied exhibited levels of antioxidant activity which were comparable to or even higher than those found for extracts of medicinal plants and orchids studied previously. It is suggested that drugs and natural food additives can be extracted from those wild orchids with high levels of antioxidant activity.

## 1. Introduction

Some chemicals, whether synthetic or natural, inhibit or delay oxidative damage to tissues by free radical, namely, superoxide anions, hydroxyl radicals and hydrogen peroxide. These chemicals are known as antioxidants and their action is known as antioxidant activity[1,2]. Plant phenolics or polyphenols include four major classes: hydroxycinnamic acids, flavonoids, anthocyanins and tannins, all of which function as antioxidants and are produced in all higher plants along with a diverse array of phytochemicals[3]. Polyphenols are commonly found in both edible and non-edible plants and mainly stored in flowering tissues, leaves, stems, barks and berries and other fruits[4]. Extracts of various parts of plants show varying levels of antioxidant activity depending on which part or parts of given plant polyphenols accumulate in. In addition

to their antioxidant activity, phytochemicals are involved in other biological and preservative activities.

Natural antioxidants, especially flavonoids, have multiple biological effects including antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic and vasodilatory actions[1]. Some natural antioxidants, such as the extracts of rosemary and sage, have already been exploited commercially, either as food additives or as nutritional supplements[5]. Other natural antioxidants, like tocopherols and ascorbic acid and their derivatives, are used as alternative antioxidants in foods. Recently, researchers have discovered that plant polyphenolic compounds can be used as additives for retaining the quality of fish and fish products[6].

Reports on the importance of ethno-pharmaceutical practices abound, as do phytochemical and pharmacological studies, include those on the antioxidant activity of Nepali medicinal plants. The literatures state that some of the orchids selected for this study are traditional medicines used by different ethnic groups of Nepal and its neighbouring countries to cure diseases and ailments. The leaves of *Luisia trichorhiza* (*L. trichorhiza*)[7] and the pseudobulbs of *Otochilus albus* (*O. albus*), for example, are said to cure chronic wounds and bone fractures, respectively[8]. *Pholidota articulata* (*P. articulata*) is used to treat skin ulcers, skin eruptions and dislocated bones, and the leaves of *Rhynchostylis retusa* (*R. retusa*) are used to

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treat rheumatism[8]. Powder made from the leaves of *Vanda cristata* (*V. cristata*) is said to be an expectorant and its paste is applied on cuts and wounds[9].

Though there are reports on the ethnomedicinal and ethnoveterinary usages of the Nepali orchid species selected for this study, there are almost no reports on their phytochemical and biological activities including their antioxidant activity. To fill in this gap, the antioxidant activity and total polyphenolic and flavonoid contents of the ethanolic extracts of the leaves and roots of *Gastrochilus acutifolius* (*G. acutifolius*), the entire plant of *Gastrochilus distichus* (*G. distichus*), the leaves and roots of *L. trichorhiza*, the pseudobulbs of *O. albus*, the whole plant of *Papilionanthe uniflora* (*P. uniflora*), the pseudobulbs of *Pinalia graminifolia* (*P. graminifolia*), the leaves and pseudobulbs of *P. articulata*, the leaves of *R. retusa*, and the leaves and stems of *V. cristata* were investigated. The antioxidant activity of these thirteen extracts was assessed using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay, their total flavonoid contents using the aluminium chloride method, and their total polyphenolics content using the Folin-Ciocalteu's phenol reagent method. Also, the relationships between DPPH free radical scavenging activity and total polyphenolics and flavonoids contents using regression equations were evaluated.

## 2. Materials and methods

### 2.1. Collection of plant materials

Nine wild orchids from different regions in Nepal were selected. Between May and November 2013, the leaves and roots of both *G. acutifolius* and *L. trichorhiza*, the pseudobulbs of *O. albus* and the leaves and stems of *V. cristata* were collected from Salyan District in the mid-western region. The leaves and pseudobulbs of *P. articulata* and the leaves of *R. retusa* were collected from Kathmandu District in the central region. And the entire plants of both *P. uniflora* and *G. distichus*, and the pseudobulbs of *P. graminifolia* were collected from Makawanpur District, also in the central region. Each material was identified and authenticated by the authors using standard literature on Nepalese orchids.

### 2.2. Drying and extraction

The above mentioned parts of each orchid were shade dried and ground into fine powders using an electric grinder. The fine powder was then extracted by Soxhlet extraction using 70% ethanol as a solvent. Then, the solvent was dried using a rotary evaporator (BUCHI Labortechnik AG, Flawil, Switzerland) at a low temperature and reduced pressure. The brown to dark pastes of the extracts were put in amber-coloured tiny glass bottles at 4 °C for further investigation.

### 2.3. Preliminary phytochemical screening

Each orchid extract was tested for the presence of alkaloids, flavonoids, saponins, steroids, terpenoids and tannins using established protocols[10,11].

### 2.4. Estimation of total polyphenolics and flavonoids contents

The total flavonoid content in each orchid extract was estimated using the standard protocol with a slight modification[12]. Each

orchid extract (2 mL, 0.5 mg/mL) was mixed with an aluminium chloride solution (1 mL, 2%) and allowed to react for 1 h at room temperature. Then, the absorbance of the mixture was measured at 415 nm using a UV spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA). The calibration curve of quercetin, which was chosen as the reference compound, was taken from a series of its solutions at increasing concentrations of 25, 50, 75 and 100 µg/mL. A mixture of 1 mL of the aluminium chloride reagent with 2 mL of ethanol instead of 2 mL of plant extract solution was used as a blank. The total flavonoid content in each orchid extract was calculated by interpolating the absorbance of mixture in a linear equation of calibration curve and was expressed in terms of milligrams of quercetin equivalent per gram (mg QE/g) of dry extract.

The total polyphenolics content was determined by a standard protocol with minor modifications[12]. Each orchid extract (0.5 mL, 1 mg/mL) was mixed with Folin-Ciocalteu's phenol reagent (2.5 mL, 10%) and aqueous sodium bicarbonate solution (2.5 mL, 7.5%) and allowed to react for about 45 min at room temperature. The absorbance of the mixture was then measured at 765 nm using a UV spectrophotometer. The calibration curve of gallic acid was obtained from a series of its solution with different concentrations of 25, 50, 75 and 100 µg/mL. The Folin-Ciocalteu's phenol reagent mixed with 0.5 mL of ethanol instead of 0.5 mL of orchid extract solution was used as a blank. The total polyphenolics content of each sample was calculated using the linear equation of the gallic acid calibration curve and was expressed in terms of milligrams of gallic acid equivalent per gram (mg GAE/g) of dry extract.

### 2.5. DPPH assay

The antioxidant activity of the orchid extracts was determined using a DPPH assay slightly modified from the standard protocol[2]. Each orchid extract was made into a series of solutions with concentrations of 50 µg, 100 µg, 200 µg, 400 µg and 800 µg per mL. The solution of orchid extract (1.5 mL of each concentration in the series) was mixed with DPPH solution (1.5 mL, 0.25 mmol/L in ethanol) to make five sample solutions and with absolute ethanol (1.5 mL) to make five blank solutions. DPPH solution (1.5 mL, 0.25 mmol/L) was mixed with absolute ethanol (1.5 mL) to make a control solution. Since quercetin was the chosen reference compound, solutions of it at various concentrations were prepared exactly as they were for the orchid extracts. All of the quercetin solutions were vigorously shaken and allowed to react for 30 min after putting them in the dark immediately after preparation. The absorbance of each solution was taken at 517 nm using a UV spectrophotometer.

The scavenging activity of the orchid extracts and the reference compound (quercetin) was calculated using the following equation: Scavenging rate =  $[1 - (A_1 - A_2)/A_0] \times 100\%$  where  $A_0$  is the absorbance of the control solution,  $A_1$  is the absorbance of the sample solution (*i.e.* in the presence of an orchid extract) and  $A_2$  is the absorbance of the blank solution (*i.e.* without DPPH).

Finally, the IC<sub>50</sub> value of each orchid extract was determined using the non-linear regression equation calculated by plotting the percentage of antioxidant activity against concentration and expressed as µg/mL.

### 2.6. Statistics and software

The total flavonoids and total polyphenolics contents were determined and the DPPH free radical assay was done in triplicate form. The values of the total flavonoids and total polyphenolics contents were presented as mean ± SD. For antioxidant activity, a single value of IC<sub>50</sub> was obtained from a regression model second or third order polynomial equation for a plot of the percentage of scavenging activity against the concentration of orchid extract. The variation in total flavonoids and total polyphenolics contents and antioxidant activity between the ethanolic extracts was analyzed by a one sample *t*-test at *P* = 0.05. A *post hoc* test using the Tukey's honestly significant difference test was carried out to determine the actual difference between the mean ± SD values of the total flavonoids and total polyphenolics contents and antioxidant activity (IC<sub>50</sub>).

The data were collected in MS Excel 2007. Version 3.1.2 of statistical software R was used to calculate IC<sub>50</sub> values and to test the variation in antioxidant activity among the extracts and against the reference compound (quercetin). The relationships between antioxidant activity and both total flavonoids and total polyphenolics contents were found by obtaining a regression equation (*y* = *mx* + *c*) in MS Excel 2007.

## 3. Results

### 3.1. Preliminary phytochemical screening

Collectively, 13 orchid samples showed the presence of all five tested phytochemical classes, such as alkaloids, flavonoids, saponins, steroids and terpenoids, and tannins, but some individual samples lacked some phytochemical classes. Only the leaves and roots of *G. acutifolius* had all four phytochemicals, but all orchid extracts had flavonoids and tannins, the phytochemicals that carry out antioxidant activity. Details are shown in Table 1.

### 3.2. Total polyphenolics and flavonoids contents

The 13 orchid extracts exhibited significant variation in total flavonoid content at *P* = 0.05 with *t* = 9.88 and *df* = 38. The leaves of *R. retusa* [(110.68 ± 4.52) mg QE/g] and the roots of *G. acutifolius* [(22.32 ± 1.10) mg QE/g] had the highest and lowest total flavonoids contents, respectively. Other than the leaves of *R. retusa*, only three orchid extracts, the entire plant of *P. uniflora* and the leaves of both

*P. articulata* and *V. cristata* had total flavonoids contents above 80 mg QE/g. All eight other extracts had total flavonoids contents below 50 mg QE/g. Details are given in Table 2.

The total polyphenolics contents of 13 orchid extracts varied significantly with *t* = 15.174 and *df* = 38 at *P* = 0.05. The highest and lowest total polyphenolics contents were observed in the stems of *V. cristata* [(69.68 ± 2.78) mg GAE/g] and the leaves of *P. graminifolia* [(11.89 ± 0.64) mg GAE/g], respectively, but the majority of extracts had values between 20 and 45 mg GAE/g. Details are provided in Table 3.

**Table 2**

Total flavonoids contents in the ethanol extracts of selected orchids.

Name of orchid	Part used	Total flavonoids contents (mg QE/g)
<i>P. graminifolia</i>	Pseudobulbs	40.63 ± 1.85 <sup>d</sup>
<i>G. acutifolius</i>	Leaves	29.09 ± 1.99 <sup>e</sup>
	Roots	22.32 ± 1.10 <sup>f</sup>
<i>G. distichus</i>	Entire plant	22.99 ± 3.28 <sup>f</sup>
<i>L. trichirhiza</i>	Leaves	48.19 ± 4.57 <sup>e</sup>
	Roots	32.87 ± 2.31 <sup>e</sup>
<i>O. albus</i>	Pseudobulbs	23.05 ± 0.30 <sup>f</sup>
<i>P. uniflora</i>	Entire plant	90.58 ± 2.82 <sup>b</sup>
<i>P. articulata</i>	Leaves	95.09 ± 0.94 <sup>b</sup>
	Pseudobulbs	24.84 ± 0.70 <sup>f</sup>
<i>R. retusa</i>	Leaves	110.68 ± 4.52 <sup>a</sup>
<i>V. cristata</i>	Leaves	83.48 ± 2.41 <sup>b</sup>
	Stems	27.96 ± 1.70 <sup>e</sup>

Total flavonoids contents with different superscripts differ at *P* = 0.05. The values are expressed as mean ± SD.

**Table 3**

Total polyphenolics contents of the ethanol extracts of selected orchids.

Name of orchid	Part used	Total polyphenolics contents (mg GAE/g)
<i>P. graminifolia</i>	Pseudobulbs	30.04 ± 4.78 <sup>d</sup>
<i>G. acutifolius</i>	Leaves	11.89 ± 0.64 <sup>e</sup>
	Roots	19.52 ± 1.36 <sup>e</sup>
<i>G. distichus</i>	Entire plant	22.29 ± 1.90 <sup>e</sup>
<i>L. trichirhiza</i>	Leaves	41.77 ± 2.99 <sup>c</sup>
	Roots	51.03 ± 0.70 <sup>b</sup>
<i>O. albus</i>	Pseudobulbs	33.04 ± 0.47 <sup>d</sup>
<i>P. uniflora</i>	Entire plant	40.29 ± 2.94 <sup>cd</sup>
<i>P. articulata</i>	Leaves	33.10 ± 1.22 <sup>d</sup>
	Pseudobulbs	57.66 ± 1.59 <sup>b</sup>
<i>R. retusa</i>	Leaves	38.93 ± 5.86 <sup>cd</sup>
<i>V. cristata</i>	Leaves	39.42 ± 3.59 <sup>cd</sup>
	Stems	69.68 ± 2.78 <sup>a</sup>

Total polyphenolics contents with different superscripts differ at *P* = 0.05. The values are expressed as mean ± SD.

**Table 1**

Photochemical constituents of selected orchid species.

Name of orchid	Plant part	Alkaloids	Flavonoids	Saponins	Steroids and terpenoids	Tannins
<i>P. graminifolia</i>	Pseudobulbs	+	+	-	-	+
<i>G. acutifolius</i>	Leaves	+	+	+	+	+
	Roots	+	+	+	+	+
<i>G. distichus</i>	Entire plant	-	+	+	+	+
<i>L. trichirhiza</i>	Leaves	+	+	-	+	+
	Roots	+	+	-	+	+
<i>O. albus</i>	Pseudobulbs	+	+	-	-	+
<i>P. uniflora</i>	Entire plant	+	+	-	+	+
<i>P. articulata</i>	Pseudobulbs	+	+	-	+	+
	Leaves	+	+	-	+	+
<i>R. retusa</i>	Leaves	-	+	-	+	+
<i>V. cristata</i>	Stems	+	+	-	+	+
	Leaves	+	+	-	-	+

+: Presence; -: Absence.

### 3.3. Antioxidant activity

Antioxidant activity varied significantly among the ethanol extracts of the 13 orchid samples ( $t = 7.3794$ ,  $df = 13$ ,  $P < 5.35e-06$ ). The stems of *V. cristata* ( $IC_{50} = 79.69 \mu\text{g/mL}$ ) and the leaves of *G. acutifolius* ( $IC_{50} = 341.79 \mu\text{g/mL}$ ) had the highest and lowest DPPH radical-scavenging activities, respectively, meaning that the ethanol extracts of the stems of *V. cristata* had the highest antioxidant activity and the leaves of *G. acutifolius* had the lowest. However, none of the orchid extracts exhibited as much DPPH free radical-scavenging activity as quercetin did, the reference compound ( $IC_{50} = 32.90 \mu\text{g/mL}$ ). The rest of the extracts had  $IC_{50}$  values ranging from  $89.18 \mu\text{g/mL}$  (the pseudobulbs of *P. articulata*) to  $209.78 \mu\text{g/mL}$  (the roots of *L. trichorhiza*). The antioxidant activity of the remaining extracts ranged as follows: leaves of *V. cristata* > leaves of *R. retusa* > leaves of *L. trichorhiza* > pseudobulbs of *P. graminifolia* > pseudobulbs of *O. albus* > entire plant of *G. distichus* > roots of *G. acutifolius* > entire plant of *P. uniflora* > leaves of *P. articulata*. Details are given in Table 4.

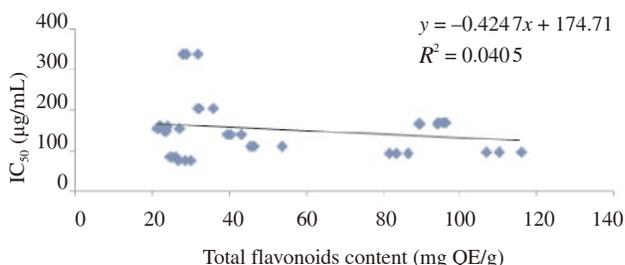
**Table 4**

$IC_{50}$  values of ethanol extracts of selected orchid samples and quercetin.

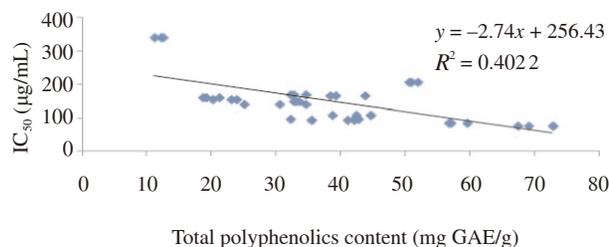
Name of orchid/compound	Part used	$IC_{50}$ ( $\mu\text{g/mL}$ )
<i>P. graminifolia</i>	Pseudobulbs	143.60
<i>G. acutifolius</i>	Leaves	341.79
	Roots	163.37
<i>G. distichus</i>	Entire plant	159.15
<i>L. trichirhiza</i>	Leaves	113.18
	Roots	209.78
<i>O. albus</i>	Pseudobulbs	152.57
<i>P. uniflora</i>	Entire plant	170.67
<i>P. articulata</i>	Leaves	172.84
	Pseudobulbs	89.18
<i>R. retusa</i>	Leaves	100.42
<i>V. cristata</i>	Leaves	98.23
	Stems	79.69
Quercetin	Reference compound	32.90

### 3.4. Relationship between antioxidant activity and total polyphenolic and flavonoid contents

The  $IC_{50}$  values for the DPPH radical-scavenging activity of the ethanol extracts of the 13 orchid samples were statistically and negatively correlated with the total polyphenol and flavonoid content of those same samples. The negative association between  $IC_{50}$  values and total flavonoid content was found using the regression equation  $y = -0.4247x + 174.71$  and  $R^2 = 0.0405$  (Figure 1). The high negative association between  $IC_{50}$  and total polyphenolic contents was determined using the regression equation  $y = -2.74x + 256.43$  and  $R^2 = 0.4022$  (Figure 2). This negative association indicated that an increase in polyphenolic and flavonoid content caused an increase in antioxidant activity.



**Figure 1.** Regression of  $IC_{50}$  with total flavonoids content.



**Figure 2.** Regression of  $IC_{50}$  against total polyphenolics content.

## 4. Discussion

### 4.1. Total flavonoids contents

The total polyphenolic and flavonoid contents in the ethanol extracts of the 13 orchid samples investigated were both similar and different from the various extracts of medicinal plants, orchids and other products previously studied. Ethanol extracts of the entire plant of *G. distichus*, the roots of *G. acutifolius*, and the pseudobulbs of both *O. albus* and *P. articulata* had greater total flavonoids contents than those in the *Pleione bulbocodioides* extracts plants [ $(1.73 \pm 0.98)$  mg QE/g] studied according to a study of Chinese medicinal plants[13]. The total flavonoids contents previously found in aqueous and methanol extracts of *Dalbergia sissoo* Roxb. [ $(45.33 \pm 0.14)$  and  $(49.41 \pm 0.49)$  mg QE/g, respectively] were comparable with the value this study found for an ethanol extract of the leaves of *L. trichorhiza*[14]. The total flavonoid content of a methanol extract of the roots of *Canthium subcordatum*, a Nigerian medicinal plant [ $(15.60 \pm 1.60)$  mg rutin equivalent/g] was considerably lower than the contents this study found for all the ethanol extracts of *G. acutifolius* and *G. distichus*, while previous findings for *Eupatorium adenphorum* [ $(63.60 \pm 8.45)$  mg rutin equivalent/g] were no higher than the value this study found for the leaves of *V. cristata*, only the fourth highest value of the 13 studied samples[15]. The total flavonoid content in a methanol extract of the leaves and stems of *Dendrobium speciosum* was less than that of all the extracts of our investigation.

The total flavonoid content in the ethanol extracts of the present study was noticeably higher than those of 10 different honey samples from Brazilian Pampa biome, which contained  $(1.8 \pm 0.4)$  to  $(4.2 \pm 0.5)$  mg QE/100 g of honey[16]. The total flavonoids contents in the pseudobulbs of both *O. albus* and *P. articulata* and the stems of *V. cristata* were the closest of the samples tested to the flavonoid content of the fruit of *Syzygium cumini* found in an earlier study[17].

### 4.2. Total polyphenolic contents

Plants important for diverse reasons, including food (fruits) and medicine, have varying total polyphenolic contents, as other products of plant origin. The present study also revealed that the total polyphenolic contents of the ethanol extracts of the 13 selected orchid samples varied significantly.

The orchids analyzed in this study had lower or moderately higher total polyphenolics contents than medicinal plants analyzed

in previous studies and they had values comparable to those of other plant extracts. The highest total polyphenolics content in this study was in the stems of *V. cristata* [(69.68 ± 2.78) mg GAE/g]. It was nearly half the content of aqueous and ethanolic extracts of the Chinese medicinal plant *Sanguisorba officinalis* [(148.09 ± 2.46) and (121.42 ± 0.78) mg GAE/g, respectively][13]. The total polyphenolic contents of the roots of *L. trichorhiza* [(51.03 ± 0.07) mg GAE/g] and the pseudobulbs of *P. articulata* [(57.66 ± 1.59) mg GAE/g] found in this study were comparable with the value for an ethanolic extract of the roots of *Nardostachys jatamansi* [(53.06 ± 2.20) mg GAE/g] found in the previous study[18]. The total polyphenolic contents of the pseudobulbs of both *P. graminifolia* and *O. albus* and the leaves of *P. articulata* tested in this study were comparable with the values for methanolic extracts of the fruit of *Syzygium cumini* [(31.20 ± 3.15) mg GAE/g], while the total polyphenolic contents of the roots of *G. acutifolius* and the entire plant of *G. distichus*, also tested in this study, were comparable with the values of the fruits of *Ziziphus jujuba* found in a previous study[17]. The total polyphenolic content of the leaves of *P. graminifolia*, the lowest in this study, was slightly higher than that in an ethanolic extract of the fibrous root of *Bletilla striata*, a traditional Chinese herb [(8.95 ± 0.56) mg GAE/g][19].

In short, the total polyphenol contents of the ethanol extracts of the 13 orchid samples in this study showed no discernible trend in comparison to the total polyphenol contents of both the medicinal and the non-medicinal plants studied previously: they are similar, higher, and lower, depending on the samples considered.

#### 4.3. Antioxidant activity

It has been established that the extracts of different medicinal and non-medicinal plants show high antioxidant activity but there is a considerable variation among them. In DPPH free-radical assays, food, fruit and medicinal plants with low EC<sub>50</sub> or low IC<sub>50</sub> have high radical-scavenging activity, thus high antioxidant activity and *vice versa*. In this study, ethanol extracts of selected orchids showed antioxidant activity. The variation of DPPH free radical-scavenging activity among orchid extracts was significant as it has been in previous studies. Extracts of *V. cristata* had the highest antioxidant activity, and those of *G. acutifolius* had the lowest.

The results of this study's assessment of DPPH radical-scavenging activity showed no definitive trend in comparison to the results found for medicinal plants in previous studies: they were relatively close and similar in many instances but also significantly different. For instance, the stems of *V. cristata*, the extract with the lowest IC<sub>50</sub> value in this study and the extracts of the leaves of the same orchid species had IC<sub>50</sub> values close to those for the extracts of the pseudobulbs of *Bletilla striata* (IC<sub>50</sub> 68.0 µg/mL), but significantly lower than the value for the extracts of the fibrous roots of the same plant (IC<sub>50</sub> 6.2 mg/mL)[19]. The IC<sub>50</sub> value for a methanolic extract of *Dendrobium speciosum*, a medicinal orchid, was less than one third of [IC<sub>50</sub> (0.026 ± 0.004) mg/mL] the lowest IC<sub>50</sub> value in our study, but the IC<sub>50</sub> value of a methanolic extract of the stems of the same species [IC<sub>50</sub> (1.15 ± 0.10) mg/mL][20] was more

than twice the highest IC<sub>50</sub> in this study for an ethanolic extract of the leaves of *G. acutifolius*. The IC<sub>50</sub> values for the leaves of *G. acutifolius* were slightly higher but close to the IC<sub>50</sub> values of an aqueous extract of the galls of *Ficus glomerata* [IC<sub>50</sub> (314.0 ± 0.5) µg/mL], a medicinal plant used in Ayurvedic and Unani traditions. But ethanolic extracts of the roots of *L. trichorhiza* in this study were lower than those of the methanolic extracts of the same plant [(220.0 ± 0.3) µg/mL][21]. All the extracts of other orchids which were not reported as being medicinal, including *P. graminifolia*, the roots of *G. acutifolius* and the entire plants of both *G. distichus* and *P. uniflora* had IC<sub>50</sub> values well below the value for a hexane extract of *Nardostachys jatamansi* [IC<sub>50</sub> (432.68 ± 13.70) µg/mL] [18]. The IC<sub>50</sub> values of the pseudobulbs of both *O. albus* and *P. graminifolia* and the entire plant of *G. distichus* were very close to the value for a hydroethanolic extract of the leaves of *Monodora myristica* [(150.66 ± 1.56) µg/mL][22].

Plant extracts with low values in the radical-scavenging assay have high antioxidant activity, and it is the high antioxidant activity that is attributed to giving a plant its biological and medicinal properties. The ethanol extracts of the orchids in this study, especially those orchids that are recorded as being medicinal, had much higher or much lower values for antioxidant activity than those of medicinal plants and orchids previously studied. The acknowledged medicinal properties of some of the orchid samples in this study may be attributable to their high antioxidant activity. In addition, those extracts of orchids not reported as having medicinal properties that had values of antioxidant activity comparable with some medicinal plants previously studied may also have medicinal properties.

#### 4.4. Relationship between antioxidant activity and total polyphenolics and flavonoids contents

In this study, the IC<sub>50</sub> values of the ethanol extracts of 13 selected orchid samples were negatively correlated with their total polyphenolics and flavonoids contents. Since a low IC<sub>50</sub> value is associated with high DPPH radical-scavenging activity, the negative correlation of low IC<sub>50</sub> values with total polyphenolics and flavonoids contents suggests that antioxidant activity correlates positively with total polyphenolics and flavonoids contents[23].

The negative association between IC<sub>50</sub> values and total flavonoids contents (regression equation  $y = -0.4247x + 174.71$ ,  $R^2 = 0.0405$ ) in this study is like that found in previous studies, but the extent of that correlation was less in this study than in previous studies. For example, in one study of traditional medicinal plants of India, the IC<sub>50</sub> value was negatively associated with total flavonoids contents at  $R^2 = 0.808$ [24]. DPPH free radical-scavenging activity measured as a percentage of scavenging was positively associated with the total flavonoids contents of both the aqueous and the ethanol extracts of Chinese medicinal plants ( $R^2 = 0.5739$  and  $R^2 = 0.476$ , respectively)[13]. The negative correlation of IC<sub>50</sub> values and total flavonoids contents means that antioxidant activity was positively related with total flavonoids contents as it was in previous studies.

This study found a moderately negative association between IC<sub>50</sub>

values and total polyphenolics contents (regression equation  $y = -2.74x + 256.43$ ,  $R^2 = 0.4022$ ), which means that there is a positive association between antioxidant activity and total polyphenolic content, as has been found in previous studies with varying degree of strongness. In a study of the traditional medicinal plants of India, the IC<sub>50</sub> value was negatively associated with the total polyphenolic content at  $R^2 = 0.766$ [24]. DPPH free radical-scavenging activity measured as a percentage of scavenging was positively associated with the total polyphenolics contents of both the aqueous and the ethanol extracts of Chinese medicinal plants ( $R^2 = 0.8582, 0.7104$ , respectively)[13].

As demonstrated above, the 13 orchid extracts of this study contained total polyphenolics and flavonoids contents comparable with, higher or lower than various other medicinal and non-medicinal plant extracts and their antioxidant activity is considerably higher or lower than the medicinal and other non-medicinal plants, including orchids studied in the past. It is suggested that the medicinal properties attributed to some orchids are due to their high antioxidant activity and all the studied orchids have potential for the discovery of drugs and natural preservatives.

### Conflict of interest statement

We declare that we have no conflict of interest.

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