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PHARMACOLOGICAL AND PHYTOCHEMICAL PROFILE OF AN ENDANGERED EPIPHYTIC ORCHID, *PELATANTHERIA INSECTIFERA* (RCHB.F.) RIDL.

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Abstract

The current investigation deals with the study of phytochemical screening, antioxidant, and anti-inflammatory activities of *Pelatantheria insectifera*, an endangered epiphytic orchid species. Four different fractions *i.e.* Metahnol, n-Hexane, Butanol, and Dichloromethane (DCM) of the leaf, stem, and root of *P. insectifera* were considered for bioactivity test following standard protocol. The qualitative test for alkaloid and ten other secondary metabolites of this orchid showed positive results for alkaloids, tannins, flavonoids, steroids, coumarins, and terpenoids. Antioxidant activity of the Butanol-1 fraction of leaf showed the highest scavenging activity of 98.95% at concentration 250 μgml^{-1} compared with the highest scavenging activity of Ascorbic acid (99.69% at concentration 250 μgml^{-1}). On the other hand, the anti-inflammatory activity of *P. insectifera* was evaluated using heat-induced albumin denaturation assay and the experiment revealed that the DCM fraction of root showed the highest inhibition measuring 73.50%, whereas Aspirin, a standard anti-inflammatory drug showed 52.12% inhibition against egg albumin denaturation. These results indicate that orchid species may prove as a promising source of bioactive agents which can be used for treating several kinds of ailments related with inflammatory complexity.

Introduction

THE FAMILY Orchidaceae is regarded as one of the largest families of plant kingdom comprising 25,000-35,000 species and 600-800 genera. In Bangladesh, orchid species are distributed mainly in the hilly areas of greater Sylhet, Chittagong, and Mymensingh district (Alam *et al.,* 1993). About 177 species of orchids under 70 genera are being reported from Bangladesh (Huda, 2007). In many countries, orchids have been used as traditional drugs for a very long time (Balkrishna *et al.,* 2020; Devi *et al.,* 2018; Jalal *et al.,* 2008; Kumar *et al.,* 2018, 2019; Lawler, 1984; Prakash and Pathak, 2019). About 85 orchid species belonging to 39 genera have exhibited various medicinal properties (Ghanaksha and Kaushik, 1999; Jhansi *et al.,* 2019; Kaushik, 2013, 2019; Vaidya *et al.,* 2000). Some common drugs such as Riddhi, Vriddhi, Jeevak, Rishbhak, Amarkand, and Rasna are prepared from orchids (Kumari and Pathak, 2020; Pathak *et al.,* 2010). Medicinal plants produce a vast array of secondary metabolites and most of these metabolites produce beneficial therapeutic effects in regulated doses. Amongst these, alkaloids, terpenoids, and phenolic group of compounds are very important (Ghani, 2003). Medicinal plants possessing bioactive properties especially thrombolytic, anti-bacterial, anti-fungal *etc*. can manage the infectious disease, fever, pre-mature ageing, cancer *etc.* (Rahman, 2011). Orchids are medicinally important because they are rich in alkaloids, flavonoids, glycosides, carbohydrates, and other phytochemical contents (Rahman and Husen, 2003). Vanillin is furnished from the dried pulpy fruit of *Vanilla planifolia*. It is used for flavouring ice-cream, chocolate, biscuits, and confectionery commodities, and also as perfumes (Rahman, 2011). Loroglossin glycoside is derived from *Loroglossum* sp. and used as fragrance in Europe. Mature rhizome of *Iris pallida* contains r-ionone and is commonly used to produce expensive cosmetics (Gazu *et al.,* 1993). Coumarin glycoside derived from *Angraecum fragrans* and *Paphiopedilum javanicum* has saponins which possesses strong foaming properties. Thus, the intake of plant derived antioxidant is involved in the prevention of degenerative diseases causing oxidative stress such as cancer, parkinson, alzheimer or atherosclerosis (Droge, 2002; Lee *et al.,* 2004; Pisochi and Nagulescu, 2011; Valko *et al.,* 2004, 2007). Pharmacological studies conducted on orchids indicated the immense potential of these plants in treatment of conditions such as neurodegenerative disorders, and are also used as anti-convulsive, anti-cancer, and anti-diabetic (Gutierrez, 2010). Therefore, considering the medicinal properties of *P. insectifera*, it was evaluated for its phytochemical analysis along with anti-oxidant and anti-inflammatory activities. The medicinal potential of this plant species would be validated which would help the scientific fraternity to formulate the ways to preserve this valuable endangered medicinal plant species and will also pave a way for further researches on this plant species.

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Material and Methods

Collection of Plant Material

Root, stem, and leaf of *P. insectifera* were collected from Kaptai National Park, Rangamati and preserved at the Herbarium of Chittagong University (HCU) (Accession number 190). Samples were thoroughly washed with water and dried in oven at 65°C for 48 hrs. It was then grinded into coarse powder by using grinding machine and stored in an airtight container for further investigation. Mixing of one part with another was carefully avoided.

Preparation of Plant Extract

Sample from each plant part (25 g) was taken for further analysis. Methanol (50 ml) was added to the 25 g of samples in a conical flask, shaken very well for 30 min, kept overnight and then shaken again and sonicated for 10 min and then filtered using Whatman filter paper No. 1. The process was repeated thrice with Methanol and the extract was then rotavaporated and the dried sample was kept as crude sample. The concentrated crude extract was fractionated into four different fractions *i.e.* Methanol, n-Hexane, Butanol-1, Dichloromethane (DCM) by following modified method of Kupchan Partitioning Scheme (1969).

Phytochemical Tests

Test for Alkaloids

For qualitative test for alkaloid, the most reliable and rapid testing method was developed by Webb (1952) and the method was slightly modified by Aplin and Canon (1971). For the qualitative test of alkaloid, five alkaloid detecting reagents were used. These are Dragendroff's reagent (D), Hager's reagent (H), Mayer's reagent (M), Wagner's reagent (W), and Tannic acid reagent (T). These reagents were prepared following the methods of Cronwell (1955).

Test for Phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous Hydrochloric acid (HCl) was taken as evidence for the presence of phlobatannins (Edeoga *et al.,* 2005).

Test for Flavonoids

A portion of the crude powdered plant sample was heated with 10 ml of ethyl acetate in a water bath for 3 min. The mixture was filtered and 4 ml of filtrate was shaken with 1 ml of dilute Ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids (Edeoga *et al.,* 2005).

Test for Saponins

About 2 g of crude powder was boiled with 20 ml of distilled water in a water bath and filtered. The filtrate (10 ml) was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The persistent of froth indicates the presence of saponins (Kapoor *et al.,* 1969).

Test for Tannins

About 0.5 g of the crude powdered samples boiled in 10 ml of distilled water in a test tube and filtered. A few drops of Ferric chloride reagent added to the filtrate. A blue-black precipitate was taken as evidence for the presence of tannins (Harborne, 1973).

Test for Terpenoids

Crude powder (0.5 g) was dissolved in 5 ml of Methanol. The extract (5 ml) was treated with 2 ml of chloroform in a test tube and 3 ml of concentrated Sulphuric acid carefully added to the mixture to form a layer. An interface with a reddish brown colouration formed if terpenoid constituent is present (Kolawole *et al.,* 2006).

Test for Steroids

Crude powder (0.5 g) was dissolved in 5 ml of Methanol. The extract (1 ml) was dissolved in 10 ml of Chloroform and equal volume of concentrated Sulphuric acid was added from sides of the test tube. The upper layer turns red and Sulphuric acid layer showed yellow with green fluorescence indicating the presence of steroids (Kolawole *et al.,* 2006).

Test for Glycosides

Crude (0.5 g) powder was dissolved in 5 ml of Methanol and 10 ml of 50% HCl was added to 2 ml of methanolic extract in a test tube. Then it was heated in a boiling water bath for 30 min and 5 ml of Fehling's solution was added to the mixture and the mixture was boiled for 5 min. A brick-red precipitate was taken as an evidence for the presence of glycosides (Harborne, 1973).

Test for Anthraquinone

Solution (2 ml) was added in Magnesium acetate. Formation of pink colour indicates the presence of Anthraquinones (Sofowara, 1993).

Test for Quinine

In 1 ml of extract, 1 ml of concentrated Sulphuric acid was added and was allowed to stand for some time to develop colour. Development of red colour shows the presence of Quinine (Sofowara, 1993).

Test for Coumarin

In 1 ml of extract, 1 ml of 10% NaOH was added and was allowed to stand for some time, development of yellow colour shows the presence of Coumarin (Sofowara, 1993).

Antioxidant activity

The antioxidant activity of the Methanolic, n-Hexane, Butanol-1, and DCM extract of the root, leaf, and stem of *P. insectifera* and the standard antioxidant Ascorbic acid were assessed on the basis of the free radical scavanging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical activity according to the method described (Cuendet *et al.,* 1997) with slight modification.

DPPH Assay

The percentage of DPPH discoloration (scavenging) activity was calculated with the help of the following formula:

$$\% \text{ of scavenging activity} = \left(\frac{A-B}{A}\right)X100$$

A, absorbance of control (DPPH solution without the sample); *B;* was the absorbance of DPPH solution in the presence of the sample. Values are presented as mean ± SE of the mean of three replicates. The % scavenging activity was plotted against log concentration and the IC_{50} (inhibition concentration 50 μgml^{-1}) value of plant extract was calculated by using linear regression analysis.

Anti-inflammatory Activity

The ability of anti-inflammatory activity was assessed by the method of Mizushima and Kobayashi (1968) with slight modification. Three for standard, three for control, and three for the each plant extract were tested. The tubes were marked accordingly. Acetyl salicylic acid (2 ml) was used as control. After cooling and filtering, the absorbance was measured with a spectrophotometer at 660 nm. The test was repeated for three times for each extracts as replicating. The anti-inflammatory activity was calculated by using the following equation:

$$\% \text{ of inhibition} = \left(\frac{A-B}{A}\right)X100$$

A, Absorbance of control (5% egg albumin solution and Methanol); *B*, Absorbance of test group (5% egg albumin solution and plant extract) or, *B*, Absorbance of standard solution (5% egg albumin solution and Acetyl salicylic acid).

Results and Discussion

Phytochemical Analysis

The results shown in Table 1 indicated that in respect to qualitative test for the determination of the presence of alkaloids, leaf of the presently investigated orchid *Pelatantheria insectifera* was found to be highly positive. Qualitative test of secondary metabolites for the determination of glycosides and coumarin showed that they were present highly in leaves whereas flavonoids, tannins, and steroids were present moderately. On the other hand, flavonoids, tannins, terpenoids, steroids, and quinine were found to be highly positive in the extract of stem. Furthermore, flavonoids and tannins were remarkably present in the extract of roots. Anthraquinone was absent in leaf, stem, and root secondary metabolites test. Marjoka *et al.* (2016) found saponins, tannins, glycosides, flavonoids, and steroids in dried sample leaf of *Acampe papillosa*. Alam (2012) investigated three terrestrial orchids for phytochemical investigation; two species *Arundina graminifolia* and *Geodorum densiflorum* gave higher positive response for alkaloids and other secondary metabolites. Likewise Akter (2013) when worked on phytochemical analysis of seven orchid species found positive results for alkaloids and other secondary metabolites. These findings are in line with the present observations.

Antioxidant Efficacy

It was evident from the results that amongst the five different concentrations of Ascorbic acid used in the study (50, 100, 150, 200, and 250 μgml^{-1}), 94.20%, 95.61%, 98.07%, 98.60%, and 99.65% scavenging activity was observed respectively. In case of the extract of leaf, the n-Hexane fraction showed the highest scavenging activity of 92.794% at the concentration of 50 μgml^{-1}. DCM fractionation part displayed maximum scavenging activity (94.91%) at the concentration of 250 μgml^{-1}. Scavenging activity was found to be peaked (94.38%) at the concentration of 50 μgml^{-1} while the Methanolic fraction was concerned. Likewise, Butanol-1 fraction part demonstrated the greatest scavenging activity (98.95%) at the concentration of 250 μgml^{-1} (Fig. 1). However, in pertaining to the leaf extract, Butanol-1 fraction showed the maximum scavenging activity whereas n-Hexane fraction showed the minimum activity (Fig. 1).

Akter *et al.* (2020) worked on the antioxidant activity of *Eria tomentosa*. In ethanol leaf extract, the n-Hexane fraction showed the highest scavenging activity of 93.95% at the concentration of 250 μgml^{-1}. DCM fraction part displayed maximum scavenging activity (91.30%) at the concentration of 250 μgml^{-1} at the concentration of 200 μgml^{-1} and lowest (70.34%) at the concentration of 250 μgml^{-1} while the Methanolic fraction is concerned. Likewise, Butanol-1 fraction part demonstrated the greatest scavenging activity of

Table 1. Qualitative test for alkaloids and the secondary metabolites of *Pelatantheria insectifera.*

Plant parts used	Secondary metabolites (% of coloration)										
	Alk.	Phl.	Flv.	Sap.	Tan.	Ter.	Str.	Gly.	Anthr.	Qui.	Cou.
Leaf	+++	+	++	+	++	+	++	+++	-	+	+++
Stem	++	-	+++	+	+++	+++	+++	-	-	+++	+
Root	+	-	+++	-	+++	++	++	-	-	++	++

Alk., Alkaloid; Phl., Phlobatannins; Flv., Flavonoids; Sap., Saponins; Tan., Tannins; Ter., Terpenoids; Str., Steroids; Gly., Glycosides; Anthr., Anthroquinone; Qui., Quinine; Cou., Coumarin; '+++' - High, '++' - Moderate, '+' - Low and '-' - Absence.

94.87% at concentration 100 µgml^{-1}. In respect to the bulb extract, the n-Hexane fraction showed the highest scavenging activity (93.17%) at the concentration of 250 µgml^{-1} (Fig. 2). The DCM fraction exhibited maximum scavenging activity of 91.46% at the concentration of 250 µgml^{-1}. Similarly Methanolic fraction displayed optimum scavenging activity (92.24%) at the concentration of 50 µgml^{-1}. On the contrary, Butanol-1 fraction demonstrated the highest value of 93.78% at the concentration of 100 µgml^{-1}. In considering the root extract, n-Hexane fraction presented the greatest scavenging activity (84-16%) at the concentration of 200 µgml^{-1}, while the DCM fraction displayed maximum scavenging activity

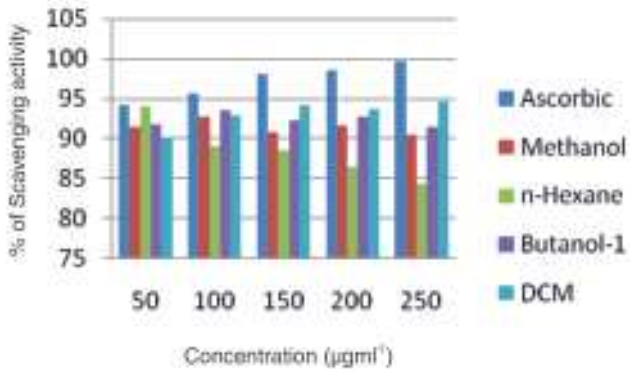

Fig. 1. DPPH free radical scavenging activity of different fraction of leaf of *Pelatantheria insectifera.*

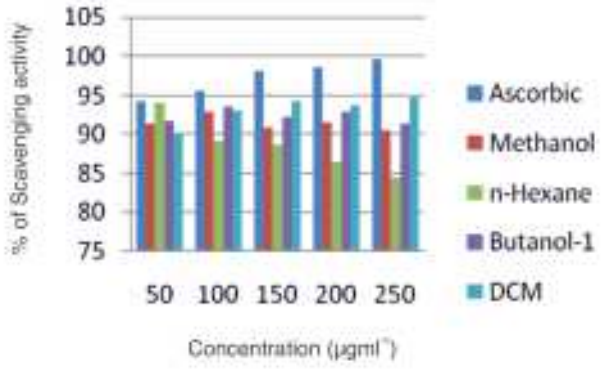

Fig. 2. DPPH free radical scavenging activity of different fraction of stem of *Pelatantheria insectifera.*

(93.79%) at the concentration of 250 µgml^{-1} (Fig. 3). Scavenging activity was found to be peaked (91.15%) at the concentration of 100 µgml^{-1} and rounded (89.29%) at the concentration of 200 µgml^{-1} when the Methanolic fraction was concerned. Similarly, Butanol-1 fraction part confirmed the highest scavenging activity of 90.06% at the concentration of 100 µgml^{-1}. Akter (2013) when investigated on anti-oxidant properties of orchid found the highest scavenging activity for root of *Rhynchostylis retusa.* Rashmi *et al.* (2015) worked on the free radical scavenging activity of selected orchids of Karnataka, India. They tested four epiphytic orchids namely *Coelogyne breviscapa, Dendrobium nutantiflorum, Luisia zeylanica,* and *Pholidota pallida* for anti-oxidant activity. Radical scavenging activity of orchid extracts was determined on DPPH free radical scavenging activity respectively. Extract of *Luisia zeylanica* exhibited stronger radical scavenging activity when compared to other orchid extracts. Chimsook (2016) narrated an antioxidant activity of *Dendrobium signatum* leaves. In his study, leaves of *D. signatum* were extracted with ethanol by maceration-called M, and by sonication-maceration for 30 and 45 min called MS30 and MS45. The antioxidant activity was measured using DPPH assay. The results showed that MS30 had the stronger free radical scavenging activity than M and MS45 and had moderate radical scavenging ability compared to Ascorbic acid. Willams and Suja (2016) worked on anti-oxidant potential of a wild epiphytic orchid, *Acampe praemorsa* of Kanyakumari district, India. DPPH radical scavenging activity of *A.*

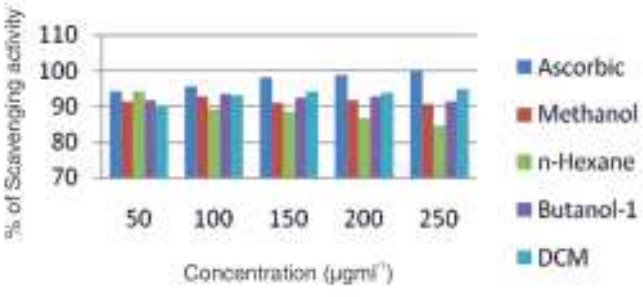

Fig. 3. DPPH free radical scavenging activity of different fraction of root of *Pelatantheria insectifera.*

praemorsa varied from the minimum inhibition of 60.37 ± 0.011% (25 µl) to the maximum inhibition of 69.74 ± 0.010 % (100 µl). Aqueous extract varied from the minimum inhibition of 56.00 ± 0.005% (25 µl) to the maximum inhibition of 58.83 ± 0.010% (100 µl). Ethanol extract varied from the minimum inhibition of 51.01 ± 0.015% (25 µl) to the maximum inhibition of 54.93 ± 0.010% (100 µl).

Anti-inflammatory Property

Inflammation can be defined as a generalized, non-specific but beneficial response of tissue to injury. It comprises a complex array of adaptive responses to tissue injury which are both local and systematic (Denko, 1992; Henson and Murphy, 1989).

The greatest inhibition of egg albumin denaturation was (54.17%), found for DCM fraction of leaf of *Pelatantheria insectifera* (Fig. 4). Butanol fraction also showed greater inhibitory activity (52.09), Methanolic fraction and n-Hexane fraction also gave inhibitory activity (44.92% and 30.42% respectively). Amin *et al.* (2011) worked on analgesic and anti-inflammatory activities of *Rhychostylis retusa* on Mice. The highest inhibition of egg albumin denaturation (39.59%) was shown for DCM fraction of stem of *P. insectifera* (Fig. 5). On the other hand, n-Hexane, Butanol, and Methanol fraction showed inhibitory activity (38.416%, 30.42% and 25.09% respectively). The greatest inhibition of egg albumin denaturation (73.5%) was shown for DCM fraction of root of *P. insectifera* (Fig. 6). Methanol fraction showed 68.75% of inhibition. Finally, least anti-inflammatory activity *i.e.* 59.17% and 41.15% was found for n-Hexane and Butanol fraction of root of *P. insectifera*. Sukumaran and Yadav (2016) worked on anti-inflammatory potential of *Dendrobium macrostachyum*. They observed that the ethanol and water extract were highly effective as albumin denaturation inhibitors (IC_{50} = 114.13 and 135.818 µgml^{-1} respectively) and proteinase inhibitors (IC_{50} = 72.49 and 129.681 µgml^{-1} respectively). Membrane stabilization was also noticeably inhibited by the stem ethanolic extract among other extracts (IC_{50} = 89.33 µgml^{-1}) but comparatively lower to aspirin standard (IC_{50}= 83.926 µgml^{-1}). Akter *et al.* (2020) while working on the anti-inflammatory activity of the leaf extract of *Eria tomentosa* showed the maximum inhibitory result for the n-Hexane fraction (89.35%) and minimum for the butanol-1 fraction (75.95%) following the sequence as n-Hexane > DCM > Methanol > Butanol-1 fraction. In case of bulb extract, n-Hexane fraction showed the highest anti-inflammatory activity (97.26%), whereas DCM fraction showed the lowest activity (76.85%) and subsequently maintained the sequence as n-Hexane > Butanol-1 > Methanol > DCM. On the contrary, the maximum anti-inflammatory activity was found to be 92.85% for n-Hexane fraction, and minimum was found to be 72.45% for Butanol-1 fraction showing the succession as n-Hexane > Methanol > DCM > Butanol-1 fraction in relation to root extract.

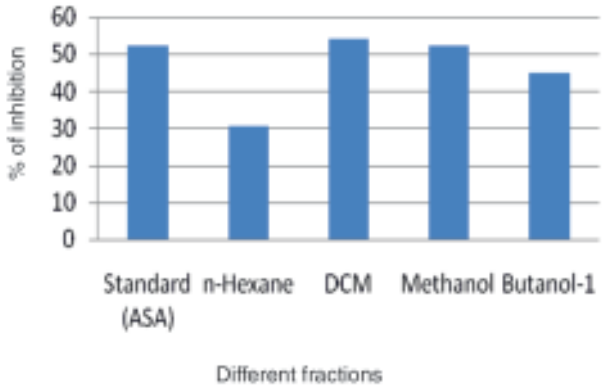

Fig. 4. Anti-inflammatory activity of leaf of *Pelatantheria insectifera* at different fractions.

Conclusion

Based on the results of the present study, it can be concluded that *Pelatantheria insectifera* leaf showed

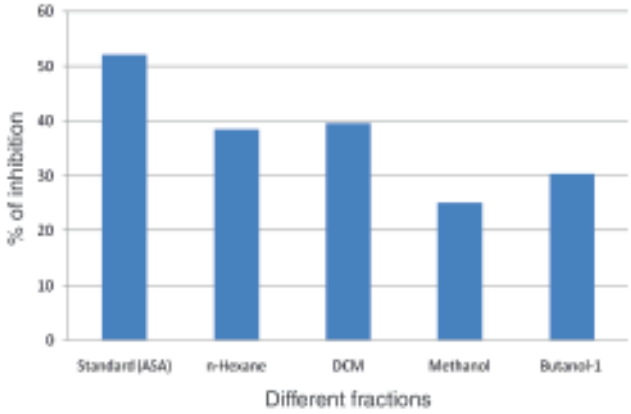

Fig. 5. Anti-inflammatory activity of stem of *Pelatantheria insectifera* at different fractions.

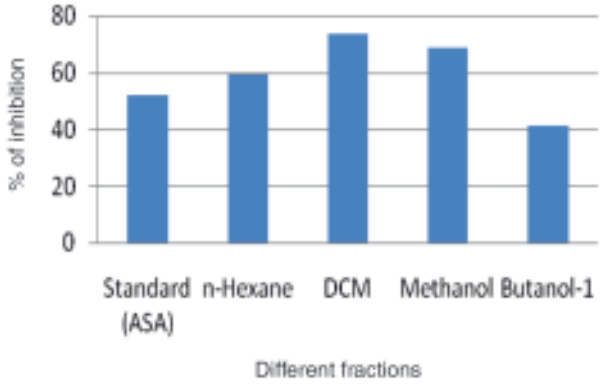

Fig. 6. Anti-inflammatory activity of root of *Pelatantheria insectifera* at different fractions.

maximum positive results for alkaloids, while stem and root showed the highest result for other secondary metabolites. Butanol-1 fraction was found to be the most effective in showing the highest scavenging activity irrespective of leaf, stem, and roots. In considering the anti-inflammatory activity, Methanol fraction of leaf of *P. insectifera* was regarded as the most efficient in comparison to others. These results indicate that orchid species may prove as a promising source of bioactive agents which can be used for treating several kinds of ailments related with inflammatory complexity.

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