International Journal of Academic Research and Development
www.multidisciplinaryjournal.in
ISSN: 2455-4197
Received: 19-04-2023, Accepted: 06-05-2023, Published: 20-05-2023
Volume 8, Issue 3, 2023, Page No. 25-29

Antioxidant and anti-inflammatory activities of an epiphytic orchid *Pholidota imbricata* Hook. f. under *In vitro* and natural conditions

Tapash Kumar Bhowmik[1*], Awishik Tripura[2], Somaya Emrog Nayma[2], Anamika Paul[2]

[1] Associate Professor, Department of Botany, Faculty of Biological Sciences, University of Chittagong, Chattogram, Bangladesh

[2] Department of Botany, Faculty of Biological Sciences, University of Chittagong, Chattogram, Bangladesh

Abstract
The seeds of *Pholidota imbricata* Hook. f. were cultured *in vitro* on agar solidified four basal media *viz*. KC, MS, MVW, PM and a huge number of plantlets were produced for used in the bioactivity test. Antioxidant and anti-inflammatory activities of naturally grown plant parts, namely leaf, pseudobulb, root and *in vitro* plantlets of *P. imbricata* were successfully compared. The moderate antioxidant activity was revealed in methanolic crude extract of leaf (IC_{50} = 208.35 µg/ml), pseudobulb (IC_{50} = 116.25 µg/ml), root (IC_{50} = 230.70 µg/ml) and *in vitro* plantlets (IC_{50} = 215.03 µg/ml) samples. Strong anti-inflammatory activity was perceived on pseudobulb (IC_{50} = 68.59 µg/ml), root (IC_{50} = 70.13 µg/ml) and *in vitro* developed plantlets (IC_{50} = 79.41 µg/ml) whereas the leaf (IC_{50}= 101.60 µg/ml) sample showed moderate anti-inflammatory activity.

Keywords: anti-inflammatory, antioxidant, *in vitro*, *Pholidota imbricata*

Introduction

Orchids are a beautiful collection of plants that thrive in a wide range of settings on earth, but they are extremely sensitive to habitat change. The family is making great efforts to unravel the biology, evolution, taxonomy, cytology, phytochemistry, hybridization and cultivation; among other topics, in order to understand the ecological, horticultural and therapeutic worth of many species (Deb and Imchen 2008) [1]. The main reasons of orchids are grown is for ornamental purposes and cut orchids are prized for both their exotic beauty and lengthy shelf life.

Orchids comprise the largest number of species of any angiosperm family, with an estimated 25,000-35,000 species worldwide (Whitten *et al.* 2007) [2]. Currently, 188 species and 72 genera from the family make up Bangladesh's flora, with 117 species belonging to 41 genera. 71 species belonging to 33 genera are terrestrial, while 33 species are epiphytic (Rahman *et al.* 2017) [3]. People of Bangladesh both rural and tribal, used different part of orchid as root, tuber, leaves, pseudobulb or whole plant in nearly 45 diseases including ailments and wounds (Hossain *et al.* 2009) [4].

The genus *Pholidota* has 46 species worldwide, ranging from the tropics and subtropics to the South West Pacific (Govaerts 2003) [5]. *Pholidota imbricata* Hook. f. is known for ethnobotanical purposes and ayurvedic practices (Bijaya 2013) [6]. The extract of the plant is found to have good antibacterial and antifungal properties against organisms like *Vibrio cholera* and *Staphylococcus aureus* (Marasini and Joshi 2012) [7]. This orchid species is used in traditional medicine. The paste of this plant species is used to treat dislocated bones with the addition of raw organic turmeric (Arditti *et al.* 1982) [8]. The whole plant is used as tonic. Root powder is used to treat cancer, juice berries is used to treat skin ulcers and skin eruptions (Arditti 1992) [9]. Juice of this plant species is applied to relieve naval pain, abdominal pain and rheumatic pain. Powder is used to induce sleep (Roy *et al.* 2007) [10]. The paste of tuber extracts of *Pholidota imbricata* is used in the treatment of stomach and rheumatic pains (Singh 2022) [11]. This study aimed to evaluate the antioxidant and anti-inflammatory activities of naturally grown plant parts with an *in vitro* sample.

Materials and methods

Collection of plant material

The mature green capsules and plant parts were collected from Naikhongchhari, Bandarban, Bangladesh.

In vitro seed germination and plantlets development

The mature *Pholidota imbricata* green capsules were cleaned under running water before being washed three to four times in sterile distilled water. The fruits were scrubbed with cotton cloth dipped in savlon and then rinsed twice or three times in distilled water. After being sterilized for 5 minutes with 0.2% (w/v) $HgCl_2$, the surfaces were rinsed twice with double distilled water. After treating the surface with 70% ethanol for 1 minute, it was cleaned 2-3 times with double sterile distilled water to disinfect it under laminar airflow.

0.8% (w/v) of agar solidified MS (Murashige and Skoog 1962) [12], PM (Phytamax Arditti, 1977) [13], MVW (Modified Vacin and Went 1949) [14] and KC (Knudson 1946) [15] was used to solidify the media and 2-3% sucrose were used for as carbon source. The pH of the media was adjusted to 5.8 in MS and 5.4 in KC, PM and MVW media using 0.1N NaOH or HCl before adding the dissolved agar. The mixture was heated in a water bath while being boiled to dissolve the agar. After that, 100 ml of each culture vessel had 50 ml of media added to it, and the vessels were autoclaved at 121 °C for 30 minutes at 15 psi pressure. The seeds were then meticulously placed within the containers as inoculum. The entire procedure had been carried out in laminar airflow cabinet.

Preparation of crude

To get rid of dust and other additional particles, all of the plant samples that were obtained were washed under running water. During a second round of washing with double-distilled water, various plant sections were separated (leaf, stem, and root). The samples were broken up into little pieces and baked to dry them. After being dried, the samples were finely powdered and soaked in methanol. The plant extract was filtered through Whatman No. 1 filter papers before being heated in a water bath (60°C) to allow the methanol to evaporate. Gradually, a crude extract was produced, and this was used for studies on the antioxidant and anti-inflammatory effects.

Test of antioxidant

According to Brand-Williams *et al*. (1995) [16] with a few minor modifications, antioxidant activity was estimated on the basis of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) to scavenge free radicals. In this process, five concentrations- 50, 100, 150, 200 and 250 µg/ml were employed.

Using the following equation, the scavenging activity against DPPH was estimated:

$$\text{Scavenging activity (\%)} = \left(\frac{A-B}{A}\right) \times 100$$

Where,

A= Absorbance of control (DPPH solution with same volume of methanol), B= Absorbance of DPPH solution in the presence of the sample (extract/ascorbic acid).

Test of anti-inflammatory

The inhibition of albumin denaturation approach, which was observed in accordance with Mizushima *et al*. (1968) [17] and Sakat *et al*. (2010) [18] with slight modification, was used to study anti-inflammatory activity. The reaction mixture contained 1% aqueous egg albumin solution and test extracts of 50, 100, 150, 200 and 250 µg/ml. The pH of the entire reaction mixture was settled at 5.60 ± 0.2.

$$\text{Percentage (\%) of inhibition} = \left(\frac{A-B}{A}\right) \times 100$$

Where,

A= Absorbance of control (5% egg albumin solution and respective solvent), B= Absorbance of test group (5% egg albumin solution and plant extract) or Absorbance of standard solution (5% egg albumin solution and acetyl salicylic acid).

Results and discussion

***In vitro* seed germination and plantlets development**

Seeds of *Pholidota imbricata* were grown on full strength 0.8% (w/v) agar solidified four basal media namely KC, MS, MVW and PM. MS basal medium gave the best performance of seeds germination followed by PM, MVW and KC media respectively.

Antioxidant activity

In this study, the results of DPPH free radical scavenging activity at five different concentrations *viz.* 50, 100, 150, 200, 250 µg/ml of leaf (30.45 ± 0.34, 39.9 ± 0.23, 44.5 ± 0.32, 50.40 ± 0.11, 52.67 ± 0.33)%, pseudobulb (38.82 ± 0.24, 42.11 ± 0.09, 60.20 ± 0.44, 68.45 ± 0.16, 71.10 ± 0.38)%, root (32.78 ± 0.76, 36.89 ± 0.65, 40.22 ± 0.40, 44.45 ± 0.29, 54.50 ± 0.37)%, *in vitro* plantlets (35.56 ± 0.42, 38.89 ± 0.19, 41.99 ± 0.85, 47.55 ± 0.15, 55.03 ± 0.26)% extract of *P. imbricata* and ascorbic acid (52.30 ± 0.72, 67.13 ± 0.47, 78.12 ± 0.56, 85.30 ± 0.49, 94.67 ± 0.23)% among showed scavenging activity respectively. The IC_{50} values of leaf, pseudobulb, root, *in vitro* plantlets extracts and ascorbic acid are 208.35, 116.25, 230.70, 215.03 and 26.09 µg/ml accordingly. The plant parts *viz.* leaf, pseudobulb, root and the *in vitro* grown sample showed moderate antioxidant activity. Rashmi *et al*. (2015) [19] worked on the free radical scavenging activity of selected orchids of Karnataka, India. They tested four epiphytic orchids namely *Luisia zeylanica*, *Pholidota pallida*, *Dendrobium nutantiflorum* and *Coelogyne breviscapa* for anti-oxidantal 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. Sukumaran and Yadav (2016) [20] studied general antioxidant potential of *Dendrobium macrostachyum* Lindl. In this study, stem and leaf extracts were assessed for its antioxidant activity by *in vitro* methods. Giri *et al*. (2012) [21] investigated *in vitro* production of phenolic compounds and antioxidant activity in callus suspension cultures of *Habenaria edgeworthii*. Chand *et al*. (2016) [22] observed the antioxidant activity of wild orchids *Rhynchostylis retusa*, *Vanda cristata* and *Gastrochilus acutifolius.*

Anti-inflammatory activity

The results of anti-inflammatory activity at five different concentrations (50, 100, 150, 200, 250 µg/ml) of leaf (40.35±0.002, 50.32±0.003, 57.46±0.001, 69.77±0.009, 76.85±0.005%), pseudobulb (46.02±0.007, 58.30±0.005, 64.70±0.004, 75.65±0.001, 88.30±0.05%), root (48.90±0.002, 53.23±0.007, 63.34±0.009, 70.33±0.005, 82.45±0.08%), *in vitro* (41.15±0.009, 58.31±0.008, 67.28±0.005, 80.55±0.001, 96.36±0.004%) extract of *P. imbricata* and acetyl salicylic acid (52.48±0.001, 67.35±0.009, 77.50±0.005, 83.91±0.002, 96.36±0.007%) among the showed inhibition activity respectively. The IC_{50} values of methanolic crude extracts of leaf, pseudobulb, root, *in vitro* and acetyl salicylic acid was 101.60, 68.59, 70.13, 79.41 and 28.41 µg/ml. Pseudobulb and root of this orchid exposed strong anti-inflammatory activity so did the *in vitro* sample. Yang *et al.* (2006) worked on *Dendrobium chrysanthum* to evaluate anti-inflammatory activity. Ramos *et al.* (2012) [24] described phytochemical profile and anti-inflammatory effect of the orchid *Catasetum macroglossum*.

Table 1: Results of antioxidant activity

	% of scavenging activity				
Conc. (µg/ml)	**Leaf**	**Pseudobulb**	**Root**	***In vitro* plantlets**	**Ascorbic acid**
50	30.45±0.34	38.82±0.24	32.78±0.76	35.56±0.42	52.30±0.72
100	39.9±0.23	42.11±0.09	36.89±0.65	38.89±0.19	67.13±0.47
150	44.5±0.32	60.20±0.44	40.22±0.40	41.99±0.85	78.12±0.56
200	50.40±0.11	68.45±0.16	44.45±0.29	47.55±0.15	85.30±0.49
250	52.67±0.33	71.10±0.38	54.50±0.37	55.03±0.26	94.67±0.23
IC_{50}	208.35	116.25	230.70	215.03	26.09

Table 2: Results of anti-inflammatory activity

	% of inhibition				
Conc. (µg/ml)	**Leaf**	**Pseudobulb**	**Root**	***In vitro* plantlets**	**Acetyl salicylic acid**
50	40.35±0.002	46.02±0.007	48.90±0.002	41.15±0.009	52.48±0.001
100	50.32±0.003	58.30±0.005	53.23±0.007	58.31±0.008	67.35±0.009
150	57.46±0.001	64.70±0.004	63.34±0.009	67.28±0.005	77.50±0.005
200	69.77±0.009	75.65±0.001	70.33±0.005	80.55±0.001	83.91±0.002
250	76.85±0.005	88.30±0.05	82.45±0.08	96.36±0.004	96.36±0.007
IC_{50}	101.60	68.59	70.13	79.41	28.41

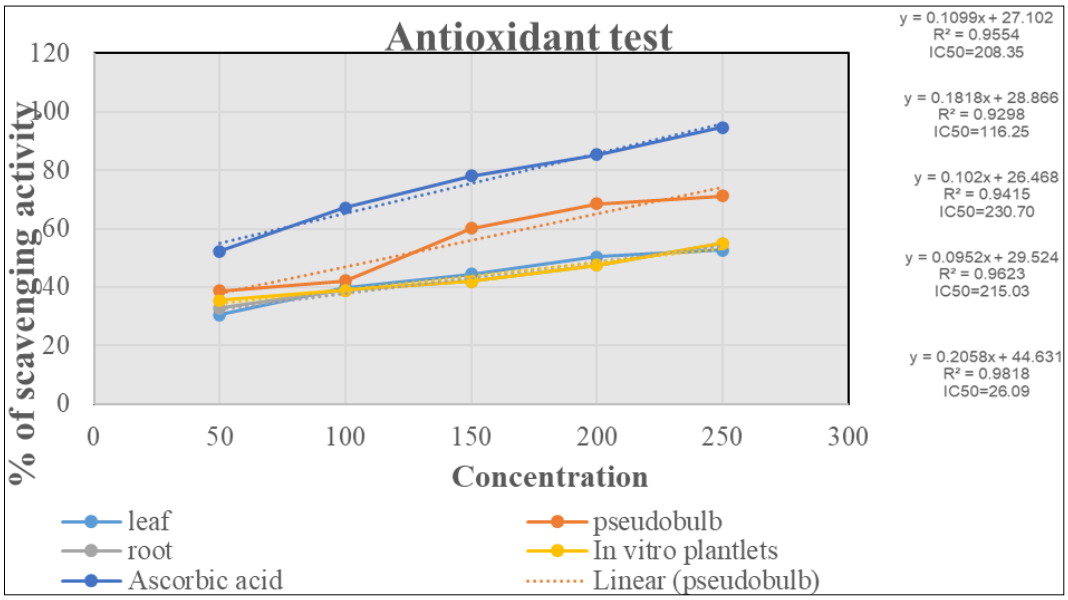

Fig 1: Scavenging activity of naturally grown plant parts *viz*. leaf, pseudobulb, root and *in vitro* plantlets of *Pholidota imbricata* and ascorbic acid (Standard)

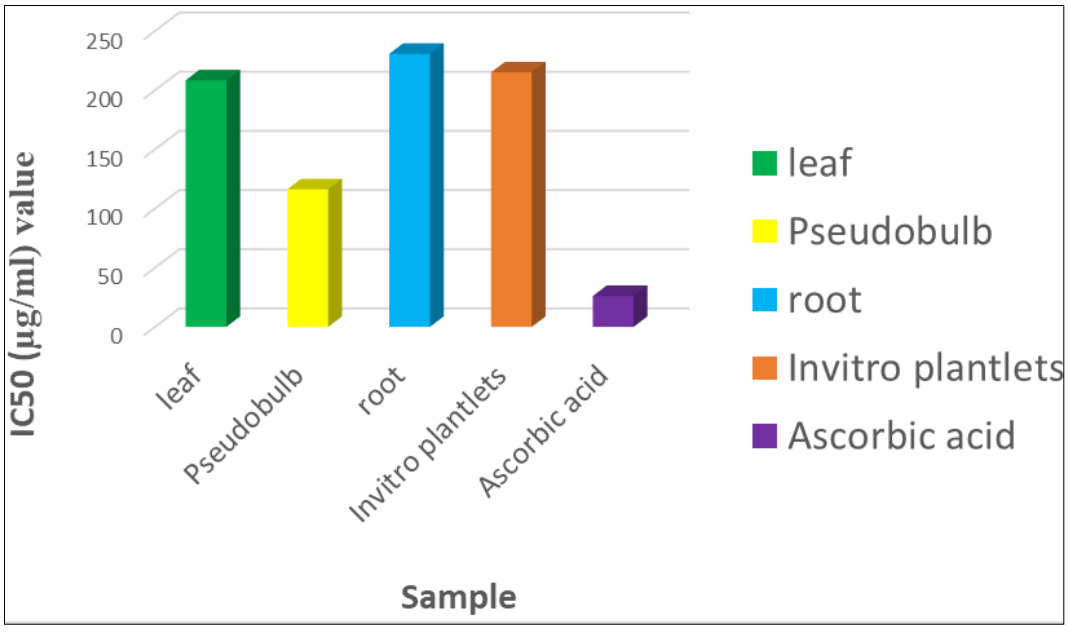

Fig 2: IC50 (µg/ml) values sample and standard solution

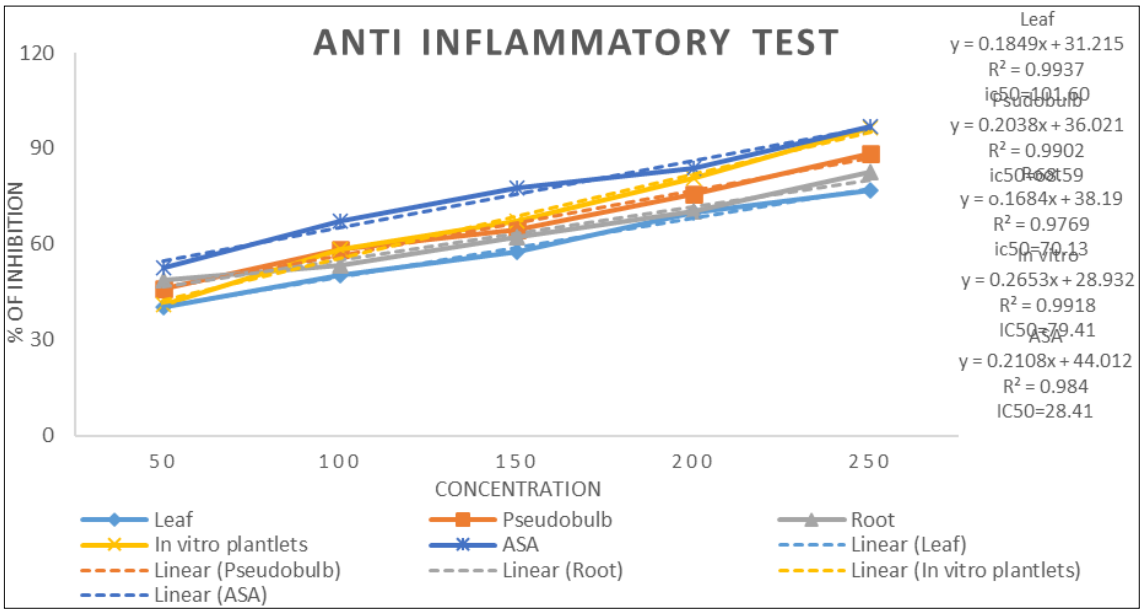

Fig 3: % of inhibition of naturally grown plant parts *viz*. leaf, pseudobulb, root and *in vitro* plantlets of *Pholidota imbricata* and acetyl salicylic acid (Standard)

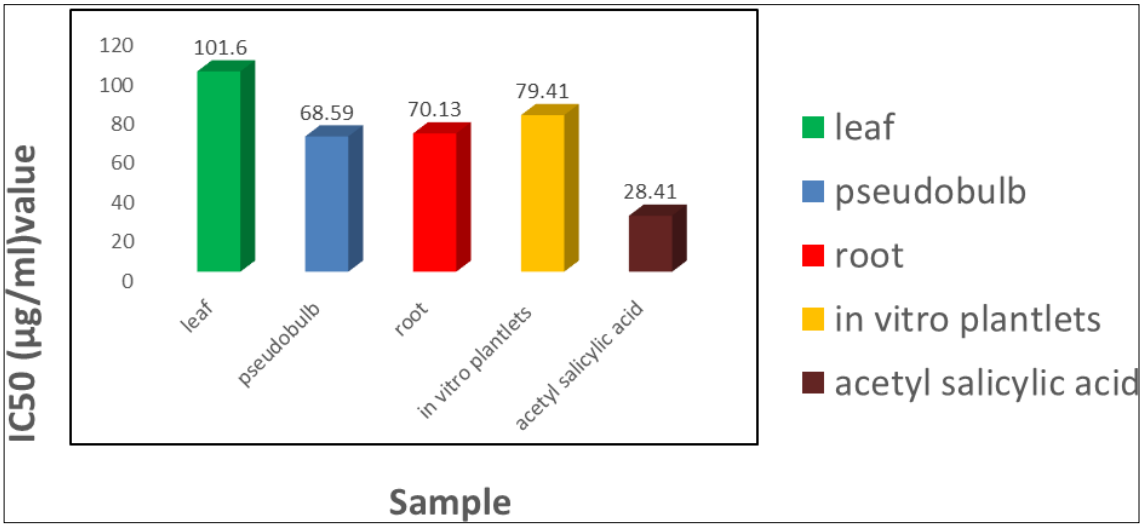

Fig 4: IC_{50} (µg/ml) values sample and standard solution

Conclusion

The moderate antioxidant activity of *P. imbricata* was found in the leaves, pseudobulbs, roots and *in vitro* plantlets. The *in vitro* sample as well as the pseudobulb and root revealed effective anti-inflammatory effects. To address pharmaceutical needs for therapeutic purposes, this orchid can be widely used.

Acknowledgement

We would especially like to thank the Laboratory of Plant Tissue Culture and Biotechnology, Department of Botany, Faculty of Biological Sciences, University of Chittagong, Bangladesh, for providing all laboratory facilities during our research.

References

1. Deb CR, Imchen T. Orchid diversity of Nagaland. Scichem Publishing House, Udaipur, India, 2008.
2. Whitten WM, Blanco MA, Williams N H, Koehler S, Carnevali G, Singer RB *et al.* Molecular phylogenetics of *Maxillaria* and related genera (Orchidaceae: Cymbidieae) based on combined molecular data sets. American Journal of Botany,2007:94(11):1860-1889.
3. Rahman MA, Huda MK, Rashid ME. Orchid species diversity in Bangladesh and their revised nomenclatural updates. Biodiversity Bulletin of Bangladesh,2017:10:1-63.
4. Hossain MM, Sharma M, Pathak P. Cost effective protocol for *in vitro* mass propagation of *Cymbidium aloifolium* (L.) Sw.–a medicinally important orchid. Engineering in Life Sciences,2009:9(6):444-453.
5. Govaerts R. World checklist of selected plant families database in ACCESS: 1-216203. The board of trustees of the Royal Botanic Gardens, Kew, 2003.
6. Bijaya P. Medicinal orchids and their uses: Tissue culture a potential alternative for conservation. African Journal of Plant Science,2013:7(10):448-467.
7. Marasini R, Joshi S. Antibacterial and antifungal activity of medicinal orchids growing in Nepal. Journal of Nepal Chemical Society,2012:29:104-109.

8. Arditti J, Clements MA, Fast G, Hadley G, Nishimura G, Ernst R. Orchid seed germination and seedling culture- A Manual. In: Orchid Biology, Reviews and Perspectives, Cornell University Press, Ithaca, New York, (ed. J. Arditti),1982:2:243-370.
9. Arditti J. Fundamentals of Orchid Biology. John Wiley & Sons, Cornell University Press, Ithaca, New York, 1992.
10. Roy AR, Patel RS, Patel VV, Yadav DS. Medicinal orchids of Meghalaya. The Journal of the Orchid Society of India,2007:21:15-17.
11. Singh B. Therapeutic Himalayan herbs: Folklore uses, bioactive phytochemicals and biological activities of medicinal orchids used by Nomads. Indian Journal of Natural Products and Resources (IJNPR),2022:13(1):94-104.
12. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum,1962:15:473-497.
13. Arditti J. Clonal propagation of orchids by means of tissue culture-a manual. Orchid biology: Review and perspective, 1977, 203-293.
14. Vacin EF, Went FW. Some pH changes in nutrient solutions. Botanical Gazette,1949:110(4):605-613.
15. Knudson L. For orchid seedlings in culture. American Orchid Society Bulletin,1946:15:214-217.
16. Brand Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. Journal of Food Science and Technology,1995:28(1):25-30.
17. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum preoteins, especially with some biologically active proteins. Journal of Pharmacy and Pharmacology,1968:20:169-173.
18. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Internation Journal of Pharmacy and Pharmaceutical Sciience,2010:2(1):146-55.
19. Rashmi K, Shweta SD, Sudeshna CS, Vrushala PS, Kekuda TRP, Raghavendra HL. Antibacterial and radical scavenging activity of selected orchids of Karnataka, India. Science, Technology and Arts Research Journal,2015:4(1):160-164.
20. Sukumaran NP, Yadav RH. General unknown screening, antioxidant and anti-inflammatory potential of *Dendrobium macrostachyum* Lindl. Ancient Science of Life,2016:35(4):240.
21. Giri L, Dhyani P, Rawat S, Bhatt ID, Nandi SK, Rawal RS, Pande V. *In vitro* production of phenolic compounds and antioxidant activity in callus suspension cultures of *Habenaria edgeworthii*: A rare Himalayan medicinal orchid. Industrial Crops and Products,2012:39:1-6.
22. Chand MB, Paudel MR, Pant B. The antioxidant activity of selected wild orchids of Nepal. Journal of Coastal Life Medicine,2016:4(9):731-736.
23. Yang H, Barros Rios J, Kourteva G, Rao X, Chen F, Shen H *et al.* A re-evaluation of the final step of vanillin biosynthesis in the orchid *Vanilla planifolia*. Phytochemistry,2017:139:33-46.
24. Ramos P, Colareda GA, Rosella MA, Debenedetti SL, Spegazzini ED, Consolini AE. Phytochemical profile and anti-inflammatory effect of the orchid *Catasetum macroglossum*. Latin American Journal of Pharmacy,2012:31(1):62-67.