International Journal of Research in Engineering, Science and Management
Volume-1, Issue-9, September-2018
www.ijresm.com | ISSN (Online): 2581-5782

Antioxidant, Antibacterial and Antifungal Activities of Ethanolic Extract of Therapeutically Important Orchid, Satyrium Nepalense D.Don

Deepak K. Singh
Department of Botony, University of Delhi, Delhi, India

***Abstract*—Drugs derived from plant are gaining interest because of fewer adverse effect, inexpensiveness and acceptance due to long of history use. We have attempted to evaluate antioxidant, antibacterial and antifungal effect of *in vivo* plant sample ethanol extract of *Satyrium nepalense*. The antioxidant activity of *in vivo* tubers extract of different dilution (0.5-10mg/l) was determined and compared by using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Concentrated tuber extract had maximum free radical scavenging activities. To determine antibacterial potential, extracts at different concentration(20, 40mg/ml) was assessed against three gram positive and three gram negative bacteria, where the tested sample expressed broad spectrum activity against both strains, higher being against gram positive followed by gram negative bacteria. Antifungal activities of extracts of different dose (20, 40mg/ml) were too examined against *Aspergillus flavus* and *Fusarium oxysporum*, where extract was effective against both fungi.**

Index Terms*—Antioxidant, Antibacterial, Antioxidant, Tubers, *Satyrium nepalense.

I. Introduction

In India, medicinal plants, especially orchids are well known for its therapeutic aids and widely used by the people either directly as folk remedies or indirectly by the pharmaceutical companies for the preparation of modern medicine (Srinivasan *et al.* 2001). Many orchids are used in traditional system of medicine for curing several ailments including microbial infection (Sahaya *et al*. 2012). Today's, interest regarding extraction of drugs from the plant goes on increasing. Medicinal plant's extract has a great potential for the making of new anti-infectious agents (Machado *et al.* 2005). Because of disease treating efficiency is retained within living system, they are more biological friendliness than totally synthetic molecules (Minhajur *et al.* 2011). According to National Health Experts, 2000 medicinal plants in India are being used for preparing medicines and use them to treat several aiments (Srinivasan *et al.* 2001). According to the World Health Organization, about 80% of the world's populations rely on herbal based alternative systems of medicine (Mazid *et al.* 2012).

Satyrium nepalense, is an endangered, medicinal, terrestrial tuberous herb of the family Orchidaceae locally known as 'Salam misiri' (Jalal *et al*. 2008). Decoction made from its tubers, root and stems are used as food supplements since ancient time (Jalal *et al*. 2008). It has an excellent remedial properties to cure diarrhea, dysentery, fever and malaria (Rao 2004; Mahendran and Bai 2009; Joshi *et al*. 2009; Saklani *et al*. 2011). Extract of plant is supposed to have aphrodisiac and anti-inflammatory properties (Mishra *et al*. 2012). Preliminary study of Mishra and Saklani (2012) has shown its antibacterial role against *Staphylococcus mutans*, *Pseudomonas aeruginosa*, *Staphylococcus aurens* and *Klebsiellla pneumonia.*
The present study describes the screening of medicinally important orchid, *S. nepalense* for the evaluation of its antioxidant, antibacterial and antifungal activities in ethanolic extract of plant samples.

II. Materials and Methods

A. Plant Material

In vivo tubers of *Satyrium nepalense* were collected from Dhanaulti, Uttarakhand, India (Latitude: 78°12'02.82″E, Longitude: 30°25'42.91″N, Altitude: 2378m) during the first week of September 2012. The botanical identity of the plants was authenticated at the Botanical Survey of India, Dehradun, Uttarakhand and the herbarium specimen of one of the collected plants was deposited in the University of Delhi herbarium (Voucher specimen no.13741, 13742).

B. Preparation of Ethanolic Crude Plant Extracts

Tubers were dried for 48 hr in an oven maintained at 50°C. These were then pulverized in liquid nitrogen. The powdered 100 and 200 mg of each sample was incubated individually for 12 hr in 5 ml of ethanol contained in 50 ml falcon tubes on a rotary shaker run at 200 rpm and maintained at 25°C. The tubes were then centrifuged at 10,000g for 20 min. The supernatant was filtered through Millipore filters (0.22µm). The filtrate containing plant extracts to be analyzed were stored at 4°C till further use.

C. Test Microorganisms

The following Gram-positive and Gram-negative bacteria and fungal cultures were used for the experiments. Gram positive bacteria: *Mycobacterium hassiacum* (3849^T), *Corynebacterium pollutisoli* ($VDS11^T$) and *Fictibacillus halophilus (AS8^T)*, Gram negative bacteria: *Pontibacter mucosus* ($PB3^T$), *Algoriphagus roseus*($W29^T$) and *Novosphingobium lindaniclasticum* ($LE124^T$); fungi: *Aspergillus flavus* (ITCC 107) and *Fusarium oxysporum* (ITCC 4755). Fungal cultures employed were obtained from Indian Type Culture Collection Centre (ITCC), *Indian Agricultural Research Institute* (*IARI)*, New Delhi, India, whereas bacterial cultures were obtained from Department of Zoology, University of Delhi, India.

D. Preparation of Growth Media

Luria-Bertani (LB) agar media was prepared for bacterial cultures, whereas potato dextrose agar (PDA) medium was used for fungal cultures. For LB agar and PDA medium, pH was adjusted to 7.5 and 5.6 respectively. Approximately 25ml of the medium was poured into 90mm sterile petri-dish. Mother plates of each culture were too maintained during subculture.

III. Antioxidant Activity

A. DPPH Radical Scavenging Assay

The antioxidant activity of the plant extract was determined by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method described by Brand-Williams et al. (1995). The stock solution of the reagent was prepared by dissolving 24 mg of DPPH in 100 ml methanol and stored at -23oC until use for the experiments. One ml each of the stock of DPPH and the extract were mixed. For the control, only methanol and the DPPH were mixed. Resulting mixtures were vortexed and incubated in dark at room temperature for 30 min. Thereafter, absorbance was recorded with a spectrophotometer (Eppendorf Biospectrometer Kinetics, Version 1.3.6.0) at 593nm. Radical scavenging activity was expressed as the percentage of inhibition and was calculated using the following formula:

$$\%\ \text{radical scavenging activity} = \frac{\text{Absorbance of the control-Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

B. Screening of Plant Samples for their Antimicrobial Activities

Antibacterial as well as antifungal activities of different concentration of crude extracts of different plant samples were tested using "disc diffusion technique" (Doughari et al. 2008).

1) Antibacterial activity

The bacterial suspension was prepared in LB broth and 150 µL of this was inoculated on each Petri plate containing solidified LB agar. They were mixed thoroughly with the help of L shaped spreader (Hi media, India) for uniform growth of bacteria. Thereafter, pre-prepared test disc/standard disc was kept on the surface of the solidified LB agar medium inoculated with bacteria. All Petri dishes inoculated with gram positive bacteria, except *Mycobacterium hassiacum*, were incubated in inverted position at 37 ± 2°C. However, all grams negative bacteria and *M. hassiacum* inoculated Petri plates were kept at 28 ± 2 °C in inverted position. Both types of bacteria were incubated for 48 hr. Antibacterial activities of the plant extract were determined by measuring clear zones of inhibition around each disc.

2) Antifungal activity

Antifungal activities of ethanolic extracts of tubers were estimated by disc plate diffusion assay method (Roberts and Selitrenikoff 1988). Fungi (*Aspergillus flavus* and *Fusarium oxysporum*) were inoculated at the middle of Petri plates (diameter, 9 cm), each containing 25 ml potato dextrose agar (PDA) medium. After incubating the cultures in dark for 48 hr at 25^{o}C, to allow spore germination and mycelia growth, the disc (6 mm, Hi Media) pre-soaked in 0, 20 and 40 mg/l ethanolic extracts of the selected plants, were placed radially on to the surface of PDA medium. Cultures were incubated in dark at 25±02^{o}C for four days. Percentage of inhibition of fungal growth was measured by using following formula:

$$\%\ \text{of inhibition} = \frac{\text{Total distance of disc from centre -Distance travelled by fungi}}{\text{Total distance of disc from centre}} \times 100$$

3) Experiment design and data analysis

All experiment was repeated twice and the results were analyzed using one way ANOVA test and significant difference between each treatment evaluated by the Duncan's multiple range test at P= 0.05 using SPSS (version 22) software package. The values followed by different superscript(s) are significantly different. The cultures were photographed was done using Canon camera (PC1234, DC 7.4v), Japan.

IV. Results

A. Antioxidant Activity

Antioxidant activity *in vivo* tubers ethanol extract of different dilution (0.5-10mg/l) was determined by DPPH method. Free radical scavenging activity of plant sample was recorded increasing in dose dependent manner. Maximum antioxidant activity (81.92%) was registered at higher concentration (10mg/ml) (Fig. 1).

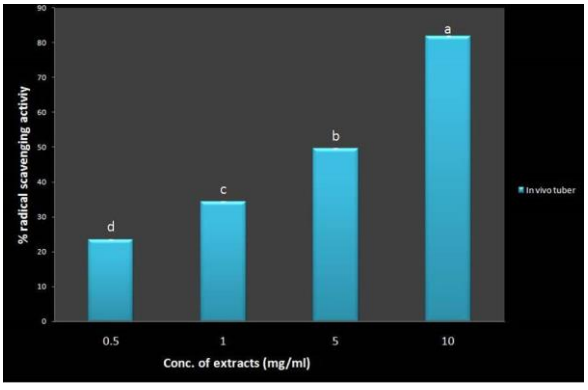

Fig. 1. Antioxidant activities of in vivo tubers of S. nepalense. Histobars with the different letter are significantly different (P = 0.05)

B. *Antibacterial Activity of in Vivo Tuber Extract*

The effect of ethanolic extracts of *in vivo* tubers of *S. nepalense* was tested on the multiplication of gram positive (*Mycobacterium hassiacum*, *Corynebacterium pollutisoli* and *Fictibacillus halophilus*) and gram negative bacteria (*Pontibacter mucosus*, *Algoriphagus roseus* and *Novosphingobium lindaniclasticum*) at two concentrations (20 and 40 mg/ml). The extract at both the concentrations inhibited the growth of all bacteria. Area of inhibitory zone was found maximum against gram positive bacteria, *Fictibacillus halophilus (126.12* mm^2). The inhibitory effect of the extract against bacteria was in a dose dependent manner except *Corynebacterium pollutisoli*, where the lower and higher concentration was almost equally effective. Among gram negative bacteria tested, *Algoriphagus roseus* was inhibited more strongly (112.5 mm^2) (Table-1).

C. *Antifungal Activity*

Different concentrations of ethanolic *in vivo* tuber extract were tested against *Aspergillus flavus*, and *Fusarium oxysporum*. Extract at both concentrations inhibited growth of fungi. Higher dose (40mg/ml) of extract was more effective than lower doses (20mg/ml) against both tested fungi. The extract at 40mg/ml significantly and adversely affected the growth of *A. flavus* (Fig. 2).

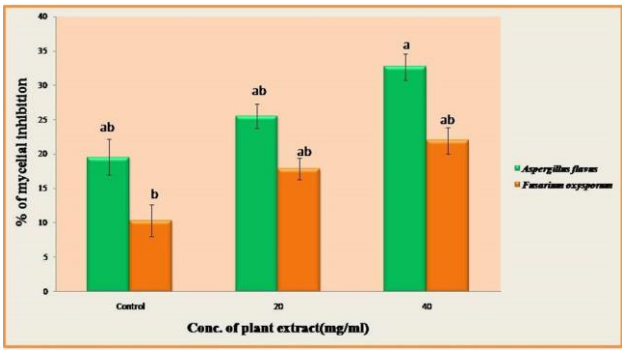

Fig. 2. Effect of different concentration of in vivo tuber extract of S. nepalense against A. flavus, and F. oxysporum. Histobars with the same letter are not significantly different (P = 0.05).

V. Discussion and Conclusion

DPPH method of detecting antioxidant activities has been extensively used as it is highly sensitive technique to detect any active ingredient at even at low concentration and can accommodate large number of samples in a short period of time (Sa´nchez-Moreno 2002). In the present investigation, different concentration (0.5-10mg/l) of an ethanolic extract of *in vivo* tubers were tested for its antioxidant activity, where an extract of higher dose exhibited maximum DPPH free radical scavenging activities. These results are in conformity with the findings of Chua *et al*. (2008), where concentrated butyl alcohol and ethanolic extract of twigs of *Cinnamomum osmophloeum* had shown maximum antioxidant activities. Likewise, bark extract and derived phytochemical had great potential to scavenge free radical in *Acacia confuse* (Tung *et al*. 2009).

Phytochemical screening, providing an idea about ethnopharmacological is supposed to be an efficient approach to prepare new anti-infective agents from the plants (Kloucek *et al*. 2005). Amongst various techniques, the disc diffusion method is widely employed technique to detect antibacterial activity of plant extract (Essawi and Srour 2000; Hossain *et al*. 2012). In the current study too, this method successfully revealed the antibacterial activity of plant extract. *In vivo* tubers were tested against three gram positive and three gram negative bacteria. Although a score of inhibition was detected against both types of tested bacteria, despite tuber extracts were more effective against gram positive than gram negative bacteria. Such difference in susceptibility and resistance may due to differences in the morphology of these two types of bacteria, where gram negative has lipopolysaccharide components in its outer phospholipidic membrane and gram positive have peptidoglycan layer (Scherrer and Gerhardt 1971; Nikaido and Vaara 1985). In contrast to current study, Ali *et al*. (2001) observed ethanolic extract of plant samples were neither effective against gram positive nor for gram negative bacteria. Our finding are in congruence with the results of Essawi and Srour 2000, where applying "disc diffusion methods", extract of different plant samples were effective against both gram positive and gram negative bacteria.

Worldwide in developing countries, post-harvest loss (up to 50%) mainly due to antibacterial and antifungal activities is a major threat to modern agriculture system. Such huge loss mainly due to lack advance technology for proper drying, handling and controlling atmosphere storage facilities (El-Ghaouth 1997).

TABLE I
Units for Magnetic Properties

Test organisms	Area of inhibition zone (mm^2)		
	In vivo tuber		
Gram(+)	**Control**	**20mg/ml**	**40mg/ml**
Mycobacterium hassiacum	0.00 ± 0.00^{f}	26.42 ± 4.45^{ef}	67.25 ± 9.99^{d}
Corynebacterium pollutisoli	0.00 ± 0.00^{f}	22.50 ± 7.26^{ef}	21.98 ± 0.00^{ef}
Fictibacillus halophilus	6.80 ± 3.41^{f}	78.76 ± 6.03^{cd}	**126.12 ± 12.71^{a}**
Gram(-)			
Pontibacter mucosus	0.00 ± 0.00^{f}	68.3 ± 16.50^{d}	79.81 ± 15.85^{cd}
Algoriphagus roseus	0.00 ± 0.00^{f}	98.39 ± 13.62^{bc}	**112.5 ± 18.82^{ab}**
Novosphingobium lindaniclasticum	0.00 ± 0.00^{f}	18.06 ± 3.93^{ef}	40.30 ± 4.98^{e}

Mean values in each column followed by the same superscript are not significantly different (P = 0.05) based on Duncan's multiple range test (DMRT).

To cope up with this serious issue, an efficient and effective protocol needs to be developed for the discovery of broad spectrum antifungal agents. In the present investigation, *in vivo* tuber extracts were tested against *Aspergillus flavus* and *Fusarium oxysporum*, where it was observed that extract inhibited mycelial growth of both fungi. These findings are parallel with the report of Magro *et al*. 2007, where they observed that *Tabebuia impetiginosa* extract at 1.14g/ml inhibited the growth of *Fusarium culmorum*, *Aspergillus candidus* and *Aspergillus niger* whereas, *Rosmarinus officinalis* exract at 1g/ml could inhibit *Fusarium culmorum* and *Aspergillus candidus* only. Alanis Garza *et al*. (2007) too reported antifungal activities of *Salvia texana* against *Aspergillus fumigatus.*

ACKNOWLEDGEMENT

Financial assistance from Department of Science and Technology, Government of India, New Delhi under DST-INSPIRE program is gratefully acknowledged. The research presented in the paper was financially supported by Research & Development Grant of the University of Delhi.

REFERENCES

[1] Alanis-Garza BA, Gonz´alez-Gonz´alez GM, Salazar-Aranda R, De Torres NW and Rivas-Galindo VM (2007) Screening of antifungal activity of plants from the northeast of Mexico. J Ethnopharm 114: 468–471.
[2] Ali MS, Azhar I, Ahmad VU, Usmanghani K (2001) Antimicrobial screening of Mimoceous plants. Pharm Biol 39:43–46.
[3] Brand-williams W, Cuvelier ME and Berset C (1995) Use of free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft Technol 28: 25-30.
[4] Chua MT, Tung YT and Chang ST (2008) Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*. Bio Technol 99: 1918–1925.
[5] Doughari JH, Elmahmood AM and Manzara S (2008) Studies on the antibacterial activity of root extracts of *Carica papaya* L. African J Microbiol Res 37-041.
[6] El-Ghaouth A (1997) Biologically-based alternatives to synthetic fungicides for the control of post-harvest diseases. – J Industrial Microbiol Biotechnol 19: 160-162.
[7] Essawi T and Srour M (2000) Screening of some Palestinian medicinal plants for antibacterial activity. J Ethnopharma 70: 343-349.
[8] Hossain MS, Sayeed MA, Sayeed MA and Chowdhury EMH (2012) Investigation on *in-vitro* cytotoxic, antibacterial and phytochemical screening of ethyl acetate extract of *Geodorum densiflorum* (Lam.) Schltr. Pharma Innovations 1:108-113.
[9] Jalal JS, Kumar P and Pangtey Y (2008) Ethnomedicinal orchids of Uttarakhand, Western Himalaya. Ethnobot Leafl 12: 1227-1230.
[10] Joshi GC, Tewari LM, Lohani N, Upreti K, Jalal JS and Tewari G (2009) Diversity of orchids in Uttarakhand and their conservation strategy with special reference to their medicinal importance. Rep Op 1: 47-52.
[11] Kloucek P, Polesny Z, Svobodova B, Vlkova E and Kokoska L (2005) Antibacterial screening of some Peruvian medicinal plants used in Calleria District J. Ethnopharma 99: 309-312.
[12] Machado TB, Leal ICR, Amaral ACF, Kuster RM, Kokis VM, Silva MG and Santos KRN (2005) Brazilian phytopharmaceuticals evaluation against hospital bacteria. Phyto Ther Res 19: 519–525.
[13] Magro A, Bastos M, Carolino M and Mexia A (2007) Antifungal activity of plant extracts. Integrated Prot Store Prod 30: 291-295.
[14] Mahendran G and Bai VN (2009) Mass propagation of *Satyrium nepalense* D.Don. A medicinal orchid via seed culture. Sci Hortic 119: 203–2.
[15] Mazid M, Khanb TA and Mohammad F (2012) Medicinal plants of rural India: a review of use by Indian folks. Indo Global J Pharma Sci 2: 286-304.
[16] Mead JW and Bulard C (1979) Vitamins and nitrogen requirements of *Orchis laxiflora* Lamk. New Phytol 83: 129-136.
[17] Minhajur RM, Alam MM, Shahriar M, Moghal MR and Siddiqui R (2011) The antimicrobial activity and brine shrimp lethality bioassay of leaf extracts of *Stephania japonica* (Akanadi). Bang J Microbiol 28: 52–56.
[18] Mishra AP and Saklani S (2012) *Satyrium nepalense*: A rare medicinal orchid of Western Himalaya (India); phytochemical screening, antimicrobial evaluation and conservation studies. Indonesian J Pharm 23: 162-170.
[19] Mishra AP, Saklani S and Parcha V (2012) Anti-inflammatory effect of methanolic extract of *Satyrium nepalense* tubers in rats using carrageenan-induced paw edema 1-3.
[20] Nikaido H and Vaara M (1985) Molecular basis of bacterial outer membrane permeability. Microbiological Reviews 1: 1-32.
[21] Roberts WK and Selitrenikoff CP (1988) Plant cell and bacterial chitinase differ in antifungal activity. J Gen Microbiol 134: 169-176.
[22] Rao AN (2004) Medicinal orchid wealth of Arunachal Pradesh news letter of Envis. Indian Med Plants 1: 1–10.
[23] Sa´nchez-Moreno C (2002) Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci Technol Int 8: 121–137.
[24] Saklani S, Mishra AP, ParchaV and Chandra S (2011) Phytochemical and antibacterial evaluation of *Satyrium nepalense* and *Saussurea simpsoniana*, the threatened medicinal herbs of Uttarakhand. J Pharm Rese 4: 3866-3870.
[25] Scherrer R and Gerhardt P (1971) Molecular sieving by the *Bacillus megaterium* cell wall and protoplast. J Bacteriology 107: 718-735.
[26] Srinivasan D, Nathan S, Suresh T and Perumalsamy PL (2001) Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharm 74: 217–220.
[27] Sahaya BS, Sarmad M, Servin PW and Chitra BD (2012) Preliminary phytochemical screening, antibacterial and antioxidant activity of *Eria pseudoclavicaulis* Blatt.—An endemic orchid of Western Ghats. Am J Pharm Tech Res 2: 519–525.
[28] Tung YT, Wu JH, Huang CY, Kuo YH and Chang ST (2009) Antioxidant activities and phytochemical characteristics of extracts from *Acacia confusa* bark Bio Technol 100: 509–514.