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IN VITRO ANTI-OXIDANT ACTIVITY OF PET-ETHER EXTRACT OF *VANDA TESSELLATA* ROXB

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ABSTRACT

Free radicals have attracted a great deal of attention in recent years. Excessive production of reactive oxygen species and oxidative stress leads to derangements of normal physiological phenomenon. To counteract the harmful effects of free radicals, antioxidant defence mechanism operates to detoxify or scavenge these free radicals. *Vanda tessellata* Roxb. (Family: Orchidaceae) have been used in the indigenous medicine such as *Ayurveda* and local traditional medical practices. However, its antioxidant potential is not explored yet. Therefore, we have decided to evaluate antioxidant activity of petroleum ether extract of *Vanda tessellata* Roxb by using established *in-vitro* antioxidant methods such as 1, 1-Diphenyl-2-Picrylhydrazil (DPPH), NO scavenging activity. *Invitro* antioxidant studies showed that Petroleum ether extract had significant NO inhibition but not DPPH free radical scavenging activity.

Keywords: Antioxidant, Petroleum ether extract, Nitric oxide, Free radical scavenging

INTRODUCTION

Vanda tessellata Roxb. (Family: Orchidaceae) is a species of orchid occurring from the Indian subcontinent to Indochina. It is a medicinal epiphytic perennial; stem 30-60 cm long, stout, scandent by the stout, simple or branching aerial roots. Leaves succulent, 15-20 cm, long, linear, recurved, complicate. Flowers in 6-10 flowered racemes, reaching with the peduncle 15-25 cm long. Sepals yellow, tessellated with brown lines and with white margins. Petals yellow with brown lines and white margins, shorter than the sepals. Lip 16 mm long, bluish, dotted with purple. Capsules 7.5-9 cm long, narrowly clavate-oblong with acute ribs.

Vanda tessellata plants have been used in the indigenous medicine such as *Ayurveda* and local traditional medical practices[1].The leaf juice is used for the treatment of certain inflammatory conditions. It is also instilled into the ear as a remedy for Otitis. The leaves in the form of a paste are applied to the body to bring down fever[2]. The roots were used in rheumatism, nervous problems, bronchitis and dyspepsia[3]. Unani practitioners hold it to be laxative and tonic to the liver. It is also used to treat hiccough, piles, and boils on the scalp. *V. tessellata* has not been evaluated in depth for its pharmacological properties, in spite of its traditional use in numerous medical conditions[4]. It is also a remedy for secondary syphilis and scorpion-sting. Juice of the leaves is given in Otitis and the paste as febrifuge. The roots possess significant anti-inflammatory activity. The plant has an alkaloid, flavonoids glycoside, tannins, β-sitosterol, γ-sitosterol and a long chain aliphatic com-

pound, fatty oils, resins and colouring matters. Roots contain tetracosyl ferrulate and β-sitosterol-D-glucoside[5]. It also enters the composition of several medicated oils for external application in rheumatism and diseases of the nervous system. Roots were reported to possess antibacterial and antitubercular properties[6]. The steroidal fraction obtained from *V. tessellata* possessed significant anti inflammatory activity against acute inflammation induced by carrageenan, serotonin and formaldehyde[7]. The methanol extract of this plant root also showed remarkable anti-inflammatory activity against carrageenan – induced oedema in rodents[8]. The traditional use indicates that various parts of this plant are likely to have several pharmacological properties. Lawler reported that several Ayurvedic type preparations containing this plant (root or whole plant) were used as aphrodisiac[9]. But still there is no valid reference to explore its antioxidant property. Thus, the present study was designed to investigate the petroleum-ether extract of *Vanda tessellata Roxb* for its antioxidant activity.

MATERIALS AND METHODS

This study was done on February 2012 at the Department of Pharmacology, Mamata Medical College, Khammam. *V. tessellata* leaves were shade dried and one kg of coarse powder was soaked in 4 litres of petroleum-ether for 3 days at room temperature. The extract was evaporated to dryness by using a rotary vacuum flash evaporator and the yield was10% w/w. The petroleum ether extract was then subjected to in vitro scavenging of the NO radical activity, 1 –Diphenyl – 2 – Picryl-hydrazil (DPPH) inhibition antioxidant assays analyzed by spectrophotometer.

Antioxidant Activity:

DPPH scavenging activity:

DPPH radical scavenging activity was done using the method of Yohozowa et al.[10] The reaction mixture containing 1ml of DPPH solution (150µM in ethanol) with different concentrations of the substance (10, 50, 100, 200, 400, 800, 1000µg/ml) was shaken and incubated in dark for 20min at room temperature. The resultant absorbance was recorded at 517nm. The percentage inhibition was calculated using the formula

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Nitric oxide scavenging activity:

The nitric oxide radical scavenging activity was done using the method of Alderson et al.[11] 3ml of reaction mixture containing sodium nitroprusside (10Mm in phosphate buffered saline) and various concentrations (10, 50, 100, 200, 400, 800, 1000µg/ml) of the extract were incubated at 37^0 c for 4 hours. To the incubation solution, 0.5ml of Griess reagent was added and the absorbance was read at 546nm. The percentage inhibition was calculated using the formula

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

RESULTS

Antioxidant activity:

The petroleum ether extract of *Vanda tessellata Roxb* showed optimum percentage of inhibition of NO but not DPPH, NO inhibition is attained at 200mg/kg. However at higher concentration the percentage inhibition is reduced due to saturation effect of the extract.

Table 1: Petroleum ether extract of *Vanda tessellata* on DPPH scavenging activity

Concentration (Mg/Ml)	%Inhibition (Mean±S.D)	
	Std (Ascorbic Acid)	Extract
10	79.23±2.46	35.76±3.04
50	81.25±1.68	40.7±2.98
100	83.52±1.96	42.48±2.04
200	86.56±2.34	48.71±1.27
400	88.15±3.14	52.07±0.58
800	92.62±2.20	41.53±1.48
1000	95.21±0.05	38.32±2.54

Table 2: Petroleum ether extract of *Vanda tessellata* on NO scavenging activity

Concentration (µg/ml)	%Inhibition (Mean±S.D)	
	Std (Curcumin)	Extract
10	32.86±2.47	43.20±O.64
50	47.64±2.15	48.08±0.85
100	58.64±1.46	59.85±2.14
200	64.00±2.37	61.50±1.48
400	78.28±2.94	57.60±O.75
800	82.47±2.48	42.47±1.64
1000	89.51±1.24	36.12±1.52

DISCUSSION AND CONCLUSION

The present study has been designed to evaluate the antioxidant activity of petroleum ether extract of *Vanda Tessellata* Roxb. Reactive Oxygen Species (ROS) are continuously produced during normal physiologic events and removed by anti-oxidant defence mechanism[12]. In pathological conditions, ROS are over produced and result in lipid peroxidation and oxidative damage. The imbalance between ROS and anti-oxidant defense mechanisms leads to oxidative modification in the cellular membrane or intracellular molecules.[13]

NO is an important physiologic messenger and effector molecule in many biological systems which include immunological, neuronal and cardiovascular tissues[14]. NO is an important signaling and effector molecule in inflammation and immunity, as it is known to couple with superoxides to form peroxynitrite. These, in turn, induce the production of prostaglandin endoperoxide synthase from the monocytes/macrophages, resulting in an enhanced synthesis of prostaglandins which are the established mediators of inflammation[15]. In the chronic synovial inflammation of arthritis, macrophages play a central role in the inflammation. The nitric oxide in the macrophages is produced as a free radical by iNOS by the catalyzation of the oxidation of the guanidino nitrogen of L-arginine, thereby converting L-arginine to L-citrulline[16].

Petroleum ether extract of *Vanda Tessellata* Roxb which was screened for its antioxidant effects, showed an NO free radical scavenging activity. However, it did not exhibit any appreciable DPPH radical scavenging activity. These differences can be explained by understanding the nature and generation of radicals as well as studying the difference in physical and chemical properties of naturally occuring antioxidants[17, 18]

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