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HUMAN RED BLOOD CELL (HRBC) MEMBRANE STABILIZING ACTIVITY OF LEAVES OF PET - ETHER EXTRACT OF *VANDA TESSELLATA* ROXB

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ABSTRACT

Vanda tessellata Roxb. (Family: Orchidaceae) have been used in the indigenous medicine such as *Ayurveda* and local traditional medical practices. In this study, membrane stabilizing properties of leaf extract of the plant was investigated on HRBC. HRBC membrane stabilizing potency was performed on human red blood cell suspension. The activity of the extracts was compared with hydrocortisone sodium which was used as the standard. The phytochemical screening revealed the presence of flavonoids and steroids. The results of the study for the first time show that the plant possesses mast cell stabilizing activity, confirming the traditional claims. Future research should focus on the isolation and identification of the active constituent responsible for membrane stabilizing activity.

Key words: Hydrocortisone, Membrane stabilizing activity**,** Petroleum ether extract

INTRODUCTION

Vanda tessellata Roxb (Family: Orchidaceae) is a species of orchid occurring from the Indian subcontinent to Indochina. *Vanda tessellata* 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 used in Otitis as ear drops. 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. The plant has an alkaloid, flavonoids glycoside, tannins, β-sitosterol, γ-sitosterol and a long chain aliphatic compound, 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]. The present study was focused on evaluating the membrane stabilization activity of pet – ether extract of *Vanda Tessellata* Roxb by using HRBC method, as it was not scientifically proven.

MATERIALS AND METHODS

This study protocol was approved by Institutional Ethical Commitee, Department of Pharmacology at Dr. V.R.K. Women's Medical College, A.P. *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 qualitative chemical investigation for the identification of phyto constituents[10] like triterpenoids, saponins, alkaloids, carbohydrates, tannins, flavonoids and glycosides using appropriate reagents. The extracts were treated with dilute hydrochloric acid and filtered. The filtrate is used in the following tests.

Test for alkaloids (Mayer's test):

The extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid.

Test for tannins:

The extract was treated with 10% lead acetate solution; appearance of white precipitate indicates the presence of tannins.

Test for flavonoids (Shinoda test):

To the extract, add 5 ml 95% ethanol, few drops of conc. HCl and 0.5g magnesium turnings. Pink coloration indicates the presence of flavonoids.

Test for saponins (froth test):

1ml of the extract was diluted to 20 ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

Test for terpenoids (Salkowski test):

Five ml of extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for carbohydrates (Molisch's test):

The extract was treated with 3ml of alpha–napthol in alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet colour ring at the junction of two liquids indicates the presence of carbohydrates

Test for glycosides (modified Borntrager's test):

To 5 ml of extract add 5ml of 5% FeCl3 and 5ml dil. HCl. Heat for 5 min. in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate the organic layer and add equal volume of dil.Ammonia. Ammonical layer shows pinkish red color.

Membrane Stabilization by Human Red Blood Cell (HRBC) Method [11]

The human red blood cell membrane stabilization method was used for this study. 10ml of fresh blood was collected from blood bank (Dr. V.R.K. Women's Medical College Hospital). The collected blood was mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. The drug samples(Pet-ether extract of *Vanda Tessellata* Roxb) ranging from a concentration of

10µg/ml -1000 µg/ml were prepared by suspending the residue in hot distilled water. The assay mixture contained the drug, 1 ml phosphate buffer, 2 ml hyposaline and 0.5 ml HRBC suspension. Prednisolone was used as the reference drug. Instead of hyposaline 2 ml of distilled water was employed as control. All the assay mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 minutes. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. The % inhibition of hemolysis was calculated = [Abs $_{control}$ –Abs $_{test}$] /Abs $_{control}$×100.

Table-1

Effect of pet-ether extract of *Vanda Tessellata* Roxb on hypotonicity induced membrane stabilization**.**

Concentrations	Mean inhibition of hemolysis (%)	
	V. tessellata Roxb.	Prednisolone
10 µg/ml	26.32%	40.32%
50 µg/ml	38.44%	73.15%
100 µg/ml	54.23%	93.85%
200 µg/ml	62.64%	99.01%
400 µg/ml	68.75%	99.03%
800 µg/ml	74.14%	99.01%
1000 µg/ml	75.22%	99.03%
IC_{50}	87 µg/ml	16.56 µg/ml

Each value represents the mean of (n=3) observations.

RESULTS

Phytochemical Screening:

The chemical tests indicate the presence of phytoconstituents like the flavonoids, tannins, saponins, terpenoids, steroids and alkaloids in the petroleum-ether extract.

The standard drug Prednisolone exhibited a dose dependent inhibition of hypotonicity induced hemolysis and showed highest protection of 99.03% at 1000µg/ml. The IC_{50} of Prednisolone for hypotonicity induced hemolysis was calculated from the concentration response curve and found to be 16.56 µg/ ml. The pet-ether extract of *Vanda Tessellata* Roxb have inhibited hypotonicity induced haemolysis of red blood cells in a concentration dependent manner when compared with the vehicle treated group. The extract exhibited a dose dependent inhibition of hemolysis and showed highest protection of 75.22% at 1000 µg/ml and The IC_{50} of extract is 87 µg/ml. (Table-1)

DISCUSSION

The present study investigated the HRBC membrane stabilizing potency of petroleum ether extract of *Vanda Tessellata* Roxb. The erythrocyte membrane resembles the lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte membrane could be extrapolated to the stabilization of lysosomal membrane [12]. Therefore as a membrane stabilizer, it interferes with the release and/or action of mediators like histamine, serotonin, prostaglandins, and leukotrienes[13]. It is well established that both Non-steroidal anti-inflammatory and steroidal ant-inflammatory drugs protect erythrocyte membranes from hypotonicity induced hemolysis. Based on the aforesaid observations of the experimental data on membrane stabilization by hypotonicity induced hydrolysis, it can be concluded that treatment with pet - ether extract of *Vanda Tessellata* Roxb caused a significant protection of erythrocyte membrane in dose dependent manner, similar to the standard drug Prednisolone.

CONCLUSION

The present study for the first time provides evidence for the mast cell stabilization activity of pet – ether extract of *Vanda Tessellata* Roxb in HRBC method. The presence of flavonoids and steroids in extract could be responsible for these activities. Further studies have to conduct on the isolation, identification and characterization of the pharmacologically potent moiety responsible for the activity.

REFERNCES

1. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. New Delhi. C.S.I.R, 1956.
2. Basu K, Das gupta B, Bhattacharya SK, Lal R, Das PK. Anti-inflammatory principles of Vanda roxburghii. Curr Sci 1971; 40-86.
3. Kirtikar PK, Basu BD. Indian Medicinal Plants. 2nd Ed. (Reprint Ed. 1975) Dehra Dun, M/s Bishen Singh Mahendra Pal Singh, 1935.
4. Madhava chetty K. et al. "*Vanda tessellata*" Flowering Plants of Chittoor District, Andhra Pradesh, India. 2008.
5. Ghani A., Rastogi Ram., Mehrota B. N. Compendium of Indian Medicinal plants. Vol. V, CDRI Lucknow and National Institute of science and communication. 757,1990-1994.
6. Das S, Bhattacharya A, Bhattacharya AK. Active constituents of Vanda roxburghii R. Br J Indian Chem Soc 1967; 44: 804-5.
7. Prasad DN, Satyawati GV, Das gupta B, Das PK. Antiniflammatory activity of the steroidal fraction obtained from Vanda roxburghii. Abstract of paper. 1st Congress of the South East Asia & Pacific Area League against Rheumatism, Bombay 1968; 68.
8. Chawla AS, Sharma AK, Handa SS, Dhar KL. Chemical studies and anti inflammatory activity of Vanda roxburghii roots. Indian J Pharmaceutical Sci 1992; 54:159-61.
9. Lawler LJ. Ethnobotany of the Orchidaceace. In: Arditti J, editor, Orchid Biology: Review and Perspectives-3. Ithaca, Cornell University Press, 1984; 27-149.
10. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. Pune, India, Nirali Prakashan 2000.
11. Shinde, U. A., Phadke, A. S., Nair, A. M., Mungaantiwar, A, A., Dikshit, V. J., & Saraf, V. O. Membrane stabilizing activity a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia*, 1994; 70: 251-257.
12. Shinde, U. A., Phadke, A. S., Nair, A. M., Mungaantiwar, A, A., Dikshit, V. J., & Saraf, V. O. Membrane stabilizing activity a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia*, 1999, 70, 251-257.
13. Braca, A., Tommasi, N. D., Bari, L. D., Pizza, P. M., Morelli, I. Antioxidant principles from *Bauhenia Terapotensis*. *Journal of Natural Products*, 2001, 64, 892-895.

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