In Vitro Antioxidant and Mast Cell Stabilizing Activity of Different Extracts of *Vanda Spathulata* Flowers

Prasad Konduri¹, Eswar Kumar Kilari² and Ravindra Babu Sajja¹,³*

¹Department of Pharmacology, Shri Vishnu College of Pharmacy, Bhimavaram, India.
²Department of Pharmacology, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.
³Department of Pharmacology, Malla Reddy Institute of Pharmaceutical Sciences, Secunderabad, India.
*Corresponding Author E-mail: ravicology@gmail.com

https://dx.doi.org/10.13005/bpj/2387

(Received: 26 September 2020; accepted: 12 January 2022)

The objective of the present work is to evaluate the petroleum ether, ethyl acetate and methanolic extracts of *vanda spathulata* on experimental models for in vitro antioxidant activity and mast cell stabilizing activity. Mast cell stabilization effect was assessed using compound 48/80 induced mast cell degranulation in rat peritoneal mast cell. The antioxidant activity was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. *Vanda spathulata* methanolic extract (VSME) at doses of 200 and 400mg/kg exhibited significant protection (p<0.01) and petroleum ether (VSPE) and ethylacetate (VSEA) extracts at the dose of 400mg/kg showed significant protection (p<0.05) against mast cell degranulation. VSME revealed better DPPH radical scavenging activity (IC₅₀ value 38.39 µg/ml) which was closely resembled to standard ascorbic acid (IC₅₀ value 33.98 µg/ml) when compared to other extracts. Phytochemical study revealed the presence of alkaloids, saponins, flavonoids, tannins, steroids and glycosides. From the results *Vanda spathulata* shows mast cell stabilizing activity mainly due to phytochemical constituents and strong antioxidant property of plant extracts.

**Keywords:** Antioxidant; Comp 48/80; DPPH; Flavonoids; Mast cell degranulation; *Vanda spathulata*.

Herbs used as medicinal purposes for many centuries. Now a days, herbal medicines are used globally as home remedies in treatment of various ailments. People rely mainly on herbal drugs to meet their primary health care needs in some developing countries. Herbal medicines are also gaining popularity as alternative and complementary therapies in many industrialized and developed countries. There has been an increase in scientific studies on herbal medicines, due to insufficient availability of reliable data still today. Mast cells are found in mucosal and epithelial tissues throughout the body. In rodents, mast cells also reside in peritoneal and thoracic cavities¹. Activated mast cells are an important source of histamine, cysteinyl leukotrienes, and prostaglandins. These mediators are central to bronchoconstriction, vasodilation, and the allergic inflammatory cascade². ROS have been shown to be associated with the pathogenesis of asthma by evoking bronchial hyper-reactivity as well as directly stimulating histamine release from mast cells and mucus secretion from airway epithelial cells³.
Vanda spathulata is an epiphytic orchid species belongs to a family Orchidaceae with golden yellow flowers. It is found in southern India and Srilanka. It favors to grow on small trees and bushes in open sun. Flowering and fruiting occur between September and January. It is also used in folk and ayurvedic medicine, dried flowers are powdered and used for a variety of illnesses such as asthma, depression and manic troubles. The leaf juice of plant used for temper the bile and frenzy abate. It is thought it may have some memory enhancing properties and its antioxidant activity has been investigated. It is also used as liver tonic.

In spite of the traditional indications, marked free radical scavenging capacity, the mast cell stabilization potential of Vanda spathulata has not been investigated. In the present study, we have investigated different extracts of Vanda spathulata on DPPH free radical scavenging activity and compound 48/80-induced induced mast cell degranulation in rat peritoneal mast cells.

**MATERIALS AND METHODS**

**Plant Material**

The flowers of Vanda spathulata was collected from the forest of Tirumala region, Andhra pradesh, India. The plants have been taxonomically identified and authenticated by Dr. K. Madhava Chetty, Department of Botany, in S.V.University, Tirupathi, Andhra Pradesh, India. A voucher specimen No: 2194 has been deposited in the herbarium of Malla Reddy Institute of Pharmaceutical Sciences, Hyderabad, India, for future reference.

**Preparation of Extracts**

Flowers of Vanda spathulata were washed thoroughly and cleaned with tap water, air dried and coarsely powdered. Powdered plant material (500g) was extracted successively with petroleum ether, ethyl acetate and methanol in a soxhlet extractor till the solvent in siphon tube of an extractor become colorless. The so obtained extract was kept in a desicator to remove moisture and stored in an amber-colored bottle at 4°C. The extracts are abbreviated as VSPE, VSEA and VSME for petroleum ether, ethyl acetate and methanolic extract of Vanda spathulata, respectively. The yields of these extracts were found to be 5.7%, 11.1%, and 15.5% respectively.

**Experimental animals**

Adult male wistar albino rats (150–200 g) were procured from Sanzyme Pvt Ltd, Hyderabad. All the Animals were maintained room temperature at 22 ± 1°C, relative humidity of 55 ± 5%, 12-hr light and dark cycle, and allowed free access to food (standard pellet diet, certified VRK laboratory animal feed, Sangli, Maharashtra, India.) and water ad libitum. All the experimental procedures and protocols were approved by the Institutional Animal Ethics Committee (Approval No: 11/MRIPS/CPCSEA-IAEC-II/Hyd/2016) of Malla Reddy Institute of Pharmaceutical Sciences, Secunderabad (Reg. No: 1662/PO/Re/S/12/CPCSEA).

**Preliminary Phytochemical Screening**

The preliminary phytochemical analysis of various extracts of vanda spathulata was performed for Alkaloids, Saponins, Tannins, Steroids and triterpenoids Flavonoids, Phenols, Glycosides, Carbohydrates, Proteins, Fixed oils & Fats, Gums & Mucilages according to published standard methods.

**Acute toxicity study**

Healthy adult swiss albino mice weighing about 20-30 g were used in this study. Acute toxicity test was performed according to OECD guideline 423.

**Compound 48/80 induced mast cell degranulation**

This method was performed on healthy adult albino rats. Different extracts (pet ether, ethyl acetate and methanol) of Vanda spathulata at doses of 100, 200 and 400mg/kg, p.o and the standard drug, disodium cromoglycate (10 mg/kg, i.p) was administered to different groups of animals each consisting of 6 animals for 4 days. On the 5th day, 2 h after the last treatment, 10 ml/kg of normal saline was injected into the peritoneal cavity of rats. After gentle abdominal massage for 90s, the peritoneal fluid containing mast cells was collected and transferred into the eppendorf test tubes containing 7-10ml of RPMI-1640 media (pH 7.2–7.4). Then the mast cells were washed three times with RPMI-1640 media by centrifugation at low speed (500–600 rpm), discarding the supernatant and re suspending the pellets of mast cells in the medium. Mast cells from the treated and control groups were incubated with 0.1ml of compound 48/80 (10µg/ml) at 37°C for 10 min in a water bath. After incubation, mast cells were
stained with 1% toluidine blue solution and percent of protection against degranulation was counted under high power microscope (45X). Percentage protection of the mast cells in the control group and the treated groups were calculated by counting the number of degranulated mast cells from total of at least 100 mast cells counted. Percent inhibition of mast cell degranulation for each treatment was calculated by following formula:

\[
\%\text{ inhibition of MCD} = \frac{1 - \frac{\text{Number of degranulated mast cells}}{\text{Total number of mast cells}}} \times 100
\]

**In vitro antioxidant activity**

**Determination of 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical scavenging Activity**

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca et al., 2003. An aliquot of 3ml of 0.004% DPPH solution in methanol and 0.1 ml of plant extract at various concentrations (5-125µg/ml) were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition activity was calculated as

\[
\%\text{ inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

**Statistical analysis**

The results obtained were expressed as mean ± SEM. Statistical analysis was performed using a one-way analysis of variance (ANOVA). Data was considered statistical significant at \( p < 0.05 \). When data was found to be very (\( p < 0.01 \)) or highly (\( p < 0.001 \)) significant, this was indicated in the results. All statistical analyses were performed using Graph Pad prism 8 software (San Diego, CA).

**RESULTS**

**Preliminary Phytochemical Screening**

The results obtained from the phytochemical tests are presented in Table 1. Preliminary phytochemical screening of different extract of *Vanda spathulata* showed the presence of alkaloids, flavonoids, glycosides, tannins, steroids & terpenoids and saponins.

**Table 1.** Effect of different extracts of *Vanda spathulata* on compound 48/80 induced rat peritoneal mast cell degranulation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg/kg)</th>
<th>% Mast cells degranulation</th>
<th>% inhibition of degranulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>Normal saline 1ml/kg</td>
<td>3.50 ±0.43</td>
<td>_</td>
</tr>
<tr>
<td>Positive control (Compound 48/80)</td>
<td>10µg/ml</td>
<td>79.50 ±3.4*</td>
<td>_</td>
</tr>
<tr>
<td>Disodium cromoglycate</td>
<td>10mg/kg</td>
<td>23.00±1.8**</td>
<td>71.06</td>
</tr>
<tr>
<td>VSPE</td>
<td>100mg/kg</td>
<td>73.54±3.9**</td>
<td>7.49</td>
</tr>
<tr>
<td>VSPE</td>
<td>200mg/kg</td>
<td>69.62±2.05**</td>
<td>12.42</td>
</tr>
<tr>
<td>VSPE</td>
<td>400mg/kg</td>
<td>60.45±2.34*</td>
<td>23.96</td>
</tr>
<tr>
<td>VSEA</td>
<td>100mg/kg</td>
<td>70.33 ± 3.1**</td>
<td>11.53</td>
</tr>
<tr>
<td>VSEA</td>
<td>200mg/kg</td>
<td>67.10± 2.7*</td>
<td>15.59</td>
</tr>
<tr>
<td>VSEA</td>
<td>400mg/kg</td>
<td>58.83 ± 1.98**</td>
<td>26.00</td>
</tr>
<tr>
<td>VSME</td>
<td>100mg/kg</td>
<td>64.50±2.64*</td>
<td>18.86</td>
</tr>
<tr>
<td>VSME</td>
<td>200mg/kg</td>
<td>43.76±1.67**</td>
<td>44.95</td>
</tr>
<tr>
<td>VSME</td>
<td>400mg/kg</td>
<td>25.32±2.48**</td>
<td>68.15</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. n=6 rats/group. Negative control: saline, Positive control: compound 48/80 (10g/ml). Standard: disodium cromoglycate (10mg/kg), VSPE: *Vanda spathulata* petroleum ether extract, VSEA: *Vanda spathulata* ethyl acetate extract, VSME: *Vanda spathulata* methanolic extract. #p<0.01: Significantly different from negative control. ** p< 0.01, *p< 0.05: Significantly different from positive control. ns: Not significant.
Acute toxicity studies

Various extract of *Vanda spathulata* at a dose of 2000mg/kg administered orally did not showed any toxicity in tested animals. No signs of observable toxicity and no mortality was seen during the period of 14 days study. Hence there is no LD₅₀ and tested plant extracts are considered safe and nontoxic. Therby the therapeutic doses for the pharmacological evaluation was 1/20th, 1/10th and 1/5th of the maximum tolerated which was then fixed to be 100mg/kg, 200mg/kg and 400mg/kg p.o of the experimental animals.

**Compound 48/80 induced mast cell degranulation**

Effect of different extracts of *Vanda spathulata* on compound 48/80 induced rat peritoneal mast cell degranulation

The group administered with compound 48/80 showed (79.50 ±3.4%) degranulation of mast cells while groups treated with disodium cromoglycate (10mg/kg), a reference standard drug significantly protect (23.00±1.8%) degranulation of mast cells. The negative control group showed 3.50 ±0.43% mast cell degranulation.

VSME at doses (100, 200 and 400mg/kg) showed an inhibition of 18.86%, 44.95% and 68.15% of mast cell degranulation respectively. VSME at concentrations 200 and 400mg/kg showed significant (p<0.01) protection against compound 48/80 induced mast cell degranulation (Table. 1and Fig.1). VSEA at doses 200 and 400mg/kg showed significant protection (15.59%, 26.00%), but lower concentration (100mg/kg) did not show any protection (11.53%) against mast cell degranulation (Table.1 and Fig.1). VSPE showed significant protection (23.96%) at 400mg/kg concentration. However, it did not show any protection at lower concentrations (100 and 200mg/kg). Histopathological changes were shown in Fig.3.

**In-vitro Antioxidant activity**

In the present study, the antioxidant activity of different extracts of *Vanda spathulata* was evaluated by determining their DPPH radical scavenging abilities and results were presented as IC₅₀ (µg/ml). The scavenging effect of Ascorbic acid, methanolic (VSME), ethyl acetate (VSEA) and petroleum ether (VSPE) extracts of *Vanda spathulata* showed concentration dependent scavenging activity on DPPH radicals. The results of scavenging activity on DPPH radicals were given in Table.2 and Fig.2. The lower the IC₅₀ value indicated a higher antioxidant activity. The mean IC₅₀ values for hydroxyl radical of VSME, VSEA and VSPE were found to be 38.39µg/ml, 61.47 µg/ml and 86.07µg/ml, respectively. The mean IC₅₀ value of ascorbic acid was found to be 33.98µg/ml.

![Degranulation by compound 48/80](image-url)

*Fig. 1. Effect of *Vanda spathulata* pet ether, ethyl acetate and methanolic extracts on compound 48/80 induced rat peritoneal mast cell degranulation. All bars represent the mean ± SEM (n=6). Negative control: saline, Positive control: compound 48/80 (10µg/ml), DSCG (standard): disodium cromoglycate (10mg/kg), VSPE: *Vanda spathulata* petroleum ether extract, VSEA: *Vanda spathulata* ethyl acetate extract, VSME: *Vanda spathulata* methanolic extract. #p<0.01: Significantly different from negative control. ** p< 0.01, *p< 0.05: Significantly different from positive control. ns: Not significant.*
IC\textsubscript{50} value of VSME was closely resembled to that of standard ascorbic acid. The order of free radical scavenging activity of different extracts of \textit{Vanda spathulata} against DPPH radical was Ascorbic acid > Methanolic extract > Ethyl acetate extract >Petroleum ether extract.

**DISCUSSION**

Mast cells are present virtually in all organs and are well known participants in allergic diseases\textsuperscript{13}. Mast cell activation occurs when the Fc\textsubscript{RI}-bound IgE is cross linked by binding to multivalent antigen. Signalling in this way stimulates release of both pre-formed mediators and the production of new inflammatory mediators. Their activation and subsequently degranulation can be elicited by not only the aggregation of cell surface-specific receptor for IgE, Fc\textsubscript{RI}, but also by the number of positively charged substances like compound 48/80\textsuperscript{14}. Compound 48/80 increases intracellular calcium level and generates ROS.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Ascorbic acid</th>
<th>VSME</th>
<th>VSEA</th>
<th>VSPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>21.67±0.67</td>
<td>17.16±0.13</td>
<td>11.2±0.81</td>
<td>6.79±0.14</td>
</tr>
<tr>
<td>10</td>
<td>33.71±0.58</td>
<td>38.12±0.67</td>
<td>16.33±0.79</td>
<td>13.43±0.269</td>
</tr>
<tr>
<td>25</td>
<td>54.89±0.88</td>
<td>47.81±1.09</td>
<td>35.48±0.82</td>
<td>25.62±0.321</td>
</tr>
<tr>
<td>50</td>
<td>64.97±0.68</td>
<td>62.30±0.54</td>
<td>47.67±0.67</td>
<td>34.88±0.11</td>
</tr>
<tr>
<td>75</td>
<td>78.95±0.33</td>
<td>76.28±1.45</td>
<td>53.85±0.76</td>
<td>48.60±0.31</td>
</tr>
<tr>
<td>100</td>
<td>88.66±0.56</td>
<td>85.56±0.88</td>
<td>76.33±0.93</td>
<td>53.31±0.54</td>
</tr>
<tr>
<td>125</td>
<td>92.40±0.92</td>
<td>89.23±0.58</td>
<td>85.10±0.43</td>
<td>67.30±0.25</td>
</tr>
<tr>
<td>R\textsuperscript{2} Value</td>
<td>0.909</td>
<td>0.906</td>
<td>0.969</td>
<td>0.974</td>
</tr>
<tr>
<td>IC\textsubscript{50} Value</td>
<td>33.98</td>
<td>38.39</td>
<td>61.47</td>
<td>86.07</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (n=3) of triplicate observations. IC\textsubscript{50} Values were obtained from the figure and R\textsuperscript{2} Values are mentioned in the table. VSME: Vanda spathulata methanolic extract. VSEA: Vanda spathulata ethyl acetate extract, VSPE: Vanda spathulata petroleum ether extract.

![DPPH Method](image_url)

**Fig. 2.** Graphical representation of \textit{Vanda spathulata} flower extracts on hydroxyl radical scavenging model. VSME: \textit{Vanda spathulata} methanolic extract. VSEA: \textit{Vanda spathulata} ethyl acetate extract, VSPE: \textit{Vanda spathulata} petroleum ether extract.
MAST CELL DEGRANULATION STUDIES

Fig. 3. Histopathological studies of effect of various extracts of *Vanda spathulata* on mast cell degranulation.

Microscopic photographs of rat peritoneal mast cells. (A) Normal control group shows normal mast cell histology, (B) Compound 48/80 (10µg/ml) treated group shows significant mast cell degranulation, (C) Disodium cromoglycate (10 mg/kg) treated group shows significant mast cell stabilization, (D) VSME (100mg/kg) treated group shows less mast cell stabilization, VSME(200mg/kg) treated group shows moderate mast cell stabilization, (F)VSME(400mg/kg) treated group shows significant mast cell stabilization, (G) VSEA (100mg/kg) treated group did not show mast cell stabilization, (H) VSEA(200mg/kg) treated group shows moderate mast cell stabilization, (I)VSEA(400mg/kg) treated group shows significant mast cell stabilization, (J) VSPE (100mg/kg) treated group did not show mast cell stabilization, (K) VSPE(200mg/kg) treated group shows less mast cell stabilization, (L)VSPE(400mg/kg) treated group shows significant mast cell stabilization.
endogenously which results in mast cell disruption to produce proinflammatory mediators\textsuperscript{15}. Numerous reports established that stimulation with compound 48/80 or IgE initiates the activation of signal transduction pathway which leads to histamine release. Degranulated mast cells release number of mediators including histamine, a potent vasoactive mediator, which may precipitate hypersensitive reactions\textsuperscript{16}. Compound 48/80 was used to induce mast cell degranulation and histamine release from RPMC. Decrease in histamine release from RPMC was taken as an index of mast cell stabilization.

Phytochemical screening of \textit{Vanda spathulata} showed the presence of saponins, flavonoids, tannins, steroids and glycosides. Saponins are reported to possess mast cell stabilizing, anti allergic and anti-histaminic activities\textsuperscript{17-19}. Tannins are reported to possess mast cell stabilizing, anti-allergic and anti-histaminic activities\textsuperscript{20}. The flavonoids also inhibited the histamine release induced by compound 48/80\textsuperscript{21}. Thus, the presence of flavonoids and Tannins in the plant extracts might be responsible for the mast cell stabilizing activity.

The extracts of \textit{Vanda spathulata} showed the attenuation of compound 48/80 induced mast cell degranulation in dose dependent fashion. The methanolic extract of \textit{Vanda spathulata} showed most significant mast cell stabilizing activity. It was found that methanol extract of \textit{Vanda spathulata} was potent inhibitor of DPPH free radical while petroleum ether extract was least active.

\textbf{CONCLUSION}

It may be concluded that \textit{vanda spathulata} possess mast cell stabilization property mediated through their phytochemical constituents and antioxidant capacity. In addition, further studies are required to clear molecular mechanisms of these plant extracts to investigate for the successful development of the drug for clinical use.

\textbf{ACKNOWLEDGMENT}

The authors are thankful to the Shri Vishnu College of Pharmacy, Bhimavaram, AU College of Pharmaceutical Sciences, Vishakhapatnam and Malla reddy Institute of Pharmaceutical Sciences, Hyderabad for providing necessary facilities to carry out the work.

\textbf{Conflict of interest}

The authors declare that they have no conflict of interest.

\textbf{REFERENCES}

11. Gupta PP, Srimal RC, Srivastava M, Singh KL and Tandon JS. Antiallergic activity of


