Antiproliferative Activity of Hydroalcoholic Extract of *Aerva Lanata* and *Lantana Camara Flowers*

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Abstract

The well-known traditional herbs *Aerva Lanata* and *Lantana Camara* are widely used in India for the management of different ailments. In the present study, flowers of *Aerva Lanata* and *Lantana Camara* were selected for screening the combined antiangiogenic and anticancer potentials, as our previous studies on individual flower extract and the literature supports those flowers have highest quantity of natural constituents. Hydroalcoholic (80%-water, 20%-alcohol) extract of *Aerva Lanata* and *Lantana Camara* flowers were prepared. Antiangiogenic activity was screened by Cell proliferation assay, which is supposed to have the highest (*in vitro*). All the groups were compared with the control group using one-way ANOVA, followed by a post hoc test, Dunnett’s test, to compare the mean of all the groups with the control mean. In the studies on human umbilical vein endothelial cells the test extract (1-100 nM) showed significant inhibition of proliferation in a dose-dependent manner was observed. *Aerva Lanata* and *Lantana Camara* in combination reported potent antiproliferatory activity in a dose dependent manner.

Keywords: *Aerva Lanata*, *Lantana Camara*, antiproliferatory, ANOVA.
I. INTRODUCTION

Anti-angiogenic agents specifically aim on dividing vessels so, are effective on many diseases caused due to abnormal excess angiogenesis; more over they treat the disease from the very root cause [1-4].

Endothelial cell (EC) structure and functional integrity are important in the maintenance of the vessel wall and circulatory functions, and most of these endothelial functions are regulated by ion channels [5,6]. The role of ion channels in the pathophysiology of diseases has been extensively discussed [7-9]. Despite their prime role in several diseases, there are very few drugs targeting specifically the ion channels as therapeutic inhibitors for the treatment of diseases caused by excessive angiogenesis. Such clinically approved ion channel modulators with well-known safety profiles may be reframed in the treatment of many diseases, saving significant time and money [10].

The traditional medicine system of India is a rich source of valuable medicinal plants but there is no scientific data reported to establish the activity of Aervalanata and Lanata Camara. Hence these plants need to be evaluated, based on their biological efficacy and chemical constituents for the drug development [10-14]. So, we have selected Aervalanata and Lanata Camara flowers, of India for the present study. The flowers were subjected to bioactivity guided isolation and screening for antiproliferatory activity in order to investigate and justify the traditional claim.

Aervalanata and Lanata Camara are mainly used as herbal medicine and in some areas as firewood and mulch [15,16]. These are also used for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atox of abdominal viscerai.4. Extracts from the leaves exhibit antimicrobial, insecticidal and nematicidal activity and also contain verbascoside, which possesses antimicrobial, immunosuppressive and antitumor activities. Several previous reports have described antifungal, antiproliferative and antimicrobial activities of L. camara [17, 18].

Although Aervalanata and Lanata Camara are used traditionally by Ayurvedic physicians, however, there is no report on the anti-angiogenic potentials by any of the active constituents isolated from Aervalanata and Lanata Camara [19-20]. Hence these plants were selected for screening antiproliferatory activity.

II. MATERIALS AND METHODS

2.1. Collection and authentication of plant part

The flowers of Aerva lanata and Lanata camara were collected from Medchal District, Hyderabad, Telangana. The plant was authenticated by Dr. K. Madhava Chetty, Department of Botany, Osmania University.

2.2. Extraction

The powdered dried flowers were used for the extraction using hydroalcoholic (80-20%) solvent system by Soxhlet extraction. The extract was condensed using a desiccator and stored in an air tight container for future use.

2.3. Endothelial cell culture

Human umbilical vein EC (HUVECs) were grown on gelatinized dishes in M199 supplemented with 15% fetal calf serum, 50 U/mL penicillin, 50 mg/mL streptomycin, 50 mg/mL gentamycin, 2.5 mg/mL amphotericin B, 5 U/mL heparin, and 150-200 mg/mL EC growth supplement. Cells were used between passages 1 and 3. Each experiment shown is derived from three independent repeats, each time using different pools (isolates) and/or passages of cells [21,22].
2.4. Endothelial cell proliferation assay
The HUVECs were seeded in 24-well plates at a density of 6000 cells/cm² and incubated overnight in Dulbecco’s modified Eagle’s medium. The cells were exposed to different concentrations of HALLC, bevacizumab, VEGF, or vehicle and allowed to proliferate for 48h. At the end of this incubation time, the cells were trypsinized, and their number was determined using a Neubauer hemocytometer [21,22].

III. RESULTS & DISCUSSION

Na+ and Ca²⁺ channels are important for cell proliferation, migration, and cord-like network formation. To further test the link between channel inhibition and anti-angiogenesis, EC-based assays triggering proliferation and mobilization were performed. In the cell proliferation assay VEGF resulted in elevated proliferation (increase of 50%), whereas in our previous study bevacizumab and the three doses of HALL showed significant inhibition of proliferation (inhibition by 50%, 80%, 70%, and 60%, respectively). The results of the present study reports that bevacizumab and the three doses of HALLC showed greater significant inhibition of proliferation (inhibition by 50%, 70%, 60%, and 50%, respectively). Aerva lanata and Lanata camara flowers in combination showed greater antiproliferative activity which was equal to the commercial product bevacizumab.

Table 1: Tables representing the effect of HALLC on Modulation of endothelial cell proliferation

<table>
<thead>
<tr>
<th>S.NO</th>
<th>TREATMENT</th>
<th>Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>100.0±1.00***</td>
</tr>
<tr>
<td>2</td>
<td>Bevacizumab(1µM)</td>
<td>50.3±1.29***</td>
</tr>
<tr>
<td>3</td>
<td>HLC (1µM)</td>
<td>68.7±1.52***</td>
</tr>
<tr>
<td>4</td>
<td>HLC (10µM)</td>
<td>78.5±1.61***</td>
</tr>
<tr>
<td>5</td>
<td>HLC (100µM)</td>
<td>50.0±1.29***</td>
</tr>
<tr>
<td>6</td>
<td>VGEF (500pM)</td>
<td>149.0±2.00***</td>
</tr>
</tbody>
</table>

All the results were expressed as mean ± standard error of mean; n=6. ***p<0.001, **p<0.01, *p<0.05 vs control, ns: Non-significance.

Figure 1: Modulation of endothelial cell responses to HALLC, bevacizumab and VEGF. Cell proliferation was determined by cell counting with a haemocytometer.
HALLCantiproliferatory action may be due to calcium channel blocking, as *A. Lanata* has calcium channel blocking action [23]. Ca2+ ions have long been known to be secondary messengers in various cellular signal resulting in angiogenesis. The fact that deprivation of extracellular Ca2+ leads to cell growth arrest in G1/S indicates that Ca2+ is required for cell cycle progression [24-27].

The anti-angiogenic action of *L. camara* is due to the presence of alkaloids, glycosides and triterpenoids which act on various cellular mechanisms resulting in angiogenesis. Now both the medicinal flowers in combination showed a synergistic activity. Modulation of EC response to HALLC was significant at all the test doses of 1µM, 10µM, and 100µM on the EC proliferation. HALLC being a strong blocker of Ca2+ ion influx and with active constituents like alkaloids, glycosides and triterpenoids, gave significant antiproliferative results. This extract serves as good chemical template that can be structurally modified for more site-specific actions for anti-angiogenic therapy [25,26].

IV. CONCLUSION

The present investigation supports our previous study which reported that *Aervalanata* and *Lanata Camara* individually had good antiproliferative action and now this study supports that both the medicinal flowers in combination showed an additive antiproliferative action.

Conflict of Interest
The authors declare no conflicts of interest.
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