**Evaluation of analgesic and anti-inflammatory activity of Alpinia speciosa K. Schum rhizomes**

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**ABSTRACT**

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**Alpinia speciosa** is an evergreen tropical perennial herb used as balsamic, diuretic, stomachic, used for cold, flu, fever, flatulence and indigestion. It is widely cultivated and distributed in most tropical and Semi-tropical areas including Brazil, Peru, the Amazon and in United States. This plant is an evergreen tropical perennial that grows in upright clumps to 8-10' tall in tropical climates. It produces fleshy rhizomes much like ginger that have a ginger like aroma. In Brazilian herbal medicine the essential oil of the leaf is used for high blood pressure and as a heart tonic. In other parts of the world the plant is considered balsamic, Diuretics and Stomachic and traditionally used for cold, flu, fevers, flatulence, stomach problems and indigestion. The rhizome was found to contain Phenolic compounds and seeds contain cardonadin and alpinetin. Considering the medicinal value of this plant, the present study was undertaken to evaluate the analgesic and anti-inflammatory activity of *Alpinia speciosa* K. Schum rhizome.

**INTRODUCTION**

In recent years, significant contribution made by herbal medicines to human health has lead to increased popular, official and commercial interest. Herbal preparation against ailments is gaining importance due to the partial rejection of synthetic drugs because of their side effects especially NSAIDS. The long term use of synthetic drugs is always feared during the treatment of chronic diseases. But this is not the case with that of herbal drugs and medicines obtained from natural sources. On the basis of the above criteria and paramount importance of herbal drug research, *Alpinia speciosa* K. Schum rhizome was selected for the study.

**MATERIALS AND METHODS**

**Plant Material**

The rhizomes of *Alpinia speciosa* were collected in the month of January from shervoroyan hill, Salem, Tamil Nadu. The plant material was identified and authenticated by Mr. A. Balasubramanian, Consultant, Central Siddha Research Institute, Salem, Tamil Nadu.

**Extraction**

The air dried and powdered rhizomes of *Alpinia speciosa* K. Schum were extracted with ethanol (90%) using soxhlet apparatus for 72 hours. The solvent was completely removed under reduced pressure to get crude extract. The extract obtained was stored in a labeled, airtight, container in a dessicator to carryout phytochemical and pharmacological studies.

**Qualitative Evaluation of Extract**

The extract was subjected to preliminary phytochemical investigation for the identification of various phytoconstituents.

**Animals**

Adult albino mice of female sex weighing 22-30gm were utilized for Acute oral toxicity study, while adult albino mice of either sex weighing 22-30gm were used for analgesic activity. Healthy wistar rats weighing 180-220gms were used for the Anti-inflammatory study. The animals were obtained from Agricultural University, Manuthy, Trissur and were housed in polypropylene cages. The animals were maintained under standard laboratory conditions (25±2°C, 12hr light and dark cycle). The animals were fed with standard diet and water adlibitum. Ethical clearance for handling of animals and the procedures used in the study was obtained from the Institutional animal ethical committee before performing the study on animals.

**Evaluation of Analgesic Activity**

**Tail Immersion Method**

Adult albino mice of either sex weighing 22-30gm were used for tail immersion method. The animals were divided into four groups of six animals each. Each group served as control received vehicle 2% w/v acacia suspension. Group II animals received the standard drug Pentazocine (5mg/kg) intraperitoneally served as a reference standard. Group III animals received ethanol extract of *Alpinia speciosa* 250mg/kg orally and Group IV animals received ethanol extract of *Alpinia speciosa* 500mg/kg orally.

The distal 2-3cm portion of mouse tail was marked and this part of tail was immersed in hot water maintained at 55°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time. The maximum cutoff time for immersion was fixed at 15 Sec to avoid injury to the tail.

The initial reaction time was taken for all animals before administration of test and standard drugs. After administration of standard drug and test extract, the withdrawal response was recorded at 15, 30, 60, 90 and 120 minutes. Mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs.

**Evaluation of Anti-Inflammatory Activity**

**Cotton Pellet - Induced Granuloma Method**

Adult healthy wistar rats weighing 180-220gms were selected for the study. They were divided into four groups of six animals each. The first group served as control group, second group served as standard and third and fourth group served for extract treatment 250mg/kg and 500mg/kg by oral administration. After administration of vehicle 2% w/v acacia suspension, the animals in group I received vechicle 2% w/v acacia suspension orally for 7 days and animals in group II received reference standard Indomethacin 10mg/kg suspended in 2% w/v acacia suspension orally for 7 days. Group III received ethanol extract of *Alpinia speciosa* 250mg/kg suspended in 2% w/v acacia suspension orally for 7 days and Group IV animals received ethanol extract of *Alpinia speciosa* 500mg/kg orally.

The initial reaction time was taken for all animals before administration of test and standard drugs. After administration of standard drug and test extract, the withdrawal response was recorded at 15, 30, 60, 90 and 120 minutes. Mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs.

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Statistical Analysis
The results were expressed as mean ± SE. The results were analyzed for statistical significance by one-way ANOVA followed by Dunnett’s test. P<0.05 were considered significant.

RESULTS AND DISCUSSION
The yield of extract obtained by soxhlet extraction using ethanol as solvent for 72 hours was found to be 13%. The preliminary phytochemical study of Alpinia speciosa rhizome extract showed the presence of Alkaloids, Phenolic compounds, Tannins, Carbohydrates, Proteins and Aminoacids and flavonoids.

In the acute toxicity study, oral administration of ethanol extract of Alpinia speciosa rhizome in doses of 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg did not produce significant changes in behaviors, breathing, cutaneous effects, sensory, nervous system responses and gastrointestinal effects in mice. No lethality or adverse signs were seen during the experimental period. During 14 days observation period, no delayed toxic signs were noted in experimental group. From the results, it was observed that ethanol extract of Alpinia speciosa rhizome is non-toxic.

Table 1. Analgesic activity of ethanol extract Alpinia speciosa K. schum rhizome by tail immersion test in mice.

<table>
<thead>
<tr>
<th>Group Treatment</th>
<th>Dose (mg/kg)</th>
<th>Basal reaction time in seconds</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (Vehicle)</td>
<td>10mg/kg</td>
<td>2.00 ± 0.33</td>
<td>2.40 ± 0.19</td>
<td>2.54 ± 0.19</td>
<td>2.62 ± 0.17</td>
<td>2.51 ± 0.12</td>
<td>2.31 ± 0.19</td>
</tr>
<tr>
<td>2. Pentazocine</td>
<td>5mg/kg</td>
<td>2.43 ± 0.19</td>
<td>4.12 ± 0.41</td>
<td>7.60 ± 0.21</td>
<td>10.78 ± 0.12</td>
<td>9.15 ± 0.12</td>
<td>6.14 ± 0.17</td>
</tr>
<tr>
<td>3. Ethanol extract of Alpinia speciosa rhizome</td>
<td>250mg/kg</td>
<td>2.20 ± 0.21</td>
<td>2.77 ± 0.42</td>
<td>3.41 ± 0.18</td>
<td>4.98 ± 0.36</td>
<td>3.96 ± 0.71</td>
<td>2.83 ± 0.72</td>
</tr>
<tr>
<td>4. Ethanol extract of Alpinia speciosa rhizome</td>
<td>500mg/kg</td>
<td>2.61 ± 0.34</td>
<td>3.10 ± 0.21</td>
<td>4.91 ± 0.39</td>
<td>6.21 ± 0.16</td>
<td>5.75 ± 0.71</td>
<td>3.71 ± 0.16</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n=6 in each group; values are significantly different from control by one-way ANOVA followed by Dunnett’s test *P<0.05 ; **P<0.01, ***P<0.001.

The effect of ethanol extract of Alpinia speciosa rhizome on cotton pellet granuloma is shown Table 2. The ethanol extract at dose levels 250mg/kg and 500mg/kg inhibited the granuloma tissue formation. The percentage inhibition was found to be 38.24% at 250mg/kg and 45.26% at 500mg/kg whereas the reference standard indomethacin 10mg/kg showed 56.22% decrease in granuloma weight. A significant reduction in the dry weight of granuloma was found in extract treated as well as indomethacin treated group compared with control group animals.

The cotton pellet granuloma method is widely used to evaluate the transludative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transuda and the dry weight of the pellet correlates with the amount of granulomatous tissues. Chronic inflammation occurs by means of the development of proliferative cells. This model is an indication of proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are the basic sources of granuloma formation. Non-steroidal anti-inflammatory drugs decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration/inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides.

The Alpinia speciosa rhizome showed significant anti-inflammatory activity in cotton pellet induced granuloma. It is thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

Preliminary Phytochemical study indicated the presence of Alkaloids, Phenolic compounds, Tannins, Carbohydrates, Proteins and Aminoacids and flavonoids which might be responsible for the analgesic and anti-inflammatory effect of Alpinia speciosa K. Schum.

Flavonoids and Phenolic compounds have been reported to have multiple biological effects such as antioxidant activity, antinociceptive activity in vivo, anti-inflammatory action, inhibition of platelet aggregation, inhibition of mast cell histamine release and inhibitory action on archidonic acid metabolism as demonstrated by in vitro and invivo tests.

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REFERENCES

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