ANTI ULCER ACTIVITY OF METHANOLIC AND AQUEOUS EXTRACTS OF LEAVES OF SAPINDUS TRIFOLIATUS. LINN.

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ABSTRACT
The methanolic and aqueous extracts of leaves of Sapindus trifoliatus were screened to evaluate anti ulcer activity in healthy rats, using ethanol induced ulcer method. The leaves of Sapindus trifoliatus were collected from the local areas of Mangalore and shade dried. The dried powdered leaves were extracted from the maceration and soxhlet extraction methods, by using both the solvents methanol and water. Healthy rats weighing 180-250 gm were taken for the experiment. The anti ulcer activity was noted after drug administration. All data were analysed with one-way ANOVA followed by Dunnets test. The both the extracts of the leaves of Sapindus trifoliatus showed significant anti ulcer activity at a dose of 400 mg/ kg body weight in both the extracts. A maximum anti ulcer activity was found at 400 mg/ kg of aqueous extract which was near equivalent to that of Sucralfate.

Keywords: Anti ulcer, Aqueous extract, Methanolic extract, Sapindus trifoliatus.

INTRODUCTION
Sapindus is a genus of about five to twelve species of shrubs and small trees in Sapindaceae, native to warm temperature at tropical regions. Sapindus trifoliatus Linnaeus (Family: Sapindaceae) a handsome tree, commonly called as soap nut tree or Indian filbert distributed in Indian Peninsula, chiefly in South India. It has been reported that Sapindus trifoliatus have shown astringent, anthelmintic, expectorant, and in the treatment of Asthma, Cholera, epilepsy, gout, rheumatism, and paralysis1,2. The seed oil is employed medicinally as well as in manufacture of soap3. β-sitosterol was isolated4 from the unsaponifiable matter of seed kernel oil. The present study was under taken to screen the anti ulcer activity of leaves of Sapindus trifoliatus because of the presence of flavonoids and sterols. They possess anti ulcer activity.

MATERIALS AND METHODS
Collection of Plant materials:
The leaves of ‘Sapindus trifoliatus’ were collected from local area around Mangalore in the month of Feb- April. It was authenticated by B.Usha Nalini M.Sc, B.Ed. HOD, Dept of Botany, St Agnes College, Mangalore-2.

Preparation of extracts:
The leaves of Sapindus trifoliatus were collected and shade dried. Then the dried leaves were powdered to get a coarse powder. The dried powdered leaves were extracted by the maceration and soxhlation process by using methanol and water respectively. Both the extracts are collected and concentrated to a dry mass by using vaccum distillation. Preliminary phytochemical tests showed presence of triterpenoids, steroids, flavonoids and carbohydrates. The suspensions of methanol and aqueous extracts were prepared by using 0.6 % of Na CMC solution.

Selection of animals:
Wistar albino rats of either sex weighing between 180 – 250 gms were obtained from NGSMP5 Panee Deralakatte Mangalore. These animals were used for the antulcer activity study. The animals were stabilized for 1 week; they were maintained in standard condition at room temp; 25±3°C, 35-60% relative humidity and 12 h light dark cycle. They had been given standard pellet diet supplied by Hindustan Lever Co. Mumbai. And water ad libitum throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal out put.

Acute toxicity studies:
Acute toxicity study was carried out in female albino rats as per staircase method5 and OECD guidelines 425. There was no mortality and no signs of toxicity were found upto 2,000 mg/ kg body weight and found to safe up to 2,000 mg/kg body weight. All the experiments were performed within the guidelines of the Institutional ethical committee of KSHEMA. Deralakatte, Mangalore. (KSHEMA /IAEC / 064/2007)

Evaluation of Anti Ulcer Activity:
Ethanol induced ulcer model:
Wister albino rats of either sex weighing in between 180-250 gms were divided into nine groups containing 6 animals in each group. Group I act as Control, (received Sod CMC 0.6% w/v in distilled water 5 ml/ kg, p.o). Group II as standard (Ranitidine suspended in 0.6% w/v sodium
CMC, 30 mg/ kg p.o). Group III as standard (Sucralfate suspended in 0.6% w/v sodium CMC, 400 mg/ kg p.o). Group IV, V, VI treated with 100, 200, 400 mg/ kg p.o. body weight of methanolic leaf extract of Sapindus trifoliatus. Group VII, VIII, IX treated with 100, 200, 400 mg/ kg body weight of aqueous leaf extract of Sapindus trifoliatus. The above treatment was given for 7 days. Food was withdrawn for 14 - 16h before performing the experiment. On the 7th day one hour after the final dose of the drug, 90 % ethanol was administered to the overnight fasted rats of all groups at a dose of 1ml per animal, irrespective of the weight of the animal through oral route. The animals were sacrificed by cervical dislocation method 6 h after the dose of ulcerogen. The stomach was removed, incised along the greater curvature and washed under the running tap water. Mucosal erosions and ulcer scores were recorded macroscopically by using hand lens (10 X). Mean ulcerative index was calculated as follows

I. Presence of edema, hyperemia and single submucosal punctiform hemorrhages.

II. Presence of submucosal hemorrhagic lesions with small erosions.

III. Presence of deep ulcer with erosions and invasive lesions.

Ulcer index = (number of lesion.I) + (number of lesion.II) X 2 + (number of lesion.III) X 3

The percentage inhibition was determined as follows

\[
\text{Percentage inhibition} = \frac{(\text{Control mean lesion index} - \text{test mean lesion index})}{\text{Control mean lesion index}} \times 100
\]

Statistical analysis:
The data were represented as Mean ± SEM. The data of antiulcer activity of leaves of Sapindus trifoliatus were analyzed by one way analysis of variance (ANOVA) followed by Dunnett’s test. 'P' value less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemicals screening of Sapindus trifoliatus indicates the presence of sterols flavonoids, tannins, glycosides and triterpenoids. In case of ethanol induced gastric ulcer method, the results of anti ulcer activity showed in table 1.

The dose of methanolic and aqueous extracts of leaves of Sapindus trifoliatus at 400 mg/kg body weight showed significant inhibition in ulcer formation at 68.94 %, 76.03 % respectively as compared to standard drug Sucralfate which showed 80.50% of protection (P<0.01). Ranitidine showed less ulcer protection for about 65.42 % when compared to Sucralfate and methanolic and aqueous extracts Ranitidine had shown less ulcer protective effect than the Sucralfate because it did not overcome the depletion of mucous induced by ethanol, which was expected since its antiulcerogenic efficacy is due to mainly to its antagonism on histaminergic activity as a consequence of the H2 receptors blockade. But sucralfate has got good ulcer protective effect than the Ranitidine because it is having the property to inhibiting the hydrolysis or depletion of mucous induced by ethanol, including stimulation of local production of prostaglandins and epidermal growth. It appears from the study that methanolic and aqueous extracts containing flavonoids and sterols effectively reduced gastric lesions produced by ethanol may have protective effect against various irritants has been called cytoprotective effect through prostaglandins. However further studies are required to evaluate the exact mechanism of action.

### Table 1: Anti ulcer activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ulcer index ± SEM</th>
<th>Percentage protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5 ml/ kg of 0.6 % CMC)</td>
<td>19.03±0.31</td>
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</tr>
<tr>
<td>Ranitidine (30 mg/ kg)</td>
<td>6.58±0.16</td>
<td>65.42 %</td>
</tr>
<tr>
<td>Sucralfate (400 mg/ kg)</td>
<td>3.71±0.92 *</td>
<td>80.50 %</td>
</tr>
<tr>
<td>Methanol Extract (100 mg/ kg)</td>
<td>16.31±0.46</td>
<td>14.29 %</td>
</tr>
<tr>
<td>Methanolic extract (200 mg/ kg)</td>
<td>12.11±0.72</td>
<td>36.40 %</td>
</tr>
<tr>
<td>Methanolic extract (400 mg/ kg)</td>
<td>6.30±0.26 *</td>
<td>68.94 %</td>
</tr>
<tr>
<td>Aqueous extract (100 mg/ kg)</td>
<td>15.71±0.46</td>
<td>17.44 %</td>
</tr>
<tr>
<td>Aqueous extract (200 mg/ kg)</td>
<td>9.31±0.72</td>
<td>51.07 %</td>
</tr>
<tr>
<td>Aqueous extract (400 mg/ kg)</td>
<td>4.56±0.36 *</td>
<td>76.03 %</td>
</tr>
</tbody>
</table>

All the values expressed as Mean ± SEM

*P<0.05, **P<0.01 when compared with control

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