Comparative Phytochemical and Biological Evaluation of Different Extracts Obtained from the Leaves of *Saraca asoka*

**Debnath M**, **Karan TK**, **Pandey JN**, **Biswa M**

**INTRODUCTION**

The Asoka tree (literally meaning “sorrow-less”) (*Saraca asoka* (Roxb.) Wilde, or *Saraca indica* L.) is a plant belonging to the Caesalpinioideae subfamily of the legume family. It is an important tree in the cultural traditions of the Indian subcontinent and adjacent areas. In Sanskrit it is known as Sita-Ashok, Anganapriya, Ashopalava, Asoka, Ashok, Asupala, Apashaka, Ashoka, Hemapushpa, Kankeli, Madhupushpa, Pindapushpa, Pindipushpa, Vanjula, Vishoka, Vichitra. In Bengali it is known as asoke. The Asoka is a rain-forest tree. Its original distribution was in the central areas of the Deccan plateau, as well as the middle section of the Western Ghats in the western coastal zone of the Indian subcontinent. The Asoka is prized for its beautiful foliage and fragrant flowers. It is a very handsome, small, erect evergreen tree, with deep green leaves growing in dense clusters. Its flowering season is around February to April. The Asoka flowers come in heavy, lush bunches. They are bright orange-yellow in color, turning red before wilting. As a wild tree, the Asoka is a vulnerable species. It is becoming rarer in its natural habitat, but isolated wild Asoka trees are still to be found in the foothills of central and eastern Himalayas, in scattered locations of the northern plains of India as well as on the west coast of the subcontinent near Mumbai. There are a few varieties of the Asoka tree. One variety is larger and highly spreading. The columnar varieties are common in cultivation. The bark of the herb is strongly astringent and uterine sedative. It acts directly on the muscular fibers of the uterus. It has a stimulating effect on the endometrium and the ovarian tissue.

**ABSTRACT:** In the present study, evaluated the analgesic and anti-inflammatory activity of the extracts of *Saraca asoka* in Swiss albino mice and Wister albino rats respectively. Dried leaves of *Saraca asoka* were extracted by petroleum ether, chloroform and methanol successively by percolation. The comparative TLC study was also done with these extracts. The extracts had been screened at the dose of 200 mg/kg body weight orally and exhibited significant analgesic and anti-inflammatory properties.

**Keywords:** *Saraca asoka* (Roxb.) Wilde; petroleum ether; chloroform; methanol; analgesic activity; anti-inflammatory activity.

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Asoka is a very valuable medicinal plant. Some ethnopharmacological uses of this plant are known. Its bark was used in metorrhagia, menorrhagia, chronic lymphadenitis and inflammation.[1] Some modern research has explored another useful activity of *Saraca asoka*. Chemoprevention of skin cancer by the flavonoid fraction of *Saraca asoka* flower has been found.[2] Potential anticancer activity of *Saraca asoka* extracts towards transplantable tumours in mice has also been reported by some other researchers.[3] *In vitro* examination of bark extraction from *Saraca asoka* has shown oxytocic activity.[4] Molluscicidal activity of *Saraca asoka* and *Thuja orientalis* against the fresh water snail *Lymnaea acuminate* has also been reported.[5] But till now no work has been reported on the leaves of *Saraca asoka*.

Present work is based on the phytochemical and biological evaluation of the different extracts obtained from the leaves of *Saraca asoka*. The TLC profile is reported for petroleum ether, chloroform and methanol extract of the leaves of *Saraca asoka*. The analgesic and anti-inflammatory effect of these extracts are also found and the details of all those data have been described here.

### MATERIALS AND METHODS

#### Plant material

The leaves of *Saraca asoka* were collected from Nadia, West Bengal in the month of January 2010 and were authenticated at Indian Botanical Garden, Howrah, West Bengal, India. A voucher specimen (voucher no. CNH/1-I/5/2010/Tech.II/182) has been preserved in our research laboratory for future reference.

#### Extraction

The shade dried crushed leaves of *Saraca asoka* (1.76 kg) were subjected to percolation by petroleum ether.

### TABLE 1:

<table>
<thead>
<tr>
<th>NAME OF EXTRACT</th>
<th>SOLVENT SYSTEM</th>
<th>Rf VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether extract</td>
<td>Benzene: Chloroform: Ethyl acetate (2:4:4)</td>
<td>(A) Rf 1 = 4/4.65 = 0.86, (B) Rf 2 = 3.3/4.65 = 0.70</td>
</tr>
</tbody>
</table>

### TABLE 2:

<table>
<thead>
<tr>
<th>NAME OF EXTRACT</th>
<th>SOLVENT SYSTEM</th>
<th>Rf VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform extract</td>
<td>Benzene: Chloroform: Ethyl acetate (2:4:4)</td>
<td>(A) Rf 1 = 4.1/4.75 = 0.86, (B) Rf 2 = 3.3/4.65 = 0.70</td>
</tr>
</tbody>
</table>

### TABLE 3:

<table>
<thead>
<tr>
<th>NAME OF EXTRACT</th>
<th>SOLVENT SYSTEM</th>
<th>Rf VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol Extract</td>
<td>Ethyl acetate : Methanol(7:3)</td>
<td>(A) Rf 1 = 5.15/5.8 = 0.88, (B) Rf 2 = 4.7/5.8 = 0.81, (C) Rf 3 = 4.3/5.8 = 0.74, (D) Rf 4 = 2.8/5.8 = 0.48</td>
</tr>
</tbody>
</table>
ether, chloroform and methanol successively with each solvent for two times (1.4 L × 2). The three extracts thus obtained were subjected to solvent evaporation. The extractive values were calculated for the extracts.

**General procedure**

The petroleum ether, chloroform and methanol extracts were subjected to a battery of chemicals tests for detection of steroids, terpenoids, tannins, glycosides, carbohydrates and saponins. TLC was carried out on silica Gel 60F<sub>254</sub>. No spraying reagent is used for visualization of the spot.

**Analgesic Activity:** The pharmacological screening of the petroleum ether, chloroform and methanol extracts of the leaves of *Saraca asoka* was carried out using standard protocols to determine the analgesic activity.<sup>[10]</sup> The crude extract was suspended in 2% dimethyl sulfoxide (DMSO) for administration to Swiss albino mice.<sup>[12]</sup>

**Acetic acid induced writhing reflex:** Thirty Swiss albino mice were divided into five groups of six mice each (n = 6). Group I received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 min. Group II, III, IV and V received aspirin and the extracts (each separately) at the dose of (200 mg/kg b.w., p.o.) respectively. 30 min after aspirin and extracts the writhing reflex was noted for the period of 15 min.

**Anti-inflammatory activity:** The pharmacological screening of the petroleum ether, chloroform and methanol extracts obtained from the *Saraca asoka* was carried out using a standard protocols for anti-inflammatory activity.<sup>[10]</sup> The crude extract was suspended 2% DMSO for administration to albino rats.

**Carrageenan induced rat paw edema:** Thirty rats were divided into five groups containing six rats in each group (n = 6). The concentration of carrageenan was selected as 0.1 ml of 1% and was injected subcutaneously 30 minutes after administration of the extracts (200 mg/kg p.o.) into the planter region of right hind paw to induce edema. The paw volume was measured initially and at 1, 2, 3 and 4 hr after injection using plethysmometer. Indomethacin 10 mg/kg was injected through i.p. route as standard drug. Percentage inhibition of edema was calculated by the formula:

$$\text{Inhibition} = \left(1 - \frac{v_t}{v_c}\right) \times 100$$

Where vt and vc indicates mean relative changes in paw volume of the test and control respectively.

**RESULTS**

**Phytochemical screening**

The phytochemical screening of petroleum ether extract has shown positive results for both terpenoids

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>DOSE</th>
<th>MEAN NO. OF WRITHINGS ± SEM</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Acetic acid)</td>
<td>10 ml/kg</td>
<td>52.83 ± 1.4</td>
<td>-</td>
</tr>
<tr>
<td>Standard (Aspirin)</td>
<td>300 mg/kg</td>
<td>17.65 ± 1.66**</td>
<td>66.59</td>
</tr>
<tr>
<td>Pet-ether extract</td>
<td>200 mg/kg</td>
<td>36.21 ± 1.22**</td>
<td>31.46</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>200 mg/kg</td>
<td>33.34 ± 1.35**</td>
<td>36.89</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>200 mg/kg</td>
<td>29.76 ± 1.54**</td>
<td>43.67</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. One way ANOVA with Tukey-Kramer multiple comparison post test.

**TABLE 4: Analgesic effect of extract on acetic-acid induced writhing in mice. (n = 6)**

**P<0.001 when compared to control.**
Comparative Phytochemical and Biological Evaluation of Different Extracts Obtained from the Leaves of *Saraca asoka*

The petroleum ether, chloroform and methanol extracts obtained from the leaves of *Saraca asoka* showed analgesic activity, which ease found to be statistically significant at higher concentration (200 mg/kg, i.p) in acute acetic acid induced writhing. Among the three extracts, the methanol extract had found to be most potent. However, the activity was less potent as compared to aspirin.

Inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular disease. A number of natural products are used in various traditional medicine systems to relieve symptoms from inflammation. The extracts obtained from the percolation of *Saraca asoka* leaves by petroleum ether, chloroform and methanol has shown significant anti-inflammatory activity in carrageenan induced inflammation model. Carrageenan induced paw edema model is known to sensitive to cyclooxygenase inhibitors. It has been used to evaluate the effect of NSAIDS, which primarily inhibits the cyclooxygenase involved in prostaglandin synthesis. There are two phases in inflammatory reaction in carrageenan-induced paw edema model in rats: first phase and second phase. The first phase, which occurs between 0 to 2.5 h after injection, has been attributed to the release of histamine or serotonin. The second phase of inflammatory reaction which is measured after 3 h is caused by the release of bradykinin, protease, prostaglandin and lysosome.

The extracts had shown a significant inhibitory effect towards anti-inflammatory activity. The petroleum ether extract had inhibited 83.74%, the chloroform extract had inhibited 85.17% the methanol extract had inhibited 86.13% which is less than standard (89.48%). But it is very close and comparable with standard indomethacin. Therefore, it can be inferred that the inhibitory effect of those three extracts on carrageenan-induced inflammation could be due

### TABLE 5: Anti-inflammatory effect of different extracts on carrageenan induced paw edema in rats. (n = 6)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>DOSE</th>
<th>1 HR</th>
<th>2 HR</th>
<th>3 HR</th>
<th>4 HR</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Carrageenan)</td>
<td>200 mg/kg</td>
<td>0.74 ± 0.08</td>
<td>1.50 ± 0.57</td>
<td>1.7 ± 0.57</td>
<td>2.09 ± 0.81</td>
<td>-</td>
</tr>
<tr>
<td>Standard (Indomethacin)</td>
<td>200 mg/kg</td>
<td>0.21 ± 0.05*</td>
<td>0.52 ± 0.05**</td>
<td>0.34 ± 0.03**</td>
<td>0.22 ± 0.03**</td>
<td>89.48</td>
</tr>
<tr>
<td>Pet-ether extract</td>
<td>200 mg/kg</td>
<td>0.31 ± 0.05*</td>
<td>0.55 ± 0.03**</td>
<td>0.42 ± 0.05**</td>
<td>0.34 ± 0.03**</td>
<td>83.74</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>200 mg/kg</td>
<td>0.29 ± 0.04**</td>
<td>0.51 ± 0.05**</td>
<td>0.39 ± 0.07**</td>
<td>0.31 ± 0.06**</td>
<td>85.17</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>200 mg/kg</td>
<td>0.25 ± 0.05**</td>
<td>0.49 ± 0.08**</td>
<td>0.37 ± 0.04**</td>
<td>0.29 ± 0.05**</td>
<td>86.13</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. One way ANOVA with Tukey-Kramer multiple comparison post test.

**P<0.001 when compared to control. *P<0.01 when compared to control.

and steroids. Its chloroform extract has shown positive response for steroids, terpenoids and carbohydrates. The methanol extract has shown positive response for condensed tannins, saponins, carbohydrates, steroids and glycosides.

**TLC PROFILE**

*Comparative TLC Study of Saraca asoka*

The TLC profile study of the different extracts of the leaf of *Saraca asoka* with details has been described below:

1. TLC study of petroleum ether extract of the leaves of *Saraca asoka* has been illustrated on Table 1 and the picture of the TLC plate has shown in Figure 1.

2. TLC study of chloroform extract of the leaves of *Saraca asoka* is illustrated on Table 2 and the picture of the TLC plate is shown on Figure 2.

3. TLC study of methanol extract of the leaves of *Saraca asoka* is illustrated on Table 3 and the picture of the TLC plate is shown on Figure 3.

The analgesic activity of the petroleum ether, chloroform and methanol extracts of *Saraca asoka* leaves were shown in the Table IV and Fig 4. The anti-inflammatory activities of the petroleum ether, chloroform and methanol extracts of *Saraca asoka* leaves were shown in the Table V and Fig 5.

**DISCUSSION**

The acetic acid induced writhing is normally used to evaluate the peripheral analgesic effect of drugs. The method is thought to be mediated through peritoneal mast cell, acid sensing ion channel and the prostaglandin pathway. Therefore it can be inferred that the inhibitory effect of the compound could be due to the inhibition of prostaglandin pathway.
to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Thus the results presented in the current study indicate that the petroleum ether, chloroform and methanol extract, extracted from the leaves of *Saraca asoka* have potent and significant anti-inflammatory activity. However a more detail study is required to identify the exact mechanisms of action.

**CONCLUSION**

The petroleum ether, chloroform and methanol extracts of the leaves of *Saraca asoka* has shown analgesic activity in Swiss albino mice, and anti-inflammatory activity in albino rats.

**ACKNOWLEDGEMENT**

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**REFERENCES**