

Antinociceptive and Antioxidant Activities of the Ethanolic Extract of *Excoecaria indica*

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The ethanolic extract of the leaves of *Excoecaria indica* has been assessed for its antinociceptive and antioxidant activities. The extract significantly and dose dependently inhibited the acetic acid induced writhing in mice (93.54% and 81.85% for 500, 250 mg/ kg body weight respectively) which was comparable to the standard drug diclofenac-Na. The extract also showed potent antioxidant activity (IC₅₀ 85.11 µg/ml).

Excoecaria indica. (Willd.) Muell. Arg (Syn. *Sapium indicum*) (Euphorbiaceae), commonly known as Batul (Bengali), is a small, semi-deciduous to evergreen tree and is found in Sunamgonj, Chittagong, Cox's Bazar, Sundarban, Barishal and other coastal areas of Bangladesh.¹ Traditionally the local people in Chalna (Khulna), use the leaves of this plant against the irritation after fish sting to relieve pain. Here the milky juice of the plant is applied on the wound area and a rapid recovery of irritation and subsequently pain are perceived. The plant is also used in fish poisoning.¹ The fruit of *E. indica* is reported to possess antimicrobial activity. Phorbol esters, sapiol (n-tetratriacontanol), sitosterol,

aliphatic esters of the tiglane nucleus (4-deoxyphorbol derivatives), Sapintoxins A, B, and D and alkaloid are known to occur in *E. indica*.²⁻⁶ As part of our on-going pharmacological screening of some selected Bangladeshi medicinal plants⁷⁻⁹, we here in, report antinociceptive and antioxidant activities of *E. Indica* leaves for the first time.

Leaves of *E. indica* were collected from the Chalna, Khulna, Bangladesh, during the month of November, 2004 and identified by experts of the Bangladesh National Herbarium, Dhaka, Bangladesh, where voucher specimen (DACB. 31118) has been deposited. Shade-dried ground leaves (200 g) were extracted by maceration with 90% ethanol (EtOH) at room temperature for 7 days. The extract was filtered and dried using a rotary evaporator at a temperature not exceeding 55 °C and the yield was found to be approximately 2.13% w/w. The antinociceptive activity was assessed on the swiss albino mice of either sex (20-25 g) obtained from the Animal House, Pharmacy Discipline, Khulna University, Khulna. The animals were housed under standard laboratory conditions and were fed with standard diet and water *ad libitum*.

Antinociceptive activity of the ethanolic extract of *E. indica* leaves was measured by acetic acid induced writhing method.⁸ The animals were orally fed with the extract (250 mg & 500 mg/kg body

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weight), vehicles (for control groups) at the specified doses. Thirty minutes after administration of the extract and the vehicle, each animal was given 0.7% (v/v) solution of acetic acid (0.1 ml/10 g body weight) intraperitoneally (i.p.) to induce abdominal contractions or writhing. Five minutes after the administration of acetic acid, the number of writhing for each animal was counted for 15 min. The number

of writhings in the control was taken as 100% and percent inhibition was calculated as follows:

$$\% \text{ Inhibition of writhing} = 100 - (\text{treated mean} / \text{control mean}) \times 100$$

For comparison, the same experiment was carried out with a positive control group treated orally with diclofenac-Na (Square Pharmaceuticals Ltd., Bangladesh) at the dose of 50-mg/kg body weights.

Table 1. Effect of *E. indica* extract on acetic acid induced writhing in mice.

Treatment	Dose ^a (mg/kg, p.o.)	Writhings ^b	Inhibition (%)
Control (1% Tween 80, 10 ml/kg, p.o.)	-	24.8 ± 2.07	-
Diclofenac-Na	50	6.8 ± 0.91**	72.58
<i>E. indica</i>	250	4.5 ± 0.39**	81.85
	500	1.6 ± 0.32**	93.54

^aAdministered 45 min before 0.7% acetic acid administration (ml/kg, i.p.)

^bCounted for 15 min, starting 5 min after acetic acid administration; values are mean ± S.E.

**P < 0.001 vs. control, Student's *t*-test; n=5.

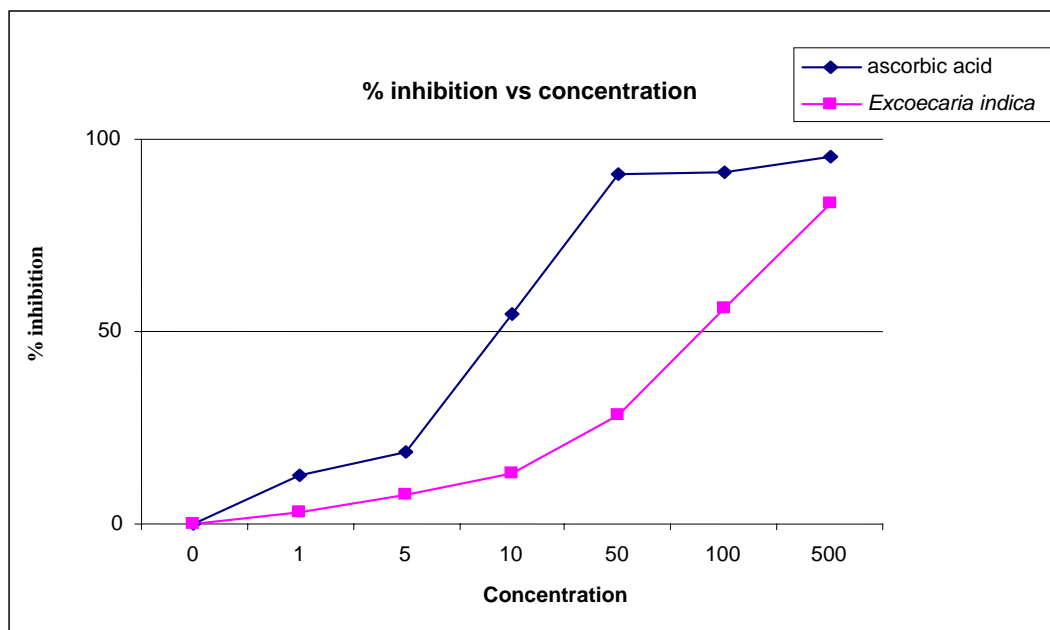


Fig. 1. DPPH scavenging assay of ethanolic extract of *E. indica* and ascorbic acid.

Qualitative antioxidant assay was performed by the standard method.¹⁰ Test samples were developed with a suitable solvent system on a TLC plate and sprayed with 0.004% methanolic DPPH (2,2-Diphenyl-1-picrylhydrazyl, 95%, Aldrich, USA)

solution using an atomizer. The positive activity was detected by the pale yellow spots on a reddish purple background. Ascorbic acid (Avocado Research Chemicals Ltd, Shore road, Heysham, Lancs) was used as the positive control. Quantitative DPPH assay

method¹¹ was adopted with suitable modifications to our particular circumstance. The DPPH antioxidant assay method is based on the ability of 2,2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. Stock solution (10 mg/ml) of the plant extract was prepared in ethanol. Serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 & 500 µg/ml. Solution of each concentration (1 ml) were mixed with DPPH (3 ml 0.004% methanolic solution) and allowed to stand for 30 min for reaction to occur. The absorbance was recorded at 517 nm. Sample of each concentration was tested in triplicate and the average absorption was taken. Ascorbic acid was used as the positive control and percent inhibition was calculated as follows:

$$\% \text{ inhibition of DPPH} = \frac{A(b) - A(s)}{A(b)} \times 100$$

where, A (b) = absorbance of blank, and A(s) = absorbance of sample.

In acetic acid induced writhing test, the extracts significantly and dose dependently suppressed the frequency of acetic acid induced writhing in mice. At the dose 250 mg/ kg body weight the extract of *E. indica* showed 81.85% writhing inhibition ($P < 0.001$) where as at 500 mg/ kg body weight the extract produced 93.54% writhing inhibition ($P < 0.001$), which were comparable to the standard drug (Table 1). Diclofenac-Na, used as the positive control exhibited a writhing inhibition of 72.58% as compared to control and the results was statistically significant ($P < 0.001$).

In the TLC-based qualitative antioxidant assay using DPPH spray, the ethanolic extracts of *E. indica* showed potent free radical scavenging properties indicated by the presence of a yellow/white spot on a purple background on the TLC plates. In the DPPH quantitative assay the ethanolic extract of *E. indica* showed potent antioxidant activity (IC_{50} , 85.11 µg/ml) while the IC_{50} of the ascorbic acid, a standard

antioxidant was found to be 8.9 µg/ml (Figure 1). All the results support the traditional uses of the *E. indica* leaves.

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