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ANTIOXIDANT & ANALGESIC ACTIVITIES OF LEAVES OF PANLATA *(Derris trifoliate)*: IN-VITRO INVESTIGATION

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Abstract

In the present study, the antioxidant and analgesic potential of the ethanolic extract of the leaves of *Derris trifoliata* was evaluated. The free radical scavenging activity of the crude extract on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined by comparing the DPPH inhibitory capacity of the extract. In the quantitative assay, *Derris trifoliata* extract displayed a free radical scavenging activity in the DPPH assay (IC_{50} = 19 µg/ml) which is comparable to that of ascorbic acid (IC_{50} = 7.80 µg/ml), a well-known standard antioxidant. The analgesic responses of the given samples of extracts were evaluated using the Tail immersion method. In the analgesic activity test, extract at dose of 200 mg/kg and 400 mg/kg exhibited significant ($P<0.05$ and $P<0.001$ respectively) inhibition of pain by 166.82 and 184.95 after 120 and 180 minutes respectively while the standard drug Diclofenac Na inhibition was found to be 217.67 after 180 minutes at a dose of 25 mg/kg body weight

Key Words: *Derris trifoliate,* DPPH, Analgesic, Tail immersion.

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Introduction

Derris trifoliata (synonyms: *D. uliginosa*, *Pongamia uliginosa*; local name: pan lota, kali lota, goali lata, kirtana) is an evergreen mangrove plant belonging to the family Fabaceae (Leguminosae) which is commonly known as the legume family, pea family, bean family or pulse family. It is probably the only common climber that grows in mangroves, especially in Sundarban (mangrove forest) of India and Bangladesh. It is a perennial climber, or a much branched climbing shrub, reaching a length of 8 meters or less. The plant has 12.5-20 cm long odd-pinnate compound leaves with 3-7 leaflets (5.7-10 cm by 3.2-5 cm) and 7.5-15 cm long white flowers that are fascicled in axillary racemes.

Several rotenoids [1,2] and glycosidic compounds [3] have been isolated from aerial parts of *D. trifoliata*. Rotenone, one of its principal secondary metabolite has low toxicity to mammals, but is extremely toxic to fish [4]. Some species belonging to the genera including *D. trifoliata* and *D. elliptica*, are commercially cultivated as sources of insecticidal rotenoids[5]. Other species including *D. scandens* and *D. indica* have been traditionally used as diuretic and antidysentery6 However, these therapeutic potentials of the plant have not been scientifically evaluated. Therefore, the present study was undertaken to investigate the antioxidant and analgesic activities of Leaves of *Derris trifoliate*

Materials and methods

Collection of plant materials and extraction

The plant selected for present work was *Derris trifoliata* Lour. (Family: Leguminosae). was collected from Sundarbans, mangrove forests during the month of November, 2011 and was authincated by the experts at Bangladesh National Herbarium accession no 29790.The collected plant parts were separated from undesirable materials or plants or plant parts and then were washed with water. They were sun-dried for one week. The plant parts were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory,China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

About 250gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 14 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through filter paper.

Experimental animal

Young Swiss-albino mice aged 4-5 weeks, average weight 20-25 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition for one week in the animal house of the Department of Pharmacy, Khulna University, Bangladesh for adaptation after their purchase. The animals were provided with standard laboratory food and tap water and libitum and maintained at natural day night cycle. All the experiments were conducted on an isolated and noiseless condition.

Free radical scavenging activity

The free radical scavenging activity of the crude extract on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined by comparing the DPPH inhibitory capacity of the extract (Brand-Williams *et al.,* 1995)[7]. A series of concentration (1, 8, 16, 32, 64, 128 and 256 µg/ml) were made with the extractives by using the respective solvents and 0.004 % (w/v) DPPH solution in methanol. Then the reaction mixtures were allowed to stand for 20 min and the absorbance was determined at 517 nm with UV-VIS spectrophotometer against methanol as blank and from these values the corresponding percentages of inhibitions were calculated by using the following equation:

$$\% \text{ inhibition} = [1- (A_{sample} \div A_{blank}) \times 100]$$

where A_{sample} and A_{blank} are the absorbance of the sample and blank respectively. Then % inhibitions were plotted against respective concentration and from the graph the IC_{50} was calculated by using Ascorbic acid as standard antioxidant.

Analgesic screening

The analgesic responses of the given samples of extracts were evaluated using the Tail immersion method (Chandrashekar KS, 2002) using a self made apparatus. In this method the mice were divided into four groups (each group containing six animals). The first group was served as control and received distilled water (orally), the second group of animals was served as standard and administered standard drug Diclofenac sodium (25 mg/kg bd wt.,orally.). The animals of remaining groups were treated with extracts (200 mg/kg and 400 mg/kg bd wt, orally). The analgesic responses of the fruit extracts were evaluated using the tail immersion method. In this procedure the albino mice were weighed and marked. They are placed into individual restraining cages leaving the tail hanging out freely. The animals are allowed to adapt to the cages for 30 min before testing. The lowest 5 cm portion of the tail is marked. This part of the tail is immersed in freshly filled water of exactly 55 ^{o}C. Within a few seconds the mice react by withdrawing the tail. A maximum immersion time of 15 sec was maintained to prevent thermal injury to the animals .The reaction time was noted at 0, 30, 60, 90, 120 and 180 minutes.

Pain inhibition percentage (PIP) = (T1- T) / T × 100. T1 = Post drug latency (reaction time after drug treatment) and T = Pre drug latency (basal reaction time).

All data were expressed as Mean ± SEM and analyzed statistically by using *t*-test. A difference was considered significant at P value less than 0.05.

Result & Discussion

In the DPPH assay quantitative assay, ***Derris trifoliata*** extract displayed a free radical scavenging activity in the DPPH assay (IC_{50} = 19 µg/ml) which is comparable (Figure 1) to that of ascorbic acid (IC_{50} = 7.80 µg/ml), a well-known standard antioxidant. However, this assay may be used to guide the fractionation and isolation of potential antioxidant compounds from this mangrove plant.

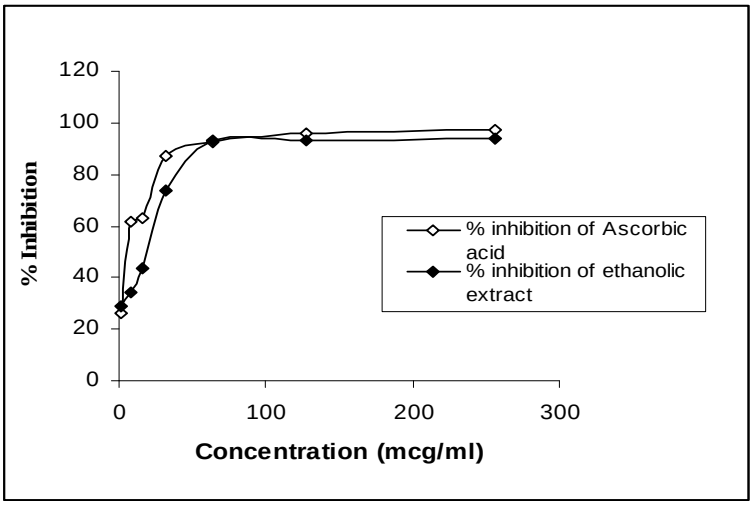

Figure 1: DPPH Scavenging Assay of *Derris trifoliata* Lour. (% inhibition Vs Concentration.)

In the analgesic activity test using tail immersion method extract at dose of 200 mg/kg and 400 mg/kg exhibited significant ($P<0.05$ and $P<0.001$ respectively) inhibition of pain by 166.82 and 184.95 after 120 and 180 minutes respectively while the standard drug Diclofenac Na inhibition was found to be 217.67 after 180 minutes at a dose of 25 mg/kg body weight (Table 1). So it can be claimed that the analgesic activity of *Derris trifoliata* extract was significant in comparison with negative control animals as the extract, at the doses of 200 and 400 mg/kg body weight showed significant analgesic activity in mice.

Table 1: Comparative % inhibition of pain of *Derris trifoliata* and Diclofenac sodium on tail immersion test

Treatment Dose (mg/kg)			% inhibition of pain			
		0 min	30 min	60 min	120 min	180 min
Diclofenac sodium(Standard)	25	0	114.65	175.25	206.06	217.67
Derris trifoliata	200	0	44.07	98.59	148.82	166.82
	400	0	72.82	126.70	184.95	182.04

Conclusion

Based on the results of the present study, it can be concluded that the leave extract possesses remarkable antioxidant and analgesic potential. However, further studies are needed to understand the exact mechanisms of antioxidant and analgesic action and to isolate the compound(s) responsible for such activity.

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