

ANTINOCICEPTIVE, ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF
DALBERGIA SPINOSA SPIKE

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Summary

For the evaluation of the Phytochemical and pharmacological activity of 80% methanolic extract of spike of *Dalbergia spinosa* this project work has been designed to investigate Phytochemical nature and selected pharmacological activity (cytotoxicity, analgesic, anti-oxidant). Phytochemical study of the extract of spike of *D. spinosa* indicated the presence of tannins, steroid, alkaloid, flavonoid and saponin types of compounds. At the 250 mg/kg body weight dose, the extract showed a moderate writhing inhibition (33.34%) and at the 500 mg/kg body weight dose, the extract showed a marked writhing inhibition (63.89%) in acetic acid induced writhing method in mice and the results were statistically significant ($P < 0.01$ and $P < 0.001$ respectively). A general toxicity of the extracts was assessed by a simple and low cost assay using brine shrimp lethality as an indicator of toxicity. The extract of *D. spinosa* spike showed marked level of general toxicity in the brine shrimp lethality assay ($LC_{50} = 15 \mu\text{g/ml}$ & $LC_{90} = 25 \mu\text{g/ml}$). In the qualitative anti-oxidant test (DPPH assay) the extract showed presence of a potent antioxidant property.

Key words: *Dalbergia spinosa*, Anti-oxidant activity, Analgesic activity, Cytotoxic activity

Introduction

Delirium, delirium tremens, fruits are tonic and antipyretic, seed oil is said to be used as cosmetic and for discharge from the ear, leaves and stem bark is used as febrifuge, emmenagogue and anthelmintic [1]. This plant occasionally grows in the Mangrove forest within the frequently inundated zones, but this species is mostly considered as back Mangroves it is distributed in the shores of the East and West Peninsula, Sundarbans and Chittagong in Bangladesh and some coastal parts of India [2]. Some chemical compounds already have isolated from *D. spinosa* neoflavonoid 2,4-dihydroxy-5-methoxy-1-(1-phenyl-2-propenyl)benzene(I) [3], 3-(3,4-dimethoxyphenyl)-5,7-dihydroxy-6-methoxy-4H-1-benzopyran-4-one [4], glucopyranoside [5], prunetin 4'-O- β -D-galactoside and 7-methyltectorigenin 4'-O- β -D-galactoside [6], dalspinin-O- β -D-galactopyranoside [5],

Dalspinosin 7-*O*- β -Dglucopyranoside [7], Dalspinosin [7]. Kurz et al (1987) reported that a spoonful of powder in a tumblerful of water is said to be sufficient to destroy, in less than half an hour, the effects of alcohol, even in cases bordering on delirium tremens [8].

Materials and Methods

Plant material collection and extraction

The barks of *Dalbergia spinosa* were collected from the Sundarbans' Mangrove Forests, Bangladesh in August 2007, and were taxonomically identified by the teachers of FWT discipline of Khulna University, Khulna (Accession number-29789). About 400 g of powdered leaves were placed in a clean, flat-bottomed glass container and soaked in 1,500 ml of 80% methanol. The container with its contents was sealed and kept for a period of 13 days with occasional shaking and stirring. The mixture was then coarsely filtered with a piece of cotton wool, followed by filtration through Whatmann filter paper. The filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract.

Drugs

Drugs employed in the study were: diclofenac sodium (Opsonin Chemical Industries Ltd, Bangladesh) and loperamide (Square Pharmaceuticals Ltd., Bangladesh).

Preliminary phytochemical analysis

The crude extracts were subjected to preliminary phytochemical screening for the detection of major chemical groups. In each test, 10% (w/v) solution of the extract in methanol was used unless otherwise mentioned in individual tests [9].

Tests for tannins

Ferric Chloride Test: A 5 ml volume of the extract was placed in a test tube and then 1 ml of 5% ferric chloride solution was added to it.

Potassium dichromate test: A 5 ml volume of the extract was placed in a test tube and then 1 ml of 10% potassium dichromate solution was added.

Test for flavonoids

A few drops of concentrated hydrochloric were added to 5 ml of the extract.

Test for saponins

A 1 ml volume of the extract was placed in a graduated cylinder and was diluted to 20 ml with distilled water and shaken gently for 15 min.

Test for gums

A 5 ml volume of the extract was placed in a test tube and then Molish's reagent and sulphuric acid were added to it.

Tests for steroids

Liebermann-Burchard test: A 1 ml volume of the extract was placed in a test tube and 2 ml of Liebermann-Burchard reagent added.

Sulphuric acid test: A 1 ml volume of the extract was placed in a test tube and 1 ml sulphuric acid added.

Tests for alkaloids

Mayer's test: A 2 ml volume of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube and 1 ml of Mayer's reagent added.

Dragendorff's test: A 2 ml volume of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube and 1 ml Dragendorff's reagent was added. Wagner's test: The extract (2 ml) and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of Wagner's reagent was added. Hager's test: A 2 ml volume of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of picric acid solution (Hager's reagent) was added.

Animals

Young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 - 65%, room temperature $25.0 \pm 2.0^\circ\text{C}$ and 12 h light-dark cycle), fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Antinociceptive activity

Antinociceptive activity of the crude extract was tested using the model of acetic acid-induced writhing in mice [10,11]. The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 mL/kg of body weight; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with the extracts at dose of 250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection in peritoneum. Then after an interval of 10 min, the number of writhes (squirms) was counted for 5 min.

Cytotoxicity test

The brine shrimps used for cytotoxicity test were obtained by hatching 5 mg of eggs of *Artemia salina* in natural seawater after incubation at about 29°C for 48 h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. Five doses of plant extract (1, 2, 4, 6, 8 and 10 $\mu\text{g/ml}$) in 5 % DMSO and/or seawater were tested. Each extract preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. The concentration of DMSO in the vials was kept below 10 $\mu\text{l/ml}$. For control, same procedure was followed except test samples. After marking the test tubes properly, 10 living shrimps were added to each of the 20 vials with the help of a Pasteur pipette [12]. The test tube containing the sample and control were then incubated at 29°C for 24 h in a water bath, after which each tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration.

Screening for In-vitro Anti-oxidant Activity

Pre-coated Silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and sprayed with 0.02% 1,1-diphenyl-2-picryl hydrazyl (DPPH) in ethanol. Bleaching of DPPH by the resolved bands is observed for 10 minutes and the color changes (yellow on purple background) were noted [13].

Results

Preliminary phytochemical analysis

Results of different chemical tests on the methanol crude leaves extract of *D. spinosa* showed the presence of alkaloids, steroids, flavonoids, saponins and tannins.

Antinoceptive

The methanolic extract of *D. spinosa* exhibited statistically significant ($p < 0.001$) analgesic effect in acetic acid induced writhing of white albino mice (Swiss-webstar strain). The extract produced about 33.3% and 63.4% writhing inhibition at the doses of 250 and 500 mg/kg-body weight respectively. The results were statistically significant ($p < 0.001$) and were comparable to the standard drug diclofenac sodium, which showed about 75.0% writhing inhibition at the dose of 25 mg/kg ($p < 0.001$).

Table 1. Effects of *D. spinosa*, spike extract on writhing effect on acetic acid induced mice.

Treatment	Dose mg/kg	Mean Writhing	% Inhibition	SD	P value
Experimental Control	10	36	---	3.847	P<0.001
Positive Control	25	8	75.0	1.414	P<0.001
Test	250	24	33.3	3.286	P<0.001
Test	500	13	64.4	1.673	P<0.001

Test sample: *D. spinosa* crude extract: 30 min after treatment, 0.7% acetic acid was injected *i.p.* 10 min after injection writhing responses was recorded for 5 min. n=5.

Cytotoxicity

In this bioassay, the crude extract of *D. spinosa* showed lethality indicating the biological activity of the compound present in the extract. Test sample showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase in concentration of the sample and plot of percent mortality versus log concentration on the graph paper produced an approximate linear correlation between them. From the graph (figure) the concentrations at which 50% mortality (LC₅₀) of brine shrimp nauplii occurred were obtained by extrapolation. The values were found about 15 µg/ml for the crude extract. The 90% mortality (LC₉₀) values were 25 µg/ml respectively.

Table 2. Toxic effect of *D. spinosa*, crude spike extract on brine shrimp.

Test Sample	Conc. (µg/mL)	No of alive shrimp			% Mortality	LC50 (µg/ml)	LC90 (µg/ml)
		Test 1	Test 2	Average			
Ethanollic Extract	0	10	10	10	0	15	25
	5	7	7	7	30		
	10	6	6	6	40		
	15	4	5	4.5	55		
	20	3	3	3	70		
	25	2	0	1	90		
	30	0	0	0	100		

Anti-oxidant

DPPH applied TLC plates were observed under UV detector both in short (254 nm) and long (360 nm) wavelength. Antioxidant components in the methanolic extract of *D. spinosa* were identified.

Discussion

Plants are employed as important source of medication in many traditional medications [14,15,16]. Since *D. spinosa* belongs to the coastal forests, part of the plant constituents may be polar in nature. Methanol was used which has a wide range of solubility in both polar and nonpolar region. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness [17].

Antinociceptive activity of the methanol extract of *D. spinosa* was tested by acetic acid-induced writhing model in mice. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algia by liberation of endogenous substances, which in turn excite the pain nerve endings [18]. Increased levels of PGE2 and PGF2 α in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid [19]. The extract produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 2). The polar compounds present in the plant extract may be responsible for the obtained antinociceptive activity. Based on this result it can be concluded that the ethanol extract of *D. spinosa* might possess antinociceptive activity.

The cytotoxic activity of the methanol extract of *D. spinosa* was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. The plant is reported to contain saponins [2]. There is growing interest in natural saponins caused as much by the scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of pharmacological activities; for instance, bactericidal, antiviral, cytotoxic, analgesic, antiinflammatory, anti-cancer and anti-allergic [20]. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of

pharmacological activities such as antimicrobial, pesticidal, antitumor, etc. [21]. The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

The in-vitro antioxidant test confirmed the presence of antioxidant compound in the methanolic extract of *D. spinosa*.

Conclusion

Finally, it could be suggested that the methanol extract of *D. spinosa* leaf possesses antinociceptive, cytotoxic and anti-diarrhoeal activities. These facts indicate the scientific basis of *D. spinosa* being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical potentialities of the plant as a medicine.

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