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ANTIBACTERIAL AND CYTOTOXIC POTENTIAL OF *DALBERGIA SPINOSA* ROXB. LEAVES

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ABSTRACT

Dalbergia spinosa Roxb. (Leguminosae) is a mangrove plant native to Asia and enjoys number of traditional uses. This paper describes the investigation of antibacterial and cytotoxic activity of the ethanol extract of *D. spinosa* leaves. Antibacterial activity of the ethanol extract of *D. spinosa* leaves was tested by disc diffusion assay and broth macrodilution assay. The extract was also tested for brine shrimp lethality. The extract showed antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria in both assays. The minimum inhibitory concentrations (MIC) against these bacteria were in the range of 250-500 µg/mL. The LC_{50} against brine shrimp nauplii was 84.14 µg/mL. The above data demonstrates that the plant has some important biological activities with sufficient potency and further studies can be performed to identify the active compound(s).

INTRODUCTION: *Dalbergia spinosa* Roxb. (Leguminosae) is a small tree up to 3-7 m tall with purplish-white flowers and brownish one seeded glabrous fruits. Leaves are of 6-9 cm long and crowded at nodes of spinous branchlets. In Bangladesh it widely grows in mangrove forest Sundarban. It is also distributed in India, Burma, Sri Lanka and Malaysia. Traditionally fruits are used as antipyretic and tonic, leaves and stem barks are used as febrifuge and anthelmintic, seed oil is used as cosmetic [1].

In previous study, the stem barks were investigated for antinociceptive activity and reported [2]. Antioxidant, cytotoxic and antinociceptive activities were screened from spikes [3]. Antimicrobial activity of roots is also reported [4]. Anti-inflammatory activity was investigated for roots and activity was strongly comparable to the indomethacin [5]. In phytochemical investigations, earlier researchers isolated several isoflavones from the stems and roots of *D. spinosa* [6]. Some chemical compounds like dalspinin, dalspinosin and dalspinin-7-*O*-*β*-*D*-galactopyranoside were isolated from this plant [5, 7].

Other chemical investigations on this plant species isolated 3-(3,4-dimethoxyphenyl)-5,7-dihydroxy-6-methoxy-4H-1-benzopyran-4-one [8], prunetin 4'-*O*-*β*-*D*-galactoside and 7-methyltectorigenin 4'-*O*-*β*-*D*-galactoside [9].

Upon literature survey it was found that no research work has been performed on leaves of *D. spinosa* and it is being used in traditional medicines in the treatment of different ailments.

That is why this plant part was selected for pharmacological investigations to evaluate anti-bacterial and cytotoxic potential.

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MATERIALS AND METHODS:

Plant material collection: Leaves of *Dalbergia spinosa* were collected from Sundarban, Sathkhira, Bangladesh in September' 2011. The plant parts were identified and authenticated by the experts at Forestry and Wood Technology Discipline (FWT), Khulna University, Khulna, Bangladesh, where a voucher specimen (Accession number- 39586) has been submitted for future reference.

Extraction: The shed dried leaves were grinded by commercial grinder into fine powder and macerated with ethanol for seven days with occasional shaking and stirring. The extract was filtered with clear cotton plug to remove plant debris and dried using rotary vacuum evaporator (Bibby RE200, Sterilin Ltd., U.K.) at 50 °C to get crude extract. The leaves yielded 17% extract of dried plant material.

Chemicals and drugs: Dimethyl sulfoxide (DMSO) was obtained from Merck, Germany. Standard vincristine sulphate was obtained from Cipla Pharmaceuticals Ltd, India. Ceftriaxone was obtained from Drug International Ltd, Bangladesh.

Bacterial strains: Bacterial strains were collected from the Microbiology Laboratory of ICDDR, B Dhaka, Bangladesh. Test microorganisms were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Shigella sonnei*, *Shigella dysentery* and *Shigella boydii*.

Antibacterial activity test by Disc Diffusion Assay: Sterile blank discs (BBL, Cocksville, USA) were impregnated with the test extract at the concentration of 500 µg/disc. Sample containing discs, standard antibiotic discs (Kanamycin 30 µg/disc, Oxoid Ltd, UK) and control discs were placed on nutrient agar medium seeded with test organism. The Petri dishes were then incubated at 37 °C for 16 h. The zone of inhibition was measured using digital slide calipers [10, 11].

Determination of Minimum Inhibitory Concentration (MIC): Minimum inhibitory concentration was determined by broth macro dilution assay with some modifications [12, 13]. In brief, selected bacterial strains were cultured on nutrient agar media at 37 °C for overnight. The bacterial colony was suspended in sterile 0.9% NaCl solution in such a way to get an absorbance of 0.1 at 620 nm (1×10^8 CFU/mL). Aliquot of 100 µL of this bacterial suspension was then mixed with 10 mL of Mueller Hinton broth to get the inoculum (1×10^6 CFU/mL). The extracts were mixed with Mueller Hinton broth with the assistance of DMSO to get a concentration of 4 mg/mL (DMSO concentration < 5%). The extract was then serially diluted in sterile capped tubes containing 1 mL each. Bacterial inoculum (1 mL) was added to each tube to get a starting concentration of 1000 µg/mL of extract in the first tube. The same procedure was also followed for standard antibiotic ceftriaxone. The tubes were then incubated for 18 h. MIC values were recorded as the lowest extract concentration with no bacterial growth. The MIC values were further confirmed with the addition of resazurin (0.01% in sterile distilled water) and incubation for 5 minutes. Pink color or discolouration of resazurin indicated bacterial growth.

Hatching shrimp: The eggs of *Artemia salina* were hatched in artificial sea water prepared by dissolving 20 g of NaCl and 18 g of table salt in 1 litre of distilled water with continuous stirring to make homogeneous solution and then filtered with cotton plug to get clear solution. Rectangular tank divided into two compartments with porous divider utilized to hatch eggs. The larger compartment was darkened and eggs were kept there to hatch at the room temperature (25-28 °C) for 24 hours with continuous supply of air by air pump and air stone was used to spread air uniformly. The nauplii were attracted by light and entered into the illuminated compartment through the small holes of divider from where they were collected by pipette.

Brine shrimp Lethality Bioassay: Brine shrimp lethality bioassay was considered to assess cytotoxic potential of the ethanol leaves extract of *D. spinosa* [14]. Samples of extract were prepared in distilled water with DMSO (not more than 0.01%) and serially diluted to get the final concentrations of 320, 160, 80, 40, 20, 10 and 5 µg/mL in 5 mL containing 10 alive nauplii in each test tube. Control was DMSO in distilled water. Anticancer drug vincristine sulphate (5, 2.5, 1.25, 0.625 and 0.312 µg/mL) was used as standard drug. After incubation at room temperature (25-28 °C) for 24 hours, the number of viable nauplii was counted and compared with control to assess lethal effect.

RESULTS:

Results of Antibacterial activity test: The leaves extract of *D. spinosa* showed a moderate zone of inhibition against some of the Gram-positive and Gram-negative bacteria included in the assay. Zone of inhibition ranged between 7 and 14 (**Table 1**).

TABLE 1: RESULTS OF THE DISC DIFFUSION ASSAY OF *D. SPINOSA* LEAVES

Bacterial strains	Diameter of zone of inhibition (mm)	
	***D. spinosa* (500 µg/disc)**	**Kanamycin (30 µg/disc)**
Staphylococcus aureus	7	18
Staphylococcus epidermidis	8	28
Streptococcus pyogenes	11	26
Salmonella typhi	9	25
Pseudomonas aeruginosa	14	19
Shigella flexneri	9	35
Shigella sonnei	10	35
Shigella dysentery	9	32
Shigella boydii	8	33

In the broth macro dilution assay, the extract inhibited the growth of all the microorganisms tested in the present investigation. The MICs obtained were between 250 and 500 µg/mL (**Table 2**).

TABLE 2: RESULTS OF THE BROTH MACRODILUTION ASSAY OF *D. SPINOSA* LEAVES

Bacterial strains	Minimum Inhibitory Concentration (MIC)	
	***D. spinosa* (µg/mL)**	**Ceftriaxone (µg/mL)**
Staphylococcus aureus	250	4
Staphylococcus epidermidis	250	1
Streptococcus pyogenes	250	2
Salmonella typhi	500	4
Pseudomonas aeruginosa	250	2
Shigella flexneri	250	1
Shigella sonnei	250	1
Shigella dysentery	500	0.5
Shigella boydii	500	0.5

Result of Brine Shrimp Lethality Bioassay: LD_{50} was calculated using probit analysis software LdP line (USA). The LD_{50} for *D. spinosa* leaves extract was found to be 84.14 µg/mL while that of vincristine sulphate was 0.64 µg/mL.

DISCUSSION: The ethanol leaves extract of *D. spinosa* was investigated for antibacterial activity against a number of Gram-positive and Gram-negative strains by disk diffusion assay based on its traditional uses in skin infections. The extract showed activity against all tested strains with strong comparable zone of inhibition as compared with standard kanamycin.

The extract also showed minimum inhibitory concentration (MIC) between 250 and 500 µg/mL against the tested strains and results were compared with standard ceftriaxone. Though disk diffusion assay is most widely used method to screen potential antibacterial activity but it cannot be sole method due to the inherent limitation of this technique. If the active compounds are non polar in nature, they may not diffuse in the polar media giving rise to low zone of inhibition or a false negative result. That is why tube dilution, broth macrodilution and other techniques can be applied to screen antibacterial activity in bigger resolution.

In the present study, we found that against *Staphylococcus aureus,* the extract showed low zone of inhibition in disk diffusion assay but comparatively MIC was lower. It also rationales the drawbacks of disk diffusion assay. Since the extract showed activity against some bacterial strains in disc diffusion assay, it is possible that more than one compound of polar and non polar nature is associated with the antibacterial activity against different organisms included in the study.

Brine shrimp lethality bioassay is most widely applied technique to assess bioactivities like enzyme inhibition, ion channel interference, antimicrobial and cytotoxic properties [14-16]. The extract showed moderate lethality against brine shrimp which demands that further cell line assay is required to assess cytotoxic activity in more sensitive way. Many scientists have already been shown correlation between cytotoxic activity and brine shrimp lethality [17-20].

CONCLUSION: Further investigations like LC-MS, GC-MS are required to isolate bioactive compounds, characterize its structure and also to identify underlying mechanisms. Cell line assay is also required to justify cytotoxic property shown in brine shrimp lethality bioassay.

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