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Antibacterial and larvicidal potentials of *Derris indica* (Lamk.) Bennet. extractives

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Abstract: Chloroform extracts of the fruit shell, leaves, root bark, root wood, seeds, stem bark and stem wood of *Derris indica* were tested for their antibacterial and larvicidal potentials. Except the seed extract all other extracts offered activity against 15 pathogenic bacteria. The fruit shell extract showed activity against *B. cereus*, *S.-β- haemolyticus* and *S. typhi*; the leaf extract against *Klebsiella* sp. only; the root bark against *B. cereus*, *B. megaterium*, *B. subtilis*, *S. -β- haemolyticus*, *S. typhi*, *S. dysenteriae* and *S. sonnei*; the root wood extract against *B. sereus*, *B. megaterium*, *B. subtilis*, *S. -β- haemolyticus*, *S. typhi*, *S. dysenteriae*, *S. shiga*, *S. sonnei*, *Klebsiella* sp. and *P. aeruginosa*; the stem bark extract against *B. cereus, B. subtilis*, *S. -β- haemolyticus* and *S. sonnei* and the stem wood extract against *B. cereus*, *B. megaterium*, *B. subtilis*, *S. -β- haemolyticus*, *S. typhi* and *S. dysenteriae*. According to the intensity of activity against the selected bacteria the *D. indica* extracts could be arranged in a descending order of root wood> root bark> stem wood> stem bark> fruit shell> leaf extract. The minimum inhibitory concentrations (MICs) of the chloroform extract of root wood of *D. indica* were 128 µg/ml against *S. -β- haemolyticus*, *B. megaterium* and *S. dysenteriae* and 64 µg/ml against *B. cereus*; for the stem wood extract 128 µg/ml against *S. -β- haemolyticus*, *B. megaterium*, *B. cereus* and *B. subtilis,* and 64 µg/ml against *S. dysenteriae*. The root bark, root wood, seed and stem wood extracts showed efficacy against the 3rd instar larvae of *Musca domestica* with LC_{50} values in a descending order of root wood (3615.92 ppm) > seed (5538.07 ppm) > stem wood (12139.40 ppm) > root bark (16721.24 ppm).

Key words: Chloroform extract, *Derris indica*, antibacterial and larvicidal activity.

Introduction

Derris, a genus of trees belonging to the family Fabaceae, inhabitants of India, SriLanka, Bangladesh, Myanmer, Thailand, Malaysia, North Australia and Polynesia, occurs in the tidal forests, often along river and canal banks, especially along the water-edges. It is a very well known medicinal plant. Alcoholic and aqueous extracts of the fresh bark and leaves of this plant are reported to exhibit remarkable antibacterial activity against *Micrococcus pyogenes* var. *aureus* (Anon, 1969). The juice of the leaves is prescribed in flatulence dyspepsia, diarrhoea and cough; it is also considered as a remedy for leprosy and the root juice is used in the treatment of gonorrhoea. A hot infusion of the leaves is used as a medicated bath for relieving rheumatic pains, and for cleaning foul ulcers and sores. The roots and seeds are known to be used as fish-poison by the aborigins of Australia (Kirtikar and Basu, 1935), and a very well known plant derived toxin rotenone has been extracted from the roots of *Derris* sp. during first half of the 19th century.

The seeds are mainly valued for the oil obtained from them, which has many industrial and medicinal uses. Powdered seed is valued as a febrifuge and tonic and used also in bronchitis and whooping cough, and the seed oil is used as a soap liniment to treat scabies, herpes and rheumatism (Burkill, 1966). The stem bark is fibrous and is used for cordage. It is also given internally in bleeding piles (Haemorrhoids). A decoction of bark is used for beri-beri. However, few reports on the biological activity of this plant, especially on insect larvae and pathogenic bacteria are available, which led us to go through this investigation. In addition an experimental endeavor had been attempted against the housefly larvae, while the housefly transmit more than 20 human and animal diseases (Hicking, 1974) and pathogens that are harmful to livestock

(Cumming & Cooper, 2000); while bacterial pandemics are not unknown to mankind. Mondal and Islam (2008) found insecticidal activity of the test plant while chloroform extracts of its different parts were screened against adult beetles of *Callosobruchus maculates* (F.).

Materials and Methods

Preparation of plant materials for extraction: The fresh leaves, fruit shell, root bark, root wood, seeds, stem bark, and stem wood of *D. indica* were collected from the campus of the University of Rajshahi, Bangladesh. After drying under shade the plant materials were powdered in a grinder separately avoiding excess heat during grinding.

Chemical extraction of the collected materials: Chloroform was selected to extract seven different parts of *D. indica* separately. The ground dried materials were extracted with sufficient amount of chloroform (500g × 1500ml × 3 times) for each of the test samples by the cool method just plunging the materials for 72 hours. Extracts, thus, obtained through filtration and evaporation of the solvent as residue kept in a refrigerator after proper labeling.

Antibacterial screening: The agar diffusion technique (Bauer *et al.,* 1966; Barry *et al.,* 1980; Vander & Vlietinck, 1991) was employed to conduct antibacterial screening. Standard antibiotic discs of Ciprofloxacin (30 µg /disc) were used for comparison. Fifteen pathogenic bacteria (six of which were gram-positive and the rest were gram-negative) were selected for the test and were cultured at the Molecular Biology Laboratory, Institute of Biological Sciences, Rajshahi University, Rajshahi. The test extracts were dissolved in respective solvents in such a manner that the desired concentrations for application in the disc have been obtained.

Determination of Minimum Inhibitory Concentrations (MICs): There are two methods for the determination of the MIC:

a) Serial tube dilution technique or turbidimetric assay, and

b) The paper disc plate technique or agar diffusion assay.

Here, the serial tube dilution technique was followed using nutrient broth medium to determine the MIC values of chloroform extracts against the gram positive bacteria, *Streptococcus-β-haemolyticus, Bacillus megaterium, Bacillus cereus* and *Bacillus subtilis*, and gram negative pathogenic bacteria, *Shigella dysenteriae*.

Preparation of inoculum: Fresh cultures of the test organisms were grown at 37°C overnight on nutrient agar medium. Bacterial suspensions were then prepared in sterile nutrient broth medium in such a manner that the suspension contained 10^7 cells/ml, which was confirmed by O.D. (0.5) measurement.

Preparation of the sample solution: The root wood and the stem extracts were selected for the MIC test and were taken into different vials in a fixed amount (2.048 mg). The broth medium (2 ml) was then added to each of the vials and agitated well to make sample solution whose concentration become 1024 µg/ml. The standard antibiotic Ciprofloxacin solution (Reiner, 1980) was prepared through the same procedure to get the concentration 512 µg/ml.

Procedure of serial tube dilution technique:

i. Twelve (12) autoclaved test tubes were taken, nine of which were marked as 1, 2, 3, 4, 5, 6, 7, 8, 9 and the rest were assigned as Cm = (medium), Cs = (medium + Compound) and Ci = (Medium + Inoculum).

ii. One ml of sterile nutrient broth medium was added to each of the 12 test tubes.

iii. One ml of sample solution was added to the first test tube and mixed well.

iv. One ml content from the first test tube was transferred by the sterile pipette to the second test tube and mixed uniformly. Then 1 ml of this mixture was transferred to the third test tube. This process of serial dilution was continued up to the ninth test tube.

v. One drop (10 µl) of properly diluted inoculum was added to each of the nine test tubes and mixed well.

vi. For the control test tube, 1 ml of the sample solution was added to the 1 ml nutrient broth,

mixed well and a half of this mixture was discarded. This was to check the clarity of the medium in presence of diluted solution of the compound.

vii. 10 µl of the inoculum was added to the control test tube Ci to observe the growth of the organism in the medium used.

viii. The control test tube Cm, containing the medium only, was used to confirm the sterility of the medium.

ix. All the test tubes were incubated at 37.5°C for 18-24 hours.

Larvicidal screening: The food was prepared with 6.25 g of wheat bran and 0.5 g of milk powder (Red Cow) and 12 ml of water as a total of 19.5 g. For *D. indica* extracts 500 mg was dissolved in 1 ml of the respective solvent (Chloroform) and mixed with the prepared food. However, being volatile, the solvent was evaporated out shortly. To have a dose-effect to calculate toxicity by probit analysis 4 other successive doses were prepared and applied through the ½ serial dilution method. Thus the concentration of the extract in the food medium was calculated as 12,820.5 ppm for the highest dose (Dose A). However, depending on the efficacy traced through pilot experiments doses made of *D. indica* root bark extract were 20,512.82, 15,384.60, 10,256.40, 5,128.20, 2,664.10 and 1,282.05 ppm; for root wood extract 15,384.60, 10,256.40, 5,128.20, 2,664.10, 1,282.05 and 641.03 ppm; for seed extract the doses were 12,820.50, 6,410.25, 3,205.13, 1,602.56, 801.28 and 400.64 ppm and for the stem wood extract 20,512.82, 15,384.60, 10,256.40, 5,128.20, 2,664.10 and 1,282.05 ppm in V/V state. For each test 10 larvae were released in the treated food medium and 3 replicates were set for each of the doses, while a control was also set for comparison. The data was read after 24 h of exposure.

Results and Discussion

Antibacterial effects: The anitbacterial activities of the test materials were determined by measuring the diameters of the zones of inhibition in terms of mm. The results are shown in Table 1. Among the 15 bacteria (6 gram-positive and 9 gram-negative) only three (*B. cereus*, *S. -β-haemolyticus* and *S. typhi*) were responsive to the fruit shell extract giving promising inhibition zones, only *Klebsiella* sp. (gram-negative) was responsive to the leaf extract giving inhibition zone; *B. cereus*, *B. megaterium, B. subtilis, S.-β-haemolyticus*, *S. typhi*, *S. dysenteriae* and *S. sonnei* were responsive to root bark extract giving promising inhibition zones; *B. cereus*, *B. megaterium, B. subtilis, S.-β- haemolyticus*, *S. typhi*, *S. dysenteriae*, *S. shiga, S. sonnei*, *Klebsiella* sp. and *P. aeruginosa* to the root wood extract giving promising inhibition zones; *B. cereus*, *B. subtilis, S.-β-haemolyticus* and *S. sonnei* were responsive to the stem bark extract giving promising inhibition zones; *B. cereus*, *B. megaterium, B. subtilis, S. -β-haemolyticus*, *S. typhi* and *S. dysenteriae* were responsive to the stem wood extract giving promising inhibition zones respectively at 200 µg/disc application, however it was only 07 mm at 50 µg/disc application for all the test organisms except *Klebsiella* sp. which offered no clear zone, while all the inhibition zones of the test materials mentioned above were compared to the inhibition zones given by the standard Ciprofloxacin 30 µg/disc and results are mentioned in the Table 1.

It is clearly evident from the above investigations that the extracts of different parts of *D. indica* are significantly active against most of the bacteria used in the screening. The larvicidal effect against the 3rd instar larvae of *M. domestica* was also found remarkable. Thus extensive studies are essential for the isolation of active compound(s) for the development of novel antibacterial, as well as insecticidal agents especially from the root wood and stem wood of this promising plant.

Table 1. Antibacterial activity of the chloroform extracts of the different parts of *D. indica* in comparison with the standard Ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm) 50 and 200 µg/disc												Ciprofloxacin 30 µg/disc
	Fruit shell		Leaf		Root bark		Root wood		Stem bark		Stem wood		
	50	200	50	200	50	200	50	200	50	200	50	200	
Gram-positive													
S. aureus	-	-	-	-	-	-	-	-	-	-	-	-	31
B. cereus	07	18	-	-	07	18	07	19	07	17	07	21	30
B. megaterium	-	-	-	-	07	18	07	21	-	-	07	21	28
B. subtilis	-	-	-	-	-	15	-	23	-	15	07	21	33
S. lutea	-	-	-	-	-	-	-	-	-	-	-	-	30
S. β .haemolyticus	07	19	-	-	07	21	07	21	07	22	07	22	31
Gram-negative													
S. typhi	-	17	-	-	07	19	-	19	-	-	07	20	35
S. dysenteriae	-	-	-	-	-	18	07	19	-	-	07	21	31
S. shiga	-	-	-	-	-	-	07	19	-	-	-	-	34
S. sonnei	-	-	-	-	07	19	07	20	-	17	-	-	33
S. boydii	-	-	-	-	-	-	-	-	-	-	-	-	34
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	33
Klebsiella sp.	-	-	-	12	-	-	-	13	-	-	-	-	31
P. aeruginosa	-	-	-	-	-	-	-	17	-	-	-	-	31
Proteus sp.	-	-	-	-	-	-	-	-	-	-	-	-	30

MIC of *D. indica* extracts against the test bacteria: The extracts found promising during the antibacterial screening have been subjected to the MIC test, especially on the test bacteria the extracts showed activity. The results have been presented in Table 2 and Table 3.

Table 2. Minimum Inhibitory concentrations of the chloroform extract of *D. indica* root wood against pathogenic bacteria.

Test tube No.	1	2	3	4	5	6	7	8	9	10	Cm	Cs	Ci	Result of MIC (µg/ml)
Nutrient broth medium (ml)	1	1	1	1	1	1	1	1	1	1	1	1	1	
Root wood extract (µg/ml)	512	256	128	64	32	16	8	4	2	1	0	512	0	
Inoculum added (µl)	10	10	10	10	10	10	10	10	10	10	0	0	10	
S. β- haemolyticus	-	-	-	+	+	+	+	+	+	+	-	-	+	128
B. Megaterium	-	-	-	+	+	+	+	+	+	+	-	-	+	128
B. cerus	-	-	-	-	+	+	+	+	+	+	-	-	+	64
S. dysenteriae	-	-	-	+	+	+	+	+	+	+	-	-	+	128

Note: "+" = Growth "-" = No growth

Table 3. Minimum inhibitory concentrations of the chloroform extract of *D. indica* stem wood against pathogenic bacteria.

Test tube No.	1	2	3	4	5	6	7	8	9	10	Cm	Cs	Ci	Result of MIC (µg/ml)
Nutrient broth medium (ml)	1	1	1	1	1	1	1	1	1	1	1	1	1	
Root wood extract (µg/ml)	512	256	128	64	32	16	8	4	2	1	0	512	0	
Inoculum added (µl)	10	10	10	10	10	10	10	10	10	10	0	0	10	
S. β- haemolyticus	-	-	-	+	+	+	+	+	+	+	-	-	+	128
B. Megaterium	-	-	-	+	+	+	+	+	+	+	-	-	+	128
B. cerus	-	-	-	+	+	+	+	+	+	+	-	-	+	128
B. subtilis	-	-	-	+	+	+	+	+	+	+	-	-	+	128
S. dysenteriae	-	-	-	-	+	+	+	+	+	+	-	-	+	64

Note: "+" = Growth "-" = No growth

Larvicidal effects: Among the seven extracts the fruit shell, leaf and stem bark extracts didn't show any activity, while the rest offered mortality to the 3rd instar larvae of *M. domestica.* The result is presented in Table 4 and illustrated in Fig. 1.

Table 4. Dose-mortality effect of *D. indica* extracts against *M. domestica* larvae.

Test extract	Time exposed	LC_{50} value (ppm)	95% Confidence limits		Regression equation	χ^2 Value
			Lower limits	Upper limits		
Root bark	24h	16721.24	9957.03	28080.65	Y=2.4438+1.178X	2.109 (4)
Root wood	24h	3615.92	2394.88	5459.52	Y=1.1554+1.080X	1.923 (4)
Seed	24h	5538.07	2485.48	12339.75	Y=2.5137+0.664X	0.842 (4)
Stem wood	24h	12139.40	7763.46	18981.91	Y=0.2793+1.156X	1.017 (4)

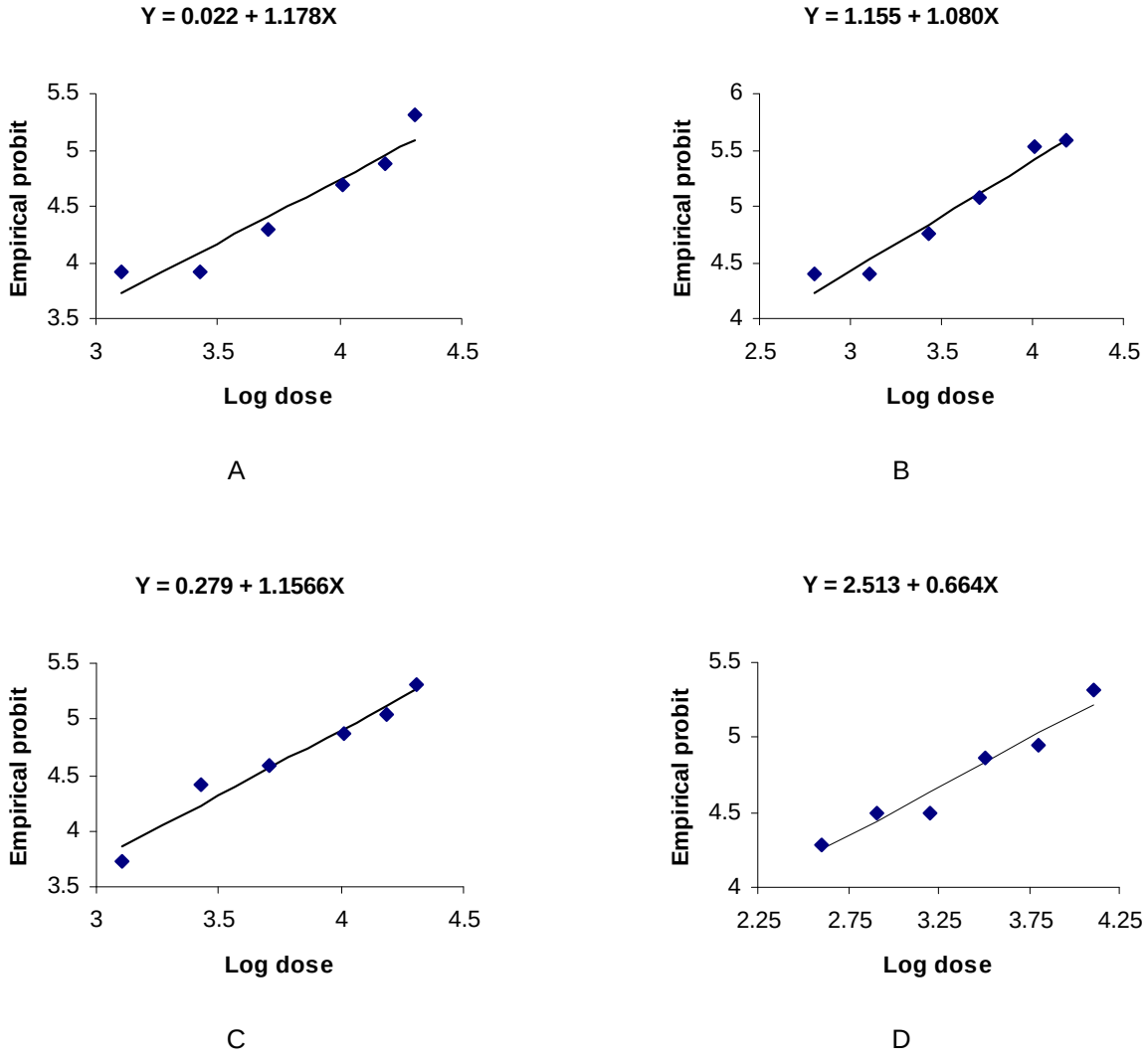

Fig. 1. Probit mortality lines of the chloroform extracts of A- root bark; B- root wood; C- stem wood and D- seeds of *D. indica* against *M. domestica* larvae.

It is clearly evident from the above investigations that the extracts of different parts of *D. indica* are significantly active against most of the bacteria used in the screening. The larvicidal effect against the 3rd instar larvae of *M. domestica* was also found remarkable. Thus extensive studies are essential for the isolation of active compound(s) for the development of novel antibacterial, as well as insecticidal agents, especially from the root wood and stem wood of this promising plant.

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