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A Study on the Effect of *Sterculia foetida* Seed Coat Extract on Multidrug Resistant Bacteria.

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ABSTRACT

At present, multidrug resistance of bacteria is a serious concern for clinicians, as most conventional antibiotics fail to show actions against MDR strains. The incidence of infection with MDR strains is increasing day by day, often with fatal outcomes. This study mainly centered on the antibacterial effect of ethanol extract of the seed coat of *Sterculia foetida*, for its possible use in MDR bacterial infections if found useful. Our study indicated that it is significantly effective as an antimicrobial agent against MDR bacteria with MIC values of the crude extract varying from 0.625 to 1.25 mg/ml. Thus this study confirms the effectiveness of this extract against bacterial drug resistance.

Keywords: MDR bacteria, *Sterculia foetida*, seed coat extract, minimum inhibitory concentration.

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INTRODUCTION

Plants, a life on Earth that helps sustain more lives had been used by humans in many ways. Some plants are used as foods like vegetables, fruits, and cereals, and medicines like aspirin from the bark of white Willow, morphine from Opium, and quinine from the South American Cinchona plant. Industrial uses of plants include the production of essential oils, waxes, resins, etc. and our daily used materials like fiber, wood, rubber, and paper. Important pesticides like nicotine and rotenone are obtained from plants [1].

Sterculia foetida is such a useful plant. The seeds of these plant (Fig. 1) are edible raw or roasted [2], it is oily and tastes like cacao but less bitter [3]. The rootstocks of young plants being a rich source of starch are eaten raw [3]. The bark and leaves are used as a remedy for constipation [4][5]. The fruit is used in the treatment of gonorrhea [5]. The seed as well as the oil from the seed has a laxative effect [6][7]. The bark yields a type of fiber [6] that is used in fancy work [8]. Gum is another useful product used in bookbinding [6].

Figure 1: Seed of *Sterculia foetida*

Sterculia foetida grows in hot tropical lowlands of elevation around 1500m [6]. The temperature and pH range for optimum growth is 18-32°C and 6-7.5 respectively [6]. This plant is dioecious thus male and female flowers grow on separate plants [6].

The bacteria in the following study are multidrug resistant. This phenomenon of resistance can be conferred by various means such as the accumulation of genes resistant to each type of antibiotics on the resistance(R) plasmid [9] or the expression of genes encoding for multidrug efflux pumps [9]. Multidrug resistance can also be caused due to other mobile gene segments like transposons or integrons [10].

MATERIALS AND METHODS

Materials

Extract of the seed coat: The fruits of the plant *Sterculia foetida* were collected from Jharkhali, Sundarbans (22.0306° N, 88.7013° E). The plant was identified by a botanist before the collection of the fruits. The seeds were separated from the fruits and seed coats were then removed from the seeds, and after thoroughly mixing 1 gram was weighed. This 1gm of seed coat was added into 5 ml of ethanol and incubated for 72 hours at room temperature.

Mueller Hinton broth: The broth powder was procured from Himedia, and 4.2gm of broth powder was added to 200ml of distilled water, mixed well, and autoclaved. After autoclaving, the sterile medium was stocked in the laboratory for the study.

Bacterial strains: The bacterial strains used were *Escherichia coli* ATCC 25922, *Escherichia coli* MDR, *Klebsiella pneumoniae* MDR, *Pseudomonas aeruginosa* MDR, *Acinetobacter baumannii* MDR, and *Salmonella typhi* MDR strain.

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METHODS

This study aimed to determine the antibacterial effect of the extract of the *Sterculia foetida* seed coat, and if antibacterial activities are present then the Minimum Inhibitory Concentration (MIC) of the extract was determined. At first, the bacterial strains were subcultured on respective mediums to obtain pure strains. All the wells of a microtiter plate were filled up with 100µl of Mueller Hinton broth. Then 100 µl of the extract was added to the first well, and thoroughly mixed and the 100 µl of this was transferred to the next well in a horizontal row, mixed again, and 100 µl of it again transferred to the next well. In this way, serial double dilution of the extract was performed up to the eighth well of the row. Thus, the concentration of the extract in the first well was 10mg/ml, and this was diluted to 5mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml, 0.156 mg/ml, and 78 µg/ml. Then one test bacterial suspension in sterile normal saline (0.5 MacFarland standard) was added in each well in 10 µl amounts.

In the next horizontal row of eight wells, the vehicle of the extracted ethanol was similarly diluted, and the test bacteria was similarly added in each well. This row was done as a control to compare the results. Other bacterial strains were similarly tested in each separate row with one control row.

After completing the procedure, the microtitre plate was gently rotated to mix the bacteria uniformly in each well. Then an initial reading at 0 hour was taken at 620nm in a Microscan reader to find out the baseline optical densities. Another reading at a similar wavelength was also taken after 24 h. Then the baseline reading of each well was deducted from the final reading of each well.

RESULTS

The extract of the seed coat of *Sterculia foetida* showed antibacterial activities against MDR bacterial strains as well as against the *E. coli* ATCC25922 strain. The MIC values against *E.coli* ATCC 25922 strain and *E. coli* MDR strain was 0.625mg/ml; however, the MIC value against all other tested bacteria namely *Klebsiella pnemoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Salmonella typhi* was 1.25 mg/ml (Fig.2-7). The most important point is that all these tested bacteria were MDR strains showing the possible utilization of this extract against resistant strains of bacteria. All these microbial species are commonly isolated strains from hospitals and thousands of persons die each year due to infection of these bacteria as conventional antibiotics often fail to act against them. It creates a scope to utilize this extract against these resistant bugs in the future.

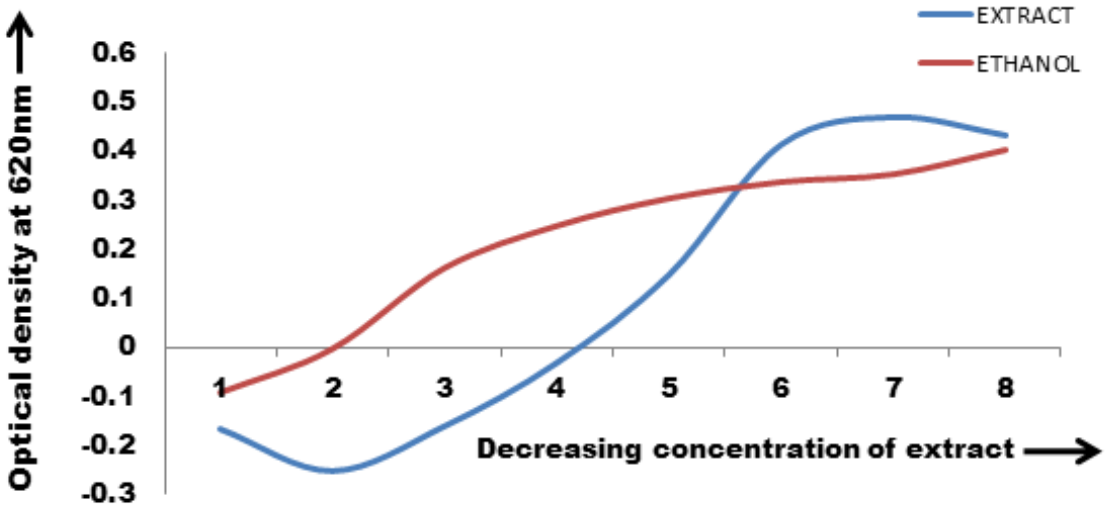

Figure 2: Showing MIC value (0.625mg/ml) of the extract against *Escherichia coli* ATCC 25922; concentration of extract: 1= 10mg/ml;2= 5mg/ml; 3= 2.5mg/ml ;4= 1.25mg/ml; 5= 0.625mg/ml; 6= 0.312mg/ml; 7= 0.156mg/ml; 8= 0.078mg/ml.

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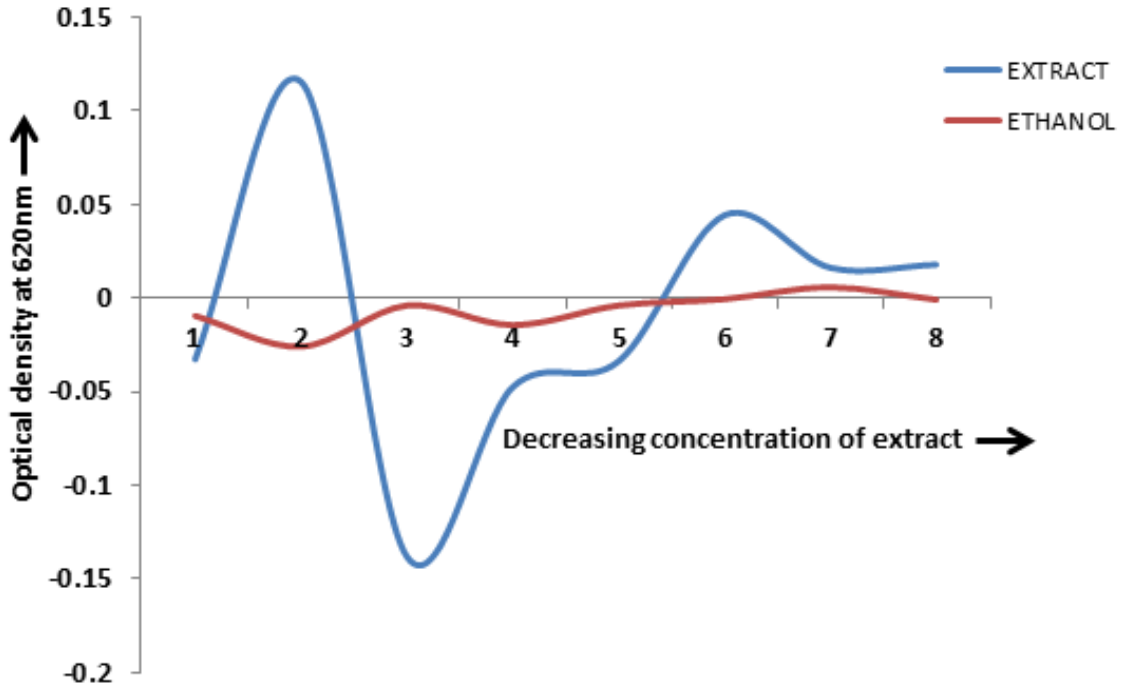

Figure 3: Showing MIC value (0.625mg/ml) of extract against *Escherichia coli* MDR; concentration of extract: 1= 10mg/ml;2= 5mg/ml; 3= 2.5mg/ml;4= 1.25mg/ml; 5= 0.625mg/ml; 6= 0.312mg/ml; 7= 0.156mg/ml; 8= 0.078mg/ml.

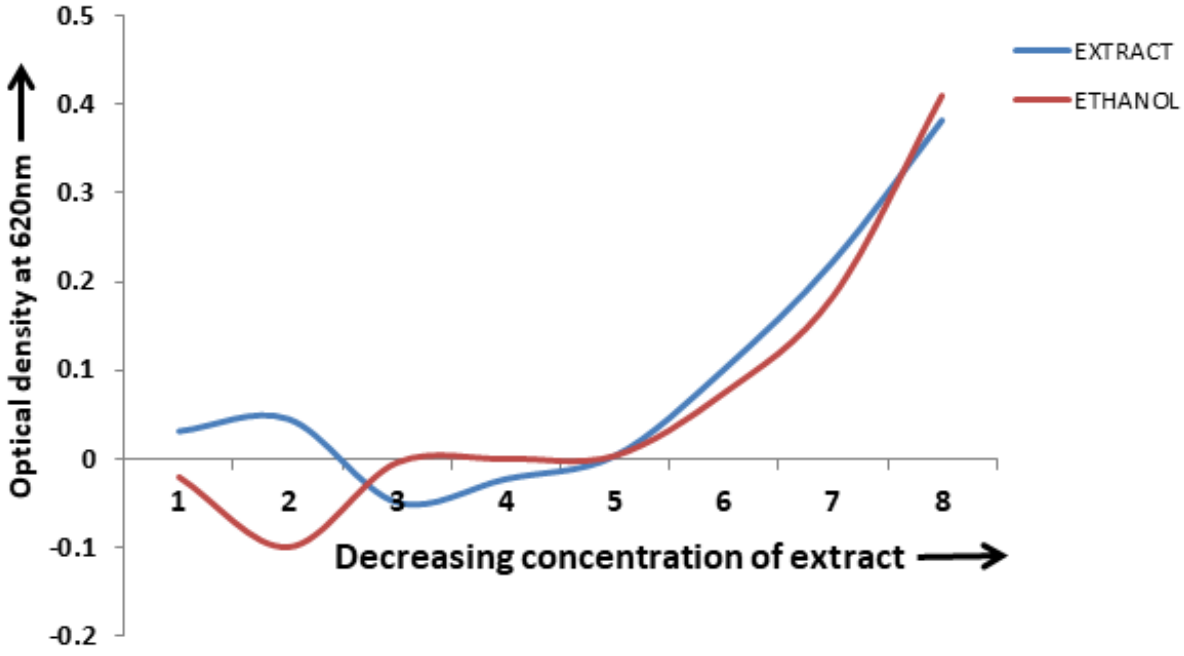

Figure 4: Showing MIC value (1.25mg/ml) of extract against *Klebsiella pneumonia* MDR; concentration of extract: 1= 10mg/ml;2= 5mg/ml; 3= 2.5mg/ml;4= 1.25mg/ml; 5= 0.625mg/ml; 6= 0.312mg/ml; 7= 0.156mg/ml; 8= 0.078mg/ml.

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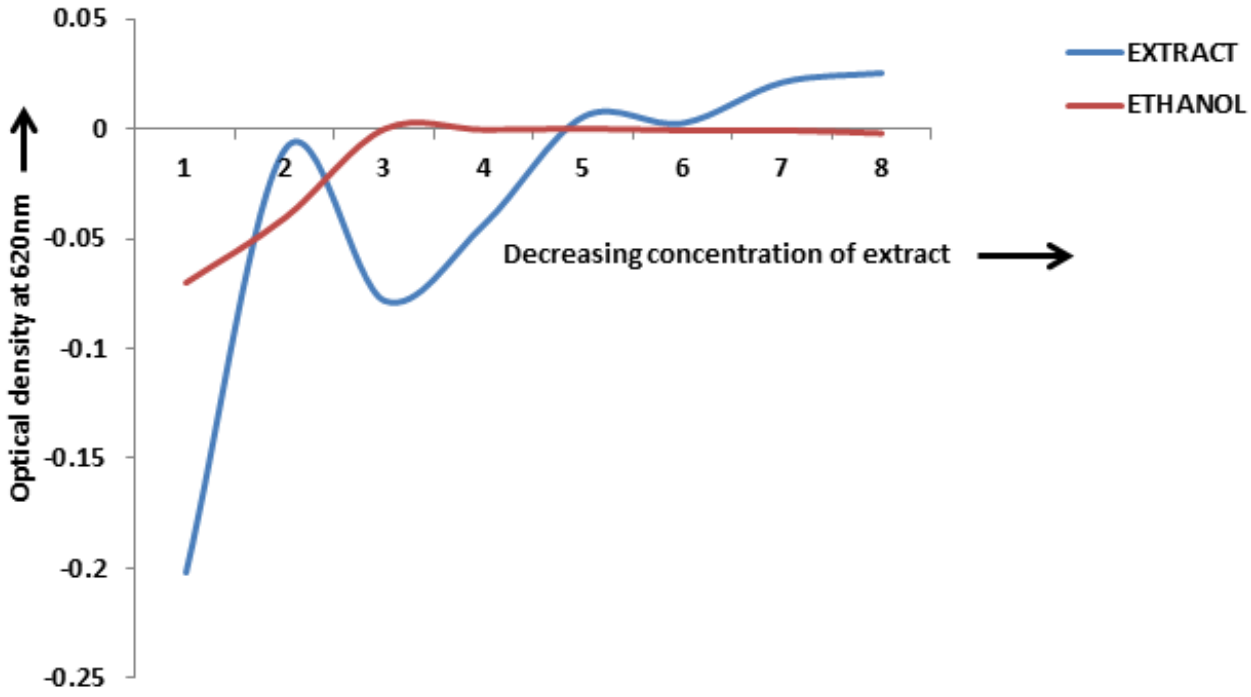

Figure 5: Showing MIC value (1.25mg/ml) of extract against *Pseudomonas aeruginosa* MDR; concentration of extract: 1= 10mg/ml;2= 5mg/ml; 3= 2.5mg/ml;4= 1.25mg/ml; 5= 0.625mg/ml; 6= 0.312mg/ml; 7= 0.156mg/ml; 8= 0.078mg/ml.

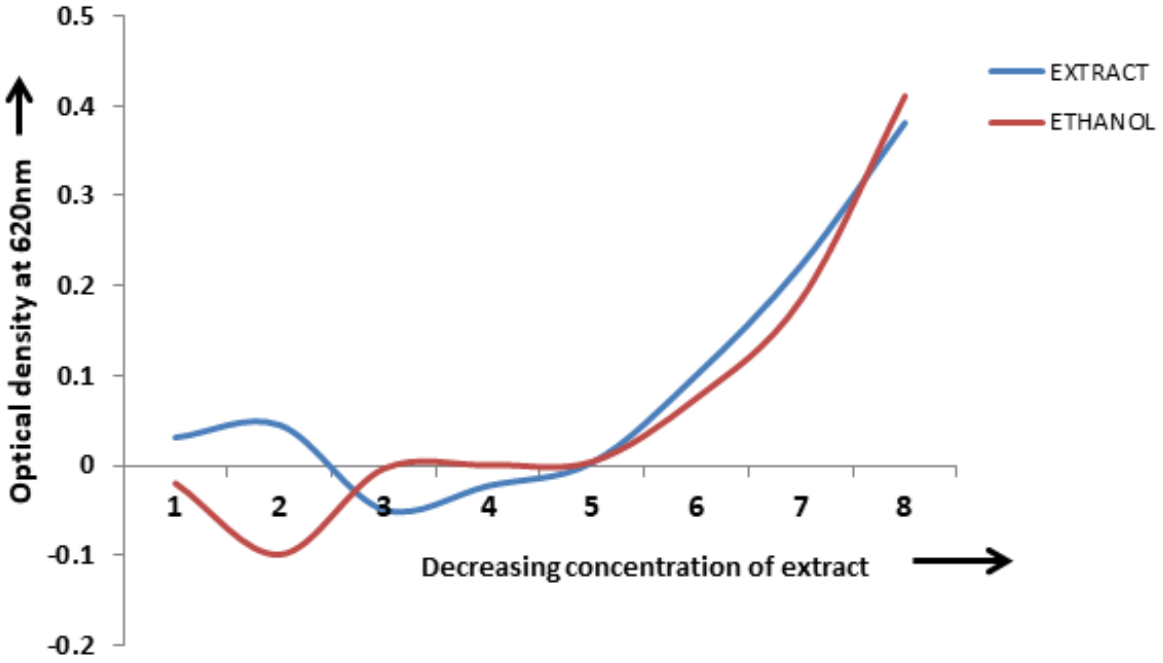

Figure 6: Showing MIC value (1.25mg/ml) of extract against *Acinetobacter baumannii* MDR; concentration of extract: 1= 10mg/ml;2= 5mg/ml; 3= 2.5mg/ml;4= 1.25mg/ml; 5= 0.625mg/ml; 6= 0.312mg/ml; 7= 0.156mg/ml; 8= 0.078mg/ml.

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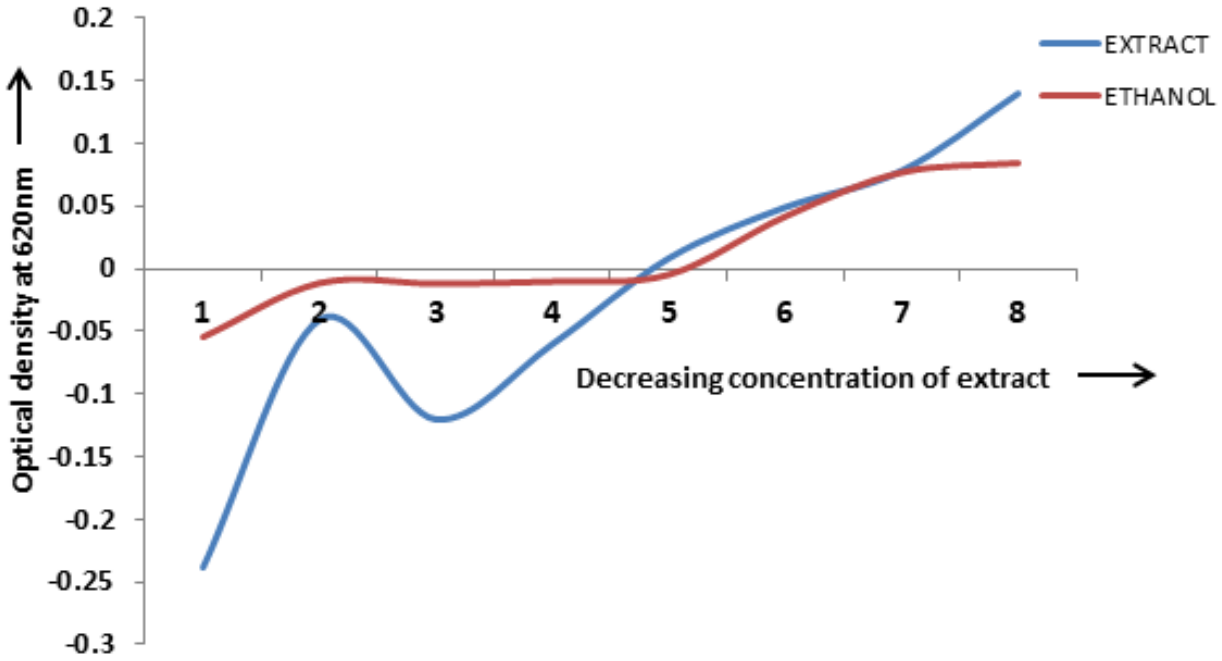

Figure 7: Showing MIC value (1.25mg/ml) of extract against *Salmonella typhi* MDR: concentration of extract: 1= 10mg/ml;2= 5mg/ml; 3= 2.5mg/ml;4= 1.25mg/ml; 5= 0.625mg/ml; 6= 0.312mg/ml; 7= 0.156mg/ml; 8= 0.078mg/ml.

DISCUSSION

Although there are some reports on the antimicrobial actions of the *Sterculia foetida* plant, so far there is no report on the antimicrobial activities of the seed coat of this plant, indicating the novelty of this work. Thus, a group of workers published the antimicrobial action of leaves of *Sterculia foetida* against various bacteria, fungi, and protozoa [11]. Another study showed that the extract from the *Sterculia foetida* plant has a potent amount of antioxidants, antimicrobial products, as well as anti-cancerous properties [12]. There are only a few studies on the antimicrobial action of seed coat extracts of other plants also. In one study antimicrobial effect of phenolic extract of soybean seed coat was observed [13]. A similar study was done with alcoholic seed coat extract of *Trachycarpus fortunei* (Chinese Windmill Palm) on multidrug-resistant bacteria which also showed a Minimum Inhibitory Concentration (MIC) of 1.25 mg/ml for most of the strains tested [14]. However, these studies were mainly done against usual microbial species, not against MDR bacteria. It appears that this extract can be used in the future to tackle MDR bacterial strains which are now rampant in all infected patients, particularly in ICU and ITU. A toxicity study of this extract is urgently needed to achieve this goal.

Conflict of interest

There is no conflicts of interest of any Author.

Author's contribution

R.H – Experiment, Manuscript writing; B.N.C and P.G – Manuscript editing, Guidance; S.D – Experiment designing, Manuscript correction

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