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Short Communication

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E-mail: bjsir07gmail.com

Antimicrobial Activity of Akanda (*Calotropis gigantea* L.) on Some Pathogenic Bacteria.

M. Ashraful Alam, M. Rowshanul Habib, Farjana Nikkon, Matiar Rahman and M. Rezaul Karim*

Department of Biochemistry and Molecular Biology, Faculty of Science, Rajshahi University, Rajshahi-6205, Bangladesh.

Abstract

The antibacterial activity of methanol extract from the root bark of Akanda (*Calotropis gigantea* L.) and its petroleum ether, chloroform and ethyl acetate fractions were investigated. Both of methanol extract and its chloroform fraction showed activity against *Sarcina lutea, Bacillus megaterium* and *Pseudomonas aeruginosa*. Petroleum ether fraction showed activity against *Bacillus subtilis* and *Shigella sonnei* whereas ethyl acetate fraction showed activity against *Pseudomonas aeruginosa* and *Escherichia coli* at 20µg/disc, 30µg/disc and 40µg/disc doses. Among the tested materials, methanol extract and its chloroform fraction showed comparatively better results. Minimum inhibitory concentration (MIC) for methanol extract and each fraction were also determined by serial dilution technique.

Keywords: Methanol extract, Akonda (*Calotropis gigantea*), Petroleum ether fraction, Chloroform fraction and Ethyl acetate fraction.

Introduction

The plant, *Calotropis gigantea* L. grows widely throughout the Indian subcontinent. The root bark of this plant is used as medicine in treatment of leprosy, piles, wounds, tumours, parasitic infections and dysentery (Kirtikar and Basu 1994). In literature, it was also reported that alcoholic root extract of *Calotropis gigantea* showed analgesic, anticonvulsant, anxiolytic and sedative effect in albino rats (Argal and Pathak 2006). No reports about antimicrobial screening of extracts and isolated bioactive compounds from *Calotropis gigantea* such as calotropin, frugoside, 4'-O-beta-D-glucopyranosylfrugoside, calotroposide A and B, giganticine, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucopyranoside, taraxasteryl acetate, 19-Nor- and 18,20-epoxy-cardenolides etc.,

* Corresponding author, Email: rezaplazad@ yahoo.com

have been drawn (Kiuchi *et al*. 1998; Kitagawa et al. 1992; Pari *et al*. 1998; Sen *et al*. 1992; Lhinhatrakool and Sutthivaiyakit, 2006). So the purpose of this study was aided to prepare methanol extract from root bark of akanda (*Calotropis gigantea* L.) and different (petroleum ether, chloroform and ethyl acetate soluble) fractions of methanol extract to assess the antibacterial activity of these test samples against some pathogenic bacteria such as those causing dysentery, burn and wound infections, food poisoning and other diseases.

Materials and Methods

Extraction procedures

The plant *Calotropis gigantea* L. was taxonomically identified by Professor A.T.M Naderuzzaman, Department of Botany, University of Rajshahi. Voucher specimen (No. 1A. Alam, collection date 15.04.2007) was kept in the Dept. of Botany, University of Rajshahi. The roots of *Calotropis gigantea* were collected during the month of April-May, 2007 from the relevant area (Meherchandi) of Rajshahi University campus. The collected root bark pieces were sun dried for 7-10 days and finally kept in an electric oven for 72 hours at 40^{O}C. After complete drying, the dried pieces were then pulverized into a coarse powder with the help of a grinding machine (FFC-15, China). The powdered plant materials (1.4 kg) were extracted with methanol by Soxhlet extractor and the extraction process was performed repeating 4 cycles. The extract was then filtered through Whatman No.1 filter paper. The filtrate was concentrated with a rotary evaporator under reduced pressure at 60^{O}C to afford crude methanol extract (40 g). The crude methanol extract (30 g) was fractionated into petroleum ether (3 g), chloroform (10 g) and ethyl acetate (2 g) fractions by solvent-solvent partitioning (Bahl and Bahl 1992).

Tests for antimicrobial activity

The methanol extract of root bark of akonda and its petroleum ether, chloroform and ethyl acetate fraction were tested for antibacterial activity by disc diffusion assay method (Vander and Vlietnck, 1991). Six pathogenic bacteria (*Bacillus megaterium* BTCC18, *Bacillus subtilis* BTCC19, *Sarcina lutea* ATCC27853, *Shigella sonnei* AJ8992, *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC25922) were collected from the Institute of Biological Science (IBSC), University of Rajshahi, Bangladesh. Standard *Kanamycin* disc (30 µg/disc) and blank disc impregnated with the respective solvent, were used as positive and negative control respectively to study the antibacterial activity. The antibacterial activity of methanol extract and its petroleum ether, Chloroform and ethyl acetate fractions were tested against six bacteria at concentrations of 20 µg/disc, 30 µg/disc and 40µg/disc.

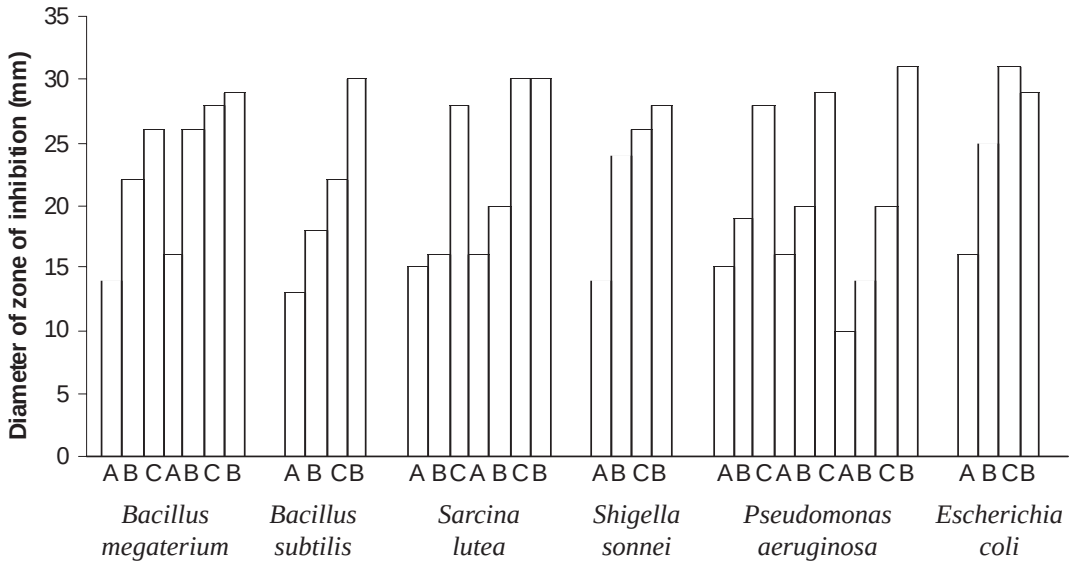
Determination of Minimum Inhibitory Concentration (MIC)

The MIC values for methanol extract, petroleum ether, chloroform and ethyl acetate fractions were determined by serial tube dilution technique (Reiner, 1982) against *Bacillus megaterium, Bacillus subtilis, Sarcina lutea, Shigella sonnei, Pseudomonas aeruginosa* and *Escherichia coli.*

Results and Discussion

The antibacterial activity of methanol extract and its petroleum ether, chloroform and ethyl acetate fractions were tested against six bacteria at concentrations of 20µg/disc, 30 µg/disc and 40µg/disc. The results obtained are graphically shown in Figure 1.

In this study we found that both methanol extract from root bark of akonda and its chloroform fraction showed remarkable activity against *Pseudomonas aeruginosa, Sarcina lutea* and *Bacillus megaterium* (Figure 2-4 and 7-9). Since *Pseudomonas aeruginosa* and *Bacillus megaterium* are commonly implicated in pus causing wounds and food poisoning, respectively, so both the methanol extract and its chloroform fraction can be used as medicine in the treat-



Where

→ Methanol extract, → Petroleum ether fraction, ■ → Chloroform fraction,

→ Ethyl acetate fraction, ■ → Kanamycin, A → 20 µg/disc, B → 30 µg/disc

and C → 40µg/disc

Fig. 1. Antibacterial activity of methanol extract and its different fractions of root bark of *Calotropis gigantea* L.

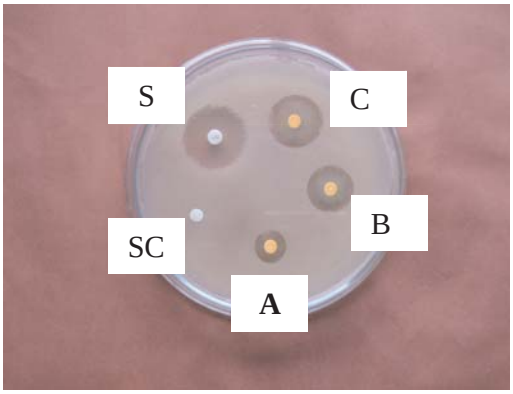


Fig. 2. Antibacterial activity of methanol extract against *Bacillus megaterium*

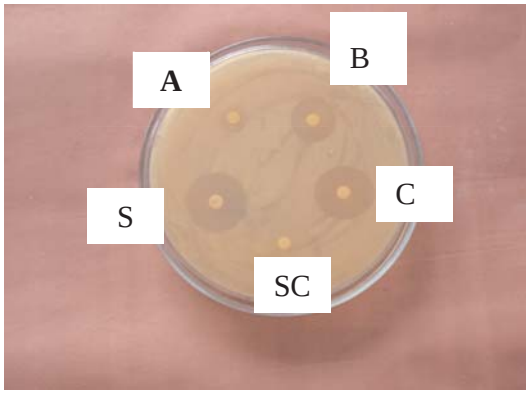


Fig. 3. Antibacterial activity of methanol extractagainst *Sarcina lutea*

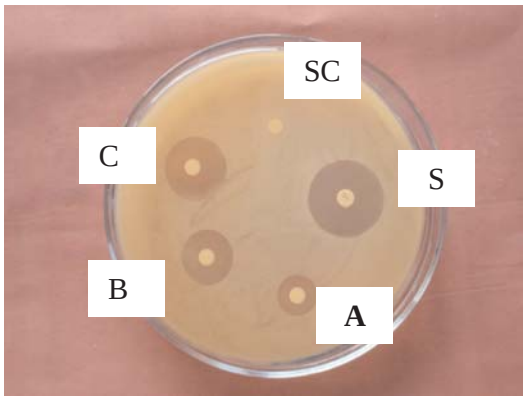


Fig. 4. Antibacterial activity of methanol extract against *Pseudomonas aeruginosa*

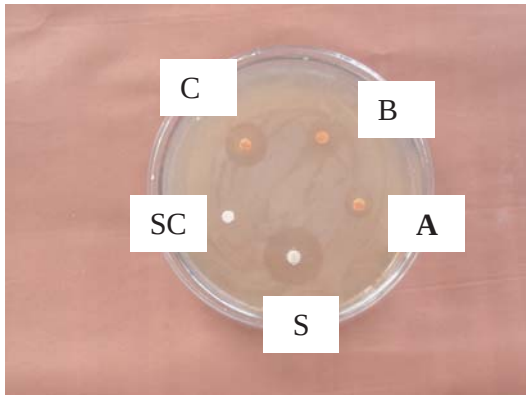


Fig. 5. Antibacterial activity of petroleum ether fraction against *Bacillus subtilis*

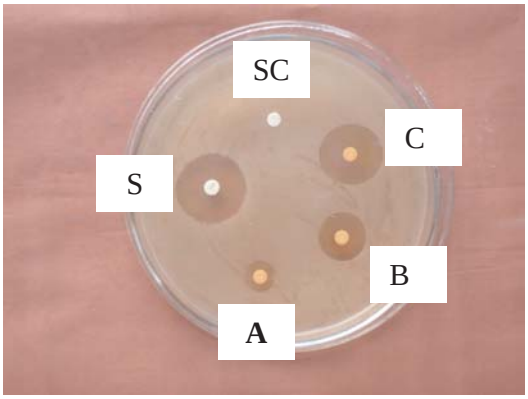


Fig. 6. Antibacterial activity of petroleum ether fraction against *Shigella sonnei*

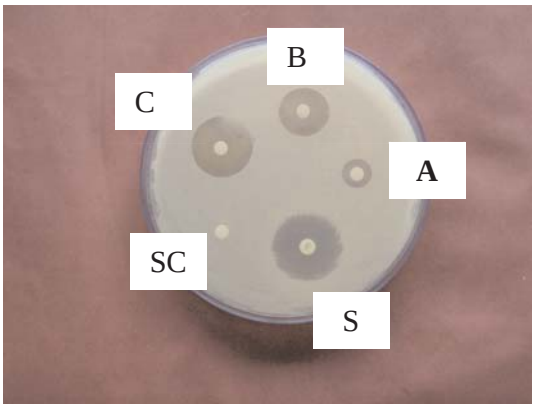


Fig. 7. Antibacterial activity of chloroform fraction against *Bacillus megaterium*

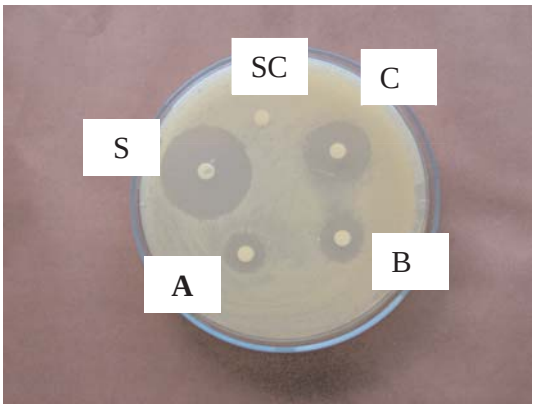


Fig. 8. Antibacterial activity of chloroform fraction against *Sarcina lutea*

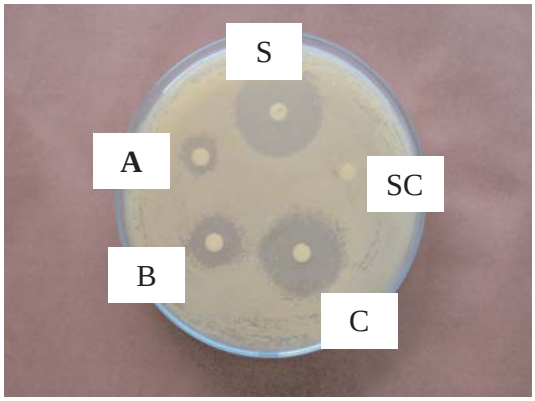


Fig. 9. Antibacterial activity of chloroform fraction against *Pseudomonas aeruginosa*

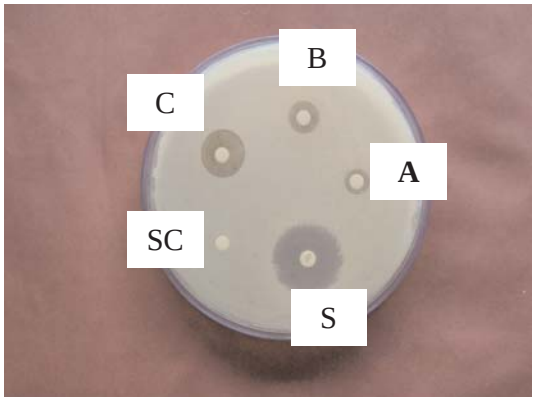


Fig. 10. Antibacterial activity of ethyl acetate fraction against *Pseudomonas aeruginosa*

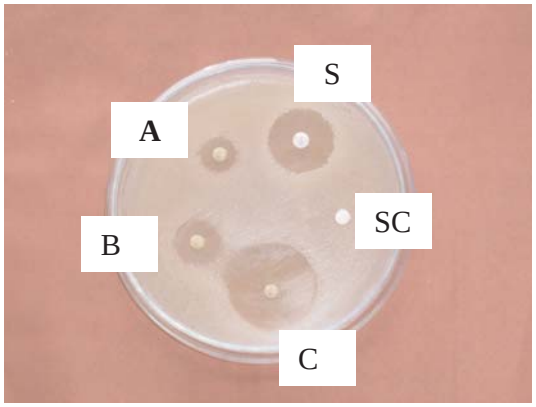


Fig. 11. Antibacterial activity of ethyl acetate fraction against *Escherichia coli*

ment of wounds and food poisoning. *Bacillus subtilis* and *Shigella sonnei* are responsible for food poisoning and shigellosis in human. Petroleum ether fraction showed activity against these two bacterial strains (Figure 5 and 6). So it can be used as medicine in food poisoning and dysentery. Ethyl acetate fraction showed activity against *Escherichia coli* (Figure 11) which can cause a moderate to severe gastroenteritis in humans. This fraction also gave activity against *Pseudomonas aeruginosa* (Figure 10).

The MIC values of methanol extract and its petroleum ether, chloroform and ethyl acetate fractions against six bacteria were shown in the Table I. Methanol extract showed moderate activity against *Pseudomonas aeruginosa* and the MIC value was 64 µg/ml. Chloroform fraction showed activity against *Bacillus megaterium* and *Pseudomonas aeruginosa* and the MIC value for this two bacterial strain was 64 µg/ml.

The lowest MIC value for petroleum ether and ethyl acetate fraction were 64 µg/ml and 32 µg/ml against *Shigella sonnei* and *Escherichia coli*, respectively.

Conclusion

A serious problem was created to human health when the microorganisms were found to resistant to the antibiotics and recently, this problem has become more evident since most of the organisms exhibit some degree of resistance to the commonly available antimicrobial and chemotherapeutic agents. The heavy uses of antibiotic like tetracycline, amphicillin and chloramphanical (Haque and Aziz 1977), nalidixic acid (Carlson *et al*. 1983) cotrimoxazole (Zaman *et al.* 1983) and most currently used antibiotic like mecillinam and ciprofloxacin contributed to the development of resistant strains of microorganisms. Moreover, powerful drugs against which antimicrobial resistance has

Table I. MICs of methanol extract and its different fractions

Bacterial strain	Methanol extract	Petroleum ether fraction	Chloroform fraction	Ethyl acetate fraction
	Minimum inhibitory concentration (µg/ml)			
B*acillus megaterium*	128	-	64	-
Bacillus subtilis	-	128	-	-
Sarcina lutea	128	-	128	-
Shigella sonnei	-	64	-	-
Pseudomonas aeruginosa	64	-	64	256
Escherichia coli	-	-	-	32

not yet been developed are unavailable and costly. So, poor people of our country cannot afford this. So there is crying need for alternative treatment for different types of diseases such as dysentery, burn, wound etc. Extract from root bark of *Calotropis gigantea* have better activity in comparison with chloroform extract from *Wedelia calendulacea* (Rashid *et al.*, 2004) which showed the highest inhibitory activity against most of the above used bacterial strains with mean zone of inhibition of 10-21 mm at 200 µg/disc. The results of this study reflect that potent antibacterial phytochemicals present in methanol extract of the root bark of akonda. Further investigation is required to isolate the active ingredients.

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