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ANTIBACTERIAL ACTIVITIES OF THE SEED EXTRACTS OF SOME INDIGENOUS PLANTS

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ABSTRACT

The chloroform and methanol extracts of seed and seed coat of *Caesalpinia bonduc* L., *Mucuna pruriens* L., *Adenanthera pavonina* L., *Terminalia bellirica* Geatn., *Syzygium cumini* L. and *Myristica fragrans* Houtt. were tested against 14 pathogenic bacteria. According to the intensity of activity against the selected bacteria the extracts could be arranged in a descending order of *M. fragrans* > *A. pavonina* > *S. cumini* > *C. bonduc* > *M. pruriens* > *T. dbelerica*. The minimum inhibitory concentrations (MIC) of the chloroform extract of seed of *Syzygium cumini* were 128 µg/ml against *Bacillus cereus* and 64 µg/ml against *S. aureus*. For the methanol extract the MIC values were 128 µg/ml against *B. cereus*, *Shigella dysenteriae* and 64 µg/ml against *B. megaterium*, *S. aureus* and *S. sonnei*.

Key words: Antibacterial activity, Indigenous plants, Seed extracts

INTRODUCTION

The plants are the natural chemical factories that synthesize innumerable compounds. The plant-derived compounds have been utilized by the human being from time immemorial in public health and pest management. Many of the test plants are native to Bangladesh and are easily available in abundant quantities. Some of these plants are well-known for their medicinal values.

Antibacterial activities of some important medicinal plants were conducted against Gram-positive and Gram-negative pathogenic bacteria. Chowdhury *et al*. (2010) studied the biological activities of two isolated compounds of methanol leaf extract of Nishinda, *Vitex negundo* L. and found that the zone of inhibition for some pathogenic bacteria was prominent when compared with kanamycin (control) sensitivity at concentration of 30 µg/disc and some extracts exhibited more prominent clear zone of growth inhibition compared to kanamycin at 100 µg/disc. Bari *et al*. (2010) reported that the chloroform and methanol extracts of the stem of *Smilax zeylanica* showed significant antibacterial activities against *Bacillus cereus* and *Salmonella typhi* when compared with

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ciprofloxacin. Bari *et al.* (2010) also investigated the effects of chloroform and methanol extracts of *Solanum torvum* Sw. on 15 human pathogenic bacteria and found that the methanolic extracts of root exhibited significant antibacterial effects. Oly *et al.* (2011) investigated the antibacterial activities of crude extracts of different parts of *Clerodendrum viscosum* Vent. in different solvents against six Gram-positive and nine Gram-negative bacteria using disc diffusion and micro broth dilution techniques. They observed promising results. Waliullah *et al.* (2014) reported that the ethanol and ethyl acetate extracts of root, leaf and stem of *C. infortunatum* L. against six Gram-positive and nine Gram-negative bacterial strains were very effective against the experimental bacteria.

Due to the notable medicinal value of the experimental plants, it was felt necessary to carry out phytochemical and antimicrobial investigation of the extracts of the said plants.

MATERIALS AND METHODS

The fresh seeds of *Caesalpinia bonduc* (Natai), *Mucuna pruriens* (Alkushi), *Adenanthera pavonina* (Rakta Chandan), *Terminalia bellirica* (Bahara), *Syzygium cumini* (Kalojam) and *Myristica fragrans* (Jayfal) were collected from the Botanical Garden, Rajshahi University and the identification of voucher specimens were confirmed at the Taxonomical Section, Department of Botany, University of Rajshahi, Bangladesh.

The plants were chopped into small pieces, dried under internal shade and powdered using a hand grinder separately. The seeds and seed coats powder were extracted with chloroform and methanol (BDH, Pooleg England) using Soxhlet's apparatus according to Feuerhake and Schmutterer (1982). The extracts obtained were stored in a refrigerator at –20°C with proper labeling.

Nutrient agar medium (Bauer *et al.* 1966) was used for determining anti-bacterial activity. Fourteen pathogenic bacteria (five Gram-positive and nine Gram-negative) were selected for the antibacterial test and were cultured at the Molecular Laboratory, Institute of Biological Sciences, Rajshahi University. The test extracts were dissolved in respective solvents in such a manner that the desired concentrations (50 and 200 µg/disc) for application in the disc have been obtained. Standard antibiotic discs of ciprofloxacin (30 µg/disc) was also used for comparison.

The serial dilution technique was followed using nutrient broth medium to determine the MIC values of the chloroform and methanol extracts against *B. cerus*, *B. megaterium*, *S. aureus*, *S. sonnei* and *S. dysenteriae*. The selected extracts were taken into different vials in a fixed amount (2.048 mg), and then broth medium (2 ml) was added to each of

the vials and agitated well to make sample solution whose concentration became 1024 μg/ml. The standard antibiotic ciprofloxacin solution 512 μg/ml (Reiner 1980) was used for comparison.

RESULTS AND DISCUSSIONS

All the crude extracts subjected to screening against a number of Gram-positive and Gram-negative bacteria showed mild to moderate toxic effects. It was clear from inhibition zones (Tables 1 - 4) that most of the extracts were effective.

The chloroform and methanol extracts of seed and seed coats of *A. pavonina*, *S. cumini* and *M. fragrans* especially at 200 μg/disc were very effective. When concentration of the extract was increased, the zone of inhibition was found to increase. The MIC results indicated that the methanolic extract of the seed coat oil has the property of inhibiting bacterial growth even at low concentrations (64 - 128 μg/disc). This probably explains the use of the extract of this plant in traditional medicines against a number of infections. So more comprehensive studies are solicited for their effective use, specially in medicine and agriculture.

The antimicrobial activity is attributed to the presence of some active constituents in the extracts. The antibacterial study of the plant extracts demonstrates that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenanian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases (Girish and Satish 2008, Toama *et al*. 1974). These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources (Li *et al*. 1994, Eruteya and Odunfa 2009).

The present data on the antibacterial activity of the plants are supported by a number of recent reports. Kannur *et al.* (2012) reported that the seed coat extracts of *C. bonduc* was more effective in controlling the inflammation. Cerqueira *et al.* (2009) extracted, purified and characterized galactomannans from non-traditional sources. Four non-traditional galactomannans were isolated from the seeds of *A. pavonina, C. pulcherrima, Gleditsia triacanthos* and *Sophora japonica*. All the galactomannans from those plants in view of their importance, e.g. in the demanding area of food industry. The finding also supports the antibacterial activity against *E. coli, B. subtilis, P. aeruginosa* and *S. aureus* and inhibitory effect on glucoamylase of ethanolic extracts isolated at different temperatures from seeds of *S. cumini* investigated *in vitro* (Meshram *et al*. 2011).

Table 1. Antibacterial activity of the seed extracts (chloroform) of *C. bonduc, M. pruriens, A. pavonina, T. dbelerica, S. cumini* and *M. fragrans* in comparison with the standard Ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm) 50 and 200 µg/disc												Ciprofloxacin 30µg/ disc
	C. bonduc		*M. pruriens*		*A. pavonina*		*T. belerica*		*S. cumini*		*M. fragrans*		
	50	200	50	200	50	200	50	200	50	200	50	200	
Gram positive													
S. aureus	-	-	-	-	-	-	-	-	-	12		07	35
B. cereus	-	07	-	-	-	-	-	07		10		09	33
B. megaterium	-	-	-	-	-	-	-	-	-	11		11	34
S. lutea	-	-	-	-	-	-	-	-	-	09		09	34
S. haemolyticus	-	-	-	-	-	-	-	-	07	09		07	33
Gram negative													
S. typhi	-	-	-	-	-	-	-	08	-	13		-	35
S. dysenteriae	-	-	-	-	-	10	-	-	-	15		11	31
S. shiga	-	-	-	-	-	-	-	-	-	13		08	34
S. sonnei	-	-	-	-	-	-	-	-	-	11		10	33
S. boydii	-	-	-	-	-	-	-	-	-	08		11	34
E. coli	-	-	-	-	-	-	-	-	-	12		10	33
Klebsiella sp.	-	-	-	-	-	-	-	-	-	10		10	31
P. aeruginosa	-	-	-	-	-	-	-	-	-	14		09	31
Proteus sp.	-	-	-	-	-	-	-	-	-	08		11	30

Table 2. Antibacterial activity of the seed extracts (methanol) of *C. bonduc, M. pruriens, A. pavonina, T. dbelerica, S. cumini* and *M. fragrans* in comparison with the standard ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm) 50 and 200l µg/disc												Ciprofloxacin 30 µg/ disc
	C. bonduc		*M. pruriens*		*A. pavonina*		*T. belerica*		*S. cumini*		*M. fragrans*		
	50	200	50	200	50	200	50	200	50	200	50	200	
Gram positive													
S. aureus	-	-	-	-	-	09	-	-	-	08	07	15	35
B. cereus	-	-	-	-	-	-	-	-	-	13	-	10	33
B. megaterium	-	-	-	-	-	-	-	-	-	-	-	13	34
S. lutea	-	-	-	-	-	-	-	-	-	13	-	11	34
S. haemolyticus		-	-	-	-	-	-	-	07	15	-	13	33
Gram negative													
S. typhi	-	-	-	-	-	-	-	-	-	14	-	13	35
S. dysenteriae	-	-	-	09	-	-	-	-	08	17	-	14	31
S. shiga	-	-	-	-	-	-	-	-	-	08	07	15	34
S. sonnei	-	-	-	-	-	-	-	-	-	10	-	13	33
S. boydii	-	-	-	-	-	-	-	-	-	10	-	13	34
E. coli	-	-	-	-	-	13	-	-	08	19	07	15	33
Klebsiella sp.	-	-	-	-	-	-	-	-	07	18	-	10	31
P. aeruginosa	-	-	-	-	-	08	-	-	-	11	-	13	31
Proteus sp.	-	-	-	-	-	-	-	-	-	09	-	11	30

Table 3. Antibacterial activity of the seed coat extracts (chloroform) of *C. bonduc, M. pruriens, A.pavonina, T. dbelerica, S. cumini* and *M. fragrans* in comparison with the standard ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm) 50 and 200 µg /disc												Ciprofloxacin 30 µg/ disc
	C. bonduc		*M. pruriens*		*A. pavonina*		*T. belerica*		*S. cumini*		*M. fragrans*		
	50	200	50	200	50	200	50	200	50	200	50	200	
Gram positive													
S. aureus	-	-	-	-	-	-	-	-	-	07		08	35
B. cereus	-	-	-	-	-	08	-	-	-	07	-	08	33
B. megaterium	-	07	-	-	-	08	-	-	-	09	-	09	34
S. lutea	-	-	-	-	-	-	-	-	-	08	-	11	34
S. haemolyticus		07	-	-	-	07	-	-	-	08	-	10	33
Gram negative													
S. typhi	-	-	-	-	-	-	-	-	-	-	-	09	35
S. dysenteriae	-	12	-	-	-	08	-	-	-	-	-	08	31
S. shiga	-	10	-	-	-	09	-	-	-	-	-	14	34
S. sonnei	-	-	-	-	-	08	-	-	-	09	-	08	33
S. boydii	-	09	-	-	-	10	-	-	-	-	-	10	34
E. coli	-	-	-	09	-	10	-	-	-	-	-	-	33
Klebsiella sp.	-	-	-	-	-	-	-	-	-	08	-	13	31
P. aeruginosa	-	10	-	-	-	10	-	09	-	09	-	11	31
Proteus sp.	-	-	-	-	-	07	-	-	-	-	-	09	30

Table 4. Antibacterial activity of the seed coat extracts (methanol) of *C. bonduc, M. pruriens, A. pavonina, T. dbelerica, S. cumini* and *M. fragrans* in comparison with the standard Ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm) 50 and 200 µg/disc												Ciprofloxacin 30 µg/ disc
	C. bonduc		*M. pruriens*		*A. pavonina*		*T. belerica*		*S. cumini*		*M. fragrans*		
	50	200	50	200	50	200	50	200	50	200	50	200	
Gram positive													
S. aureus	-	10	-	-	-	-	-	-	-	10	08	20	35
B. cereus	-	13	-	-	-	07	-	-	-	08	-	14	33
B. megaterium	-	-	-	-	-	13	-	-	-	-	07	16	34
S. lutea	-	12	-	-	-	11	-	-	-	10	07	18	34
S. haemolyticus		10	-	-	07	17	-	10	-	14	-	12	33
Gram negative													
S. typhi	-	08	-	-	07	15	-	-	-	09	-	10	35
S. dysenteriae	-	10	-	08	-	10	-	-	-	10	-	13	31
S. shiga	-	09	-	-	-	10	-	-	-	-	-	14	34
S. sonnei	-	-	-	-	-	14	-	-	-	10	07	18	33
S. boydii	-	13	-	-	-	09	-	-	-	-	-	14	34
E. coli	-	09	-	07	-	14	-	-	-	11	-	13	33
Klebsiella sp.	07	15	-	-	-	13	-	-	-	12	-	13	31
P. aeruginosa	-	-	-	-	-	11	-	09	-	-	-	14	31
Proteus sp.	-	-	-	-	-	14	-	-	-	10	-	08	30

The most promising extracts were subjected to evaluate their minimum inhibiting concentration (MIC) especially on the test bacteria on which the extracts showed activity. The results have been presented in Tables 5 and 6.

Table 5. Minimum inhibitory concentrations of the methanol extract of *Syzygium cumini* against five 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	
Seed extract (µg/ml)	512	256	128	64	32	16	8	4	2	1	0	1024	0	
Inoculum added (µl)	10	10	10	10	10	10	10	10	10	10	0	0	10	
B. cerus	-	-	-	+	+	+	+	+	+	+	-	-	+	128
B. megaterium	-	-	-	-	+	+	+	+	+	+	-	-	+	64
S. aureus	-	-	-	-	+	+	+	+	+	+	-	-	+	64
S. sonnei	-	-	-	-	+	+	+	+	+	+	-	-	+	64
S. dysenteriae	-	-	-	+	+	+	+	+	+	+	-	-	+	128

+ = Growth - = No growth

Table 6. Minimum inhibitory concentrations of chloroform extract of *Syzygium cumini* against five 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	
Seed extract (µg/ml)	512	256	128	64	32	16	8	4	2	1	0	1024	0	
Inoculum added (µl)	10	10	10	10	10	10	10	10	10	10	0	0	10	
B. cerus	-	-	-	+	+	+	+	+	+	+	-	-	+	128
B. megaterium	-	-	-	+	+	+	+	+	+	+	-	-	+	128
S. aureus	-	-	-	-	+	+	+	+	+	+	-	-	+	64
S. sonnei	-	-	-	+	+	+	+	+	+	+	-	-	+	128
S. dysenteriae	-	-	-	+	+	+	+	+	+	+	-	-	+	128

+ = Growth - = No growth

It is clearly evident from the investigations that both the chloroform and methanol extracts of different indigenous plants are significantly active against most of the bacteria used in this screening. Thus extensive studies are essential for the isolation of active compound(s) for development of novel antibacterial agents especially from the seed of the promising plants.

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REFERENCES

Bauer, A. W., W. M. M. Kirby, J. C. Sheries and M. Turek. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **45**: 493-496.

Bari, M. A., W. Islam and A. R. Khan. 2010. Antimicrobial and cytotoxic activity of *Smilax zylanica* L. (Liliaceae). Prof. M. Iqbal Choudhary: Personality and Achievements (Edited by Prof. Atta-ur-Rahman and Mahmood Alam), International Center for Chemical and Biological Sciences, University of Karachi, Pakistan**.** pp. 19-24.

Bari, M. A., W. Islam**,** A. R. Khan and A. Mandal. 2010. Antibacterial and antifungal activity of *Solanum torvum* (Solanaceae). *Int. J. Agric. Biol.* **12**: 386-390.

Cerqueira, M. A., A. C. Pinheiro, W. S. Bartolomeu, Souza, M. P. Alvaro, Lima, Clara Ribeiro, C. Miranda, J. A. Teixeira, R. A. Moreira, M. A. Coimbra, M. P. Goncalves and A. A. Vicente 2009. Extraction, purification and characterization of galactomannans from non-traditional sources. *Elsevier. Carbohydrate Polymers* **75** : 408-414.

Chowdhury, N. Y., W. Islam and M. Khalequzzaman 2010. Biological activities of isolated compounds from *Vitex negundo* leaf. *J. Bio-sci.* **18**: 57-63.

Eruteya, O. C. and S. A. Odunfa 2009. Antimicrobial properties of three spices used in the preparation of soya condiment against organisms isolated from formulated samples and individual ingredients. *Afr. J. Biotechnol*. **8**: 2316-2320.

Feuerhake, K. J. and H. Scmutterer. 1982. Einfache Verfahren Zur Ge Winnung and Formulating Von Niemsamenextrakten and Deren Wirking auf Varschiedene Schadinsekten. *Z. Pftkrankh Pflschutz.* **89**: 737-747.

Girish, H. V. and S. Satish. 2008. Antibacterial activity of important medicinal plants on human pathogenic bacteria, Comparative Analysis. *World J. Appl. Sci*. **5**: 267-271.

Kannur, D. M., M. P. Paranjpe, L. V. Sonavane, P. P. Dongre and K. R. Khandelwal. 2012. Evaluation of *Caesalpinia bonduc* seed coat extract for anti-inflammatory and analgesic activity. *J. Advanced Pharmaceutical Technol. Res.* **3**:171-175.

Li, X. Z., D. M. Livermore and H. Nikaido. 1994. Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa*: Resistance to tetracycline chloramphenicol and norfloxacin. *Antimicrobial Agents and Chemotherapy* **38:** 1732-1741.

Meshram, G. A., S. Sunil, Yadav, S. Dattatraya, P. Bhavana and S. Deepak. 2011. Antibacterial study and effect of ethanolic extracts of *Syzygium cumini* seeds powder on Glucoamylase *in vitro*. *J. Pharm. Sci. & Res.* **3**: 1060-1063.

Oly, W., W. Islam, P. Hassan and S. Parween. 2011. Antimicrobial activity of *Clerodendrum viscosum* Vent. (Verbenaceae). *Int. J. Agric. Biol.* **13**: 222-226.

Waliullah, T. M., A. M. Yeasmin, A. Ashraful, W. Islam and H. Parvez 2014. Antimicrobial potency screening of *Clerodendrum infortunatum* Linn. *Int. Res. J. Pharm.* **5**(2): 57-61.

Reiner, R. 1980. *Antibiotics: An introduction.* F. Hoffmann -La Roche and Company Ltd. Switzerland. pp. 21- 25.

Toama, MA., TS. El-Alfy and HM. El- Fatatry. 1974. Antimicrobial activity of the volatile oil of *Nigella sativa* Linneaus seeds. *Antimicrobial Agents and Chemotherapy* **6:** 225-226.

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