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Antimicrobial Screening of *Cassia fistula* and *Mesua ferrea*

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The antibacterial and antifungal activities of various solvent extracts (400 μg disc^{-1}) of *Cassia fistula* and *Mesua ferrea* were tested against fourteen pathogenic bacteria and six fungi respectively. Crude extracts of both *Cassia fistula* and *Mesua ferrea* exhibited moderate to strong activity against most of the bacteria tested. The antibacterial activities, on overall consideration, of *Mesua ferrea* extracts were not so enough as those of *Cassia fistula* extracts. On the other hand, the antifungal activities of *Cassia fistula* and *Mesua ferrea* exhibited by all the crude extracts were not prominent.

Key words: *Cassia fistula*, *Mesua ferrea*, antimicrobial screening

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INTRODUCTION

Many microorganisms can cause several diseases and now, in this world of modern science, man can face any challenge against any disease. But in spite of the tremendous advancement of medical science and technology, diseases are the leading health problem particularly in the under privileged population in the remote rural areas in the developing countries.

Cassia fistula and *Mesua ferrea* are frequently planted on city roads and avenues in almost all the districts of Bangladesh and used in traditional medicine for several indications from the time immemorial.

Root of *Cassia fistula* is described as a laxative, useful in fever, heart disease, retained excretions, biliousness, etc. In the Makhzan-El-Adwiya, the pulp of fruits is described as lenitive, useful for relieving thoracic obstructions and heat of blood and is a safe aperient for children and women. Externally, it is said to be a good application for gout, rheumatism, etc. A poultice made of leaves is said to relieve the chilblains. It has been beneficially used in facial paralysis and rheumatism when rubbed into the affected parts. Internally, it is given as a derivative in paralysis and brain affections[1].

Bark of *Mesua ferrea* is given in the treatment of cough, dysentery, vomiting, sore throat and fever. Flowers are astringent and stomachic and are used for cough, especially when attended with much expectoration. A paste, made of the flowers, with butter and sugar is used in bleeding piles and burning of the feet. The leaves and flowers in combination with other drugs are recommended for the treatment of snake bite and scorpion sting. In North Canada, oil of seeds of this plant is used as an embrocation in rheumatism and found useful in the treatment of itch[2].

Due to the fact that these plants are very useful, as found by above mentioned reports and the fact that little information cited in the literature[3] is available on their biological activities, there is a need to find out more about the potentiality of these plants as an antimicrobial agent. The present study is, therefore, designed to assess the potency of the plant extracts on selected microorganisms. It has been expected that the present work on antimicrobial screening of the plant materials of *Cassia fistula* and *Mesua ferrea* will lead to the scientists a clinical success concerning the killer diseases.

MATERIALS AND METHODS

The plants *Cassia fistula* and *Mesua ferrea* were collected from the Rajshahi University campus, Bangladesh. Organisms used in present studies were collected from the Department of Pharmacy, Rajshahi University, pure cultures of which were previously collected from the Institute of Food and Nutrition, University of Dhaka and also from ICDDR, Bangladesh. All solvents used for this study were redistilled and purified. Other chemicals, including culture media used were of analytical grade unless otherwise specified.

Plant samples: The plant materials of *Cassia fistula* and *Mesua ferrea* were individually washed with water, dried and pulverized into fine powder by a grinding machine and then stored in airtight container. 200 g of each of powdered plant materials of stem bark, leaves and pods of *Cassia fistula* was extracted with ethyl acetate at room temperature, solvent of the extract was evaporated under a vacuum. The extract was then washed with light petroleum ether to yield petroleum ether extract. Residue left after extraction with ethyl acetate was again extracted with methanol in the same procedure. On the other hand, 150 g of each of powdered plant materials of stem bark, leaves and seeds of *Mesua ferrea* was subjected to extract successively with light petroleum ether, chloroform and ethanol at room temperature following the same procedure.

Antimicrobial screening: *In vitro* antibacterial and antifungal screening were performed with crude petroleum ether, ethyl acetate and methanol extracts of *Cassia fistula* and also with crude petroleum ether, chloroform and ethanol extracts of *Mesua ferrea* against 14 pathogenic bacteria (5 g positive and 9 g negative) and 6 pathogenic fungi by the standard disc diffusion method[4-6]. Nutrient agar medium was used for determining antibacterial activity whereas potato dextrose agar medium (PDA) was selected for antifungal screening. Standard antibiotic discs of Kanamycin (30 μg $disc^{-1}$) and Fluconal (50 μg $disc^{-1}$) were also used for comparison in antibacterial and antifungal tests, respectively.

The crude extracts were dissolved in sufficient amount of the respective solvents, so that each 15 μl of solutions contained 400 μg of the test materials. The antimicrobial activities were determined by measuring the diameter of the inhibitory zones in mm using a transparent scale. The diameters of the zones of inhibition by the samples were then compared with the diameter of the zone of inhibition produced by the standard antibiotic disc used.

RESULTS AND DISCUSSION

Petroleum ether, ethyl acetate and methanol extracts obtained from *Cassia fistula* stem bark showed mild to strong activity against most of the tested bacteria. The results were compared with those of Kanamycin as a

Table 1: Antibacterial activities of different extracts of stem bark and leaves of *Cassia fistula*

	Diameter of zone of inhibition in mm						
Test organisms	PECB	EACB	MCB	PECL	EACL	MCL	STK
Gram positive							
Bacillus subtilis	15	18	11	9	10	0	20
Bacillus megaterium	10	14	11	9	8	12	14
Streptococcus β- haemolyticus	14	14	14	7	12	16	14
Streptococcus aureus	14	11	0	9	6	0	35
Sarcina lutea	15	15	12	12	8	0	20
Gram negative							
Shigella sonnei	14	18	10	10	7	0	13
Escherichia coli	11	11	8	9	12	0	30
Klebsiella species	11	17	11	9	10	0	16
Shigella shiga	14	14	8	10	9	0	20
Shigella boydii	23	11	8	9	8	0	35
Shigella flexneriae	10	16	9	9	10	0	15
Shigella dysenteriae	15	14	15	14	0	0	30
Salmonella typhi	12	19	9	9	10	9	19
Pseudomonas aeruginosa	8	12	8	7	10	12	15

PECB= Petroleum ether extract of *Cassia fistula* stem bark (400 μg disc^{-1}); EACB= Ethyl acetate extract of *Cassia fistula* stem bark (400 μg disc^{-1}); MCB= Methanol extract of *Cassia fistula* stem bark (400 μg disc^{-1}); PECL= Petroleum ether extract of *Cassia fistula* leaves (400 μg disc^{-1}); EACL= Ethyl acetate extract of *Cassia fistula* leaves (400 μg disc^{-1}); MCL= Methanol extract of *Cassia fistula* leaves (400 μg disc^{-1}); and STK= Kanamycin (30 μg disc^{-1})

Table 2: Antibacterial activities of different extracts of *Cassia fistula* pod and *Mesua ferrea* seeds

	Diameter of zone of inhibition in mm						
Test organisms	PECP	EACP	MCP	PEMS	CMS	EMS	STK
Gram positive							
Bacillus subtilis	12	16	7	9	7	0	20
Bacillus megaterium	16	11	16	7	7	11	14
Streptococcus β- haemolyticus	11	16	13	11	13	7	14
Streptococcus aureus	11	10	12	8	7	7	35
Sarcina lutea	16	16	10	0	0	0	20
Gram negative							
Shigella sonnei	16	15	12	7	0	8	13
Escherichia coli	13	10	14	10	8	9	30
Klebsiella species	16	15	11	7	7	0	16
Shigella shiga	13	13	10	0	0	0	20
Shigella boydii	13	13	12	7	0	0	35
Shigella flexneriae	14	13	11	8	0	0	15
Shigella dysenteriae	16	12	9	8	7	0	30
Salmonella typhi	15	14	14	9	7	0	19
Pseudomonas aeruginosa	14	13	12	12	13	0	15

PECP= Petroleum ether extract of *Cassia fistula* stem pod (400 μg disc^{-1}); EACP= Ethyl acetate extract of *Cassia fistula* stem pod (400 μg disc^{-1}); MCP= Methanol extract of *Cassia fistula* stem pod (400 μg disc^{-1}); PEMS= Petroleum ether extract of *Mesua ferrea* seeds (400 μg disc^{-1}); CMS= Chloroform extract of *Mesua ferrea* seeds (400 μg disc^{-1}); EMS= Ethanol extract of *Mesua ferrea* seeds (400 μg disc^{-1}); and STK= Kanamycin (30 μg disc^{-1})

standard antibiotic. Of the three extracts, only methanol extract did not show any activity against gram-positive *Streptococcus aureus*. Petroleum ether extract displayed excellent activity against gram-negative *Shigella boydii* (23 mm) whereas ethyl acetate extract showed strong activity against gram-positive *Bacillus subtilis* (18 mm) and gram-negatives *Shigella sonnei* (18 mm), *Klebsiella species* (17 mm) and *Salmonella typhi* (19 mm). But the activities, on overall consideration, of methanol extract were not so enough as those of petroleum ether and ethyl acetate extracts. It is also evident that the extracts of *Cassia fistula* leaves were found to be mild active against most of the bacterial strains (Table 1). Although, of the three extracts, methanol extract is inactive against most of the bacteria tested but it showed strong activity against gram-positive *Streptococcus β-haemolyticus* (16 mm). On the other hand, petroleum ether extract was active against all the tested bacteria and ethyl acetate extract displayed activity against all the bacterial strains except *Shigella dysenteriae*.

All the three extracts (petroleum ether, ethyl acetate and methanol) from *Cassia fistula* pod displayed moderate activity against most of the bacteria tested while the extracts (petroleum ether, chloroform and ethanol) of *Mesua ferrea* seeds were little active against most of the tested bacteria (Table 2). In this screening work, no extract of *Cassia fistula* was found to be inactive against any organism but the organisms such as gram-positives

Table 3: Antibacterial activities of different extracts of stem bark and leaves of *Mesua ferrea*

	Diameter of zone of inhibition in mm						
Test organisms	PEMB	CMB	EMB	PEML	CML	EML	STK
Gram positive							
Bacillus subtilis	12	14	12	9	7	0	20
Bacillus megaterium	13	10	11	9	8	0	14
Streptococcus β- haemolyticus	10	11	8	14	10	10	14
Streptococcus aureus	14	16	13	13	11	7	35
Sarcina lutea	10	11	10	10	9	9	20
Gram negative							
Shigella sonnei	11	9	10	11	8	7	13
Escherichia coli	15	19	12	10	11	9	30
Klebsiella species	14	14	10	11	12	9	16
Shigella shiga	14	13	9	9	11	7	20
Shigella boydii	11	13	9	10	11	0	35
Shigella flexneriae	8	8	7	12	8	8	15
Shigella dysenteriae	8	9	8	12	12	7	30
Salmonella typhi	14	13	10	14	13	8	19
Pseudomonas aeruginosa	13	11	14	9	14	0	15

PEMB= Petroleum ether extract of *Mesua ferrea* stem bark (400 μg disc^{-1}); CMB= Chloroform extract of *Mesua ferrea* stem bark (400 μg disc^{-1}); EMB= Ethanol extract of *Mesua ferrea* stem bark (400 μg disc^{-1}); PEML= Petroleum ether extract of *Mesua ferrea* leaves (400 μg disc^{-1}); CML= Chloroform extract of *Mesua ferrea* leaves (400 μg disc^{-1}); EML= Ethanol extract of *Mesua ferrea* leaves (400 μg disc^{-1}); and STK= Kanamycin (30 μg disc^{-1})

Table 4: Antifungal activities of different extracts of stem bark and leaves of *Cassia fistula*

	Diameter of zone of inhibition in mm						
Test organisms	PECB	EACB	MCB	PECL	EACL	MCL	STF
Penecillum notatum	0	0	8	9	13	9	14
Aspergillus niger	0	20	0	8	7	9	16
Trichoderma viride	8	12	9	10	7	0	13
Aspergillus flavus	0	10	10	7	10	9	10
Candida albicans	11	13	0	11	10	7	12
Hensinela californica	12	14	9	0	0	0	10

PECB= Petroleum ether extract of *Cassia fistula* stem bark (400 μg disc^{-1}); EACB= Ethyl acetate extract of *Cassia fistula* stem bark (400 μg disc^{-1}); MCB= Methanol extract of *Cassia fistula* stem bark (400 μg disc^{-1}); PECL= Petroleum ether extract of *Cassia fistula* leaves (400 μg disc^{-1}); EACL= Ethyl acetate extract of *Cassia fistula* leaves (400 μg disc^{-1}); MCL= Methanol extract of *Cassia fistula* leaves (400 μg disc^{-1}); and STF= Fluconal (50 μg disc^{-1})

Table 5: Antifungal activities of different extracts of *Cassia fistula* pod and *Mesua ferrea* seeds

	Diameter of zone of inhibition in mm						
Test organisms	PECP	EACP	MCP	PEMS	CMS	EMS	STK
Penecillum notatum	9	13	10	10	9	8	14
Aspergillus niger	0	0	0	11	8	7	16
Trichoderma viride	7	0	12	9	9	0	13
Aspergillus flavus	11	9	10	0	0	0	10
Candida albicans	8	12	11	8	0	8	12
Hensinela californica	0	10	0	0	9	10	10

PECP= Petroleum ether extract of *Cassia fistula* stem pod (400 μg disc^{-1}); EACP= Ethyl acetate extract of *Cassia fistula* stem pod (400 μg disc^{-1}); MCP= Methanol extract of *Cassia fistula* stem pod (400 μg disc^{-1}); PEMS= Petroleum ether extract of *Mesua ferrea* seeds (400 μg disc^{-1}); CMS= Chloroform extract of *Mesua ferrea* seeds (400 μg disc^{-1}); EMS= Ethanol extract of *Mesua ferrea* seeds (400 μg disc^{-1}); and STF= Fluconal (50 μg disc^{-1})

Table 6: Antifungal activities of different extracts of stem bark and leaves of *Mesua ferrea*

	Diameter of zone of inhibition in mm						
Test organisms	PEMB	CMB	EMB	PEML	CML	EML	STF
Penecillum notatum	10	9	11	11	8	7	14
Aspergillus niger	14	12	13	8	10	9	16
Trichoderma viride	9	7	10	10	11	11	13
Aspergillus flavus	0	0	0	0	0	0	10
Candida albicans	8	7	8	10	9	10	12
Hensinela californica	10	9	8	9	0	0	10

PEMB= Petroleum ether extract of *Mesua ferrea* stem bark (400 μg disc^{-1}); CMB= Chloroform extract of *Mesua ferrea* stem bark (400 μg disc^{-1}); EMB= Ethanol extract of *Mesua ferrea* stem bark (400 μg disc^{-1}); PEML= Petroleum ether extract of *Mesua ferrea* leaves (400 μg disc^{-1}); CML= Chloroform extract of *Mesua ferrea* leaves (400 μg disc^{-1}); EML= Ethanol extract of *Mesua ferrea* leaves (400 μg disc^{-1}); and STF = Fluconal (50 μg disc^{-1})

Sarcina lutea and *Shigella shiga* were resistant to all the extracts of *Mesua ferrea*. In comparison, the extracts of *Mesua ferrea* seeds exhibited very lower activity against most of the bacteria tested than those obtained from *Cassia fistula* pod.

All the three extracts of *Mesua ferrea* stem bark showed moderate activity against most of the tested bacteria and no extract was found to be inactive against any organism. Chloroform extract of *Mesua ferrea* stem bark displayed strong activity against gram-positive *Streptococcus aureus* (16 mm) and gram-negative *Escherichia coli* (19 mm). On the other hand, the extracts of *Mesua ferrea* leaves were found to be mild to moderate active against most of the bacterial strains (Table 3). Results also indicated that petroleum ether and chloroform extracts were found to be active against all the tested organisms and the activities, on overall consideration, of ethanol extract were not so enough as those of petroleum ether and chloroform extracts.

Crude extracts obtained from stem bark and leaves of *Cassia fistula* showed mild to moderate activity against most of the fungal strains (Table 4). Among them, Ethyl acetate extract of stem bark displayed excellent activity (20 mm) against *Aspergillus niger* and found to be inactive against only *Penecillum notatum*. All the three extracts of *Cassia fistula* leaves have no activity against *Hensinela californica*.

It is evident that all the extracts of *Cassia fistula* pod and *Mesua ferrea* seeds were not significantly active against most of the fungal strains (Table 5). The fungus such as *Aspergillus niger* was resistant against all the crude extracts of *Cassia fistula* pod whereas *Aspergillus flavus* was resistant against all the extracts of *Mesua ferrea* seeds. Moreover, all extracts of *Cassia fistula* and *Mesua ferrea* were active against *Penecillum notatum*.

All the extracts obtained from stem bark and leaves of *Mesua ferrea* displayed mild to moderate activity against all the tested fungi except *Aspergillus flavus* and *Hensinela californica* (Table 6). All the extracts of *Mesua ferrea* did not show any activity against *Aspergillus flavus* while *Hensinela californica* was resistant against chloroform and ethanol extracts of *Mesua ferrea* leaves.

From the above experimental results, it is found that the activities of methanol extracts of stem bark and leaves of *Cassia fistula* were not sufficiently enough compared to those of petroleum ether and ethyl acetate extracts respectively against most of the bacteria tested and petroleum ether extract of stem bark displayed highest activity against *Shigella boydii* (23 mm). On the other hand, ethanol extracts of stem bark and leaves of *Mesua ferrea* showed lower activities than those of petroleum ether and chloroform extracts respectively against most of the bacteria tested and chloroform extract of stem bark displayed highest activity against *Escherichia coli* (19 mm). In comparison, the activities of petroleum ether and ethyl acetate extracts (400 μg disc^{-1}) of *Cassia fistula* stem bark were close to those of same extracts (200 μg disc^{-1}) of *Oroxylum indicum* root bark[7] against most of the tested bacteria. Moreover, methanol extract (400 μg disc^{-1}) of *Cassia fistula* leaves was inactive against most of the tested bacteria and inactivities was also comparable with the same extract (200 μg disc^{-1}) of *Oroxylum indicum* root bark[7]. All the crude extracts of *Cassia fistula* pod showed higher activities than those of *Mesua ferrea* seed and almost similar activities by the extracts (except petroleum ether extract) of *Vanda roxburghii* root[8] against most of the tested bacteria. Antifungal activities of extracts of both the plants *Cassia fistula* and *Mesua ferrea* were not significantly enough against most of the tested fungal strains.

It may, therefore, be concluded from the above investigation that the crude extracts obtained from various portions of *Cassia fistula* and *Mesua ferrea* may be used enough as drug to treat the disease caused by those bacteria, which are sensitive to the above mentioned samples. But before use in human being isolation of pure compound, toxicological study and clinical trial in animal model should be carried out thereafter. However, further and specific studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

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REFERENCES

1. Blatter E. and W.S. Millard, 1954. Some beautiful indian trees. The bombay natural history society, India , 2nd Ed., pp: 19-25.
2. Kirtikar, K.R. and B.D. Basu, 1995. Indian medicinal plants, International book distributors, Dehradun, India, 1: 274-277.
3. Perumal S.R., S. Ignacimuthu and A. Sen, 1998. Screening of 34 Indian medicinal plants for antibacterial properties. J. Ethnopharmacol, 62: 173-182.

4. Barry, A.L., 1980. Procedures for testing antimicrobial agents in agar media. In: antibiotic in laboratory medicine. (V. Lorian Ed.). Williams and Wilkins Co. Baltimore, USA, pp: 1-23.
5. Bauer, A.W., W.W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol., 45: 493-496.
6. Rios, J.J., M.C. Reico and A. Villar, 1988. Antimicrobial screening of natural products. J. Enthopharmacol., 23: 127-149.
7. Uddin K., A. Sayeed, A. Islam, A.A. Rahman, S. Khatun, G.R.M. Astaq Mohal Khan and M.G. Sadik, 2003. Biological activities of extracts and two flavonoids from *Oroxylum indicum* Vent. (Bignoniaceae). OnLine J. Biol. Sci., 3: 371-375
8. Ahmed F., A. Sayeed, A. Islam, S.M. Abdus Salam, G. Sadik, M.A. Sattar and G.R.M. Astaq Mohal Khan, 2002. Antimicrobial activity of extracts and a glycoside from *Vanda roxburghii* Br. Pak. J. Biol. Sci., 5: 189-191