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Preliminary Antimicrobial Activity and Cytotoxicity of Leaf Extracts of *Mesua nagassarium* (Burm.f.)

[Actividad antimicrobiana preliminar y citotoxicidad de extractos de hojas de *Mesua nagassarium* (Burm.f.)]

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Abstract

In the present study the *in vitro* antimicrobial activity, along with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), of different extracts of leaves of *Mesua nagassarium* were evaluated against 13 pathogenic microorganisms. The methanol extract and its pet-ether and carbon tetrachloride soluble fractions showed the highest antimicrobial activity. The carbon tetrachloride soluble fraction showed the maximum inhibition zone of 24.33 mm against *Bacillus megaterium* with MIC and MBC values of 7.81 µg/ml and 250 µg/ml, respectively. Ciprofloxacin (30 µg/disc) was used as standard antimicrobial agent. In the Brine shrimp lethality bioassay, the crude methanol extract and its carbon tetrachloride soluble fraction showed significant cytotoxicity with LC_{50} of 2.99 and 1.74 µg/ml, respectively as compared vincristine sulphate (LC_{50} value 0.543 µg/ml).

Keywords: *Mesua nagassarium*, extracts, antibacterial activity, cytotoxocity..

Resumen

En el presente estudio se evaluó la actividad antimicrobiana *in vitro*, incluyendo la concentración inhibitoria mínima (CIM) y la concentración bactericida mínima (CBM), de diferentes extractos obtenidos de hojas de *Mesua nagassarium* en 13 microorganismos patógenos. El extracto metanólico y sus fracciones solubles en éter de petróleo y tetracloruro de carbono, mostraron la mayor actividad antimicrobiana. La fracción de compuestos solubles en tetracloruro de carbono mostró la zona de inhibición máxima de 24.33 mm en *Bacillus megaterium* con valores de CIM y and CBM de 7.81 µg/ml y 250 µg/ml, respectivamente. Como agente antimicrobiano estándar se utilizó ciprofloxacina (30 µg/disco). En el bioensayo de mortalidad de Brine shrimp el extracto metanólico y su fracción soluble en tetracloruro de carbono mostraron importante citotoxicidad con CL_{50} de 2.99 y 1.74 µg/ml, respectivamente, comparadas con el sulfato de vincristina (CL_{50} 0.543 µg/ml).

Palabras Clave: *Mesua nagassarium*, extractos, actividad antibacteriana, citotoxicidad.

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INTRODUCTION

Mesua nagassarium (Bengali - nagesar, nageswar) is a medium-sized or fairly large evergreen tree up to 36 m tall. A mixture of pounded kernels and seed oil isolated from this plant is used for poultice as wounds. The seed-oil is used for treating itch and other skin eruptions, dandruff and rheumatism (Orwa et al., 2009). The flowers are known to be useful for the treatment of severe colds, bleeding haemorrhoids, dysentery with mucus, excessive thirst, excessive perspiration, cough and digestion, rheumatism and iron induced lipid peroxidation (Yadav *et al* ., 2010; Konwarh *et al.*, 2010). Phenolic extract of seed oil of *M. nagassarium* revealed potent antiasthmatic effect (Bhide, 1977). As a part of our ongoing program to investigate the unexplored bioactivity of traditionally used medicinal plant of Bangladesh we studied the antimicrobial and cytotoxicity of leaves of *M. nagassarium* were evaluated.

MATERIALS AND METHODS

Plant Material

The leaves of *Mesua nagassarium* were collected from Dhaka in March 2010. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no. DACB- 35158). The powdered plant sample (600 gm) was soaked in 2.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator at low temperature (40-45 ºC) and reduced pressure. The concentrated methanolic extract (MES) was partitioned by modified Kupchan method (Van Wagenen et al., 1993) and subsequent evaporation of solvents yielded pet-ether (PESF), carbon tetrachloride (CTCSF), dichloromethane (DCMSF) and aqueous (AQSF) soluble fractions which were used for the experimental processes.

Antimicrobial screening

The disc diffusion method (Bauer et al., 1966, Rahman and Rashid, 2008) was used to test antimicrobial activity of the extractives against thirteen bacteria (Table-1), collected as pure cultures from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh. Solutions of known concentration (400 µg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette and the residual solvents were completely evaporated. Discs containing the test materials (400 µg/disc according to disc diffusion method (Bauer et al., 1966, Rahman and Rashid, 2008) were placed on to nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of Ciprofloxacin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4º C) for 24 hours to allow maximum diffusion of the test materials and Ciprofloxacin. The plates were finally incubated at 37º C for 24 hours to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiment was carried out in triplicate and the mean value was taken.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

Minimum inhibitory concentrations are important to monitor the activity of new antimicrobial agents (Jennifer, 2001) and is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism (Turnidge et al., 2003). The MIC was determined for MES, PESF and CTCSF extractives by serial tube dilution technique (Jennifer, 2001). Thirteen test tubes were taken, ten of which were marked as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and the rest three were assigned as T_M (only medium), T_{MC} (Medium +extractive solution) and T_{MI} (medium + inoculum). In all the test tubes containing the calculated amount of broth media, the sample from the mother solution was added with serial dilution which gave different sample solution having concentration ranged from 1000 µg/ml to 0 µg/ml. Lastly the inoculum was added to the test tubes, shaken using rotamixer and incubated at 37 ºC for 24 hours. The control test tube $T_{M,}$ containing the medium only was used to ascertain the sterility of the medium. After 24 hours the test tubes were checked for the microbial growth and the clear test tubes compared to McFarland turbidity standards were marked for the MIC determination. Serial dilution of the liquid medium and the sample is inoculated with inoculum whose turbidity was compared with 0.5 Mcfarland standards. The lowest concentration (highest dilution)

of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). At this dilution the antibiotic is bacteriostatic. The minimum bactericidal concentration (MBC) was determined by sub-culturing the broth media into fresh agar media from each tube in which no growth was visible. The growth of one colony indicated a 99.8% fall in viable count (Brumitt et al., 1984).

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay (Meyer et al., 1982, McLaughlin et al., 1998) technique was applied for the determination of general toxic property of the plant extractives. DMSO solutions of the samples were applied against *Artemia salina* in a 1-day *in vivo* assay. For the experiment, 4 mg of each of the methanol extract as well as pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions of methanol extract were dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ml) were obtained by serial dilution technique using DMSO. Vincristine sulphate (10 µg/ml, 5 µg/ml, 2.5µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml, 0.0390 µg/ml) was used as positive control.

RESULTS AND DISCUSSION

The crude methanolic extract of leaves of *M. nagassarium* (MES) and its pet-ether (PESF), carbon tetrachloride (CTCSF), dichloromethane (DCMSF) and aqueous (AQSF) soluble partitionates were subjected to antimicrobial screening at 400 µg/disc. Among the extractives, MES, PESF and CTCSF exhibited very strong antimicrobial activity (Table 1).

The carbon tetrachloride soluble fraction (CTCSF) revealed the highest inhibition against microbial growth having zone of inhibition ranged from 19.33 mm to 24.33 mm. The maximum zone of inhibition produced by CTCSF was found to be 24.33 mm against *B. megaterium.* This partitionate also showed significant antimicrobial growth having zone of inhibition 22.67 mm against *S. paratyphi* (Table 1). The methanol extract also demonstrated significant inhibition of microbial growth having zone of inhibition ranging from 19.3 mm to 23.0 mm. This extract exerted highest inhibitory activity against *B. megaterium* (having zone of inhibition of 23.0 mm). Relatively less polar compounds revealed better antimicrobial activity as the pet-ether and carbon tetrachloride soluble partitionates showed significant antimicrobial activity than the aqueous and chloroform soluble partitionates of methanol extract.

As MES, PESF, CTCSF showed better inhibitory activity against the microorganisms, the minimum inhibitory concentration (MIC) required to inhibit the growth of organisms were measured in this study. The MIC value of methanol extract (where no bacterial growth was observed in broth media) was found to be 7.81 µg/ml and the MBC value (where no bacterial growth was observed in agar media) was found to be 250 µg/ml against *B. megaterium* (Table 2). No microbial growth was observed in the test tubes T_M (containing medium only) and T_{MC} (medium + test sample, no inoculum) indicating that the medium and the test sample were not contaminated by microorganism and the total investigation was performed properly in the sterile condition. Microbial growth was observed in the test tube T_{MI} (medium + inoculums) revealing the fact that there was no problem with the subcultured microorganisms.

In case of brine shrimp lethality bioassay, the lethality of the methanol extract (MES) and its pet-ether (PESF), carbon tetrachloride (CTCSF), dichloromethane (DCMSF) and aqueous (AQSF) soluble fractions were evaluated against *A. salina*. (Table 3) shows the results of the brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, vincristine sulphate (VS). The carbon tetrachloride soluble fraction (CTCSF) showed potent cytotoxic activity having LC_{50} of 1.74 µg/ml as compared to 0.453 µg/ml for vincristine sulphate.

Table 1: *In vitro* antibacterial activity of the extracts from *M. nagassarium* leaves

Test microorganisms	Diameter of zone of inhibition (mm)					
	MES	**PESF**	**CTCSF**	**DCMSF**	**AQSE**	**Ciprofloxacin**
Gram positive bacteria						
Bacillus cereus	21.3±0.57	21.3±2.08	22.33±2.08	10.7±1.15	10.3±2.08	40.33±3.78
B. megaterium	23.0±1.0	20.0±1.73	24.33±1.53	12.0±1.73	8.67±0.58	44.66±1.52
B. subtilis	20.3±1.52	21.0±1.73	22.67±1.15	12.3±2.08	10.3±0.58	40.66±2.08
Staphylococcus aureus	20.3±1.52	20.7±1.15	22.0±1.0	10.3±0.57	9.67±0.58	43.0±1.73
Sarcina lutea	21.0±1.0	21.3±3.51	21.67±2.08	11.0±1.0	11.3±1.53	41.0±2.0
Gram negative bacteria						
Escherichia coli	20.0±1.73	20.3±0.58	21.33±1.15	10.0±1.0	9.67±0.57	44.67±1.53
Pseudomonus aeruginosa	21.3±1.52	20.0±0	20.0±2.0	10.3±0.57	11.0±1.73	44.0±2.65
Salmonella paratyphi	21.7±2.08	22.3±2.08	22.67±2.52	9.67±0.57	10.7±2.08	44.33±2.08
S. typhi	19.3±2.08	19.3±1.15	19.33±3.06	9.67±0.57	12.3±2.08	43.66±1.52
Shigella boydii	22.7±2.51	20.7±3.07	22.0±2.65	9.67±0.57	11.7±1.53	44.33±2.08
S. dysenteriae	20.7±1.15	20.7±1.53	19.67±2.08	10.0±2.0	10.7±3.06	41.33±1.52
Vibrio mimicus	21.3±1.15	22.3±2.31	23.0±1.0	11.0±1.73	11.7±2.08	43.33±2.88
V. parahemolyticus	21.7±2.08	22.7±2.52	22.67±2.31	11.0±1.0	12.0±2.0	44.0±1.73

Values are expressed as mean ± S.D. (n=3)
MES= Methanol extract, PESF= Pet-ether soluble fraction, CTCSF= Carbon tetrachloride soluble fraction, DCMSF= dichloromethane soluble fraction, AQSF= aqueous soluble fraction.

Table 2: Determination of MIC and MBC (µg/ml) of the extracts from *M. nagassarium* leaves

Test microorganisms	Determination of MIC and MBC (µg/ml)							
	MES		PESF		CTCSF		Ciprofloxacin	
	MIC	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
Gram positive bacteria								
Bacillus megaterium	7.81	250	15.62	250	7.81	250	1.25	2.5
Bacillus subtilis	31.25	500	15.62	250	62.5	500	0.62	1.25
Staphylococcus aureus	31.25	500	15.62	500	7.81	250	0.31	0.62
Sarcina lutea	15.62	500	15.62	500	7.81	250	1.25	5.0
Gram negative bacteria								
Escherichia coli	62.5	1000	31.25	500	31.25	250	1.25	2.5
Pseudomonas aeruginosa	31.25	500	62.5	500	15.62	500	0.62	2.5
Salmonella typhi	62.5	1000	31.25	500	31.25	500	0.31	1.25
Shigella dysenteriae	31.25	500	31.25	250	62.5	500	0.31	2.5
Vibrio parahemolyticus	31.25	1000	15.62	500	15.62	500	0.62	2.5

MIC = Minimum inhibitory concentration, MBC= Minimum bactericidal concentration.

Table 3: Cytotoxic activity of different partitionates of *M. nagassarium.*

Sample	Cytotoxic activity (LC_{50} mg/ml)
VS	0.453 ± 0.002
MES	2.99 ± 0.188
PESF	4.83 ± 0.025
CTCSF	1.74 ± 0.11
DCMSF	12.64 ± 1.03
AQSF	7.86 ± 0.09

The average values of three calculations are presented as mean ± S.D. (standard deviation); VS = Vincristine sulphate; MES = Methanolic extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; DCMSF= dichloromethane soluble fraction; AQSF = Aqueous soluble fraction of the methanolic extract of *M. nagassarium*

CONCLUSIONS

The results of *in vitro* microbial screening of *M. nagassarium* indicated that the methanol extract and its pet-ether (PESF) and carbon tetrachloride (CTCSF) soluble partitionates have strong antimicrobial activity and the isolation of active compounds is underway.

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