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ORIGINAL ARTICLE

Anti-Bacterial and Cytotoxicity Properties of the Leaves Extract of Nahar (*Mesua ferrea*) Plant

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Ahmed Idris Adewale, Mohamed Elwathig Saeed Mirghani, Suleyman Aremu Muyibi, Jamal Ibrahim Daoud, Mikail Maryam Abimbola: Anti-Bacterial and Cytotoxicity Properties of the Leaves Extract of Nahar (*Mesua ferrea*) Plant

ABSTRACT

The increasing bacterial resistance to antibiotics and the growing interest in human health have resulted into an increasing need for the exploration of both the essential oils and other plant extracts in the food and pharmaceutical industries. This work, as part of on-going work on the leaves of Nahar (*Mesua ferrea*) plant, was aimed at evaluating the antibacterial activity, minimum inhibitory concentration as well as the cytotoxicity of the leaves extract. The dry leaves were grinded and extracted in an oven shaker set at 37°C and 200rpm for 24 hours using ethanol and methanol as solvents. The agar disc diffusion method was used for the evaluation of antibacterial property of the leaves extract, micro broth dilution was employed for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), while Brine shrimp (*Artemia salina*) lethality bioassay was made use of for the cytotoxicity assay. Ethanol gave higher extract's yield (6.20%) than methanol. The extract showed a remarkable antibacterial property against all the selected microbes (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus*) with the inhibition zones ranging from 16.0±0.5 mm to 18.0±0.5 mm for all the tested bacteria. The MIC range of 2.5-0.625 mg/mL with MBC value of 5 mg/mL was obtained for the gram-negative bacteria while MIC range of 1.3- 0.313 mg/mL with MBC value of 2.5 mg/mL was obtained for the gram-positive bacteria. The leaves extract was found to be toxic to the Brine shrimps with LC_{50} of 500ppm (µg/mL) suggesting that the extracts may contain bioactive compounds of potential therapeutic and prophylactic significance.

Key words: Nahar *(Mesua ferrea)* leaves extract, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Cytotoxicity Assay.

Introduction

The exploration of both the essential oils and other plant extracts in the food and pharmaceutical industries have gained more attention, and thus is as a result of the waxing interest in human health, concern over pathogenic and spoilage microorganisms in foods, increase in outbreaks of food borne disease and increasing bacterial resistance to antibiotics among others (Adebolu and Salau, 2005; Haddad and Dezashibi, 2007; Sharif *et al*., 2010). Nahar *(Mesua ferrea)* is a species in the family Guttiferae (Clusiaceae). The plant is named after the heaviness of its timber and cultivated in tropical climates for its form, foliage, and fragrant flowers. It is native to tropical Sri Lanka but also cultivated in Assam, southern Nepal, Indochina, and the Malay Peninsula (Dilip and Rupanjali, 2006 & Dutta *et al*., 2007). Brine shrimp lethality bioassay is a recent development in the assay procedure of bioactive compounds which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal, AIDS, etc.) of the compounds (Majid *et al*., 2004). The outstanding results obtained by the group from the antimicrobial assay of the oil extract of Nahar seed kernel and the availability of the leaves through out the year made us to explore the potential of Nahar leaves (NL).

This work, as part of on-going work on the leaves of Nahar (*Mesua ferrea*) plant, was aimed at evaluating the antibacterial activity, minimum inhibitory concentration as well as the cytotoxicity of the leaves extract.

Materials and Methods

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Materials

The NL samples used in this study were obtained from Mahallah Nusaibah area of the International Islamic University Malaysia (IIUM), Gombak campus. The plant material was identified and authenticated by plant taxonomists; Dr. Richard Chung and Mr. Kamarudin Saleh of Forest Research Institute Malaysia (FRIM) and voucher specimen (PID 010111-01) was deposited in the herbarium. All chemical reagents used were of analytical grade from Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MO, USA).

Leaves pretreatment and sample preparation:

Fresh samples of NL were collected, washed and subjected to drying at 45°C for 2 days using a laboratory oven (Memmert, Germany). The dried leaves were then grinded using a laboratory blender (Waring Products Division, Torrignton C.T., USA). The samples were stored at 4°C inside a laboratory chiller.

Extraction of Nahar leaves:

The dry ground leaves powder was extracted in an oven shaker using ethanol and methanol as solvents. For each extraction set-up, the NL powder (20.0±0.01g) was put in conical shake flask, then 100 mL of solvent was added, the flask was then put in an oven shaker (INFORS, AG-CH-4103 BOTTMINGEN) set at 37°C and 200rpm for 24 hours. The resulting sample were then poured into a tube and centrifuged at 4°C, 4000rpm for 10 minutes. The solvents were then dried off in a rotavapor (Buchi, Switzerland), using a vacuum controller (V-850), rotavapor (R-215), heating bath (B-491) at 40°C, distillation chiller (B-741) and vacuum pump (V-700). The extracts obtained were weighed and then dissolved in DMSO in the ratio 1:2 and stored at -20°C.

Disc-diffusion assay:

Dilutions of the bacterial cultures were done at (OD_{625} = 0.1) to obtain a bacterial suspension of 10^8 CFU/mL. Petri plates containing 20 mL of Luria-Bertani nutrient agar were inoculated with 100 µL of bacterial culture. Agar was prepared following the manufacturer's instructions. The agar solution was mixed, sterilized at 121°C for 15 minutes. After, sterilization, the agar solution was cooled to about 40-50°C before it was poured into 100 x 15 mm Petri dishes. The plates were allowed to dry at room temperature. Discs (6 mm in diameter) were then impregnated with about 10 µL of the various NL extracts and the controls (Ahmed *et al*., 2011). The tested organisms were two gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa)* and two gram-positive *(Bacillus subtilis* and *Staphylococcus aureus*) bacteria. They were obtained from the Microbiology Laboratory, IIUM. Similar assay was carried out using DMSO and the two solvents (negative controls) and chloramphenicol, gentamycin, streptomycin, tetracycline and vancomycin (positive controls). The antibacterial activity was quantified by determining the zone of inhibition, in millimeters, around the paper discs. Tests were performed twice and average diameter of the zones was determined and reported.

Minimum Inhibitory Concentration (MIC) and Minimum bactericidal Concentration (MBC):

The broth dilution method was employed for the determination of the MIC and MBC using the procedures reported by Denis and Kumar (1998), with little modifications, were used for the broth dilution method and determination of the minimum inhibitory concentration of the oil. In the procedure, thirteen screw-capped test tubes (13 mm x 100 mm) were sterilized and numbered individually. One mL of Luria-Bertani broth was introduced into tubes 2 to 11. To tube 12, 2.0 mL of Luria-Bertani broth, was introduced; 1 mL of the oil was pipetted into tube 1 and 2 and capped, it was vortexed for 5 seconds; 1.0 mL was withdrawn from the contents of tube 2 and transferred to tube 3, after capping the tube and mixing by shaking the contents, 1.0 mL from the contents of tube 3 was withdrawn and transferred to tube 4, the tube was capped, shaken and mixed well. This process was continued until 1.0 mL was withdrawn from tube 10 and subsequently added to tube 11, capped and shaken. One mL of the diluted inoculum was introduced into tubes 1 to 11 and to tube 13. To tube 13, 1.0 mL of the antibiotic standard was added. The tubes were incubated at 37°C for 18 to 24 hours. After incubation the tubes were examined for bacterial growth. These were seen as either clear solutions, less turbid solutions or solutions containing whitish pellets at the bottom of the tubes. Petri plates containing 20 mL of Luria-Bertani nutrient agar were then inoculated with 100 µL from the various tubes. The tube with the lowest concentration of the extract at which no growth and which gave clear solution with no turbidity was reported as the MBC against the organisms, while those with reduced bacterial growth and less turbidity were reported as MIC.

Cytotoxicity bioassay:

The brine shrimp lethality bioassay was carried out on the methanol extracts using standard procedure described by Ayo *et al.* (2007), with little modification. Briefly, brine shrimp (*Artemia salina* Leach) eggs were hatched in a hatching chamber filled with fresh artificial sea water. The chamber was kept under illumination using a fluorescent bulb for at least 48 h for the eggs to hatch into shrimp larvae. 30 mg of the extract was dissolved in 3 mL of DMSO, and from this stock solution, 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.90 and 1.95 µg/mL were prepared by serial dilution. Each concentration was tested in triplicate, giving a total of 30 test-tubes for each sample. A control containing 5 mL of DMSO solvent was also used. The final volume of the solution in each test-tube was made up to 5 mL with sea water immediately after adding shrimp larvae. The test-tubes were maintained under illumination. Survivors were counted after 24 hours and the average percentage death at each dose was determined. The LC_{50} value also evaluated.

Results:

NL crude extract yields:

The average yields of the crude extract obtained from the oven shaker extraction after the solvents were dried off are shown in Table 1.

Table 1: Yields of NL crude extract

Solvents	Yields of crude extract (g)
Ethanol	6.71±0.25
Methanol	6.09±0.22

The results showed that ethanol gave higher crude extract's yield (about 10.2%) than methanol. This confirmed the earlier report of Wang and Helliwell (2001) that ethanol is superior to methanol and acetone for extracting biologically-active components (e.g., flavonoids) from tea. Besides, ethanol is considered as safe (GRAS solvent).

Antibacterial activity:

The extract showed a remarkable antibacterial property against all the selected microbes (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus*) with the inhibition zones ranging from 16.0±0.5 mm to 18.0±0.5 mm for all the tested bacteria (Table 2).

The result obtained from the MIC and MBC determinations showed that the active extracts were found to be both bacteriostatic and bactericidal with the gram-positive bacteria showing less resistance.

Table 2: Inhibition zones diameter in **mm** (Chl. = Chloramphenicol, Gen. = Gentamycin, Str. = Streptomycin, Tet. = tetracycline and Van. = Vancomycin)

Bacteria	Ethanol extract	Methanol extract	Chl.	Tet.	Str.	Gen.	Van.	DMSO	Methanol	Ethanol
E. coli	17.5±0.5	18.0±0.5	23.0±0.5	20.0±0.5	24.0±0.5	19.0±0.5	0	0	0	0
P. aeruginosa	17.0±0.5	17.5±0.5	23.0±0.5	23.0±0.5	20.0±0.5	21.0±0.5	19.0±0.5	0	0	0
S. aureus	17.0±0.5	16.0±0.5	25.0±0.5	26.0±0.5	20.0±0.5	23.0±0.5	20.0±0.5	0	0	0
B. subtilis	18.0±0.5	18.0±0.5	24.0	26.0	21.0	24.0	20.0	0	0	0

The MIC range of 2.5-0.625 mg/mL with MBC value of 5 mg/mL was obtained for the gram-negative bacteria while MIC range of 1.3-0.313 mg/mL with MBC value of 2.5 mg/mL was obtained for the gram-positive bacteria, it could be deduced from this that the Gram-positive bacteria appeared to be more sensitive, more susceptible and less resistant, while the Gram negative bacteria are less sensitive, less susceptible and more resistant. This also justifies the fact that Gram negative bacteria have an outer membrane consisting of lipoprotein and lypopolysaccharide, which is selectively permeable and thus regulates access to the underlying structures (Chopra and Greenwood, 2001).

Brine-Shrimp Lethality Potential Bioassay:

The results of Brine shrimp lethality bioassay of methanol extract of NL were summarized in Table 3. The extract was found to be moderately cytotoxic to the Brine shrimps at high concentration with LC_{50} of 500ppm (µg/mL). In toxicity evaluation of plant extracts by brine shrimp lethality bioassay, Ayo *et al.* (2007) reported

that LC_{50} values lower than 1000 µg/mL is considered bioactive. Therefore, the methanol extracts of *M. ferrea* leaves may have some significant biological activity.

Table 3: Brine shrimp lethality bioassay of methanol extract of NL

Concentration (µg/mL)	1000	500	250	125	62.50	31.25	15.63	7.81	3.91	1.95
No. of shrimps per test sample	20	20	20	20	20	20	20	20	20	20
No. of survivors	8	10	12	12	14	16	16	18	20	20
No. of death	12	10	8	8	6	4	4	2	0	0
Percentage mortality (%)	60	50	40	40	30	20	20	10	0	0

LC_{50} = 500ppm (µg/mL)

The results of this present work may be of importance in the elucidation of the potential and medicinal uses of the extracts. The characterization and identification of the bioactive compounds in the extract, however, are challenges of future works.

Conclusion:

The antibacterial and cytotoxicity activity of Nahar leaves extracts, found in this study, may explain some of the traditional medicinal uses of the plants. These could also be of particular interest in relation to find out its untapped efficacy and can also be a potential of chemically interesting and biologically important drug candidates.

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