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ANTIMICROBIAL AND CYTOTOXIC ACTIVITY ASSESSMENT OF THE AQUEOUS METHANOLIC AND PET-ETHER EXTRACT OF THE LEAVES OF *MESUA FERREA*

H.M. Arif Ullah*[1], Syed Mohammed Tareq [2], Irfanul Huq [1], Muhammad Borhan Uddin [3] and Md. Salauddin [3]

Department of Pharmacy, North South University [1], Dhaka, Bangladesh
Department of Pharmacy, Faculty of Science and Engineering, Southern University [2], Bangladesh
Department of Pharmacy, International Islamic University [3], Chittagong, Bangladesh

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Correspondence to Author:

H. M. Arif Ullah

Lecturer, Department of Pharmacy, North South University, Dhaka, Bangladesh

E-mail: arifpha@ymail.com

ABSTRACT: The purpose of the study is to evaluate the antimicrobial and cytotoxic activity of methanolic and pet-ether extract of leaves of *Mesua ferrea*. The antimicrobial activity was investigated against eight pathogenic Gram-positive and Gram-negative bacterial species at three concentrations (100, 300, 500 µg/disc) by using disc diffusion method. Both extracts showed moderate antibacterial activity against six bacterial species among eight and the activity was increased with increasing concentration of the sample. The maximum antimicrobial potential was obtained against *Bacillus subtilis, Bacillus megaterium and Staphylococcus aureus* in case of both extracts. The cytotoxic effect of the extracts was assayed on *Artemica salina* leaches. The LC_{50} values were found to be 7.94 µg/mL for methanolic extract and 3.98 µg/mL for pet-ether extract compared to 0.451 µg/mL for standard drug vincristine sulphate. The study confirms that the plant extracts have moderate antimicrobial activity and considerable amount of cytotoxic activity.

INTRODUCTION: A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs [1]. This definition of Medicinal Plant has been formulated by WHO (World Health Organization). The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as Medicinal Plants.

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Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties. When a plant is designated as Medicinal, it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation.

Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine.

Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way indigenous medicinal plants play significant role of an economy of a country. South Asian countries have a large number of valuable medicinal plants naturally growing mostly in fragile ecosystems that are predominantly inhabited by rural poor and indigenous community.

In Bangladesh, 5,000 species of angiosperm are reported to occur [2]. The number of medicinal plants included in the Materia Medica of traditional medicine in this subcontinent at present stands at about 2,000. More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh [3]; Dhaka, Rajshahi, Sylhet and Chittagong division are rich in medicinal plants [3].

Furthermore, The development drug resistance in human pathogens against commonly used antibiotics has necessitate a search for new antimicrobial substance from other sources including plant screening of medicinal plants for antimicrobial activities is important for finding potential new compounds for therapeutic uses. Recent advances in cancer chemotherapy, Phytochemical play an important role in cancer chemotherapeutic drugs. A search for new anti-cancer drugs has taken many different approaches. The brine shrimp lethality bioassay is efficient, rapid and inexpensive tests that require only relatively small amount samples [4].

Present study focuses on *in vitro* evaluation of antimicrobial activity and cytotoxic potentiality of the methanol and pet-ether fraction from the leaf of *Mesua ferrea*.

In the present study the following attempts has been made to investigate -

1. The antimicrobial activity of ethyl acetic extract of *Mesua ferrea* against various pathogenic microorganisms (bacteria) by paper disc diffusion method [5] which is based on the ability of antibiotics to diffuse from a confined source through the nutrient gel and create a concentration gradient. The antimicrobial activity of the extracts was determined by measuring the diameter of zone of inhibition expressed in millimeters (mm) [6].
2. The brine shrimp lethality bioassay has a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity [4, 7]. Therefore, the cytotoxic activity screening of extract was carried out with a view to assess the presence of antitumor activity of that extracts. The aim of this method is to provide a front-line once the active compounds have been isolated.

   Since the introduction of this assay method in 1982 [8], this *in vivo* lethality test has been successively employed for bioassay-guide fractionation of active cytotoxic and antitumor agents such as trilobacin from the bark of *Asimina triloba* [9], cis-annonacin from *Annona muricata* [10] and ent-kaur-16-en-19-oic acid from *Elaeoselinum foetidum* [11].

MATERIALS AND METHODS:

Plant material and preparation of Extracts: *Mesua ferrea* leaves were collected from Chittagong Forest research Institute, Bangladesh in the month of April. The plant was identified by the Department of Botany, Chittagong University, Bangladesh. The leaves were dried at room temperature and grounded the powder and passed through 40# sieve. The powder (400 gm) was extracted successively in Soxhlet apparatus using petroleum ether and methanol. The sediments were filtered and filtrates were dried at 40°C in oven to get dried petroleum ether extract and methanol extract of leaves.

RESULTS:

Antimicrobial activity assay: Eight bacterial species (four Gram positive and four Gram negative) were taken for the test and were collected from Department of Microbiology, University of Chittagong.

These were sub cultured and maintained in the nutrient agar medium (Merck, Germany). Paper discs of 6 mm diameter were prepared from sterile Whatman no. 1 filter paper. Varying concentrations of extracts in methanol were applied to these discs and subsequently dried. A blank disc was prepared by applying only methanol to the disc and then drying it. For positive control, standard kanamycin discs (30 µg/disc) were used.

The sample, standard and the blank disc were placed on the agar plates pre inoculated with the test microorganisms. These plates are kept at 4ºC for 24 hours to allow sufficient diffusion of materials to the surrounding media. Then the plates were inverted and kept in incubator for 24 hours maintaining the temperature at 37ºC.

Antimicrobial activity of the samples was assessed by measuring the zone within which the sample diffused and prevented any growth of bacteria thereby maintaining a clear circular zone. The zone of inhibition given by the sample and standard were measured with the help of scale in millimeter unit.

TABLE 1: THE ZONES OF INHIBITION AGAINST SELECTIVE BACTERIA FOR THE METHANOL SOLUBLE LEAF EXTRACT OF *MESUA FERREA*

Name of the Bacteria	Zone of inhibition			
	Kanamycin disc (30µg/disc) (mm)	*Mesua ferrea* (Methanol extract)		
		100 µg/disc (mm)	300 µg/disc (mm)	500 µg/disc (mm)
Bacillus subtilis	29	-	8	13
Bacillus Megaterium	27	-	9	12
Salmonella typhi	30	-	-	10
Shigella dysenteriae	28	-	-	11
Pseudomonas aeruginosa	27	-	-	11
Satphylococcus aureus	30	-	9	14
Bacillus cereas	31	-	-	-
Escherichia coli	29	-	-	-

Zones of inhibition equal to or below 7mm were considered insignificant and were not shown in the table.

TABLE 2: THE ZONES OF INHIBITION AGAINST SELECTIVE BACTERIA FOR THE PET-ETHER SOLUBLE LEAF EXTRACT OF *MESUA FERREA*

Name of the bacteria	Zone of inhibition			
	Kanamycin disc (30µg/disc) (mm)	*Mesua ferrea* (pet-ether extract)		
		100 µg/disc (mm)	300 µg/disc (mm)	500 µg/disc (mm)
Bacillus subtilis	30	7	10	13
Bacillus Megaterium	29	-	9	14
Salmonella typhi	27	-	8	10
Shigella dysenteriae	29	-	-	9
Pseudomonas aeruginosa	28	-	-	10
Satphylococcus aureus	30	-	10	14
Bacillus cereas	33	-	-	8
Escherichia coli	30	-	-	-

Zones of inhibition equal to or below 7mm were considered insignificant and were not shown in the table.

Brine Shrimp Cytotoxicity Assay: Cytotoxicity testing was done according to [8]. Artificial sea water was prepared by dissolving measured amount of sea salt (Pure NaCl) in distilled water and then the pH was adjusted to 8.5 using 1N Sodium hydroxide (NaOH). This artificial sea water was used to hatch the eggs. For the test, 4 mg of each of the extracts were dissolved in DMSO and solutions of varying concentrations (500, 300, 100, 50, 25, 12.5, 6.25 & 3.125 µg/mL) were obtained with serial dilution technique using sea water. All of these solutions were added to pre-marked test tubes each containing 10 brine shrimp nauplii in sea water. After addition of extract, each test tube contained 5 mL of total content.

A positive control group was prepared in the same way with 7 different concentrations (20, 10, 5, 2.5, 1.25, 0.625 & 0.3125 µg/mL) of standard vincristine sulfate dissolved in DMSO. These solutions were added to pre marked test tubes each containing 10 brine shrimp nauplii in simulated sea water. For negative control, 100 µL DMSO was added to each of three pre marked test tubes containing 5 mL of simulated sea water and 10 shrimp nauplii. After 24 hours the test tubes were inspected using a magnifying glass and the number of survivors was counted. The percent (%) mortality was calculated for each dilution, the concentration mortality data were analyzed by using Microsoft Excel.

The effectiveness or the concentration–mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. The results of brine shrimp lethality bioassay are shown in **Table 3**. Test samples showed different mortality rate at different concentration.

The mortality rate of brine shrimp napulii was found to be increased with the increase with the concentration of the sample. The median lethal concentration (LC_{50}) was calculated. The LC_{50} value is 7.94 µg/mL for methanolic extract of leaves and 3.98 µg/mL for pet-ether extract.

TABLE 3: LC_{50} VALUES OF THE TEST SAMPLES OF *MESUA FERREA*

Test samples	Regression line	R^2	LC_{50} (µg/mL)
VS	y = 30.799x + 60.645	97.29	0.451
MELMF	y = 30.997x + 21.805	0.9821	7.94
PELMF	y = 26.7x + 34.91	0.9247	3.98

VS = Vincristine sulphate; MELMF = Methanolic extract of leaves of *Mesua ferrea*; PELMF = Pet-ether soluble fraction of the leaves of *Mesua ferrea*

The relations of log concentration of extracts and standard vincristine sulphate with the percent mortality of brine shrimp are presented in the **Table 4 and Figure 1, Figure 2 and Figure 3**.

TABLE 4: EFFECT OF METHANOLIC EXTRACT (MELMF), PET ETHER EXTRACT (PELMF) AND POSITIVE CONTROL VINCRISTINE SULPHATE ON BRINE SHRIMP NAUPLII

Test sample	Conc. µg/mL	Log of Conc.	% of mortality	LC_{50} µg/mL
Methanolic extract (MELMF)	3.13	0.496	35	7.94
	6.25	0.796	45	
	12.5	1.097	55	
	25	1.398	70	
	50	1.699	80	
	100	2.000	85	
	300	2.477	100	
	500	2.699	100	
Pet ether extract (PELMF)	3.13	0.496	40	3.98
	6.25	0.796	55	
	12.5	1.097	70	
	25	1.398	75	
	50	1.699	90	
	100	2.000	90	
	300	2.477	100	
	500	2.699	100	
Vincristine sulphate	0.3125	-0.505	40	0.451
	0.625	-0.204	50	
	1.25	0.097	70	
	2.5	0.398	80	
	5	0.699	80	
	10	1.000	90	
	20	1.301	100	

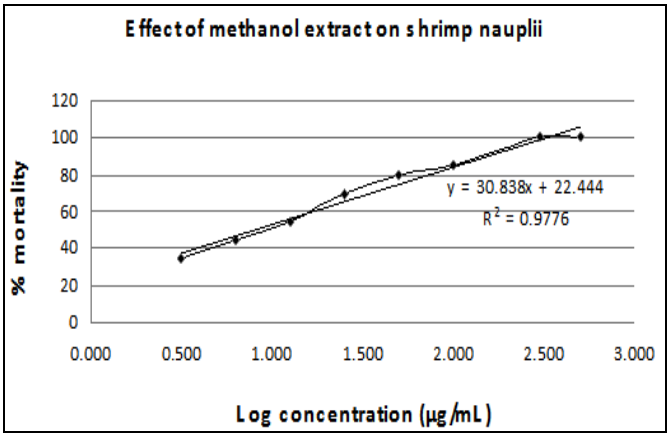

FIGURE 1: PLOT OF % MORTALITY AND PREDICTED REGRESSION LINE OF METHANOLIC EXTRACT

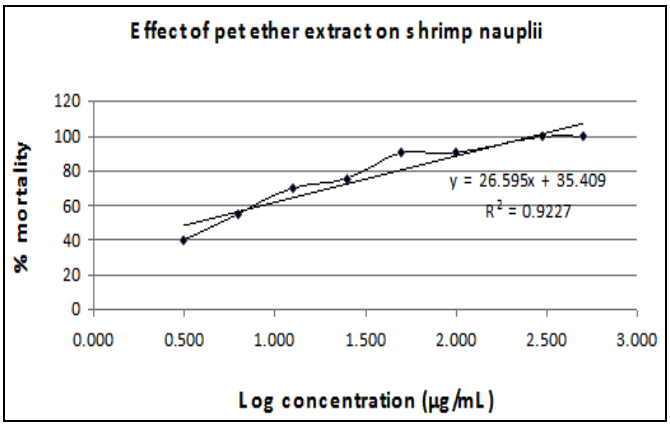

FIGURE 2: PLOT OF % MORTALITY AND PREDICTED REGRESSION LINE OF PET ETHER EXTRACT

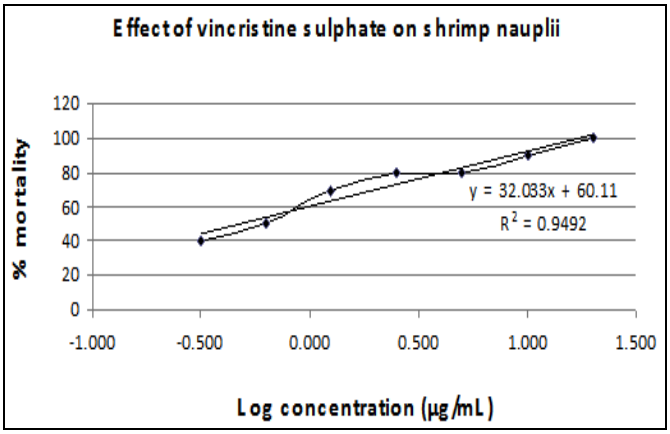

FIGURE 3: PLOT OF % MORTALITY AND PREDICTED REGRESSION LINE VINCRISTINE SULPHATE

DISCUSSION: The data presented here indicates that the plant extract has antimicrobial activity against some selective microorganisms; in this case *Bacillus subtilis, Bacillus megaterium and Staphylococcus aureus*. It is of particular interest that all of the bacteria against which activity was observed are of Gram positive in nature. Therefore, it can be assumed that the plant has specific inhibitory activity against some Gram positive bacteria.

Both aqueous methanolic and pet-ether extract of *Mesua ferrea* possess highly cytotoxic activity. It was observed that about 80% (8 died out of 10 shrimps) were died when the applied extract concentration is 50 µg/mL for both extracts. But when the applied concentration was 300 µg/mL all brine shrimps were dead (20 died out of 20 shrimps). LC_{50} value was 7.94 µg/mL for methanolic extract of leaves and 3.98 µg/mL for pet-ether extract of leaves of *Mesua ferrea*.

CONCLUSION: From the above studies, it can be concluded that the traditional plants may represent new sources of stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethno medical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethno botany and other biological actions for drug discovery.

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