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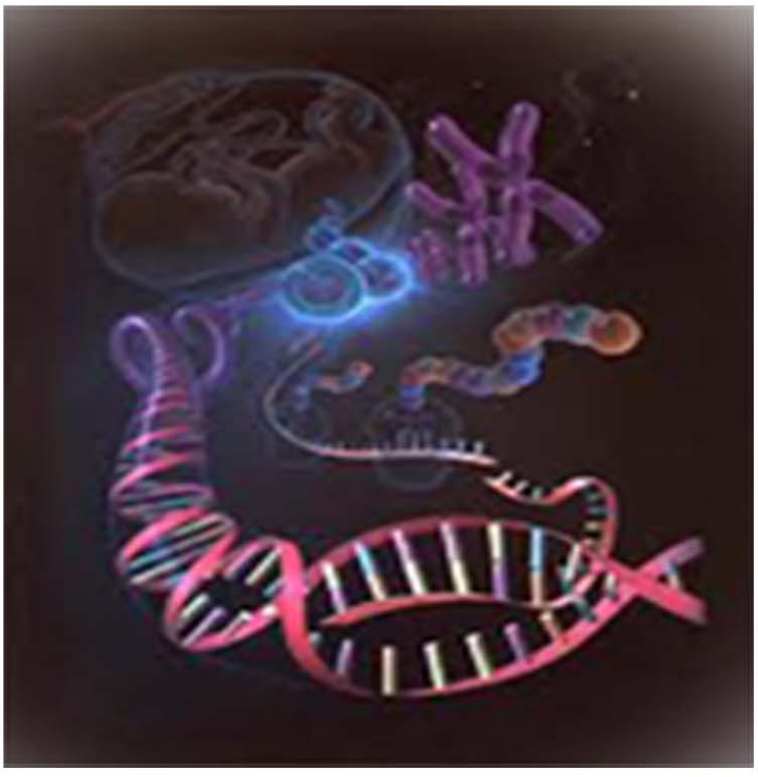

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Research Paper

ANTIBACTERIAL AND CYTOTOXIC ACTIVITIES OF THE LEAF EXTRACT OF *MURRAYA KOENIGII*

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The investigation was conducted with methanolic leaf extract of *Murraya koenigii* for its antibacterial and cytotoxic activities. Antibacterial activity of the extract was evaluated against various Gram-positive and Gram-negative bacteria using disk diffusion technique. For cytotoxic activity, brine shrimp lethality bioassay was performed to estimate LC50 values. In our preliminary screening, the n-hexane, ethyl acetate and dichloromethane soluble fractions of the crude methanolic extract of *Murraya koenigii* were subjected to antibacterial activity and brine shrimp lethality bioassay. The ethyl acetate and dichloromethane soluble partitionate of the methanol extract exhibited the mild to moderate antibacterial activity. The ethyl acetate extract showed antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Gram positive), and dichloromethane extract showed antibacterial activity against *Pasteurella multocida* and *Proteus vulgaris* (Gram negative). And the n-hexane soluble partitionate exhibited strong cytotoxicity having LC_{50} of 1.250 μg/mL.

Keywords: *Murraya koenigii*, Rutaceae, Antibacterial activity, Cytotoxic activity, Brine shrimp lethality bioassay

INTRODUCTION

Many plants are used as folk medicines to infectious diseases such as urinary tract infections, diarrhea, cutaneous abscesses, bronchitis and parasitic diseases (Ahmad *et al*., 1998). Due to the indiscriminate use of antibacterial drugs, the microorganisms have developed resistance to many commercial antibiotics. Therefore, investigation of the chemical compounds within medicinal plants has become desirable (Ahmad *et al*., 1998).

Murraya koenigii (Bengali name: Meetha neem, Family: Rutaceae) is an aromatic shrub or small tree found throughout India and mainly cultivated for its aromatic leaves. Leaves are used as a condiment in the preparation of curry powder, pickle, chutney, sausages and seasonings (Anonymous, 1962; and Hiremath *et al*., 1998).

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The flavor and fragrance of leaves were retaining even after drying (Omankutty Amma *et al*., 1984). Leaves relieve nausea, indigestion, vomiting; eaten as a cure for diarrhea and dysentery (Anon. 1962; Ghani, 2003). The leaves are highly valued as seasoning in southern and west-coast Indian cooking, and Sri Lankan cooking, especially in curries.

The leaves of *Murraya koenigii* are also used in Ayurvedic medicine. It is reported to possess anti-diabetic (Arulselvan *et al*., 2006), antioxidant (Arulselvan *et al*., 2007), anti-inflammatory (Ghani, 2003), hepatoprotective (Ghani, 2003) and hypolipidemic activities (Iyer and Uma, 2008). Recently Syam *et al*., 2011 reported that girinimbine, a carbazole alkaloid isolated from this plant, inhibited the growth and induced apoptosis in human hepatocellular carcinoma, HepG2 cells (Syam *et al*., 2011)

The purpose of the present study was to evaluate *Murraya koenigii* leaves as potential source of natural antibacterial agents. As a part of our continuing study on chemical and biological characterization of different plants, attempt was made this time to investigate the antibacterial activity of *Murraya koenigii* against different Gram-positive and Gram-negative bacteria (Mothana, 2005). The cytotoxic activity of the plant materials was performed by using brine shrimp lethality bioassay which was proposed by Michael (Michael *et al*., 1956) and modified by Solis (Solis *et al*., 1993)

MATERIALS AND METHODS

Plant Material

The leaves of the *Murraya koenigii* were collected from Gazipur, Bangladesh in February 2010. The specimens of the plant were submitted to the Herbarium of Botany Department, University of Dhaka and taxonomically identified and authenticated by the experts.

Extraction and Isolation

The leaves (1000 g) of *Murraya koenigii* was extracted with 2.5 L of methanol for 7 days and filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated with a rotary evaporator. An aliquot (4.0g) of the concentrated aqueous methanol extract was fractionated by the modified Kupchan portioning protocol (Van Wagenen *et al*., 1993) into *n*-hexane, ethyl acetate, and dichloromethane. Subsequent evaporation of the solvent afforded was, *n*-hexane (1.2 g), ethyl acetate (1.24 g), dichloromethane (0.80 g).

Antibacterial Screening

The antibacterial activity of the extractives was determined by the disc diffusion method (Bauer *et al*, 1966). The antibacterial strains used for the experiment (Table 1) were collected as pure cultures from the Institute of Nutrition and Food Sciences (INFS) University of Dhaka. The extractives were dissolved separately in chloroform and methanol as required and applied to sterile filter paper discs at 300 g/disc and carefully dried to evaporate the residual solvent. Standard kanamycin (30g/disc) discs were used as positive control.

Cytotoxicity Evaluation

For cytotoxicity screening, the *n*-hexane, ethyl acetate, and dichloromethane soluble materials of crude methanol extract were separately dissolved in DMSO. The test samples were then applied against brine shrimp, *Artemia salina* in a 1-day *in vitro* assay (McLaughlin *et al*., 1998 and Culkin, 1965). Artificial sea water was prepared

as described by Culkin (Culkin, 1965) with slight modification of chemical composition. 4 mg of each of the extractives was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.78125 μg/mL were obtained by serial dilution technique. Vincristine sulfate and DMSO were used as the positive control and negative control respectively. The median lethal concentration (LC50) of the test samples after 24 hrs of exposure were determined from a plot of % of the dead shrimps against the logarithm of the sample concentration.

Statistical Analysis

For each of the extractive, three samples were prepared for each of the bioassay. The zone of inhibition and LC_{50} were calculated as mean ± SD (n=3) for the antimicrobial screening and brine shrimp lethality bioassay respectively.

RESULTS AND DISCUSSION

Antibacterial activity of *M. koenigii*

Different partitionates of methanol extract of *M. koenigii* were tested for antibacterial activities against a number of gram-positive and gram-negative bacteria. Among the partitionates, ethyl acetate and dichloromethane soluble fraction of the methanol extract exhibited mild to moderate antibacterial activity (Table 1). The ethyl acetate soluble fraction demonstrated moderate antibacterial activity against *Bacillus cereus* and *staphylococcus aureus* having the diameter of

Table 1: Antibacterial Activity of Ethyl Acetate and Dichloromethane Soluble Partitionate (CTSF) of Methanol Extract of *M. koenigii* at 300 μg/disc

Test Microorganisms	Diameter of Zone of Inhibition (mm)		
Gram Positive Bacteria	**Ethyl Acetate**	**Dichloromethane Fraction**	**Kanamycin**
Bacillus cereus	11	8	40
Bacillus megaterium	6	7	38
Bacillus subtilis	20	6	40
Staphylococcus aureus	24	7	50
Sarcina lutea	7	8	35
Gram negative bacteria	**Ethyl Acetate**	**Dichloromethane Fraction**	**Kanamycin**
Escherichia coli	9	11	45
Pseudomonas aeruginosa	7	9	47
Salmonella paratyphi	11	7	38
Salmonella typhi	8	10	38
Pasteurella multocida	8	19	34
Proteus vulgaris	10	22	48
Vibrio mimicus	9	7	50
Vibrio parahemolyticus	8	8	38

Table 2: Results of Cytotoxicity Screening of *M koenigii*

Sample	LC50 (µg/mL)	Regression Equation	R^2
Vincristine sulphate	0.812	y = 33.219x + 52.781	0.9717
HSF	1.250	y = 28.381x + 16.745	0.9503
EASF	11.622	y = 22.344x + 52.665	0.9260
DCMSF	12.280	y = 24.371x + 37.745	0.9816

Note: HSF = Hexane soluble fraction of methanol extract, EASF = ethyl acetate soluble fraction of methanol extract, DCMSF =Dichloromethane soluble fraction of methanol extract.

zone of inhibition of 20 mm and 24 mm respectively. The dichloromethane soluble fraction showed mild antibacterial activity against *Pasteurella multocida* and *Proteus vulgaris* with zone of inhibition of 19 mm and 22 mm respectively.

Brine shrimp lethality bioassay of *M. koenigii*

Table 2 shows the results of the brine shrimp lethality assay after 24 hr exposure to the samples and the positive control vincristine sulfate. The positive control, compared with the negative control (sea water) was lethal, depicting significant mortality to the shrimp. The median lethal concentration (LC_{50}) of the test samples after 24 hr was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the graph by means of regression analysis.

The ethyl acetate and dichloromethane soluble partitionates showed less cytotoxicity than n-hexane fraction. Comparison with positive control, vincristine sulfate indicated that cytotoxicity exhibited by *n*-hexane was promising and further bioactivity guided investigation should be conducted to find out the antitumor and pesticidal compounds.

CONCLUSION

The ethyl acetate and dichloromethane soluble fractions of crude methanol extract of *M. koenigii* showed moderate antibacterial activity whereas the n-hexane soluble fractions demonstrated potent cytotoxic activity. These support the traditional uses of this plant in various infectious diseases. The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates.

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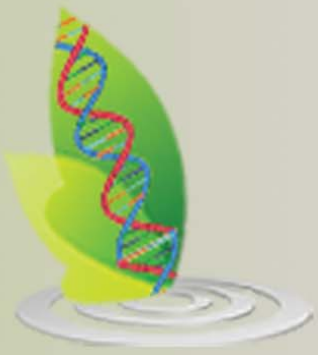

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