

## ANTIBACTERIAL AND CYTOTOXIC ACTIVITY OF *MEYNA SPINOSA* ROXB. STEM

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### Abstract

*Meyna spinosa* Roxb., a medicinal plant enjoys its use in the traditional medicine in Bangladesh for the treatment of a number of ailments. The present study was undertaken to investigate the antibacterial and cytotoxic activity of the ethanol extract of *Meyna spinosa* stem. Antibacterial activity was investigated against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Shigella dysenteriae* by disc diffusion and broth macrodilution assay. In disk diffusion assay, the extract inhibited all the microorganisms except *E. coli*. Minimum inhibitory concentration (MIC) of the extract was 1000 µg/ml for *S. aureus*, *S. pyogenes* and *E. coli*, whereas 500 µg/mL for *S. dysenteriae*. For cytotoxicity test, the extract was subjected to brine shrimp lethality bioassay. The LD<sub>50</sub> of *M. spinosa* stem extract was found to be 40 µg/mL. Findings of the study justify the use of the plant in traditional medicine and suggests for further investigation.

**Key words:** *Meyna spinosa*, Antimicrobial activity, Brine shrimp lethality.

### *Meyna spinosa* Roxb. Gövdesinin Antibakteriyel ve Sitotoksik Aktivitesi

*Meyna spinosa* Roxb., Bangladeş'te bazı rahatsızlıkların tedavisinde halk arasında kullanılan tıbbi bir bitkidir. Bu çalışmada *Meyna spinosa* gövdesinin etanol ekstresinin antibakteriyel ve sitotoksik aktiviteleri incelenmiştir. Antibakteriyel aktivite disk difüzyon ve broth makrodilüsyon yöntemleri kullanılarak *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* ve *Shigella dysenteriae*'ye karşı incelenmiştir. Disk difüzyon yönteminde ekstre *E. coli* hariç tüm mikroorganizmaları inhibe etmiştir. Ekstrenin minimum inhibitör konsantrasyonu (MİK) *S. aureus*, *S. pyogenes* ve *E. coli* için 1000 µg/ml iken, *S. dysenteriae* için 500 µg/mL olarak bulunmuştur. Sitotoksikite testi için, ekstreye tuzlu su karidesi letal testi uygulanmıştır. *M. spinosa* gövde ekstresinin LD<sub>50</sub> değeri 40 µg/mL olarak bulunmuştur. Çalışmanın sonuçları bitkinin halk arasında kullanımını doğrulamakta ve daha ileri çalışmalara gerek duyulmaktadır.

**Anahtar kelimeler:** *Meyna spinosa*, Antimikrobiyal aktivite, Brine shrimp letalite.

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## INTRODUCTION

*Meyna spinosa* Roxb. ex Link (Syn: *Vangueria spinosa* Roxb.) (Rubiaceae) is a thorny bushy shrub growing in hot and humid climate with a slightly acidic to neutral (pH 6.3-7.3) soil condition. The plant has straight, sharp spines and whorled green leaves arranged in opposite manner. Flowering season starts in late spring and lasts until early summer. It is distributed in India, Bangladesh, Nepal and also found in the plain lands of Java and Myanmar. In Bangladesh it is known as 'Moyna'. Fruits of *M. spinosa* are reported to contain sugar, gum and tannic acid whereas the seeds contain esters of palmitic, stearic, oleic and linoleic acids. Fruits are used in the treatment of fever, inflammation, biliary complaints and hepatic congestion. Leaves are used in bone fracture and in the treatment of diphtheria (1, 2). The plant is also reported to be used traditionally in the treatment of skin irritation, abortion and renal diseases (3, 4). In a previous study, the ethanol extract of *M. spinosa* leaf showed antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The extract also showed synergistic effect when given in combination with doxycycline and ofloxacin against the tested organisms except *P. aeruginosa* (5). As reported by the same authors, bioactivity guided phytochemical investigation of the leaf extract led to the isolation of (-)-epicatechin-3-O- $\beta$ -glucopyranoside as the active compound against the aforementioned bacterial strains (6).

The present study was undertaken to investigate the stem of *M. spinosa* for possible antibacterial and cytotoxic activity.

## EXPERIMENTAL

### *Plant material*

The stem of *M. spinosa* was collected from Satkhira, Bangladesh and identified by the experts at Bangladesh National Herbarium, Dhaka, Bangladesh where a voucher specimen (DACB 35447) has been submitted for future reference. The necessary plant parts were carefully cleaned and separated from other parts of the plant as well as from undesirable materials. After cutting into small pieces, it was dried under shade with ample aeration. After complete drying, the plant material was grinded into a coarse powder with the help of a suitable grinder. The powdered plant material was weighed using an electric balance, kept in a suitable airtight container and then stored in a dark, cool and dry place for further use.

### *Extraction*

The powdered plant material was macerated in 95% ethanol for three days with occasional shaking. It was then filtered through a piece of clean, white cloth and then through a cotton plug to remove the plant debris. The filtrate was evaporated using a rotary vacuum evaporator at a temperature of 50° C to yield the crude extract.

### *Test microorganism*

Two Gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes*, two Gram-negative bacteria, *Escherichia coli* and *Shigella dysenteriae* were taken for the test. The bacterial strains used for this investigation were obtained from the bacterial stocks preserved in animal cell culture laboratory of Biotechnology and Genetic Engineering discipline, Khulna University, Bangladesh.

### *Antibacterial assay*

The antibacterial activity was investigated using two methods: disc diffusion and broth macrodilution assay (7-9). Reference microorganisms from the stock were streaked onto nutrient agar plates and the inoculated plates were incubated overnight at 37°C. Using a sterile loop, small portion of the subculture was transferred into test tube containing nutrient broth and incubated (2-4 h) at 37°C until the growth reached log phase. Nutrient agar media seeded with standard inoculum suspension

was poured in Petri-dishes and allowed to solidify. Discs (BBL, Cocksville, USA) impregnated with extract (500 µg/disc), standard antibiotic disc (Tetracycline 30 µg/disc, Oxoid Ltd, UK) and blank (solvent ethanol) discs were placed on the Petri-dishes with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Finally the inoculated plates were incubated at 37° C for 18 h and the zone of inhibition was measured in millimeters.

The broth macrodilution assay was carried out to determine the minimum inhibitory concentration (MIC). Stock suspension of the extract was prepared in nutrient broth with tween-80 concentration not exceeding 5%. Serial dilution of the stock was carried out to obtain six different concentrations (4, 2, 1, 0.5, 0.25, and 0.13 mg/mL) in six vials containing 1 mL each. The same procedure was followed for the standard antibiotic solution of ceftriaxone to obtain six different concentrations (8, 4, 2, 1, 0.5 and 0.25 µg/mL) in six vials containing 1 mL each. Then 1 ml of freshly grown inoculum was added to each vial and incubated at 37° C for 12 h. After incubation period, the vials were checked for turbidity and the lowest concentrations of the extract/standard showing no turbidity were regarded as the MIC of the test substance.

#### *Brine shrimp lethality bioassay*

In this assay the eggs of *Artemia salina* were hatched for 24 h at room temperature (25-30 °C) in artificial sea water (20 g NaCl and 18 g table salt in 1L of distilled water) to obtain nauplii (shrimp larvae). Sample dissolved in DMSO was added in test tubes in such a way that the each contain 4 ml of sea water with sample concentrations of 5, 10, 20, 40, 80, 160 and 320 µg/mL with the solvent concentration no more than 5%. Same procedure was followed for the standard drug chloramphenicol. The final volume for each test tube was adjusted to 10 mL with artificial sea water and 10 living nauplii in each. The process also includes control test tubes containing 10 living nauplii in 10 mL of artificial sea water. After a period of 24 h, the test tubes were observed and the number of survived nauplii in each test tube counted and the results were noted. The percentage of dead nauplii in the test and standard group was established by comparing with that of control group (10, 11).

## RESULTS

In disc diffusion assay, the stem extract of *M. spinosa* showed moderate antibacterial activity against the test microorganisms except *E. coli* (Table 1). The highest zone of inhibition was 10 mm against *S. aureus*, 5 mm against *S. pyogenes* and *S. dysenteriae*. Zone of inhibition for the standard tetracycline discs ranged between 11 to 15 mm.

**Table 1.** Results of the disc diffusion assay of *M. spinosa* stem extract.

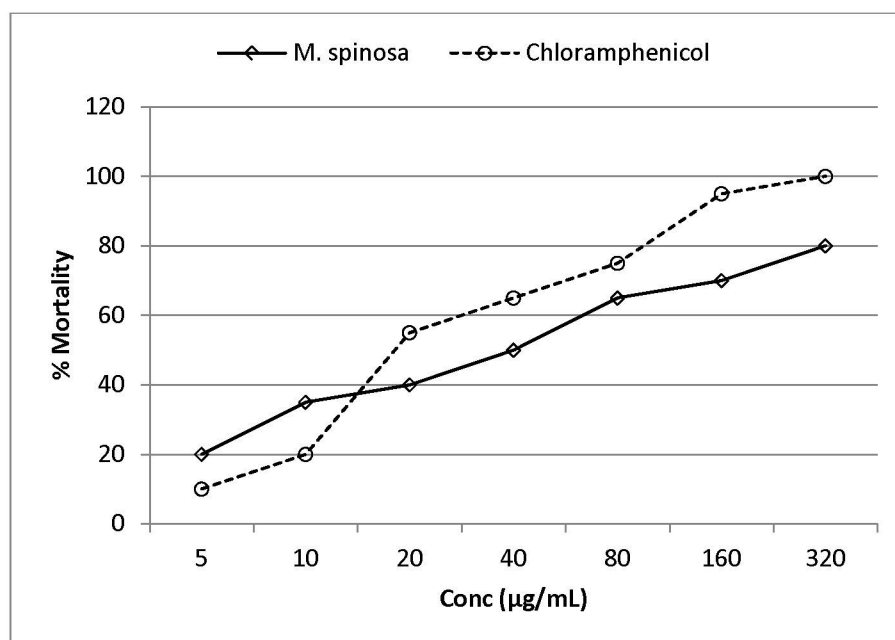
Bacterial strain	Diameter of zone of inhibition (mm)	
	<i>M. spinosa</i> stem extract (500 µg/disc)	Tetracyclin (30 µg/disc)
<i>Staphylococcus aureus</i>	10	15
<i>Streptococcus pyogenes</i>	5	15
<i>Escherichia coli</i>	0	14
<i>Shigella dysentery</i>	5	11

The data obtained from broth macro dilution assay for determining MIC is presented in Table 2. The MIC against *S. aureus*, *S. pyogenes* and *E. coli* was obtained at 1000 µg/mL. For *S. dysenteriae* the MIC was recorded at 500 µg/mL. The MIC of ceftriaxone was recorded as 1 µg/mL against *S. aureus*, *S. pyogenes*, *E. coli* and 0.5 µg/mL against *S. dysenteriae*.

**Table 2.** MICs of *M. spinosa* stem extract.

Bacterial strain	Minimum Inhibitory Concentration (MIC)	
	<i>M. spinosa</i> stem extract ( $\mu\text{g/mL}$ )	Ceftriaxone ( $\mu\text{g/mL}$ )
<i>Staphylococcus aureus</i>	1000	1
<i>Streptococcus pyogenes</i>	1000	1
<i>Escherichia coli</i>	1000	1
<i>Shigella dysentery</i>	500	0.5

In the brine shrimp lethality bioassay, the percent mortality the nauplii caused by the test extract, as well as chloramphenicol is represented in Figure 1. The  $\text{LD}_{50}$  was calculated by probit analysis software LdP (LdP Line software, USA) and was found to be  $40 \mu\text{g/mL}$  for *M. spinosa* stem extract whereas  $24 \mu\text{g/mL}$  for chloramphenicol.

**Figure 1.** Mortality of nauplii by *M. spinosa* stem extract in brine shrimp lethality bioassay.

## DISCUSSION

The stem extract showed moderate antibacterial activity in both the assays. Although the extract did not show antibacterial activity against *E. coli*, but inhibited the same microorganism in broth marco dilution assay. However, the MIC was obtained at a higher concentration ( $1000 \mu\text{g/mL}$ ) than the extract content in the disc ( $500 \mu\text{g/mL}$ ). Therefore, concentration may play a role for the observed activity in latter experiment. Antibacterial activity offered by non polar compound(s) may also be a reason as it may fail to diffuse in agar media to exhibit antibacterial activity in disc diffusion assay (12). Antibacterial activity of *M. spinosa* stem extract as observed in the present study is relatively low than that of the leaves reported previously by Chatterjee et al (1, 2). A difference in inoculums size

used for the assay can lead to variable results for a given sample. In the present study we adjusted to keep the inoculum size as close to the recommended standard of  $5 \times 10^5$  CFU/mL (9).

The brine shrimp lethality bioassay is a rapid, simple and easily mastered technique. This is a popular method for identifying biologically active compounds present in a crude extract since it does not require aseptic techniques, inexpensive and very small amount of test material is needed (13). Result of the present study indicates that the stem extract of *M. spinosa* might have compounds with biological activity with actions like enzyme inhibition, ion channel interference, antimicrobial, pesticidal and/or cytotoxic activity (14-16). In the present study, both the test extract and chloramphenicol showed a gradual increase in percent mortality of the shrimp nauplii with the increase in concentration. The LD<sub>50</sub> obtained for the extract was relatively low than that of chloramphenicol. However, it is still high as a crude extract and infers that there may be one or more compounds present in the extract having biological activity.

## CONCLUSION

The present study provides a rationale for the use of *M. spinosa* in traditional medicine in Bangladesh. Further studies like HPLC and LC-MS can be carried out to confirm whether the observed activity of the stem is due to the presence of (-)-epicatechin-3-O- $\beta$ -glucopyranoside, the compound responsible for the antimicrobial activity of the leaves. A detailed investigation may also provide identification of compound(s) in relation to the cytotoxic activity of the extract.

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