



ISSN: 0975-833X

RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIBACTERIAL PROFILE OF FRUITS OF MEYNA SPINOSA ROXB

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ARTICLE INFO

Article History:

Received 20th February, 2015
Received in revised form
23rd March, 2015
Accepted 24th April, 2015
Published online 31st May, 2015

Key words:

Bioactive compounds,
Paper disc diffusion,
Agar streak dilution,
Antibacterial activity,
MIC.

ABSTRACT

Meyna spinosa Roxb, a medicinal plant enjoys its use in traditional medicine in all over India for the treatment of a number of ailments. In Bengali it is called as Monkata. It is a wild common plant distributed in India, Bangladesh and in both northeastern tropical Africa and tropical Asia. The main objective of the present research work was to determine various bioactive compounds and to evaluate the *in vitro* antibacterial activity of different fruit extracts of *Meyna spinosa Roxb* against different gram positive and gram negative bacteria. Based on this, a new series of constituents had been planned to extract by Ethanol (E1), Methanol (E2), Ethylacetate (E3) and Chloroform (E4) from the fruits of *Meyna spinosa Roxb*. The *in-vitro* antibacterial activity was carried out by Paper disc diffusion method and MIC was determined by Agar streak dilution method. The results displayed that the various extracts of fruits of *Meyna spinosa Roxb*, had a potential antibacterial activity against different gram positive and gram negative bacteria with an MIC range of 9-22 µg/ml (gram positive organisms) and with an MIC range of 10-26 µg/ml (gram negative organisms). The MIC values of various extracts (E1, E2, E3 and E4) of fruits of *Meyna spinosa Roxb* for different gram positive bacteria were found to be *S. aureus* (MIC: 9-18 µg/ml), *B. subtilis* (MIC: 10-21 µg/ml), *S. typhi* (MIC: 11-22 µg/ml) and *S. epidermidis* (MIC: 12-21 µg/ml). The extracts (E1, E2, E3 and E4) of fruits of *Meyna spinosa Roxb* were active against all the tested gram negative microorganisms with the range of MIC values for *P. aeruginosa* (MIC: 10-20 µg/ml), *P. fluorescens* (MIC: 12-25 µg/ml) and *E. coli* (MIC: 11-26 µg/ml) and *V. cholerae* (MIC: 12-23 µg/ml).

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Citation: Asish Bhaumik, Prasenjit Das, Dr. Tajendra Bhakta, Rabindra Tripura and Sravaya, S, 2015. "Phytochemical Screening and Evaluation of Antibacterial Profile of Fruits of *Meyna spinosa Roxb*", *International Journal of Current Research*, 7(5), 16188-16191.

INTRODUCTION

Meyna is a genus of flowering plants in the Rubiaceae family. *Meyna spinosa Roxb*, a medicinal plant enjoys its use in traditional medicine in all over India for the treatment of a number of ailments. In Bengali it is called as Monkata. It is a wild common plant distributed in India, Bangladesh and in both northeastern tropical Africa and tropical Asia. Based on morphology, *Meyna* has been suggested as likely candidate for inclusion in *Canthium* subgenus *Canthium* (Bridson, 1992). In 2004, a molecular phylogenetic study showed that *Meyna tetraphylla* is related to *Canthium* and the transfer to *Canthium* was suggested (Lantz and Bremer, 2004).

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Free radical scavenging and antioxidant potential of the methanol, ethyl acetate, and petroleum ether extracts of *Meyna spinosa* leaves were investigated using several *in vitro* and *ex vivo* assays, including the 2,2-diphenyl-picrylhydrazyl radical scavenging, superoxide anion scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, hydrogen peroxide scavenging activity, metal chelating assay, and reducing power ability method. Total antioxidant activity of the extracts was estimated by the ferric thiocyanate method. Inhibition assay of lipid peroxidation and oxidative hemolysis were also performed to confirm the protective effect of the extracts. Total phenolic and total flavonoid contents of the extracts were estimated using standard chemical assay procedures. The results displayed a direct correlation between the antioxidant activity and the polyphenolic content of the extracts, which may be the foremost contributors to the antioxidant activity of the plant (Sen et al., 2013). The leaves

of *Meyna spinosa* possessed a potential anti diabetic and anti hyperlipidemic activity. The study investigated the antidiabetic and hypolipidemic action of *Meyna spinosa* leaf fractions. Effect of methanol, ethyl acetate and petroleum ether fraction (75 and 150 mg/kg p.o.) from *M. spinosa* leaf methanol extract was evaluated in high fat diet-alloxan induced type 2 diabetic rats after 21 days treatment. Glibenclimide (5 mg/kg), methanol and ethyl acetate fraction at 150 mg/kg exhibited 57.7, 63.4 and 53.8% reduction in serum glucose level after 21 days. Fractions demonstrated significant decrease in triglycerides, total cholesterol, low density lipid, very low density lipid, α -amylase level, and increase in body weight, high density lipid level in diabetic rats. Fractions showed significant ($p < 0.05$) hypoglycemic effect in glucose loaded animals but not in normal rats. Petroleum ether fraction did not produced significant effect. Result validated the claim made by folk medicinal uses and confirmed the antidiabetic potential of methanol and ethyl acetate fraction of *M. spinosa* (Saikat Sen *et al.*, 2013). *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 μ g/ml for *S. aureus*, *S. pyogenes* and *E. coli*, whereas 500 μ g/ml for *S. dysenteriae*. For cytotoxicity test, the extract was subjected to brine shrimp lethality bioassay. The LD50 of *M. spinosa* stem extract was found to be 40 μ g/mL. Findings of the study justify the use of the plant in traditional medicine and suggests for further investigation (PERVIN *et al.*, 2012).

The literature survey revealed that the Consuming fruits and vegetables of all kinds have long been associated with a reduced risk of many diseases. Many studies have suggested that increasing consumption of fruits of *Meyna spinosa* Roxb reduces the risk of obesity and overall mortality, diabetes, heart disease and promotes a healthy complexion and hair, increased energy, overall lower weight, improving vision, relieving constipation etc. The literature survey displayed that the fruits of *Meyna spinosa* Roxb were possessed a wide range of pharmacological activities viz Antioxidant; Anticancer; Antidiabetic; Antimicrobial; Antiinflammatory; Antihyperlipidemic etc. The main aim and objective of the present work is to evaluate the antibacterial profile of the fruits of *Meyna spinosa* Roxb and based on this a new series of constituents have been planned to extract by Ethanol (E1), Methanol (E2), Ethylacetate (E3) and Chloroform (E4) from the fruits of *Meyna spinosa* Roxb.

MATERIALS AND METHODS

Chemicals and drugs

The all chemicals used for the extraction and phytochemical screening were of LR and AR grade. Standard drugs

Tetracyclin (TCLN) and Gentamicin (GENTA) were purchased from Local Retail Pharmacy Shop and solvents and other chemicals were used from Institutional Store and were of AR grade. Bacterial cultures *Staphylococcus aureus* (ATCC 9144), *Bacillus subtilis* (ATCC 6633), *Salmonella typhi* (ATCC 700931), *Streptococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 27853), *Pseudomonas fluorescens* (ATCC13525), *Escherichia coli* (ATCC 25922) and *Vibrio cholerae* (ATCC 14035) were provided by the Biotechnology Lab of the CLBMCP, Chennai and maintained on Nutrient agar slant was maintained at 40C.

Methodology for extraction

Weigh 20 g of fruits of *Meyna spinosa* Roxb and paste (ripen can be mashed to prepare a paste) into a 250 ml round-bottomed flask. Add 50 ml of ethanol and 60 ml of dichloromethane. Heat the mixture under reflux for 10 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 150 ml each with sodiumchloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating. Same procedure is followed for the extraction of E2; E3 and E4 extracts (Raj and Bansal, 2009).

Preliminary Phytochemical screening (Dandiya and Sharma, 2004; Devala Rao, 2006; Jaswant Kaur, 2010; KOKATE *et al.*, 2012)

Preliminary phytochemical screening of various extracts (E1, E2, E3 and E4) of fruits of *Meyna spinosa* Roxb had shown the presence of following bioactive compounds which were confirmed by their specific qualitative confirmatory chemical tests: Proteins and amino acids, Carbohydrates, Glycosides, Alkaloids, Terpenoids, Saponins, Phytosterols, Flavanoids and phenolic compounds, Gum and mucilage etc.

Evaluation of invitro antibacterial profile by Paper disc diffusion method (Gaud and Gupta, 2004)

The sterilized (autoclaved at 120°C for 30 min) medium was inoculated (1 mL/100mL of medium) with the suspension [105 cfu ml (colony forming unit per milliliter)] of the microorganism (matched to McFarland barium sulphate standard) and poured in Petri dish to give a depth of 3-4mm. The paper impregnated with the test compounds (25, 50, 100, 150 μ g/ml in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1hr at RT and incubated at 37°C for 24 hr for anti-bacterial activity respectively. Tetracyclin and Gentamicin (100 μ g/disc) were used as a standard drugs for gram-positive and gram-negative bacteria respectively. The observed zone of inhibition was compared with standard drugs.

Determination of MIC by Agar streak dilution method (Hawkey, 1994)

MIC of the extracts was determined by agar streak dilution method. A stock solution of the extracts (100 μ g/ml) in

Dimethyl formamide were prepared and graded quantities of the test extracts were incorporated in specified quantities of molten nutrient agar medium. A specified quantity of the medium containing the extract was poured into a Petri dish to give a depth of 3-4mm and allowed to solidify. Suspension of the micro-organism were prepared to contain approximately 10⁵ cfu/ml and applied to plates with serially diluted extracts in Dimethyl formamide to be tested and incubated at 37° C for 24hr. for bacteria and fungi. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary Phytochemical screening of various extracts (E1, E2, E3 and E4) fruits of *Meyna spinosa* Roxb had shown the presence of various bioactive compounds such as carbohydrates aminoacids and peptides phytosterols carotenoids and polyphenols (higher concentration) and alkaloids.

Table 1. For Zone of Inhibition (mm) of Gram-positive Bacteria by various Extracts (E1; E2; E3 and E4) of fruits of *Meyna spinosa* Roxb

Extracts	S. aureus				B. subtilis				S. typhi				S. epidermidis			
	Concentration of E1; E2; E3 & E4 (µg/ml)															
	25	50	100	150	25	50	100	150	25	50	100	150	25	50	100	150
Zone of inhibition(mm) at different concentration																
E1	5	10	15	17	3	9	17	19	6	7	16	18	4	8	18	20
E2	4	9	16	16	5	9	18	20	5	8	17	20	4	9	19	21
E3	6	8	13	15	6	10	16	19	5	9	18	21	6	10	20	22
E4	4	12	20	24	5	11	19	22	4	10	19	22	5	9	19	23
TCLN	28 (100µg/ml)				28 (100µg/ml)				28 (100µg/ml)				28 (100µg/ml)			

Table 2. For Zone of Inhibition (mm) of Gram-negative Bacteria by various Extracts (E1; E2; E3 and E4) of fruits of *Meyna spinosa* Roxb

Extracts	P. aeruginosa				P. fluorescense				E.coli				V. cholerae			
	Concentration of E1; E2; E3 & E4 (µg/ml)															
	25	50	100	150	25	50	100	150	25	50	100	150	25	50	100	150
Zone of inhibition(mm) at different concentration																
E1	5	9	14	19	3	9	16	19	6	7	16	18	4	8	18	20
E2	4	8	16	18	5	7	15	20	5	8	17	20	4	9	17	21
E3	6	7	12	16	6	11	14	17	5	9	15	19	6	10	16	22
E4	4	11	20	22	5	12	19	21	4	10	19	22	5	9	20	23
GENTA	28 (100µg/ml)				28 (100µg/ml)				28 (100µg/ml)				28 (100µg/ml)			

Antibacterial screening

The extracts E1, E2, E3 and E4 were (25, 50, 100 and 150µg/ml) were screened for antibacterial activity by paper disc diffusion method. From the data shown in Table 1 and 2 the observations were made as followed: Most of the extracts executed moderate to good antibacterial activity against the tested micro-organisms. When compared to standard drugs Tetracyclin (TCLN), the extracts E1, E2, E3 and E4 were found to exhibit good Anti-bacterial activity against various gram positive bacteria. The order of antibacterial spectrum was given as below: E4 > E1 > E2 > E3 etc. When compared to standard drugs Gentamicin (GENTA), the extracts E1, E2, E3 and E4 were found to exhibit good Antibacterial activity

Table 3. For MIC of various extracts of fruits of *Meyna spinosa* Roxb against different gram positive bacteria

Extracts	Minimum Inhibitory Concentration (MIC) [µg/ml]			
	S. aureus	B. subtilis	S. typhi	S. epidermidis
E1	14	15	17	13
E2	15	18	15	16
E3	18	21	22	21
E4	9	10	11	12
TCLN	0.2	0.2	0.2	0.2

Table 4. For MIC of various extracts of fruits of *Meyna spinosa* Roxb against different gram negative bacteria

Extracts	Minimum Inhibitory Concentration (MIC) [µg/ml]			
	P. aeruginosa	P. fluorescense	E. coli	V. cholerae
E1	15	17	14	12
E2	18	20	22	19
E3	20	25	26	23
E4	10	12	11	13
GENTA	0.5	0.5	0.5	0.5

against various gram negative bacteria. The order of antibacterial spectrum was given as below: E4 > E1 > E2 > E3 etc. The MIC of the extracts (E1, E2, E3 and E4) was screened by agar streak dilution method.

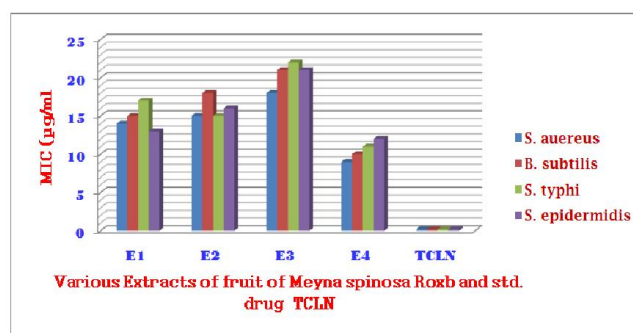


Fig. 1. Graphical representation of MIC of various extracts of fruit of *Meyna spinosa* Roxb and std. drug Tetracyclin (TCLN) against different gram positive bacteria

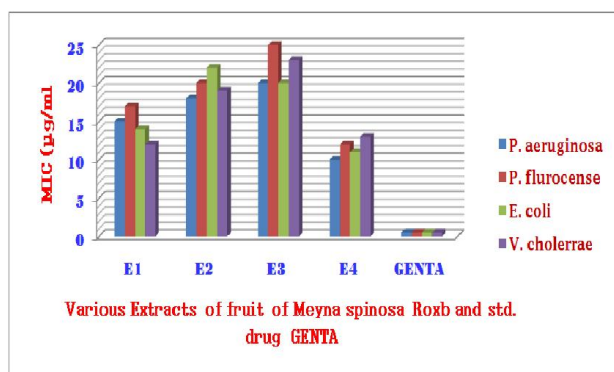


Fig 2. Graphical representation of MIC of various extracts fruit of *Meyna spinosa* Roxb and std. drug GENTA (Gentamicin) against different gram negative bacteria

From the data shown in Table 3 and 4 observations were made as followed: All the extracts (E1, E2, E3 and E4) exhibited moderate to good antibacterial activity with an MIC range of 9-22 µg/ml (gram positive organisms) and with an MIC range of 10-26 µg/ml (gram negative organisms). The MIC values of various extracts (E1, E2, E3 and E4) of fruits of *Meyna spinosa* Roxb for different gram positive bacteria were found to be *S. aureus* (MIC: 9-18 µg/ml), *B. subtilis* (MIC: 10-21 µg/ml), *S. typhi* (MIC: 11-22 µg/ml) and *S. epidermidis* (MIC: 12-21 µg/ml). The extracts (E1, E2, E3 and E4) of fruits of *Meyna spinosa* Roxb were active against all the tested gram negative microorganisms with the range of MIC values for *P. aeruginosa* (MIC: 10-20 µg/ml), *P. fluorescense* (MIC: 12-25 µg/ml) and *E. coli* (MIC: 11-26 µg/ml) and *V. cholerae* (MIC: 12-23 µg/ml).

Conclusion

From the present study it was concluded that most of the extracts executed moderate to good antibacterial activity against tested microorganisms (Gram positive and Gram negative bacteria) with an MIC range of 9-22 µg/ml (gram positive organisms) and with an MIC range of 10-26 µg/ml (gram negative organisms). The MIC values of various extracts (E1, E2, E3 and E4) of fruits of *Meyna spinosa* Roxb for different gram positive bacteria were found to be *S. aureus* (MIC: 9-18 µg/ml), *B. subtilis* (MIC: 10-21 µg/ml), *S. typhi* (MIC: 11-22 µg/ml) and *S. epidermidis* (MIC: 12-21 µg/ml).

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