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PharmTech

International Journal of PharmTech Research
CODEN(USA): IJPRIF ISSN : 0974-4304
Vol.1, No.3, pp 679-681 , July-Sept 2009

ANTIBACTERIAL ACTIVITY OF METHANOLIC FRUIT EXTRACT OF *RANDIA DUMETORUM* LAMK

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ABSTRACT : The goal of this study was to determine the preliminary antibacterial activity of Methanolic extract of *Randia dumetorum* Lamk. (Xeromphis spinosa Thumb.) belonging to family Rubiaceae toward some phytopathogenic bacteria. The antibacterial activity of the extract was done on some standard and wild pathogenic bacterial strains such as *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis Escherichia coli* and *Salmonella typhi*. The testing was done by the agar cup plate method using sterile top agar. Zone of inhibition of extract (50, 100 and 150 mg/ml) was compared with that of standard Amoxicillin (0.5 and 1 mg/ml) prepared in DMSO. The extract shows potential antibacterial properties comparable with that of standard amoxicillin against the organisms tested. The methanolic extract of *R. dumetorum* displayed a concentration related antibacterial activity. The results show that the inhibition of the bacterial growth was more pronounced on *Escherichia coli* as compared to the other tested organisms.
Keywords: Antibacterial activity, *Randia dumetorum,* cup plate method, *Xeromphis spinosa* Thumb.

INTRODUCTION

The most critical problem related with health and causes of mortality rates in society is infectious disease[1]. Since last decade, there has been an increasing evidence of bacterial and fungal infections due to population explosion, pollution, changed environmental conditions, wastes from different sources, which may affect food with perfect nutrition value. This factor causes less immunogenecity in human beings and animals. This fact coupled with the resistance developed in microorganisms to allopathic agents, antibiotics with increased toxicity in human being and animal during prolonged treatment with several antimicrobial drugs[2]. These facts motivate us to find out a new herbal moiety, which can act as an antimicrobial biomolecules to treat opportunistic microbial infections[3]. This paper reports the in-vitro antimicrobial screening of methanolic extracts of *R. dumetorum* against clinical isolates of six bacterial species.

In Indian system of medicine *Randia dumetorum* (*Xeromphis spinosa* Thumb.) belonging to family Rubiaceae is an important medicinal plant and popularly known as emetic nut. It is found in waste places & jungles all over India, extending northwest to the Bias river & ascending to outer Himalaya to 4000 ft[4]. Literature survey reveals that the fruit is bitter, sweet; heating, aphrodisiac, emetic, purgative, carminative, antipyretic; cures abscess, ulcers, inflammations, wounds, tumors, skin diseases and have antibacterial activity. The pulp of fruit is believed by many practitioners to also have anthelmintic properties, and also used as an abrortificient as folklore remedy[4-6]. But the data of systemic and scientific antimicrobial study is not available so far. Therefore it was our intention to study the antibacterial profile of *R. dumetorum* by using different microbial species.

MATERIALS AND METHODS

Plant collection and authentication

Fruits of *Randia dumetorum* were collected during November from Botanical garden of M.S.U. Baroda and were identified by Head of Botany department, M. S. University, Baroda. A voucher specimen has been deposited in the museum of department of Pharmacognosy, M.S.U. Baroda. Voucher specimen (PH-805) was deposited in the herbarium of Pharmacy Department of M.S.U. Baroda.

Plant preparation and extraction

The fruits were dried in sunlight and reduced to a coarse powder. Then the powder was subjected to soxhlet extraction with methanol for 72 hours at a temperature of 50-60ºC. The extract was concentrated and the solvent was completely removed. They were freeze dried and stored in the vacuum dessicator until further use.

Preliminary phytochemical screening[7]

Preliminary phytochemical screening revealed the presence of phytosterol, polyphenol, saponins, flavonoids and carbohydrates.

Microorganisms

Standard cultures of following microorganisms were obtained from Food and Drug Laboratory, Baroda. The microorganisms were identified by staining techniques. The organisms were maintained by sub culturing at regular intervals in nutrient agar medium.

Gram +ve bacteria:*Staphylococcus aureus*
Staphylococcus epidermidis
Bacillus cereus
Bacillus subtilis

Gram -ve bacteria:*Escherichia coli*
Salmonella typhi

Preparation of inoculums

The suspension of all organisms were prepared by inoculating one colony of the strain in 20 ml of nutrient broth in conical flask and incubated at 37^{o}C for 24 hours to activate the strain. The suspension is adjusted such that it contained approximately 1 x 10^{6} cells/ml. It was obtained by calculating the cells by Neubers chamber. Nutrient agar (HiMedia) was prepared for the study.

Culture medium

The medium was prepared by dissolving 13 gm of nutrient broth in 1000ml of distilled water pH to (7.3 ± 0.2), and subjecting it to sterilization in an autoclave at 121^{o}C for 15 min.

Antimicrobial Agent

The reference standard amoxicillin was procured as gift sample from Hindustan Antibiotics Ltd., Pune.

Determination of minimum inhibitory concentration

The molten nutrient agar media was prepared and distributed in Mc cartney bottles, 20 ml each, prier to sterilization. A measured amount of the methanol extract was added to each bottle in such a manner that the final concentration per ml of the agar medium was 0 (control), 5, 15, 25, 50 and 100 mg. the final mixture was poured individually into 100 mm sterile petriplates.

For uniform diffusion of the drug throughout the medium, the nutrient agar plates containing different concentrations of the drug were refrigerated overnight at 4˚ C and then dried for 24h at 37˚ C before inoculation. One loopful (loop diameter – 2mm) of an over night grown bacteriological culture of the test organism at concentration ~ 10^{6} colony forming units (cfu/ml) was placed in all the petriplates marked by checkerboard technique[8]. The spot inoculated plates were incubated at 37˚ C for 24h and then observed for any growth of microorganisms. The minimum concentration of extract which prevent bacterial growth was taken as MIC (Table 1). The antibacterial growth was observed by formation of bacterial colony or turbidity around the inoculum's spot.

Determination of zone of inhibition by cup plate method[9]

The antibacterial activity of methanolic extract was performed using Agar cup-plate method. 20ml of sterile nutrient agar medium was pored into sterile petri-dishes and allowed to solidify. The petri dishes were incubated at 37^{o}C for 24 hours to check for sterility. The medium was seeded with the organisms by pour plate method using sterile top agar (4 ml) contained 1 ml culture. Bores were made on the medium using sterile borer. Dried methanolic extract of fruits of *Randia dumetorum* was dissolved in Dimethyl sulfoxide (DMSO) to obtained different concentration (50, 100 and 150 mg/ml) and sterilized by filtration through a Whatman filter paper no. 1, and 0.1 ml of the different concentrations of extract were added to the respective bores. 0.1ml of Amoxicillin at a concentration of (0.5 mg/ml, 1mg/ml) was taken as standard reference. The plates were incubated overnight at 37^{o}C with appropriate positive and negative controls. The petri-dishes were kept in refrigerator at 4^{o}C for ½ hour for diffusion. After diffusion the petri-dishes were incubated at 37^{o}C for 24 hours and zone of inhibition were observed and measured. Dimethyl sulfoxide was used as the control.

RESULT AND DISCUSSION

The observations of the MIC study has been tabulated in table 1 and it was found that the minimum inhibitory concentration for methanolic fruit extract against *E. coli* is 15 mg/ml, where as for *Salmonella typhi, Staphylococcus aureus* and *Staphylococcus epidermidis* it was 25 mg/ml and for, *Bacillus cereus* and *Bacillus subtilis* were inhibited at 50 mg/ml. From the data it is evident that the methanolic extracts is active against both Gram positive and bacteria but more active against Gram negative at low concentration. The results of zone of inhibition of the methanolic fruit extract and comparison with standard antibiotic amoxicillin were recorded in Table 2. The result shows that the methanolic fruit extract of *R. dumetorum* displayed concentration dependent antibacterial activities. It indicates that *R. dumetorum* shows antibacterial activity towards all six investigated phytopathogenic bacteria. The highest antibacterial activity was found towards *E. coli*, while it was less active against *S. aureus*. The compounds responsible for this antimicrobial property were not investigated. However preliminary phytochemical analysis of the methanolic extract revealed the presence of phytosterol, polyphenol, saponins, flavonoids and carbohydrates. The antimicrobial potency of the plant may be attributed to the single or combined effect of the above mentioned chemical groups. The methanolic fruit extract of *R. dumetorum* had impressive antibacterial and could lead to the discovery of new antibiotics. This becomes more relevant as the current antibiotics in use are fast loosing effectiveness due to emergence of resistant microorganisms. The isolation of components of fruits of *R. dumetorum* methanol extract is in progress as very potent antimicrobial agents.

Table 1. Determination of MIC of methanolic fruit extract of R. dumetorum against different bacteria

Name of bacteria	Growth in nutrient agar containing different concentration of extract in mg/ml					
	0	5	15	25	50	100
S. aureus	+	+	+	–	–	–
S. epidermidis	+	+	+	–	–	–
B. subtilis	+	+	+	+	–	–
B. cereus	+	+	+	+	–	–
E. coli	+	+	–	–	–	–
S. typhi	+	+	+	–	–	–

'0' – Control (without extract); '+' – Growth; '-' – No growth

Table 2. Antibacterial activity of Amoxicillin and fruits methanolic extract

Micro organism	Zone of inhibition in mm				
	Extract Conc. mg/ml			Amoxicillin Conc. mg/ml	
	50	100	150	0.5	1
S. aureus	12 ± 0.42	16 ± 0.53	18 ± 0.17	20 ± 0.31	26 ± 0.54
S. epidermidis	14 ± 0.51	15 ± 0.18	16 ± 0.57	22 ± 0.42	24 ± 0.72
B. subtilis	7.5 ± 0.22	10 ± 0.92	11.5 ± 0.21	13 ± 0.61	15.5 ± 0.16
B. cereus	6 ± 0.31	7.5 ± 0.15	8 ± 0.43	12.5 ± 0.48	15 ± 0.52
E. coli	20 ± 0.73	21 ± 0.41	23 ± 0.61	21 ± 0.15	25 ± 0.68
S. typhi	14.5 ± 0.48	17 ± 0.63	21.5 ± 0.12	19 ± 0.23	23 ± 0.71

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