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Antimicrobial potential of *Ficus religiosa* L. against Multidrug resistant bacterial isolates

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ABSTRACT

Introduction

Bacterial diseases account for high proportion of health problems in the developing countries. The emergence of antimicrobial resistant pathogens has resulted in increased morbidity and mortality as well as health care costs.

Objective of study

The study was conducted with an objective to find out antimicrobial potential of *Ficus religiosa* L. against clinically important MDR strains of *Staphylococcus aureus*, *Enterococcus faecalis, Klebsiella pneumoniae* and *Acinetobacter baumannii*.

Material and Methods

Hexane, chloroform, methanol and aqueous extracts of *F. religiosa* L. leaves were employed for testing antimicrobial activity by using agar well diffusion method or cup plate method MIC (µg/ ml) was determined .

Results

Methanol extract was maximum inhibitory in case of *S. aureus*; followed by hexane extract and chloroform extract but aqueous extract did not show antimicrobial activity. Maximum activity was observed for chloroform extract followed by hexane, aqueous and least for methanol extract against *E. faecalis*. Chloroform and hexane extract showed more activity as compared to aqueous and methanol extracts in case of *A. baumannii*. In *K. pneumoniae*, maximum activity was shown by aqueous extract followed by hexane, chloroform and methanol extracts.

Conclusion

The present study suggests the use of *F. religiosa* L .in the treatment of various diseases caused by multidrug resistant bacteria. Further, the antimicrobial potential of this plant must be explored more and more in order to develop an alternate therapy for the treatment of infections caused by antibiotic-resistant bacteria.

Keywords: *F.religiosa* L., infections, multidrug resistant, agar diffusion assay, MIC, Phytotherapy

INTRODUCTION

Plants generally produce many secondary metabolites which are bio-synthetically derived from primary metabolites and constitute an important source of many pharmaceutical drugs. *Ficus religiosa* L.(*F. religiosa*) belongs to the family Moraceae, commonly known as Peepal tree and has many medicinal properties.[1] It contains tannins, phenols, saponins, sugars, alkaloids, methionine, terpenoids, flavonoids, glycosides, proteins, separated amino acids, essential and volatile oils and steroids. Phenolic acids, tannins, and flavonoids have anti-carcinogenic and anti-mutagenic effects. Flavonoids present in high amounts have antibacterial and antioxidant effects. Flavonoids interact with a number of cellular signalling pathways and improve brain function, prevent oxidative stress and apoptosis, protect against endothelial barrier dysfunction and injury, improve the cognitive function and decrease the neurode generation. Tannins serve as a natural defense mechanism against microbial infections and cellular oxidative damage, including lipid peroxidation.[2] The rich phytochemical content of leaves of *F. religiosa* is the subject of increasing scientific interest because of its possible beneficial effects on human health. The leaves of the plant show antioxidant, wound healing, anticarcinogenic, antimutagenic, antimicrobial, analgesic and anti-inflammatory activity.[3]

A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity. There are several reports on the antimicrobial activity of different herbal extracts. Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections. In several studies, it is shown that leaves extract of *F. religiosa* contains antimicrobial compounds.[4]

The increasing antibiotic resistance to commonly used antibiotics exhibited by pathogens has led to the screening of several medicinal plans for their potential antimicrobial activity. The increasing trend in development of antibiotic resistance could be attributed to frequent, unnecessary and indiscriminate usage of antibiotics and longer duration of hospitalization.[5] Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains.[6]

Objective of the study

The study was conducted with an objective to find out antimicrobial potential of *F. religosa* against clinically important multidrug resistant (MDR) strains of *Staphylococcus aureus*, *Enterococcus faecalis, Klebsiella pneumoniae* and *Acinetobacter baumannii.*

MATERIAL AND METHODS

The study was conducted in Centre for Interdisciplinary Biomedical Research and in Bacteriology Laboratory in the Department of Microbiology, Adesh Institute of Medical Sciences & Research (AIMSR), Adesh University Bathinda. The study was approved by Institutional Research Committee and Ethics Committee of Adesh University.

Plant Material

Dried Leaves of *Ficus religosa* L. were procured from a certified and authorized Herb Store in the Majith Mandi, Sri Amritsar. Identity of dried leaves was confirmed through NISCAIR, New Delhi. (Letter No:. NISCAIR/RHMD/CONSULT/2018/3236-37-4)

Solvent Extraction of plant material

Hexane, Chloroform and Methanol were employed for Soxhlet extraction of active ingredients using Soxhlet apparatus and finally the drug was boiled with distilled water to obtain water extract.[7]

Recovery of solvents

Solvents from extracts were recovered under reduced pressure using rotary vacuum evaporator and the dried extracts were preserved in a vacuum dessicator containing anhydrous silica gel.[8]

Drying of residual mass

Extracts were filtered, concentrated using rotary vacuum evaporator, and dried in an oven at 40-50 ºC. The dried extracts were preserved in a vacuum desiccator over fused calcium chloride. [8]

Phytochemical screening

All the leaf extracts were screened for different classes of phytoconstituents using specific standard reagents.[9]

Bacterial strains

The bacterial strains each of four clinically important multidrug resistant (MDR) bacteria: *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Acinetobacter baumannii* were obtained from Department of Microbiology, Adesh Institute of Medical Sciences and Research (AIMSR). The MDR strains isolated from clinical samples are shown in Table 1.

Table1 : Bacterial MDR strains isolated from various sources(clinical samples)

Name of isolate	Source of Isolates	Source of Isolates	Source of Isolates
S. aureus	Pleural fluid	Pus	Pus
E. faecalis	Endotracheal secretions	Pus	Pus

K. pneumoniae	Endotracheal secretions	Pus	Urine
A. baumannii	Endotracheal secretions	Endotracheal secretions	Blood

Identification and antibiogram of Bacterial isolates

Confirmation and antimicrobial susceptibility of isolates was carried out by using Vitek 2 Compact system (Biomereiux®). Identity of Gram positive bacterial isolates (*S. aureus and E. faecalis*) was confirmed by using GP card and their antibiogram was obtained by using P628 card. Identity of Gram negative bacterial isolates (*K. pneumoniae* and *A. baumannii*) was confirmed by using GN card. The antibiogram for *K. pneumoniae* and *A. baumannii*was obtained by using N 280 card and N281 card respectively.

Stock solutions

Stock solutions of extracts were prepared by dissolving the extracts in DMSO. These solution were then used to prepare test solutions of desired range of concentrations.

Test solutions

Test solutions of extracts were prepared in DMSO to produce solutions of various concentrations ranging from 50 to 1000µg/ml. Following concentrations were prepared: 50 ,100 ,200 ,500 ,1000 µg/ml.

Preparation of inoculums

To prepare inoculum, a loopful of isolated colony of MDR test strains was taken and inoculated into nutrient broth and incubated at 37□C for 6-4hrs. This cell suspension was used to prepare inoculum (Conc. = 10^8 CFU/ml). The cell suspension was standardized to obtain CFU= 10^8 per ml using Densitometer (Biomerieux®).This concentration is equivalent to 0.5 Mc Farland conc.; ideal to be used for assaying antimicrobial activity of plant extracts.

Antimicrobial assay
Cup-plate or cylindrical plate method

The Mueller Hinton agar medium was poured into sterile petri-plates and allowed to solidify. Inoculum of test microorganism was then spread on the surface of agar plate by using sterile cotton swab. Holes of 6 mm in diameter were cut in the medium with sterile cork borer. The volume of solutions added to each cavity or cylinder was kept uniform to fill the holes. 50 microliters of solutions of each concentrations of extracts prepared in DMSO were added in the cavities or cylinder prepared in a solid medium using micropipette under strict aseptic conditions in the laminar flow bench. The plates were left for 1 to 2 hours at room temperature so as to provide a sufficient pre-incubation diffusion period which in turn minimize the effects of variations in time between the applications of different solutions. All the plates were then incubated for about 18 to 24 hours at 37□C. The zones of inhibition obtained after incubation were considered the basis of measurement of antimicrobial activity. Diameter of any resultant zone of inhibition including well size was measured in millimeters. DMSO and Distilled water were used as vehicle control and negative control respectively. The minimum concentration of the extract/s showing a clear zone of inhibition was recorded as MIC of that particular extract on a particular bacterial strain. [9,10]

RESULTS

Phytochemical Constituents of *F. religiosa*

Hexane extract of *F. religiosa* showed the presence of fixed oils & fats, terpenoids & triterpenoids. Chloroform extract showed presence of alkaloids, terpenoids & triterpenoids, phytosterols, flavonoids. Alkaloids, phenolic compounds & tannins, glycosides were present in methanolic extract. Aqueous extract showed presence of carbohydrates, proteins and amino acids phenolic compounds & tannins.

Antimicrobial activity of various extracts

Hexane extract of *F. religiosa* had maximum inhibitory effect against *A. baumannii* followed by *K. pneumoniae, E. faecalis and S. aureus*. Chloroform extract of *F. religiosa L.*hadmaximum inhibitory effect against *A. baumannii* followed by *K. pneumoniae.* Lesser antimicrobial effect was observed in *E. faecalis* followed by *S. aureus* isolates. Methanol extract of *F. religiosa L.*hadmaximum inhibitory effect against *A. baumannii* followed by *K. pneumoniae, S. aureus* and *E. faecalis* isolates. Aqueous extract of *F. religiosa* had maximum inhibitory effect against *A. baumannii* followed by *K. pneumoniae.* Lesser inhibitory effect was seen for *E. faecalis* and no inhibitory effect was observed for *S. aureus* isolates. The results of antimicrobial activity (zone size obtained in millimetres) of various extracts of *F. religiosa* are shown in Table 2.

Table2: Antimicrobial activity (Zone size obtained in millimetres) of various extracts

Type of extract	Concentration (µg/ ml)	*S. aureus*	*E. faecalis*	*K. pneumoniae*	*A. baumannii*
Hexane	50	NIL	NIL	13	17
	100	NIL	NIL	13	18
	200	NIL	NIL	14	18
	500	14	16	15	19
	1000	16	18	16	20
	50	NIL	12	12	17
	100	NIL	13	13	19

Chloroform	200	NIL	14	14	19
	500	13	16	16	20
	1000	14	16	16	20
Methanol	50	NIL	NIL	10	15
	100	NIL	NIL	12	16
	200	NIL	20	12	17
	500	16	21	13	18
	1000	17	22	15	19
Water	50	NIL	NIL	14	16
	100	NIL	NIL	15	16
	200	NIL	NIL	15	17
	500	NIL	14	16	18
	1000	NIL	16	18	19
Dmso	NIL	NIL	NIL	NIL	NIL
Distilled water	NIL	NIL	NIL	NIL	NIL

DISCUSSION

Pathogenic microorganisms are one of the major causes of health problems in humans and animals and their contagious nature make it difficult to control. In pre antibiotic era, microbial infections were the major cause of untimely death in humans. Soon after the discovery of antibiotics, death rate of microbial infection has significantly decreased, even though, drug resistant microorganisms remain a major threat for human beings. Therefore, newer antimicrobial compounds with low/no side effects are desirable for pharmaceutical applications. Higher trees synthesize a variety of phytochemicals compounds as secondary metabolites to protect themselves from the microbial infections and environmental stress conditions.The current study was aimed to carry out the to check invitro antibacterial activity of *F. religiosa* against MDR bacteria. There are various studies which have been published to check the antimicrobial activity in *F. religiosa* but only few studies have been done to check the antibacterial potential of various plants against MDR strains.

In a study by Jahan et al, *F. religiosa L.*extracts showed moderate activity against antibiotic resistant and antibiotic sensitive *S. aureus* isolates. The antimicrobial activity may be due presence of glycosides, phenols and tannins.[11]
Ramakrishna and Hariparsad, conducted study using methanol and diethyl ether extracts of *F. religiosa L.* on *E. coli*, *P. aeruginosa* and *S. aureus*. Concentrations 100, 200, 300 and 400 µg/ml were used for the assay. Methanol extract inhibited all the three bacterial species with zone diameters: *S. aureus*- 28 mm, *E. coli*- 24 mm, *P. aeruginosa*- 22 mm. Diethyl ether extracts did not show any antibacterial activity against any of the bacterial species.[12]
In a study by Supriya and Harshita, *S. aureus* and *E. coli* were subjected to petroleum ether, chloroform and methanol and water extracts of *F. religiosa*. It was found that chloroform extract showed good antimicrobial effect against *E. coli* and *S. aureus* with zone size of 16 mm which was more as compared to methanol and water extracts. Petroleum ether extracts did not show any activity.[13]

Salem et al. published that aqueous and ethanolic extracts of *F. religiosa* show high antimicrobial activity against MDR bacteria – *S. aureus* and *E. coli* with MIC of 500 µg/ ml. Chloroform extract showed high antibacterial effect against infectious bacteria *S. Typhi*, *S. Typhimurium*, *P. vulgaris* at concentration of 20 µg/ ml. Methanolic extract had moderate antibacterial activity against *P. aeruginosa*, *E. coli* , *S. aureus* and *P. vulgaris*.[14]

Tambekar et al. reported that aqueous and methanolic extracts of *F. religiosa L.*showed high antibacterial potential against Gram negative bacteria- *E. coli* and *P. vulgaris*.[15]
Rajiv and Sivaraj, found that aqueous extracts of *F. religiosa* showed presence of many phytochemical alkaloids, phenolic compounds, sugars, terpenoids, tannins, glycosides and flavonoids. They reported that methanolic extract had higher activity than chloroform and aqueous extract whereas hexane extract did not show any antibacterial activity against Gram positive and Gram negative bacteria.[16]

Manimozhi et al. reported that methanolic extract of *F. religiosa* showed more antibacterial activity against *S. aureus* whereas hexane extract did not exhibit any activity against pathogenic bacteria.[17]
Various other studies which include Preethi et al, Uma et al, Nair and Chanda, Hemaiswarya et alalso reported that methanolic, aqueous and chloroform extracts of *F. religiosa L.*showed high antibacterial effect against *S. aureus*, *K. pneumioniae* and *E. coli*. [18,19,20,21]

Prakash et al. reported that aqueous and methanolic extract of *F. religiosa L.*were found inhibitory against *E. coli* with zone size of 10 mm and 12 mm respectively indicating that methanolic extract was more inhibitory as compared to aqueous extract.[22]
In a study by Chavan et al. leaf extract of *F. religiosa L.*in various solvents like- Petroleum ether, acetate, ethanol and water were tested for antimicrobial activity against *E. coli*, *B. subtilis*, *S. aureus* and *P. aeruginosa*. All types of extracts were found to be inhibitory for *S. aureus* at minimum concentration of 100 µg/ ml.[23]

The findings of this study show similarity with Ramakrishna and Hariparsad, Supriya and Harshita, Salem et al. as in all these studies also methanol and chloroform extracts of *F. religiosa* were inhibitiory for *S. aureus.* However, in the present study, aqueous extracts did not show any antimicrobial against *S. aureus* which is contrary to the results reported by Salem et al.[12,13,14]
Tambekar et al reported that aqueous and methanol extracts were more inhibitory against Gram negative bacteria as compared to Gram positive bacteria which finds concordance with the present study.[15]

The results of this study are also in line with many other studies with an important finding that all types of extracts

of *F. religiosa L.*possess antibacterial activity against variety of pathogenic bacteria.[19,20,21,22,23]

CONCLUSION

It is inferred from the current findings that phytoconstituents along with some new microbicidal agents present in the plant extracts of *F. religiosa L.* reflects the high anti-microbial potential of this plant. The indiscriminate use of antibiotics resulted in the emergence of a number of resistant bacterial strains, and the antimicrobial compounds from plants may inhibit bacteria by a different mechanisms than the currently used antibiotics and may have clinical value in the treatment of resistant microbial strains which are posing very serious threat to the health care system and become a challenge for the clinicians nowadays . This study strongly suggests the possibility of this plant as an important source of antimicrobial drug development specially against MDR bacterial strains.

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