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Research Article

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In vitro studies on antibiotic activity of *Ficus religiosa* fruits extract against human pathogenic bacteria

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ABSTRACT

Effect of ethanolic extract of F. religiosa fruits extract was studied against two gram positive bacteria (Staphylococcus epidermidis and Staphylococcus aureus) and two gram negative bacteria(Pseudomonas vulgaris and Klebsiella pneumonia). The minimum inhibitory concentration of extract effective against S. epidermidis and K. pneumonia was 15 mg/ml while the minimum inhibitory concentration for the S. aureus and P. vulgaris was 30 mg/ml. At 15 mg/ml concentration of extract K. pneumonia showed more sensitivity (inhibition zone 21 mm) than S. epidermidis (inhibition zone 19 mm). At 30 mg/ml concentration P. vulgaris showed more sensitivity (inhibition zone 12 mm) than S. aureus (inhibition zone 9 mm).Present observations indicate that the extracts possess antibiotic activity. Further scientific evaluation is needed to screen active phytochemical from this important plant to use it for production of new antibiotics.

Keywords: *F. religiosa*, Phytochemistry, Antibacterial, Antibiotic, Antimicrobial.

INTRODUCTION

The frequency of life-threatening infections caused by pathogenic microorganisms are constantly harmingthe health [1-2]. The clinical efficacy of existing antibiotics is being challenged by the emergence of multi-drug resistant pathogens. The increasing prevalence of multi-drug resistant strains of bacteria and the recent emergence of strains with reduced susceptibility to antibiotics evoked the need to search new antibiotics [3].

S. epidermidis is a gram-positive bacterium which is part of the normal human skin and mucosal flora [4] and is often resistant to antibiotics, including penicillin, amoxicillin, and methicillin [5].Although *S. epidermidis* is not usually pathogenic, patients with compromised immune systems are at risk of developing infection [6].*S. aureus* is a gram positive bacterium found in the human respiratory tract and on the skin.It is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning [7].*P. vulgaris* is a gram-negative bacterium that inhabits the intestinal tract of humans and animals and known to cause urinary tract infections and wound infections [8].*K. pneumoniae* is a gram-negative found in the normal flora of the mouth, skin, and intestine and, can cause destructive changes to human lungs if aspirated [9].

Medicinal plants are naturally gifted with invaluable bioactive compounds which form the backbone of traditional medicines [10]. Indian medicinal plants have been known to have many important secondary metabolites such as alkaloids, phenolic compounds, etc., which impart the antimicrobial properties [3].The *Ficus religiosa* is one of the important Indian religious medical plant.Its stem, leaves, roots and fruits are known to possess several medical properties [11].In Ayurveda, *F. religiosa* belongs to a class of drugs called rasayana. Rasayana are rejuvenators, antioxidants and relieve stress in the body [11].

Indian medicinal plants have been exploited for treatment of human diseases by different ethnic groups in different parts of the world since the dawn of civilization. But the traditional cultures without proper scientific evidence are not able to understand the importance of plant species for treatment of human diseases. The present study focused on the screening of*F. religiosa*fruits extract for antibiotic activity againsttwo Gram positive bacteria (*S.epidermidis*and *S. aureus*) and two Gram negative bacteria (*P. vulgaris*and *K. pneumonia*).

EXPERIMENTAL SECTION

Reagents

The reagents used during the study were of analytical grade and procured from standard laboratory suppliers.

Collection of Plant Material

The Fruits of *Ficus religiosa* were collected from the Kurukshetra University campus, Kurukshetra ($29^0$6'N, $76^0$5'E). The plant and sample specimen were identified by a taxonomist from Department of Botany.

Preparations of Plant Extract

In order to avoid any alternation/degradation of biologically active ingredientin fresh extract, the ethanol extract of the dried fruit was used. The collected fruits were dried in the oven at 40^0C temperature for 48 hours. The dried fruits were grinded to make fine powder. After weighing the powder was macerated in absolute ethanol i.e. 100 g / 250 ml, w/v and stirred using magnetic stirrer for one day at room temperature. Extract was then flittered through Whatman filter paper No 1. After filtration, the ethanol was evaporated from the extract by heating at 55^0C in water bath for 12 hrs. The resulting partially solid extracts were stored at -20^0C for future experimentation [10-11].

In-vitro Antibiotic Activity

An antibiotic is a substance that kills or inhibits the growth of micro-organisms such as bacteria, fungi or protozoans. Antibiotic drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antibiotic substances used on nonliving objects or outside the body [12].

Antibiotic Activity Investigation Method

There are three types of method for investigation of antibiotic activity [13-14]. During the present study Cup Plate Diffusion Technique was used.

Cup Plate Diffusion Method

All the glassware and the petri plates were sterilized by dry heat in an oven at 160^0C for one hour. Nutrient agar was prepared in distilled water. The nutrient agar was poured in sterile petri plates aseptically and allowed to solidify at room temperature. All the petri plates were flooded with 0.1 ml of the standardized culture. The holes of 7 mm were bored aseptically using sterile cork-borer. The agar plugs were taken out carefully so as not to disturb the surrounding medium. The holes were filled completely with desired extract and kept in incubator at 30^0C for 48 hours. After this the Petri plates were observed for the antibacterial activity and zone of inhibition was measured.

Minimum Inhibitory Concentration

Minimum Inhibitory Concentration of extracts that showed antibacterial activity was determined by micro dilution technique as described by the National Committee for Clinical Laboratories standards (NCCLS) [23]. The bacteria inoculums were prepared in 5 ml nutrient broth and incubated at 37°C. The final inoculums were of approximately 106 CFU/ml. Controls with 0.5 ml of culture medium without the extract samples and other without microorganisms were used in the tests. Tubes were incubated at 37°C for 24 h. The activity was measured as a function of turbidity at 660 nm. Lack of turbidity was further confirmed by pouring suspension aliquot of 0.1 ml into pre-sterilized Petri dishes with nutrient agar medium. The tests were conducted in triplicate.

Bacterial Culture

Gram positive: *S. epidermidis and S. aureus*
Gram negative: *P. vulgaris and K. pneumonia*

Composition of Nutrient Agar Media

Yeast/meat/beef extract - 10 g
Peptone -10g
Sodium chloride - 5 g
Agar - 20 g
Distilled water - 1000 ml

Procedure

Plates are prepared with nutrient agar media medium of about 4 mm layer. Sterile non-toxic cotton swab on a woodenapplicator dipped in prepared inoculums and rotated soakedswab firmly against the upper inside wall of test tube. Streakthe entire agar surface of the plate with a swab two to threetimes, turning plate at 60^0 angle between each streaking. Theinoculums allowed drying for 5-15 minutes with lid in place.

Properly bore the plate with borer and disc is applied forstandard drug. Ciprofloxacin (10 µg/ml) was used forstandard antibiotics for activity being most resistant in bothgram-positive and gram-negative species and inhibitsbacterial protein synthesis by binding to the subunit of theribosome. Twoconcentration 15 mg/ml and 30 mg/mlof extract wereprepared. The extractswere then inoculated for 24-48 hours and zones ofinhibitionwere recorded [8-9].The antimicrobial activity was recorded bymeasuring the width of the clear inhibition zone around thedisc using zone reader (Zone Size interpretative Scale).

RESULTS

The ethanol extracts of *F. religiosa* fruits extract showed zone of inhibition against the human pathogens with varied diameter. The MIC values obtained for the extracts suggested that the plant extracts were bactericidal at lower concentration and bacteriostatic at higher concentration. The minimum inhibitory concentration of extract effective against *S.epidermidis*and *K. pneumonia*was 15 mg/ml while the minimum inhibitory concentration for the *S. aureus*and*P. vulgaris*was 30 mg/ml (Table 1).

Table 1: Effect of plant extract on bacterium

Sr. No.	Bacterial Culture	Zone of Inhibition at 15 mg/ml of extract (mm)	Zone of Inhibition at 30 mg/ml of extract (mm)	Zone of Inhibition at 10.0 µg/ml of Ciprofloxacin(mm)
1	*K. pneumonia*	15	--	21
2	*S. epidermidis*	14	--	19
3	*P. vulgaris*	--	12	21
4	*S. aureus*	--	9	18

At 15 mg/ml concentration of extract*K. pneumonia*showed more sensitivity (inhibition zone 21 mm) (Figure 1) than *S.epidermidis* (inhibition zone 19 mm) (Figure 2).

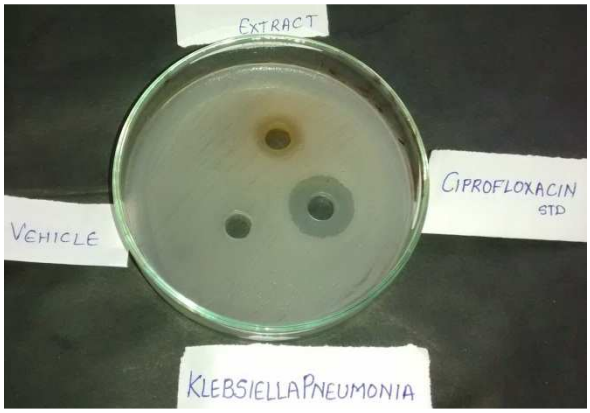

Figure 1:*K. pneumonia*

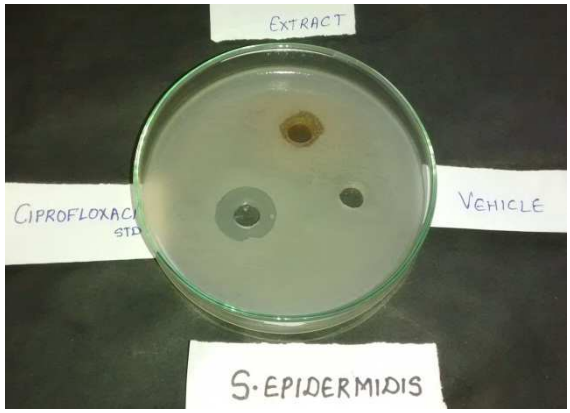

Figure 2:*S. epidermidis*

At 30 mg/ml concentration *P. vulgaris* showed more sensitivity (inhibition zone 12 mm) (Figure 3) than *S. aureus* (inhibition zone 9 mm) (Figure 4).

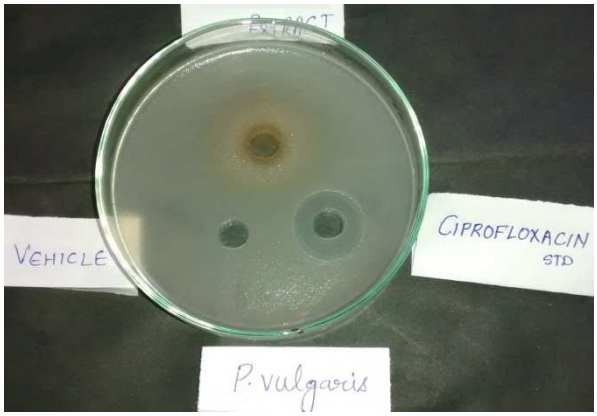

Figure 3:*P. vulgaris*

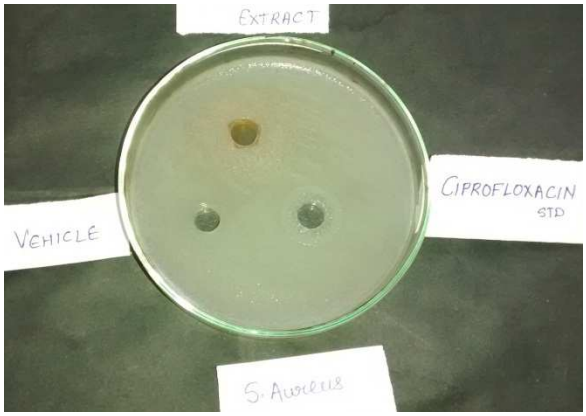

Figure 4:*S. aureus*

The result of present findings indicates that *F. religiosa* fruits extract possesses antibiotic activity against *K. pneumonia, S.epidermidis,P. vulgaris,* and *S. aureus*.

DISCUSSION

Plants are important source of potentially useful chemicals for the development of new chemotherapeutic agents. The first step towards this goal is generally the in vitro antibiotic activity assay [21]. Several workers have reported that many plants possess antibiotic properties including the parts i.e. flower, bark, stem, leaf, etc. Recently, a number of plants have been reported for antibiotic properties across the world [22].

It had been shown that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria [24]. Rathish *et al.* reported the usage of ethanol as a solvent for the preparation of plant extract for antibiotic studies [23]. In the present study, in vitro antibiotic efficiency of the ethanolic extracts of *F. religiosa* fruits extract was quantitatively assessed on the basis of zone of inhibition. Various concentration of extract exhibited varying degree of inhibitory effect against the selected bacterial human pathogens.

In the present study, the analysis of the growth inhibition activity by the disk diffusion method showed that *F. religiosa* possess antibiotic activity against *K. pneumonia, S.epidermidis,P. vulgaris,* and *S. aureus*. The present results support the earlier studies on different extract of stem bark and leaves of *Ficus religiosa*which known to possess the antibacterial activity [9, 15-11].The aqueous extract of the *F. religiosa* stem bark which show antimicrobial activityagainst the*Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Aeromonas hydrophila, Staphylococcus aureus, Streptococcus pyogenes, Aspergillus niger*and *Candida albicans*[15].However methanolic extract of fruits was effective in contrast to the methanolic extract of stem[17].

The antibacterial activity of *F. religiosa* leaves had tested against various bacteria like *P. vulgaris, E. coli, B. subtilis, S.aureus, Pseudomonas aeruginosa* and *K. pneumonia*[9, 15-17]. The current findings supports the earlier work on chloroform extract of *F. religiosa* leaves extractwhich inhibited the growth of various Salmonella species, *P. vulgaris, E. coli, B. subtilis* and *K. pneumonia* [15-16]. Present findings contradict the earlier reports on diethyl ether and methanol extracts of *F. religiosa* leaves which showed maximum inhibition on *S.aureus* followed by *E.coli* and *Pseudomonas aeruginosa*[9].

In present study the MIC values for the extracts indicate that extracts were bactericidal at lower concentration and bacteriostatic at higher concentration which was contradictory to earlier studies on three plants *Mentha longifolia, Melissa officinalis*and *Rosa damascena* in which the plant extracts were bacteriostatic at lower concentration and bactericidal at higher concentration [26]. Therefore it can be logically deduced that *F. religiosa* fruits have better bactericidal potential than *M. longifolia, M. officinalis*and *R.damascene,* thereby indicating that the bactericidal potential of the plant is worth exploring.

The *F. religiosa* fruits extract known to possess several phytochemical like n-Hexadecanoic acid, Hexadecanoic acid, 12-Octadecadienoic acid, 12, 15-Octadecatrienoic acid, Octadecanoic acid and Butyl 9,12,15octadecatrienoatet [10-11, 18-20]. It is yet to be established which of phytochemical fraction or cocktail of phytochemicals is responsible for the antibacterial activity.

CONCLUSION

Present studies opines that *F. religiosa* possess the anti-bacterial activity against the*S. epidermidis, S. aureus, P. vulgaris and K. pneumonia*bacteria. The screening active phytochemical fraction from this important plant to is required for production of new antibiotics.

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