IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
October 2020 Vol.:16, Issue:4

Antimicrobial Activity of *Azadirachta indica* and *Coscinium fenestratum* against *Staphylococcus aureus* and MRSA

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Submission: 20 September 2020
Accepted: 26 September 2020
Published: 30 October 2020

Keywords: *Azadirachta indica*, *Coscinium fenestratum*, Antimicrobial, MRSA, *Staphylococcus aureus*

ABSTRACT

Multi-drug resistance (MR) is a global public health concern. The infections caused by multidrug resistant microorganisms had been increased all over the world. According to the pharmaceutical studies, approximately 10% to 20% of plants are used in western and eastern medical stream. While among those well-known herbal plants *Azadirachta indica* and *Coscinium fenestratum* are main resources for synthesize of many antimicrobial drugs. These plants have been used for medicinal purposes from ancient time. However, the water extract of these plants against the human pathogens specifically *Staphylococcus aureus* and Methicillin resistant S. *aureus* (MRSA) are little or not known. The present study focused on evaluation of the antimicrobial activities of water extracts of two medicinal plants against *Staphylococcus aureus* and Methicillin resistant *S. aureus* (MRSA) using disk diffusion method under normal condition of the microbiology laboratory. The results of Minimum inhibition concentration (MIC) determination showed that the extract ranged from 0.1 – 0.3 mg/ml had MIC against both bacteria effectively. The high effectiveness of extract was found with aqueous extract of bark of *Azadirachta indica* for both tested bacteria. Low MIC is an indication of high efficacy of the plant extract. However, 0.1mg/ml of all extracts are well adequate to control at least the minimum growth of both tested organisms. The antimicrobial susceptibility testing showed that all extracts demonstrated significant activity against S. *aureus* and MRSA. Comparatively *Coscinium fenestratum* (stem) and *Azadirachta indica* (leaves) aqueous extracts showed high antimicrobial activity against S. *aureus* (16 mm at 0.1 mg/ml and 0.4 mg/ml) respectively. The MRSA growth was inhibited maximum by *Azadirachta indica* (leaves) (15.00 mm at 0.3mg/ml) and followed by *Coscinium fenestratum* (stem) (14.16mm at 0.2mg/ml). Generally, the inhibition zones are ranged from 7 – 16 mm in diameter for all extracts. The maximum inhibition caused by the bark extract of *Azadirachta indica* was with S. *aureus* (09 mm at 0.4 mg/ml) and MRSA (10.02 mm at 0.2mg/ml) which are considered to be 'moderate inhibition'. The antimicrobial assay by disk diffusion method revealed that the aqueous extracts of both medicinal plants exhibited effective and broad spectrum activity at low concentrations against the growth of S. *aureus* and MRSA.

1. INTRODUCTION

Global prevalence of infectious diseases caused by human pathogens is a major health problem in all over the world [1, 2]. In recent years the use of medicinal plants become popular for curing these health concern since many medicinal plants are possessing antimicrobial, antifungal, antioxidant etc.[3]. The plant extract, which is also called as natural product because of the unknown availability either as standardized extract or as pure compound although they are potential source of health care. According to pharmaceutical studies, approximately 10% to 20% of plants are used in western and eastern medical stream. While among those herbal plants *Azadirachta indica* and *Coscinium fenestratum* are main resources for synthesize antimicrobial drugs [4]. These plants have been used for medicinal purposes from ancient time. Also, people prefer to use the medicinal plant as much as safe manner including the form of consumption. However, the water extract of these plants against the human pathogen specifically against very important bacteria such as *Staphylococcus aureus* and Methicillin resistant *S. aureus* (MRSA) are little or not known. The present study focused on evaluation of the antimicrobial activities of water extracts of two medicinal plants against the most important human pathogenic bacteria; *Staphylococcus aureus* and Methicillin resistant *S. aureus* (MRSA) using disk diffusion method under normal condition of the microbiology laboratory.

2. MATERIALS AND METHOD

2.1 Selection of test microorganisms

Clinically isolated *S. aureus* (ATCC 26923) and Methicillin resistant *S. aureus* (MRSA) (ATCC25923) were collected from microbiology laboratory, General Hospital, Ampara, Sri Lanka.

2.2 Plant materials preparation

The fresh and healthy leaves and barks of *Azadirachta indica* (Neem) were obtained from the surrounding environment of the Faculty of Applied Sciences, SEUSL. Cut stems of *Coscinium fenestratum* (Venivel, Maramanjal) were purchased from Ayurveda shop. The neem plant's leaves and barks were washed under running tap water followed by sterilized distilled water. After that, fresh plant leaves and barks were shade dried for 2-3 weeks under room temperature

(31°C±0.7) until well dried for grinding[1]. Since the cut-stems of *Coscinium fenestratum* were dried enough during the purchase, they were directly used for grinding into fine powder after cutting into small pieces. The dried plant materials of both plants were ground into a fine powder separately using a mechanical grinder available in Ayurveda hospital, Ampara and that was further sieved through a 100mm sieve. The fine powder was transferred into airtight dark coloured containers and stored at 4°C for further experiments.

2.3 Extraction from plant parts

All parts of both plants in quantities of 1g, 2g, 3g and 4g were taken and mixed with 100ml of sterilized distilled water separately. The soaked mixtures were covered with aluminum foil and left at the orbit shaker at 150 rpm for overnight at room temperature. The extracted solution was filtered through a filter paper (Whatman No.42) and centrifuged at 5000 rpm for 7 min. The supernatant was collected to obtain a clear filtration without crude particles. The extracted solutions were kept in the refrigerator at 4°C in sterile bottles covered with aluminum foil until use.

2.4 Bacteria inhibition assay

2.4.1 Determination of Minimum inhibitory concentration (MIC)

The MIC value was determined by measuring the minimum zone of inhibition caused by the antimicrobial activity of the crude extract.[5] Sterilized susceptibility test discs (6 mm in diameter, Himedia; LOT: 0000384322) were soaked in 100 µl of different concentrations of the effective plant extract (0.1, 0.2, 0.3 and 0.4 mg/ml) separately for 1 hour. Mueller-Hinton agar (Sigma-Aldrich, USA, LOT: 0000378040) was poured into sterile petri dishes and seeded with bacterial suspensions of both tested bacterial (Specifically, 0.1ml of standardized inoculum (0.5×10^7cfu/ml = 0.5 McFarland's turbidity standard). The loaded filter paper discs with different concentrations of the effective plant extract were placed on the Mueller-Hinton agar plates. The plates were kept in the refrigerator at 4ºC for 2 hrs and then incubated at 37 ºC for 48 hrs. The MIC was determined for each type of plant part extract against each type of bacterium by considering the concentration of crude extract which caused minimum inhibition of growth of tested bacteria (at least ≥7 mm zone of inhibition).

2.4.2 Anti-bacterial assay

As stated above, the antibacterial assay was conducted for each pathogen using different extracts from tested plants. A 100µl of each extract was used to soak the sterilized discs for 1 hr and then discs were kept on the inoculated Mueller-Hinton agar plates using sterile forceps. The plates were incubated for 48 hours at 37°C. Commercially available Gentamycin discs (10µg – LOT:421874) were used as positive control while repeating each test at least with three replicates. After incubation, the diameter of the inhibition zones were measured in mm and recorded.[6]

2.4.3 Data analysis

Data were analyzed using one way ANOVA and groups were compared using DMRT. P value 5% ($P<0.05$) was considered significant.

3. RESULTS AND DISCUSSION

3.1 Minimum Inhibitory Concentration (MIC)

The results of MIC determination showed that the extract ranged from 0.1 – 0.3 mg/ml had the MIC against both bacteria (Table 1). The high effectiveness of extract was found with aqueous extract of bark of *Azadirachta indica* for both tested bacteria. Low MIC is an indication of high efficacy of the plant extract while high MIC may indicate less efficient or possible development of resistance by the microorganisms to the antimicrobial.[7] However, 0.1mg/ml of all extracts are well adequate to control at least the minimum growth of both tested organisms.

Table No. 01: Minimum Inhibitory concentration (MIC) of *Azadirachta indica* (dry leaves, bark) and *Coscinium fenestratum (*stem) aqueous extracts (mg/ml) against MRSA and *Staphylococcus aureus.*

	Azadirachta indica		***Coscinium fenestratum***
Bacteria	**Leaf**	**Bark**	**Stem**
Staphylococcus aureus	0.3±0.013a*	0.1±0.005a*	0.3±0.012a*
MRSA	0.1±0.002a*	0.1±0.001b*	0.1±0.030a*

Values represents the mean±SD * Significant at P<0.05.

3.2 Antimicrobial activity of aqueous extracts

The plant have traditionally provided a source of hope for novel drug compounds, as plant herbal mixture have made large contributions to human health and wellbeing. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment.[8] The antimicrobial susceptibility testing shows that all the extracts demonstrated significant activity against *Staphylococcus aureus* and MRSA (Table 2). Comparatively *Coscinium fenestratum* (stem) and *Azadirachta indica* (Leaves) aqueous extracts showed high antimicrobial activity against *Staphylococcus aureus* (16 mm at 0.1 mg/ml and 0.4 mg/ml) (Table 2 and Figure 1) respectively.

Table No. 02: Antibacterial activity of *Azadirachta indica* dry leaves, bark aqueous extracts and *Coscinium fenestratum* bark aqueous extracts against MRSA and *Staphylococcus aureus.*

Con. of extract mg/ml	**Inhibition zone diameter (mm)**						
	***Azadirachta indica* (leaves)**		***Azadirachta indica* (bark)**		***Coscinium fenestratum* (stem)**		**Positive control (Gentamycin)**
	S. *aureus*	MRSA	S. *aureus*	MRSA	S. *aureus*	MRSA	
0.1	16.00±3.61a	08.00±0.01b	07.00±0.05d	07.00±0.00a	15.00±0.03a	09.00±0.02b	18.33±0.33a
0.2	15.30±0.57a	13.16±0.76d	07.00±0.04b	10.02±0.50c	15.30±0.57a	14.16±0.05c	18.33±0.33c
0.3	15.00±1.00b	15.00±0.05c	07.80±0.01c	10.00±0.00b	15.00±1.00a	14.00±0.60c	18.33±0.33b
0.4	16.00±0.00c	15.00±0.01b	09.00±0.02c	08.00±0.00b	16.00±0.00a	14.00±0.02c	18.33±0.33d

The letters represent with mean±SD are at P<0.05.

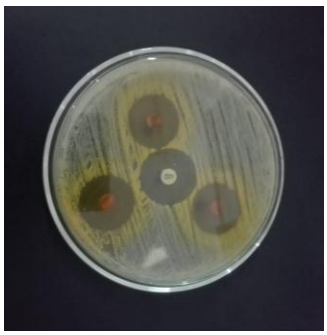

Figure No. 01: Antimicrobial activity (inhibition zone -16mm) of *Coscinium fenestratum* against *Staphylococcus*

Figure No. 02: antimicrobial activity (inhibition zone – 9mm) of *Azadirachta indica* bark aqueous extract against *Staphylococcus aureus*

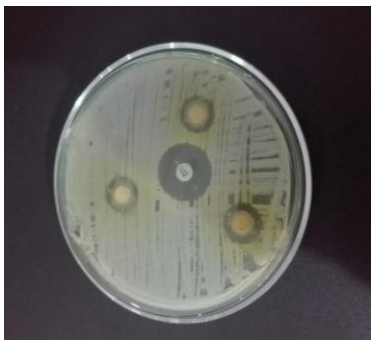

Figure No. 03: antimicrobial activity (inhibition zone – 9 mm) of *Coscinium fenestratum* against *MRSA*

The MRSA growth was inhibited maximum by *Azadirachta indica* (leaves) (15.00 mm at 0.3mg/ml) and followed by *Coscinium fenestratum* (stem) (14.16mm at 0.2mg/ml). Generally, the inhibition zones are ranged from 07 – 16 mm in diameter for all extracts tested (Figures 1, 2 and 3). The maximum inhibition caused by the bark extract of *Azadirachta indica* was with *Staphylococcus aureus* (09 mm at 0.4 mg/ml) and MRSA (10.02 mm at 0.2mg/ml) which are considered to be 'moderate inhibition' (Table 2 and Figure 2) of growth of both bacteria when compare to other types of extracts. The antimicrobial assay by disk diffusion method revealed that the aqueous extracts of both medicinal plants exhibited effective and broad spectrum activity at low concentrations against the growth of S. *aureus* and MRSA. The present investigation corroborates with studies of many authors.[4, 8]

4. CONCLUSION:

The antimicrobial assay by disk diffusion method revealed that the aqueous extracts of both medicinal plants exhibited effective and broad spectrum activity at low concentrations against the growth of S. *aureus* and MRSA.

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