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Antibacterial Activity of Extracts of *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* Against Antibiotic-resistant *Staphylococcus cohnii*

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ABSTRACT

Background: The antibacterial activity of plant extracts and phytochemicals analysis was evaluated with antibiotic susceptible and resistant micro-organisms. In addition, of the possible coefficient effects when associated with antibiotics were studied. Extracts from the following plants *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* were utilized. **Materials and Methods:** Collected clinical samples were inoculated in different media and the plates were incubated at 37°C for 48 hr. After incubation to identified Colony Morphology, Microscopic Observation and biochemical characterization and molecular identification of the Selected Bacterial isolate as *Staphylococcus cohnii*. Extracts from the following plants were *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* were utilized. Antimicrobial Susceptibility Assay and Minimal inhibitory concentration tests performed. **Results:** Ethanol, methanol and chloroform extracts of *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* gave the maximum zones of inhibition of 20 mm, 9 mm in ethanol, 25 mm to17 mm in methanol and 24 mm to 12mm in chloroform extract and MIC values of 1.03 to 0.51 mg/ml, 1.00 to 0.06 mg/ml and 1.03 to 0.60 mg/ml respectively, against *Staphylococcus cohnii*. Methanol extracts of *Solanum xanthocarpum* gave the maximum zones of inhibition of 25 mm followed by ethanol 20 mm and 24 mm chloroform extracts. The highest MIC values 0.06 mg/ml in methanol extract respectively and 0.51mg/ml in ethanol and 0.60mg/ml in chloroform extract against *Staphylococcus cohnii*. The result indicated that all extracts exhibited antibacterial activity against *Staphylococcus cohnii*. **Conclusion:** The methanol extract showed greater activity than ethanol and chloroform extracts. Among various extracts, only the methanol extract show potential agents against bacterial *Staphylococcus cohnii* strain greater than standard Vancomycin test control. Thus, the extract of *Solanum xanthocarpum* could be used to treat microbial wound infection. *Solanum xanthocarpum* have the potential to be developed as antibacterial agents, especially against *Staphylococcus cohnii* strain.

Keywords: *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose, Staphylococcus cohnii*.

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INTRODUCTION

Hospital associated infection (HAI) or Nosocomial infections are developed with the hospitals as major sources of infectious agents and were estimated to infect at least 7 to 10% of all inpatients during their admission.[1] Moreover, hospital environments renders added risks to patients with weakened health conditions more susceptible to infections by antibiotic resistant opportunistic pathogens.[2] The World Health Organization (WHO) issued its first ever list of antibiotic-resistant 'priority pathogens', the most prevalent and antibiotic resistant bacterial pathogens associated with nosocomial infections.[3] Infection in a wound delays healing and may cause wound break down, herniation of the wound and complete wound dehiscence. Therefore, the knowledge of the causative agents of Nosocomial infection will assist in the control and prevention of such infection and also help in selecting theoretical antimicrobial therapy for the control of causative micro-organisms.

Antibacterial resistance is defined as the resistance of bacteria to treat with most of the antibiotics that was originally found to be inhibition of wound infection caused by pathogenic micro-organism. This means most of the antibiotics become ineffective against pathogenic bacteria allowing severe infections to persist in patients, increased risk of patient's health condition the worse clinical outcomes and finally death. In fact, on average, the mortality rate for patients affected in many infection particularly diabetic patients affected in wound infections caused by resistant and non-resistant bacteria is less than half of that of people with a resistant form of the same infection. Antibiotic resistance is present worldwide and new resistance mechanisms are continuing to emerge, strongly increasing the highly risk of spread of resistant strains. Those antibiotic resistance represents a threat to global public health and represents a major economic issue in worldwide, due to the higher health care costs of necessary treatments and the increased duration of illness, treatment and potential hospitalization when compared with pathogenic micro-organisms, common diabetic wound infections. It has been scientifically proven that the indiscriminate and inappropriate use of antibiotics has accelerated the emergence of multidrug-resistant strains. In addition, poor sanitary conditions and inadequate food-handling encourage the further more spread of antimicrobial resistance. Considering that antibacterial resistance is a serious problem, driven by many interconnected factors, the World Health Organization suggests a series of concerted and coordinated actions.[4]

Even though pharmacological industries have produced a number of new antibiotics to resistant and non-resistant micro-organisms, but resistance to multidrug by micro-organisms has increased. In general, most of the micro-organisms have the genetic ability to mutant and getting resistance against antibiotics, which are utilized as therapeutic agents. Such a fact is cause for concern, because most of the patients

in hospitals who have suppressed immunity and due to new microbial strains, which are modified multidrug resistant. In consequence, new infections can occur in patients in hospitals resulting in high mortality.[5]

The antimicrobial properties of medicinal plants have been investigated against microbial disease by a number of researchers in worldwide, especially in America. In India, Argentina, a research tested many known medicinal plants used for therapeutic treatments for multi-drug resistance micro-organisms. Among the bioactive compounds extracted from these medicinal plants, twelve plants inhibited the growth of *Staphylococus aureus*, ten medicinal plants inhibited *Escherichia coli* and four plants inhibited on fungal species such as *Aspergillus niger* and also reported that the most effective compound was one plant extracted from *Tabebuia impetiginosa*. The antimicrobial properties of bioactive compounds exist from *Parthenum argentatum* against *Candida albicans, Torulopsis, Hansemula, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was detected.[6] Work done was observed that the active compounds extracted from nine known plants in Uruguai did not give any activity against some yeasts like *C. albicans* and *Saccharomyces cerevisiae*, but inhibited the some bacterial growth of *Bacillus subtilis*, *E. coli* and *P. aeruginosa*.[5]

Effects of phytochemicals were conducted and it was observed the antibacterial activity of anacardic acid treated with *Staphylococcus aureus, Brevibacterium ammoniagenes, Streptococcus mutans* and *Propionibacterium acnes*. The plant extract was tested the bactericidal activity of anacardic acid and totarol on methicillin resistant *S. aureus* (MRSA) and the synergistic effect of anacardic acid compounds associated with methicillin antibiotic.[7]

Hence, more studies investigated to the use of medicinal plants as used therapeutic agents should be accentuate, especially herbal medicines used to many microbial illness to control of antibiotic resistant microbes. The aim of this research was to evaluate the potential of medicinal plant extracts and phytochemicals on resistant bacterial strains as well as multi-drug resistant bacteria, which were isolated from infected patients in hospital environment. Moreover, we investigated the synergistic effects of plant extracts with antibacterial activity in association with antibiotics against drugs resistant bacteria.[5]

MATERIALS AND METHODOLOGY

Isolation of Nosocomial pathogen

Wound samples were collected from infected patients in Government Hospital, Namakkal, Tamil Nadu, India. The samples were collected in a sterile swab, immediately transported and inoculated on nutrient broth and selective media like Eosin Methylene Blue Agar, MacConkey Agar, Nutrient Agar, Cetrmide agar and Mannitol Salt Agar plate incubatedat 37°C for 48 hr.

Identification of the isolated micro-organisms

The isolated organisms were identified by morphological examination and biochemical characterizations. Morphological test, Gram's staining, Endospore Staining, Motility test. Biochemical test Indole test, methyl red test, Voges Proskar test, citrate utilization test, catalase test, oxidase test nitrate reduction test and Carbohydrate fermentation test by standards methods Bergey's Manual of Systematic Bacteriology.[8] 16S rRNA sequencing was carried out to identify the species.

Molecular Identification of the Isolates

Phylogentic Analysis

The partial 16S rRNA sequences was retrieved on NCBI server (http://blast.ncbi.nlm.nih.gov/Blast) using BLAST tool.

Maintenance of Bacterial Isolate

The isolated bacterial culture was maintained in Nutrient Agar slants and stored at 4°C to 7°C for future use. Subculture was performed every ten days interval.

Collection and Preparation of Plant Extract

For the present study three plants such as *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* were collected from different locations of Kollihills, Namakkal, Tamilnadu, India.

The collected plants were authenticated by Botanical Survey of India (BSI-Southern Circle) - Government of India, Coimbatore, Tamilnadu. Authentications of plants are endorsed by reference letter No: BSI/SRC/5/23/2019-Tech/3136. The Voucher specimens were deposited in Department of Microbiology, Muthayammal College of Arts and Science, Rasipuram, Tamil Nadu, India.

The collected plants were washed with running tap water. After cleaning of the medicinal plants were allow to drying under shade and to avoid protect from surrounding contamination and dust present in the area. The drying was done in a room for about ten to fifteen days, without any exposure to direct sun light. After completely drying the plants, obtain uniform sized powder use mixer grinder and ensure to fine powder and enhance the plant powder for better extraction process.[9]

Soxhlet Extraction

Each plant powder measured (10gms) were extracted with 100 ml of ethanol, methanol and chloroform, on water bath at 70°C for 2 hr. The extracted samples were centrifuged at 2500 rpm for 15 min and filtrate was stored in sterile bottles at 5°C for further use. The concentration was prepared diluting the solid residue with Dimethyl Sulfoxide (DMSO). These plant extracts were used for the phytochemical analysis such as mayers test for alksloids, Molish's test for Carbohydrates, Froth test for Saponins, Alkaline reagent test for Flavonoids etc,. And *in vitro* bioassays of bioactive compounds and antimicrobial activity.

Phytochemical Screening

Phytochemicals are a large group of chemical compounds like Alkaloids, Flavonoids, Phytosterols, Phenols, Tannins, Diterpenes naturally occurring in plants, fruits and vegetables. Bioactive compounds conferring color, flavor, aroma and texture. These compounds have been developed over thousands of years of evolution to defend organisms from the effects of free radicals, viruses, bacteria, protozoa and fungi. They are widely distributed in leaves, fruits, vegetables, roots, stems, legumes, whole grains, nuts, seeds, fungi, herbs and spices and in plant-based beverages such as wine and tea. Major classes of phytochemicals (Diterpenes, tannins, alkaloids, phenolic compound, cyanogenic glycosides, cardiac glycosides, sugars, Phytosterols, saponins and flavonoids) were determined by standard qualitative methods described by.[10,11]

Antibiotic sensitivity test

The sensitivities of the isolated bacterial species against different antibiotics were tested based on the disc diffusion (Kirby–Bauer) technique (Table 1).[12]

Antimicrobial Susceptibility Assays (Disk Diffusion method)

The antibacterial susceptibility was initially test by the agar disk diffusion method. Four concentrations (25, 50, 75, 100 µl) of each plant extracts were prepared with 10% DMSO. Bacteria cell suspensions were adjusted to 0.5 McFarland turbidity standards to prepare 1×108 bacterial/ml

inoculum. Each bacterial cell suspension was swabbed on Mueller-Hinton agar plates and the plates were then allowed to dry for 5 to 10 min. The sterile filter paper disks (Whatman No. 1, diameter-6mm) were soaked in each plant extract. The extract-soaked filter paper disks were then placed on the inoculated in to Mueller-Hinton agar plates. Vancomycin (30μg) disk was used as the positive control. The plates were incubated at 35 ± 2°C for overnight. After incubation, measure the zones of inhibition were recorded.

Minimal Inhibitory Concentration (MIC) of plant extracts against antibiotic resistant *Staphylococcus cohnii*

The antibacterial activity was assessed by Minimal inhibitory concentration of the following plants such as *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* methanol, ethanol and chloroform extracts were prepared. Plant extracts that gave a maximum inhibition for the disk diffusion assay method were used to determine MIC using the micro plate dilution method.[13] Serial dilutions for 5-fold of the plant extracts were prepared with 10% DMSO, yielding seven serial dilutions of the each plant extracts. The bacterial cell suspension were prepared in Mueller-Hinton broth and the turbidity was adjusted to approximately 0.5 by McFarland turbidity standard to prepare 1×108 bacteria/ml. 150 μl of plant extract was added to each well. 50 μl of bacterial cell suspension was added to each well. Vancomycin drug was used as the positive control. Microtiter plates were incubated at 35±2°C for 24hr. Antibacterial activity was assessed by spectrophotometer at 630nm.

RESULTS

Isolation and Identification

Totally 10 wound samples were collected from infected patients. One predominant bacterial isolate were selected for further studies. The Colony Morphology, Microscopic Observation and biochemical character the Selected Bacterial isolates was identified as *Staphylococcus cohnii.*

Molecular Identification of the selected isolates

The isolate suspected to be *Staphylococcus cohnii* was subjected to molecular identification by 16S ribosomal RNA gene sequencing and the isolate was confirmed as *Staphylococcus cohnii* strain NP-23. The gene sequencing was submitted to the NCBI and Accession number (MN209917) was obtained Figure 1.

Table 1: Antibiotic Sensitivity Test for *Staphylococcus cohnii*.

Name of the antibiotics	*Staphylococcus cohnii*		
	Zone of inhibition (mm)		
	Sensitive	Intermediate	Resistance
Amikacin	-	-	10
Ampicillin	-	17	-
Ciproflaxacin	22	-	-
Clindamycin	23	-	-
Erythromycin	-	19	6
Methicillin	-	-	8
Vancomycin	25	-	-
Tetracycline	-		6
Penicillin	24	-	-

Antibiotic Sensitivity Test for *Staphylococcus cohnii*

The results for antibiotic sensitivity test of the *Staphylococcus cohnii* were summarized in Table 1.

Preliminary Phytochemical screening of selected medicinal plants

In the present study, when performed qualitative tests for phytochemicals in *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose*, a number of photochemical shows positive results in their specific tests tabulated in Table 2.

Antibacterial Activity of Selected medicinal Plants against *Staphylococcus cohnii*

The antibacterial activities of selected medicinal plant extracts according to the zone of inhibition ranged between 9 and 25mm. Maximum zone of inhibition was observed from methanol extract of *Solanum xanthocarpum* (25mm) and minimum zone of inhibition was given by the ethanol extract of *Capparis spinose* (9mm) Table 3 and Plate 1. However, due to the different concentrations of plant extracts, only effectiveness of the plant extracts cannot be accurately compared by comparing the respective diameters obtained in the disc diffusion assay. Hence, MIC was used to determine the only effectiveness of the antibacterial activity against plant extracts accurately.

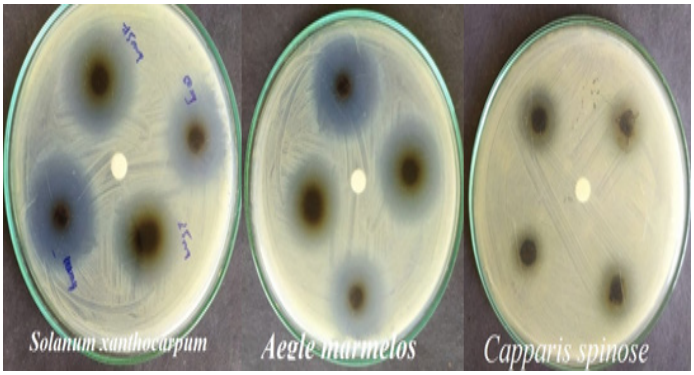
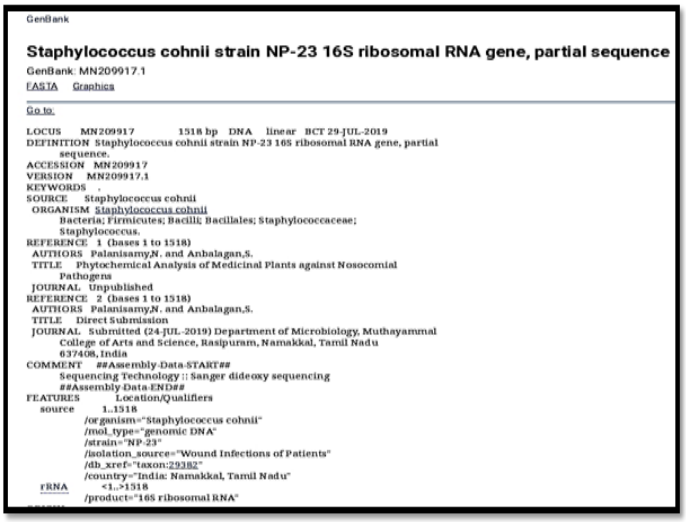


Plate 1: Antibacterial Activity of Selected medicinal Plants against *Staphylococcus cohnii.*


GenBank

Staphylococcus cohnii strain NP-23 16S ribosomal RNA gene, partial sequence

GenBank: MN209917.1

FASTA Graphics

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  JOURNAL   Unpublished
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Figure 1: 16S rRNA sequence of *Staphylococcus cohnii.*

Table 2: Phytochemical Screening of plant extracts.

S.NO	Phytochemicals	*Solanum xanthocarpum*			*Aegle marmelos*			*Capparis spinose*		
		Methanol	Ethanol	Chloroform	Methanol	Ethanol	Chloroform	Methanol	Ethanol	Chloroform
1.	Flavonoid	+++	+++	-	+++	+++	-	+++	+++	-
2.	Alkaloids	+	+	+	++	+	+	+	+	+
3.	Saponin	+++	+++	-	+++	+++	-	+++	+++	-
4.	Phenolic compound	++	+	++	+++	+	++	++	+	++
5.	Sterol	+	+	+	++	+	+	+	+	+
6.	Triterpenoids	++	++	-	++	++	-	++	++	-
7.	Protein and Amino acids	-	-	-	+	-	-	-	-	-
8.	Carbohydrates	+	+	+	++	+	+	+	+	+
9.	Oils and fats	-	-	-	+	-	-	+	-	-
10.	Tannin	++	++	+	-	-	-	-	-	-
11.	Resins	++	+	-	+++	++	+	+++	++	+

+++: Abundantly present, ++: Moderately present, +: Present, -: Absent.

Table 3: Effect of different solvents on the antibacterial activity of selected plants against *Staphylococcus cohnii*.

Plants Name	Ethanol				Methanol				Chloroform			
	25 µl	50 µl	75 µl	100 µl	25 µl	50 µl	75 µl	100 µl	25 µl	50 µl	75 µl	100 µl
Solanum Xanthocarpum	13	15	17	20	19	20	22	25	-	-	-	-
Aegle marmelos	11	13	14	17	15	17	19	23	18	19	21	24
Capparis spinose	9	11	11	10	12	13	17	20	12	12	14	15

Minimum Inhibition Concentration (MIC) of plant extracts

The MIC values obtained from *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* plants exhibited antibacterial activity ranged between 0.003 and 2.4 mg/ml (Table 4). Ethanol, methanol and chloroform extracts of *Solanum xanthocarpum* gave MIC of 1.01mg/ml, 0.06mg/ml and 1.03mg/ml respectively, *Aegle marmelos* gave MIC values of 0.51 mg/ ml, 0.42 mg/ml and 0.60 mg/ml, respectively, against *Staphylococcus cohnii*. Methanol and chloroform extracts of *Capparis spinose* gave MIC values of 1.0 mg/ml and 0.72 mg/ml, respectively, against *Staphylococcus cohnii*. Therefore, the maximum antibacterial activity was observed for the methanol extract of *Solanum xanthocarpum* against *Staphylococcus cohnii*.

Table 4: Minimal Inhibitory Concentration (MIC) of plant extracts against antibiotic resistant *Staphylococcus cohnii*.

Plants Name	Minimal Inhibitory Concentration (mg/ml)		
	Ethanol	Methanol	Chloroform
Solanum Xanthocarpum	1.01	0.06	1.03
Aegle marmelos	0.51	0.42	0.60
Capparis spinose	-	1.00	0.72

DISCUSSION

Increasing number of multi-drug resistance micro-organisms in human beings and animals as well as unwanted side effects of certain antibiotics has stimulate enormous interest to search for new antimicrobial herbal drugs of plant origin.[14] Similar studies on phytochemical analysis have been carried out by different groups. Presence of alkaloids, saponins, tannins, flavonoids, glycosides, coumarins, flavonoids, carbohydrates and protein were recorded in the present study. These findings are in accordance to a same study by Yadav and Agarwala,[15] who used different methods of extraction of plant parts such as leaves, stems, roots etc,. To produce a marked difference in the yield and time of extraction and their findings provided evidence that crude ethanol extract and some organic solvent extracts of these tested plants contain medicinally important bioactive compounds alkaloids, saponins, tannins, flavonoids, glycosides, coumarins, flavonoids, carbohydrates and protein and it justifies their use in the traditional herbal medicines for the treatment of many diseases.

Poonkothai and Saravanan[16] reported methanolic extracts give maximum zone of inhibition (13mm, 10mm, 8mm), followed by chloroform extract (10mm, 8mm, 4mm) and aqueous extract (4mm, 2mm) of *A. marmelos* were found to have maximum inhibitory activity against *Proteus mirabilis* and the zone of inhibition coincides with tetracycline. The other bacteria exhibited minimum zone of inhibition against the different plant extracts. Similar result, Thakur *et al.*[17] reported to the cinnamon extracts was no inhibition on Gram-negative bacteria, but ginger extract more effective on gram negative bacteria. Cinnamon has many bioactive compounds including carbohydrates, protein, alkaloids, phenols, flavones, tannins, quinones, terpenoids and glycosides known to hold antibacterial activity. Mujeeb *et al.*[18] determined the phytochemical evaluation and determination of Bioactive Components mainly alkaloids, phenols and flavones from *Aegle marmelos* plant. *Aegle marmelos* has been recognized as a bioactive components of traditional medication for the treatment of various human and animal's ailments. The crude ethanolic extracts of *A. marmelos* release the several bioactive compounds with the highest

quantity of flavonoids, alkaloids and phenols etc., Our study was also found to correlate with the results of on phytochemicals extracted from the leaves of *S. xanthocarpum*.[19] Rana *et al.*[20] reported methanolic extract of *Solanum xanthocarpum* was found to be maximum inhibition against *S. aureus* at (18mm at 100%), (15mm at 70%), (13mm at 50%), (11mm at 30%), followed by *E. coli* at (15mm at 100%) followed by (13mm at 70%), (10mm at 50%), (9mm at 30%) and *Y. pestis* at (15mm at 100%), (13mm at 70%), (10mm at 50%), (9mm at 30%) and minimum inhibition in *Pseudomonas aeruginosa* (14mm at 100%), (12mm at 70%), (10mm at 50%) and (9mm at 30%). The present study was concluded from the results that methanolic as well as acetone leaf extract of *S. xanthocarpum* were maximum effective on gram positive *Staphylococcus aureus* which is considered as a serious human pathogen causing diabetic wound infections. Possible reason for this antibacterial activity of *S. xanthocarpum* are presence of bioactive compounds such as alkaloids, phenolics and flavanoids in its leaves.[21] Razik[22] reported Hexane extract of *Capparis spinosa* was less effective than methanol extract effective against tested bacteria. Solvents (negative controls) used for preparation different concentrations showed no activity against wound pathogens. Kubmarawa *et al.*[23] reported that Ethanolic extracts of 50 medicinal plant species were screened for their antibacterial activity against gram negative bacteria like *Escherichia coli, Pseudomonas aeruginosa,* some gram positive bacteria such as *Bacillus subtilis, Staphylococcus aureus* and yeast as *Candida albicans*. The results indicated totally 50 plant extracts, among the fifty plant extract 28 plant extracts inhibited the bacterial growth of minimum one or more pathogens. But four plant extracts showed a maximum antibacterial activity against gram positive bacteria. Phytochemical investigation revealed the presence of tannins, alkaloids, saponins, flavonoids glycosides and essential oils from plant extracts. Majority of phytochemical components are known to produce the therapeutic activity like antimicrobial and antioxidant etc. The present findings support the applicability of *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* in traditional system for its claimed uses and can be recommended by the scientific community as an accessible alternative to synthetic antibiotics. This study is a preliminary evaluation of antibacterial activity of *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* and isolation of the compounds responsible for antibacterial activity would be taken up later.

CONCLUSION

In the present study was concluded that antibacterial activity of *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* against antibiotic resistant *Staphylococcus cohnii* the presence of active component like saponins, flavonoids and resin from methanol and ethanol extracts. Secondary metabolites are the classes of bioactive compounds which are known to show curative activity against several human diseases and therefore could explain the use of traditional medicinal plant for treatment of some illnesses. In conclusion, the study findings support the use of these three plants *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* Methanol extract 100µl most effective treatment of wound infectious diseases caused by antibiotic resistant *Staphylococcus cohnii*. The future study are focused on separation of the bioactive compounds in *Solanum xanthocarpum, Aegle marmelos* and *Capparis spinose* extracts which could be used to development of new antimicrobial drugs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

HAI: Hospital acquired infection; **MIC:** Minimal Inhibitory concentration; **MRSA:** Methicillin resistance *Staphylococcus aureus*; **DMSO:** Dimethyl sulfoxide.

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