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Evaluation of ANTI-MRSA Activity of Moringa Oleifera Seeds, Glycyrrhiza Glabra Stems and Jasminum Sambac Leaves

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Abstract:- The aim of the present study was to asses anti-MRSA (Methicilin resistance Staphylococcus aureus) activity of Moringa oleifera seeds, Glycyrrhiza glabra stems and Jasminum sambac leaves. Antimicrobial activities of ethanol, methanol, hot water and cold water extracts of Moringa oleifera seeds, Glycyrrhiza glabra stems and Jasminum sambac leaves were tested against Methicilin resistance Staphylococcus aureus (MRSA) by agar well diffusion method.

Method

Antibacterial activity of all the extracts of Moringa oleifera seeds, Glycyrrhiza glabra stems, and Jasminum sambac leaves were determined by agar well diffusion method at the concentration 500 mg/ml. The controls used were standard antibiotic discs of amoxillin (30mcg), ampicillin (30mcg), oflaxine (5mcg), methicillin (15 mcg) and cefpodoxime (15mcg).

Results

After incubation at 37^0C for 24 hours zones of inhibition were measured.

Conclusion

These findings suggested methanol extract of *Glycyrrhiza glabra* stems showed high antibacterial activity against Methicilin resistance *Staphylococcus aureus* (MRSA).

Keywords:- *MRSA, Agar well diffusion, minimum inhibition concentration and antibacterial activity.*

I. INTRODUCTION

Treatment of infections caused by the *Staphylococcus aureus* has become more difficult because of the emergency of multi-drug resistance isolates (Chambers *et al*.,2009 and Franklin,2003). Methicilin resistance *S.aureus* (MRSA) presents problems for the patients and healthcare facility staff whose immunity is compromised, or who have open access to the bodies via wounds, catheters or drips. The infection spectrum ranges from superficial skin infections to more serious diseases such as bronchopneumonia (Enright *et al*.,2002).

Failure of antibiotics to manage infections caused by multidrug resistance (MDR) pathogens, especially by MRSA, has triggered much research efforts for finding alternative antimicrobial approach with high efficiency and less resistance developed by the microorganisms (Harbottle *et al*.,2006).

Antibiotic resistance is the ability of a microorganism to with stand the effects of an antibiotic. The extensive use of antibiotics over the last 50 years has led to the emergency of bacterial resistance and dissemination of resistance genes among the pathogenic microorganisms. *Staphylococcus aureus* is one of the most important pathogens that can cause suppuration, abscess formation, a variety of pyogenic infection and even fatal septicemia in human being. MRSA is still considered as an emerging pathogen and public health threats results from the spread of hospital-acquired as well as community –acquired MRSA (Franci *et al*., 2015).

Staphylococcus aureus is an important cause of serious infections in both hospitals and community. Methicilin resistant *S. aureus* (MRSA) include those strains that have acquired a gene giving them a resistance to methicillin antibiotics and essentially all other beta-lactam antibiotics. MRSA was first reported in 1961, soon after methicilin was introduced into the human medicine to treat penicillin resistant staphylococci. This group of organism has emerged as a serious concern in human medicine. Although these organisms cause the same type of infections as other *S. aureus*, hospital-associated strains have become resistant to most common of antibiotics, and treatment can be challenging (Boucher *et al*., 2008).

Many plants have been investigated scientifically for antimicrobial activity and large number of plants products have been shown to inhibit growth of pathogenic bacteria. Many of the plants have been investigated for the novel drugs or templates for the development of new therapeutic agent (Samy *et al*., 2010).

II. MATERIALS AND METHODS

A. Collection of plant materials

Glycyrrhiza glabra stems and *Moringa oleifera* seeds were purchased from traditional medicine shop and *Jasminum sambac* leaves were collected from Ellispettai, Erode, Tamilnadu India. Collected plant material was washed with water and dried at room temperature. The dried sample was homogenized into a fine powder and used as a raw material and stored in air tight container for further use.

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Jasminum sambac leaves


Glycyrrhiza glabra stems


Moringa oleifera seeds

B. Extraction of plant materials

- *Hot Aqueous extraction*

5.0 gram of dried powder of *Glycyrrhiza glabra, Jasminum sambac* and *Moringa oleifera* was suspended in 50 ml of water and mixture was soaked for 24 hours. The suspended solid was filtered through whatman No.1 filter paper and kept in water bath at 80˚C for 2 hours. The dried crude extracts were stored at 4˚C for further use.

- *Cold Aqueous extraction*

5.0 gram of dried powder of *Glycyrrhiza glabra, Jasminum sambac* and *Moringa oleifera* was suspended in 50 ml cold distilled water and mixture was soaked for 24 hours. The suspended solid was filtered through whatman No.1 filter paper and the dried crude extracts were stored at 4˚C for further use.

- *Solvent extraction*

5.0 gram of dried powder of *Glycyrrhiza glabra, Jasminum sambac* and *Moringa oleifera* suspended in 100 ml of solvents (Aqueous, Ethanol and Methanol) and the mixture was soaked for 24 hours. The suspended solid was filtered though whatman No.1 filter paper and kept in water bath at 80˚C for 2 hours. The dried crude extracts were stored at 4˚C for further use.

C. Bacterial strain isolation

A total of 5 Strains of wound pathogens such as *S. aureus* were isolated from patients infected with wounds. Isolates were maintained at 4ºC in nutrient media.

D. Isolation of pathogens

An aseptically collected wound samples were inoculated with the help of standard inoculating loop on the EMB, Mac conkey, and Nutrient Agar. The plates were incubated for 24 hours at 37˚C. Following incubation, the growth of bacterial colonies was observed and results were recorded.

E. Identification of pathogens

The isolated pathogens were identified on the basis of Gram's reaction and biochemical characteristics (Mac Faddin, 1980) and results were identified with the help of Bergey's Manual of systematic Bacteriology.

F. Antibiotic susceptibility Test (Disk diffusion method)

Muller Hinton Agar was prepared and the medium was sterilized by autoclaving at 121^0C for 15 minutes at 15 psi pressure and was used for tests. Sterile agar was poured aseptically into sterile petridishes (20 ml each) and the plates were allowed to solidify at room temperature in sterile condition. After that the plates were seeded with bacterial strains by streaking evenly on to the surface of the medium with a sterile cotton swab. Antibiotic discs of Ampicillin, Methicllin, Amoxillin, Oflaxine, and Cefpodoxime were placed on to the agar with flamed forceps. The plates were incubated at 37^0C for 24 hours and then the zones of inhibition were measured. The results were read by the presence or absence of zone of inhibition (Baker *et al*., 1983).

G. Agar well diffusion assay

Antibacterial activity of all the extracts of *Moringa oleifera* seeds, *Glycyrrhiza glabra* stems, and *Jasminum sambac* leaves were determined by agar well diffusion method at the concentration 500 mg/ml using Muller Hinton Agar (Himedia). The defatting was diluted in dimethyl sulphoxide (DMSO).Pure DMSO was taken as the control. The experiment was performed three times. Microbial growth was determined by measuring the diameter of the zone of inhibition (Nair *et al*., 2005).

H. Minimum inhibition concentration (MIC) – Micro dilution

Minimum inhibition concentration was done by the lowest concentration of the *Glycyrrhiza glabra* stems and *Moringa oleifera* seeds extract to access the bactericidal and bacteriostatic effect. The test was performed in 96 well microtiter plates. Micro titer plate wells from each column in row 1 were marked and 100µl (500mg/ml) of stock (cold aqueous *Moringa oleifera* and Methanol extract of *Glycyrrhiza glabra*) was added. 50µl of sterile distilled water was added to rows 2-12. Two fold serial dilutions were performed by transferring 50µl of solution from row 1 to 2, using a multichannel pipette. This was repeated down the row 2 to 12. 40µl of double strength nutrient broth and 10µl of bacterial culture was added to all the wells in separate column, so the final concentration of the inoculum in all the wells. To prevent dehydration, the plates were covered with a plastic cover and then incubated at 37ºC for overnight. The bacterial growth was determined after addition of 40µl of 2, 3, 5 Tri Phenyl Tetrazolium Chloride Red (0.02mg/ml) (Kumarasamy *et al*.,2005).

III. RESULTS AND DISCUSSION

A. Isolation and identification of pathogens

Various biochemical tests were done to identify the pathogen isolated from wound samples. The result obtained is given in the Table -1. From 15 bacterial strains isolated from wound samples, all the strains were identified as

Staphylococcus aureus based on the morphological and biochemical characters.

NAME OF THE TEST	RESULT
Gram stain	Gram positive
Indole test	-
Methyl red test	+
Vogus proskover test	_
Citrate test	+
Catalase test	+
Nitrate reduction test	+
TSI-Glucose	+
TSI-Mannitol	+
TSI-Lactose	+
TSI-Sucrose	+

Table 1. Biochemical characterization of *staphylococcus aureus* isolated from clinical wound samples

'+' indicates Presence and '-' indicates Absence

B. Antibiotic susceptibility test against Staphylococcus aureus

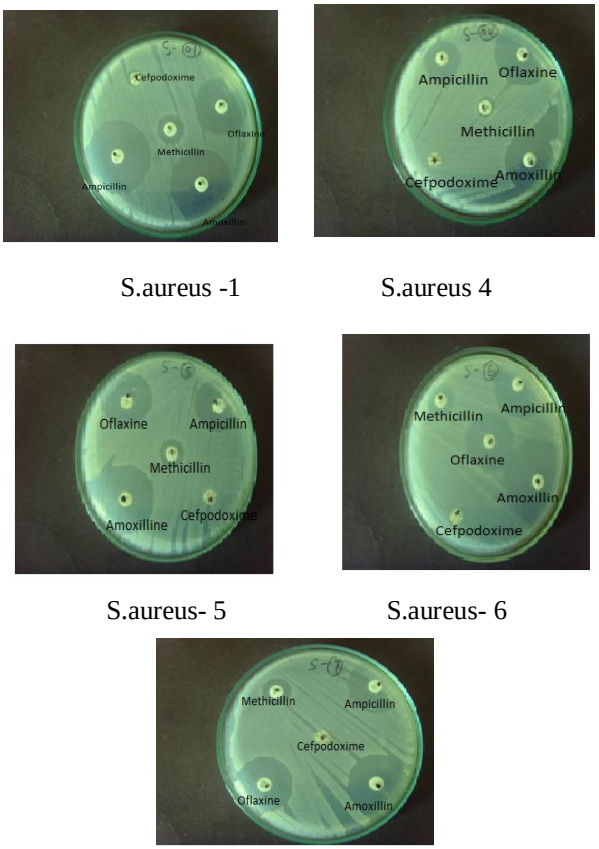
Methicillin displayed a high level of resistance. Of laxine, Amoxillin and Ampicillin shown a high level of sensitivity against *Staphylococcus aureus* strains. Results were shown in the table below.

Out of these 15 strains 5 were selected according to drug resistant and named as *Staphylococcus aureus* strains 1, 4, 5, 6, and 7. Methicillin displayed a high level of resistance followed by cefpodoxime, but of laxine, Amoxillin and Ampicillin shown a high level of sensitivity against *Staphylococcus aureus* strains. *S.aureus* -5 strain shown high percentage of MAR (Muti Antibiotic Resistance index) index of 60% followed by *S.aureus* -4 strain and *S.aureus* -7 with 40% MAR(Muti Antibiotic Resistance index) index. The results were shown in Table 2.

All 5 isolates were shown to be Multi Drug Resistant (MDR) strains; resistant to at least 3 out of 5 antibiotics. Five different antibiotic resistance patterns were identified, most of wound isolates showed multiple antibiotic resistances in the study area, which may be due to large portion of the bacteria isolate being previously exposed to several antibiotics.

Strain	Ampicillin	Methicillin	Amoxillin	Oflaxine	Cefpodoxime	Antibiotic Resistant Patterns	Multi Antibiotic Resistance Index%
S.aureus-1	S	S	S	S	R	C	20%
S.aureus-4	S	R	S	S	R	M –C	40%
S.aureus-5	R	R	S	S	R	A-M –C	60%
S.aureus-6	S	S	S	S	R	C	20%
S.aureus-7	R	S	S	S	R	A- C.	40%

Table 2. Antibiotic resistance patterns of *S.aureus* isolated from clinical wound samples
R-Resisitant, **S**-Senstive



S.aureus -1 S.aureus 4

S.aureus- 5 S.aureus- 6

S.aureus -7

Fig 1:- Antibiotic Resistant Patterns (Disk Diffusion Assay)

C. Agar well diffusion assay of Moringa oleifera seeds, Glycyrrhiza glabra stems and Jasminum sambac leaves extract

The 500mg/ml concentrations of the extracts of *Moringa oleifera* and *Glycyrrhiza glabra* were found to have better antimicrobil activity compared with methicillin. *Jasminum sambac* leaves extracts did not show any antimicrobial activity against Staphylococcus aureus strains 1, 4, 5, 6 and 7.*Jasminum sambac* leaves extracts displayed negative results with no inhibition zone of inhibition .

Moringa oleifera seeds shown antimicrobial activities in both strains (*S.aureus-4* and *S.aureus-5*) .Cold extract showed high activity of 19 mm zone of inhibition, methanol extract showed 15mm zone of inhibition to *S.aureus*-4 strain , whereas ethanol and hot aqueous extracts failed to inhibit growth of *S.aureus* .In *S.aureus-5* strain, methanoic extract of *Moringa oleifera* seeds showed 6 mm zone of inhibition and 7mm for cold aqueous extract respectively, whereas ethanol and hot aqueous extract did not show up any kind of inhibition. The results were shown in the Table- 3.

Glycyrrhiza glabra stems extracts have displayed a very high antimicrobial activities where by zones of inhibition to *S.aureus*-4 strain was read as 19 mm and 21 mm from ethanol and methanol extract respectively, but hot aqueous and cold extracts did not show any inhibition and encountered high growth of *S.aureues*-4 strain. *Glycyrrhiza*

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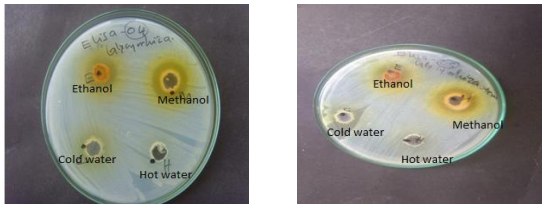
glabra extract shown 18mm and 20mm inhibition zones to *S.aureus*-5 from ethanol and methanol extracts respectively (Table-4).

The agar well diffusion assay showed that, Cold extracts of *M. oleifera*, Methanol and Ethanol extract of *Glycyrrhiza glabra* showed high antimicrobial activities against *S.aureus-4* and *S.aureus-5*.

Strain	Name of the Plant extract (500mg/ml)	Zones of inhibition in (mm) by various plant extracts			
		Ethanol	Methanol	Hot	Cold
S.aureus -4	*M. oleifera seeds*	-	15	-	19
	G.glabra stems	19	21	-	-
	J.sambac	-	-	-	
S.aureus -5	*M. oleifera seeds*	-	6	7	-
	G.glabra stems	18	20	-	-
	Jasmine sambac	-	-	-	-

Table 3. Antibaterial activity of a plant extracts against methicillin resistance strains of *S.aureus*

The experimental results obtained from the study illustrates that methanolic extracts of *Glycyrrhiza glabra* found to be more effective to control the pathogens growth compared to less effective inhibition by ethanol extract, also *Moringa oleifera* seeds shown some efforts to fight against MRSA through methanol and cold extract.

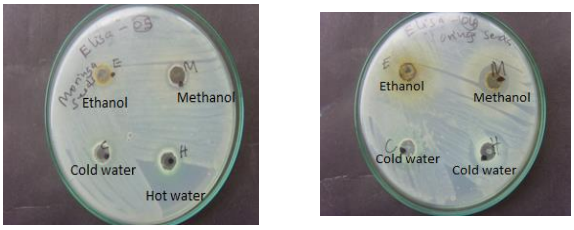


S.aureus-4 S.aureus-5

Fig 2:- Antibactreial acvity of *Glycyrrhiza glabra* stems on *S.aureus*-4 strain and *S.aureus*-5 strain

S.aureus-4 S.aureus-5

Fig 3:- Antibactreial acvity of *Moringa oleifera seeds* on *S.aureus*-4 strain and *S.aureus*-5 strain



S.aureus-4 S.aureus-5

Fig 4:- Antibactreial acvity of *Jasinum sambac leaves on S. aureus*-4 strain and *S.aureus*-5 strain

D. Minimum inhibitory concentration

Minimum inhibitory concentrations (MIC) of methanol extract from *Glycyrrhiza glabra* stems against *S.aureus*-4 strain was 0.49mg/ml and Minimum inihibitory concentrations (MIC) value of cold extract from *Moringa oleifera* seeds against *S.aureus*-4 strain was 250mg/ml. Results were shown in the Table-4.

S.No	MIC concentration (500mg/ml)	Cold Extracts of *Moringa oleifera* seeds	Methanol Extracts of *Glycyrrhiza glabra* stem
1	500	-	-
2	250	-	-
3	125	+	-
4	62.5	+	-
5	31.25	+	-
6	15.63	+	-
7	7.81	+	-
8	3.91	+	-
9	1.95	+	-
10	0.98	+	-
11	0.49	+	-
12	0.244	+	+

Table 4. Minimum Inhibitory Concentrations (MIC) of plant extract against MRSA *S.aureus*-4 strains isolate

'+' indicates Presence of growth of *S.aureus*
'–'Indicates Absence of growth of *S.aureus*

The Minimum inhibition concentration of Methanol extract of *Glycyrrhiza glabra* against *S.aureus*-5 strainwas found to be 3.91mg/ml and minimum inhibitory concentration of cold extract from *Moringa oleifera* against *S.aureus-5* was found to be 31.25mg/ml (Table-5).

S.No	MIC concentration (500mg/ml)	Methanol extracts of *Glycyrrhiza glabra stems*	Cold extracts of *Moringa oleifera* seeds
1	500	-	-
2	250	-	-
3	125	-	-
4	62.5	-	-
5	31.25	-	-
6	15.63	-	+
7	7.81	-	+
8	3.91	-	+
9	1.95	+	+
10	0.98	+	+
11	0.49	+	+
12	0.244	+	+

Table 5. Minimum Inhibitory Concentrations (MIC) of plant extract against MRSA *S.aureus*-5 strains isolate

(+) Indicates presence of growth of *S.aureus*
(-) Indicates absence of growth *S.aureus*

Methanol extracts of *Glycyrrhiza glabra* has a strong ability to fight against *S.aureus* at its very low concentration.

IV. CONCLUSION

From the above study it is concluded that the *Glycyrrhiza glabra* methanol extract showed the maximum antimicrobial activity in comparison to other extracts. These findings suggested *Glycyrrhiza glabra* methanol extract as a promising antibacterial candidate for the superficial MRSA infection.

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