In Vitro Study of Antimicrobial Activity of *Salacia chinensis* Against Methicilin Resistance *Staphylococcus aureus* (MRSA)

S. Manju[1] & K. Moorthy[2*]

[1]*Ph.D. Research Scholar (Category B), Bharathiar University, Tamilnadu, India.*

[2] *Associate Professor, Dept. of Microbiology, Vivekanandha College of arts and Sciences for women, Tiruchengode, Tamilnadu, India.*

[*]Corresponding author: K.Moorthy[2]

Abstract: ***Due to the rapid development of microbial resistance to antibacterial agents, it has become crucial to screen effective, safe, cheap and available therapeutic agents from various medicinal plants to find potential antibacterial effects. In the present study, MRSA isolates were procured from clinical laboratory and inhibited with solvent extracts of Salacia chinensis. Among the two solvents, methanol showed highest phytochemical compounds than chloroform extract. Alkaloids Sterols, Terpenoids and Quinones were commonly observed from both extracts. The better inhibitory activity was observed while using methanol extract and most of the isolates were suppressed with 7.5mg of concentration. This plant extracts which proved to be potentially effective can be used as antibacterial agents against MRSA.***

Keywords: *Salacia chinensis,* MRSA, phytochemicals, antimicrobial activity, and agar well diffusion

INTRODUCTION

Antibiotic resistances are public health problem in the both developed and developing countries. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. This incident was mostly promoted by incorrectly prescribed antibiotics, long duration of antibiotic therapy also contribute the development of resistance. In most cases, compared to infections that are easily treated with antibiotics, drug-resistant infections require long-term and/or expensive treatment, prolong hospital stays, require additional doctor visits and healthcare use, and cause greater disability [1].

Among the different types of resistant mechanism, Beta-lactamases are the most important mechanism of drug resistance among Gram-negative bacteria [2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) are resistant to all available penicillins and other Beta -lactam antimicrobial drugs. In India, Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a challenge for all healthcare institutions and this leads to serious endemic and epidemic MRSA infections [3]. In this situation, urgently we need investigate for newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. Concerning the above facts, it is worthwhile to screen plants, which have the above properties to synthesize new drugs.

Salacia chinensis L. which belongs to the family Celestraceae (spike- thorn family), is a small erect or straggling tree or large woody, climbing shrub, native to India including Andaman and Nicobar Islands [4]. The phytocompounds of Mangiferin, a xanthone glucoside is present in *S. chinensis* [5], which compounds had pharmacological properties including antidiabetic, antihyperglycemic, analgesic, antimicrobial, antioxidant, and cardiotonic [6]. There were fewer study findings on the antimicrobial activity of *S.chinensis*, hence our focus of the present study was evaluate the antibacterial activity of *S.chinensis* against MRSA isolates.

MATERIALS AND METHODS

Collection of isolates

A total of MRSA were procured from clinical laboratory and confirmed with selective media and biochemical tests. The confirmed isolates were used to assess the antimicrobial properties.

Collection of plant leaves and preparation of extract

The leaf of *S.chinensis* was collected from local area and collected leaves were watery washed with distilled water and finally dried in shade. The dried plant material of each plant species was grounded into fine powder to pass 100 mm sieve. The 10 gm of the finely powdered plant was filled in the thimble of the extraction apparatus and dropped into the soxhlet tube and extracted for 8 hrs over heat. Extraction was carried out individually with 250ml of methanol and chloroform. The obtained extracts (condensed vapour) were subsequently concentrated and dried using Rotary vacuum evaporator at 60°C under reduced pressure. The solvent was distilled off and then the dried crude residues were aseptically weighed and re dissolved in DMSO and stored at -20°C in a sterile, labelled, airtight container until further analysis.

Qualitative analysis of phytochemicals

The all solvent extracts of *S.chinensis,* were tested for the presence alkaloids, carbohydrates, flavonoids, phenolics, saponins, tannins, quinones, steroids, terpinoids and proteins as per the method described by Harborne and Kokate [7&8].

Antibacterial activity of *S.chinensis*

The sterilized Mueller hinton agar (MHA) was poured into each Petri plate (90 mm diameter) and allowed to solidify. The plates were incubated with freshly prepared inocula (*S.aureus*) which were swabbed over the entire surface of the medium, rotating the plate 60 degrees after each application by using a sterile cotton swab, to ensure the spread of the tested microbes on the surface of the plate completely. One wheel of 6mm diameter was bored with the medium of each plate with the help of sterile cork-borer. Different concentration of plant extracts were filled each well with the help of micropipette [9]. The ampicillin (10µg/ml) and DMASO were used as positive control. After 24 h, antibacterial activity was determined by measurement of diameter zones of inhibition (mm) (against the test organisms) around each of the extracts and the antibiotics.

Minimum inhibitory concentration

The Teh *et al*., [10] procedure was followed with some modification. Overnight nutrient broth culture was prepared. The culture was adjusted to obtain turbidity comparable to that of the turbidity of McFarland 0.5 standard. The inoculums thus prepared expect to obtain 10^6 CFU/ml. The various concentrations of plant extracts were prepared directly in a microtiter plate containing nutrient broth. The Resazurin was added in each well of the microtiter plate and was incubated at 37°C for 24hrs. The colour change in the well was then observed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

RESULTS AND DISCUSSION

The solvent extracts of *S.chinensis* were subjected to qualitative analysis of phytochemicals. The predominant count was observed in methanol extract than chloroform extract, which extract had 6 phytoconstituents including Alkaloids, Carbohydrates, Saponins, Sterols, Terpenoids and Quinones in case of chloroform extract Alkaloids, Flavonoids, Sterols, Terpenoids and Quinones. In 2017, Ghadage et al [11] observed the Terpenoids observed from the leaf and other part of the *S.chinensis.* Ngo and Scarlett [12] observed the predominant phytoconstituents from stem and root of *S.chinensis,* however, the result of this research is supported by recent study of Priya *et al* [13], and they were also observed the above mentioned phytochemicals on leaf extract.

The rise of multidrug resistant isolates is a serious threat and more difficult to treatment, it leads to long hospital staying and too much of cost. Nowadays, plant products have been proven to be effective antimicrobial agents due to their phytochemicals, and because they have few or negligible side effects, they are considered superior to synthetic antibiotics. For example the plant containing terpenes, phenolics, defensins, essential oil, are potential antimicrobial agents. This plant product can be a good substitute for antibiotics and can effectively deal with a multi drug resistance problem [14]. With this in mind, in this study we examined whether solvent extracts of *S. sinensis* can inhibit MRSA isolates. The MRSA isolates were collected from clinical laboratory and confirmed with selective media and biochemical tests.

Presently two solvents extracts were subjected to antibacterial activity, among them, methanol showed better inhibitory, which exhibiting the zone of inhibition was ranged from 10.33±1.14mm to 18±1.63mm. Among the 11 isolates, sputum isolates of *SAS25* and *SAS33* were highly suppressed, and two isolates were not inhibited. The inhibitory activity was started while using 2.5mg of

concentration against 3 isolates. The 80% of isolates were suppressed while using 7.5mg of concentration (Table 1).

Table 1. Antibacterial activity of methanol extract of *Salacia chinensis*

S.No	Isolates name	Con. of extract (mg)				Ampicillin	DMSO
		2.5	5	7.5	10		
1.	*SAp11*	-	11±1.632	15.33±2.05	17.66±2.05	-	-
2.	*SAp12*	-	-	-	-	-	-
3.	*SAS8*	-	11±0.816	14±1.632	17.33±2.05	-	-
4.	*SATS7*	-	12±1.63	14.66±1.24	17.66±2.05	-	-
5.	*SAB4*	-	11.66±1.24	14.66±1.24	16.66±1.24	-	-
6.	*SAu7*	10.33±1.24	12.66±2.05	12.66±2.05	17.66±2.05	-	-
7.	*SAp17*	-	12±1.63	14.66±1.24	18±1.63	-	-
8.	*SAu11*	-	-	10.33±1.24	11.66±1.24	-	-
9.	*SAS21*	-	-	-	-	-	-
10.	*SAS25*	11.66±1.24	14±1.63	16±1.63	18±1.63	-	-
11.	*SAS33*	10.33±1.14	12.66±2.05	14.66±1.24	18±1.63	-	-

± Standard deviation

In case of chloroform extract, zone of inhibition was ranged from 9±0.81mm to 14.33±1.24mm. Among the various isolates, urine isolate of *SAU7* and pus isolate of *SAP17* were highly suppressed and 4 isolates were highly resistance by chloroform extract. Among the 11 isolates 64% of were suppressed while using 7.5mg of concentration (Table 2). While using the standard agents of ampicillin and DMSO, no one isolates were suppressed. Presently MIC also subjected with titter well plant method for both solvent extracts. Whereas in methanol extract, 1.5mg of concentration was MIC for *SAu7, SAS25* and *SAS33* isolate. In case of chloroform extract, 6.5mg of concentration was MIC for *SAS8, SAu7* and *SAp17* isolates.

Table 2. Antibacterial activity of chloroform extract of *Salacia chinensis*

S.No	Isolates name	Con. of extract (mg)				Antibiotic	DMSO
		2.5	5	7.5	10		
1.	*SAp11*	-	-	9±0.81	12±1.63	-	-
2.	*SAp12*	-	-	-	-	-	-
3.	*SAS8*	-	-	11.3±1.2	12±1.63	-	-
4.	*SATS7*	-	-	9.3±1.24	10.3±1.24	-	-
5.	*SAB4*	-	-	-	10.3±1.24	-	-
6.	*SAu7*	-	-	12±1.62	14±1.63	-	-
7.	*SAp17*	-	-	12±1.6	14.33±1.24	-	-
8.	*SAu11*	-	-	-	-	-	-
9.	*SAS21*	-	-	-	-	-	-
10.	*SAS25*	-	-	-	-	-	-
11.	*SAS33*	-	-	-	9±0.81	-	-

± Standard deviation

According to literature, no one was study the antibacterial activity of leaf extract of *S.chinensis* against MRSA isolates. However, previous study by Banu et al., [15] has shown that extracts of various solvents from the whole plant of *S. sinensis* act against the MTCC isolation of *S.aureus.* In 2013 Moorthy *et al* [16] also reported that ethanolic extract of *S.chinensis* was active against to MTCC isolates of *S.aureus.* These pharmacological and medicinal potential of this plant are associated with the presence of certain bioactive molecules.

The results obtained from the current study suggest that the extracts of *S.chinensis* possess significant antibacterial properties against MRSA. Further research on the separation of active compounds and the interaction of active plant extracts can provide a better source for the development of new antimicrobial therapeutic agents.

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