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### Anti MDRSA activity of *Rhizophora apiculata* and *Avicennia marina* – An in-vitro study

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#### ABSTRACT

A total of 100 Multi-drug resistant *Staphylococcus aureus* (MDRSA) strains, isolated from the clinical specimens received in the department of Microbiology, Rajah Muthiah Medical College, Annamalai University, Tamil Nadu, India, were subjected for screening of anti-MDRSA activity of the aqueous, ethanol and methanol extracts of the leaf part of the mangrove plants i.e., *Rhizophora apiculata* and *Avicennia marina* with appropriate positive control (Amikacin). Agar well diffusion method was used in the in-vitro anti-bacterial assay to screen the crude extracts of the selected mangrove plant leaves. The study results revealed the promising anti-bacterial activity (anti-MDRSA) of the *Rhizophora apiculata* and *Avicennia marina* leaves' crude extract against the MDRSA strains of the clinical sources. The low level anti-MDRSA activity was expressed by the aqueous extracts and the high level anti-MDRSA activity was shown by the ethanolic and methanolic extracts of *Rhizophora apiculata* and *Avicennia marina* respectively. From this we conclude and suggest that the ethanolic and methanolic extracts of these mangrove plant species can be preferred when one wants to perform an in-vitro anti-bacterial assay with the leaf extracts of *Rhizophora apiculata* and *Avicennia marina*, and can avoid testing with the aqueous extract by which one can save time, energy and economy and the *Rhizophora apiculata* and *Avicennia marina* leaves may be considered as one of the major sources for the production of the effective drugs to control the multi-drug resistant infections with MDRSA strains.

**Key Words:** Anti-MDRSA activity, *Rhizophora apiculata*, *Avicennia marina*,, *Staphylococcus aureus*

## INTRODUCTION

Staphylococcus aureus is one of the most important pathogens that cause suppuration, abscess formation and a variety of pyogenic infections even fatal septicemia in human beings. Due to the development of methicillin resistance among S.aureus isolates, treatment of this infection has become a problematic one.<sup>1</sup> Methicillin resistant Staphylococcus aureus (MRSA) is a frequent cause of nosocomial pneumonia and other pyogenic infections among humans and animals. In recent years, drug resistance to human pathogenic bacteria and fungi has been commonly reported from world over. Therefore, the increasing prevalence of multidrug resistant strains of micro-organisms and the recent appearance of strains with reduced susceptibility to antibiotic raises an urgent need to search for new sources of antimicrobial agents.<sup>2</sup>

Reference with the past year records of our Microbiology diagnostic laboratory, we also acknowledge the reports and the statements of the researchers all over the world, who published about the increasing percentage of the emergence of multi-drug resistant bacterial strains with different types of human infections among which the multi-drug resistant Staphylococcus aureus stands first. Plants are the richest source of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization.<sup>2</sup> Mangroves are a rich source of several bioactive compounds. Secondary metabolites like alkaloids, phenolics, steroids, terpenoids have been characterized from mangroves and have toxicological, pharmacological and ecological importance.<sup>6,7</sup> These plants have been extensively used in the traditional medicine and different studies have been performed and pointing their activity against human, animal and plant pathogens but very few snapshots are available on their antibacterial activity.<sup>3</sup> There seems to be still more reduced numbers of research publications about the antibacterial activity of the mangrove plant species especially Rhizophora apiculata and Avicennia marina against the multi-drug resistant Staphylococcus aureus strains of the clinical source with more and new informations. Mangroves are used in traditional medicine for the treatment of many diseases.<sup>4</sup> It has

been reported that the mangrove plants have also been proved for antiviral, antibacterial and antiulcer properties.<sup>4,5</sup> Numerous research-works have been performed with different bacterial species from clinical source and its susceptibility pattern towards the mangrove plant extracts. Many research publications are available with informations about the antibacterial activity of MRSA, still it remains inadequate. Furthermore, the south-Indian research publications from the medical faculty, the mangrove research is in the stage of infancy. From our Diagnostic laboratory records, we could observe quite noticeable percentage of the MDRSA, which seems to be resistant to the following single drug oxacillin, vancomycin or resistant to both of these drugs and these strains showing very good susceptibility to other drugs such as Amikacin, Gentamicin, Ciprofloxacin, Cefotaxim, Ofloxacin etc. From this, we understand that the Staphylococcus aureus strain which shows resistance to oxacillin or vancomycin alone need not be considered as the multidrug resistant strain. In this present study we have selected and included, the S.aureus strains which had shown resistance to five or more antibiotics which includes oxacillin alone or both oxacillin and vancomycin. In view of getting new and more information, we step into the present study.

## MATERIALS AND METHODS

### MDRSA USED

Totally hundred (100) multi drug resistant Staphylococcus aureus (MDRSA) strains resistant to 5 or more antibiotics were used in this study. The MDRSA isolated from different clinical specimens received in the division of microbiology, Rajah Muthiah Medical College, Annamalai University, Tamil Nadu, India, were used to assess the in-vitro antibacterial activity of the crude leaf extracts of Rhizophora apiculata and Avicennia marina mangrove plants.

### STANDARDIZATION OF MDRSA INOCULUMS

The fresh cultures (24 hrs old) of the MDRSA was used in the anti-bacterial assay study. Each MDRSA strain on Nutrient Agar slope (stock cultures) were sub-cultured on Nutrient Agar plates and overnight culture was harvested (1 loopful) and mixed in 2ml of Muller Hinton broth. Further the MDRSA bacterial suspension was

adjusted to McFarland opacity No: 0.5 ( $10^8$  cells per ml) and this freshly prepared and standardized MDRSA inoculum was used in the in-vitro anti-bacterial assay.

#### **MANGROVE PLANTS USED IN THIS STUDY**

The mangrove plant species *Rhizophora apiculata* and *Avicennia marina* was used in this study. Fresh mangrove leaves of *Rhizophora apiculata* and *Avicennia marina* were collected from Pichavaram sea shore with the aid of the qualified staff from the Centre for Advanced Study, faculty of Marine biology, Annamalai University, Tamil Nadu, India.

#### **AQUEOUS EXTRACT**

The method of Dhayanithi et al. 2012 was used, with slight modifications to prepare the crude aqueous leaf extracts of *Rhizophora apiculata* and *Avicennia marina*. The mangrove leaves were harvested and thoroughly washed with tap water once and twice with sterile distilled water. Then the washed leaves were weighed for 100gms and crushed with the help of electronic mixer by adding 300 ml of sterile distilled water and frozen to  $-20^\circ$ , thawed and frozen repeatedly for three times and blended and the extract was filtered through a sterile cotton cloth. The filtrate was then centrifuged at 5000 rpm for 20 min and the supernatant was collected and stored at  $4^\circ\text{C}$  till used.

#### **ETHANOL AND METHANOL EXTRACTS**

The method of Dhayanithi et al. 2012 was used with slight modifications to prepare the ethanol and methanol crude extracts of *Rhizophora apiculata* and *Avicennia marina*. To prepare the ethanol and methanol extracts, each 50 grams of crushed leaf materials of *Rhizophora apiculata* and *Avicennia marina* species was soaked in 200 ml of 80% ethanol and methanol separately, and left for 72 hrs at room temperature ( $35^\circ\text{C}$ ). The mixture was stirred at 24 hrs interval using a sterile glass rod. Then

the extract was filtered through Whatman No. 1 filter paper (Himedia, Bombay - India) and the filtrate (160 ml) obtained was concentrated up to 20 ml by using rotary evaporator at  $45^\circ\text{C}$ . The extract was stored at  $4^\circ\text{C}$  until used.

#### **AGAR WELL DIFFUSION METHOD**

The method of Dhayanithi et al. 2012 was used in this study. The Muller Hinton agar plates were inoculated with the standardized MDRSA inoculums and wells were made by using the sterile micro-tips (6mm). The test agents (mangrove plant leaf extracts) of  $50\ \mu\text{l}$  quantity was filled in the wells and incubated for 24 hours at  $37^\circ\text{C}$ . The plates were examined for the zone of inhibition which indicates the anti-bacterial (anti MDRSA) activity of the mangrove plant extracts and the results were recorded.

#### **RESULTS AND DISCUSSION**

There has been an increase in the number of mangrove plant research in the field of marine biology as well as in other fields of life sciences, specially to screen the antimicrobial such as antibacterial<sup>1</sup>, antiviral<sup>3</sup> and antifungal<sup>8</sup> activity of the mangrove plant extracts of various parts like mangrove plants leaves, bark etc. Many research documents indicating the different types of bioactive compounds present in the parts of these mangrove plants and hold medicinal and economical values. There is a continuous and urgent need to discover new antimicrobials with diverse chemical structures and novel mechanism of action for new and re-emerging infectious diseases. In the present study the bioactive compounds especially having anti-MDRSA activity of the mangrove plant species; *Rhizophora apiculata* and *Avicennia marina* leaves were subjected to the different extractants and were screened for their anti-MDRSA activity against the multi-drug resistant *Staphylococcus aureus* of the clinical source.

**Table 1:** Susceptibility pattern (%) of the MDRSA to the leaf extracts of *Rhizophora apiculata* and *Avicennia marina* with different extractants

Sl. No.	Mangrove Plants	MDRSA n=100			
		Aqua	Etha	Meth	+ Control
1	<i>Rhizophora apiculata</i>	60%	100%	100%	100%
2.	<i>Avicennia marina</i>	70%	100%	60%	100%

**MDRSA** – Multi-drug resistant *Staphylococcus aureus*

**Aqua** – Aqueous extract.

**Etha** – Ethanol extract.

**Meth** – Methanol extract

**+ control** – Amikacin.

Our study results revealed the facts of the degree or the extent of the *Rhizophora apiculata* and *Avicennia marina* leaf extracts' anti-MDRSA activity with different extractants. Out of 100 MDRSA strains tested, the anti-MDRSA activity of the aqueous extracts of *Rhizophora apiculata* and *Avicennia marina* was recorded with 60%

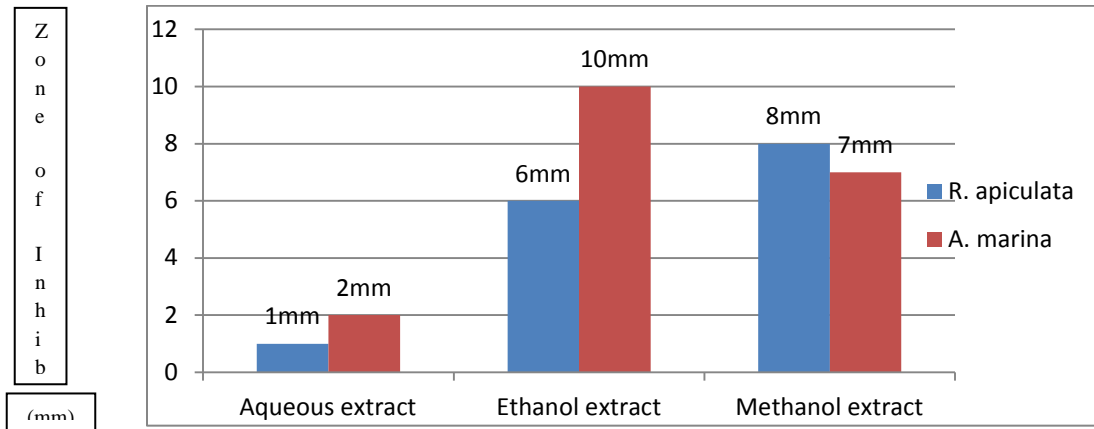
and 70% of the MDRSA strains. While 100% MDRSA strains were responded to the ethanol extracts of both *Rhizophora apiculata* and *Avicennia marina*. About 100% and 60% MDRSA strains responded to the methanol extracts of *Rhizophora apiculata* and *Avicenniamarina* respectively.

**Table 2:** Anti-MDRSA activity of the Mangrove plant leaf extracts against the MDRSA of different clinical specimens (zone of inhibition-mm)

Sl No:	Extracts	Zone of inhibition (mm)		
		Minimum & Maximum	MDRSA Urine	MDRSA Pus
<b>I Ethanol</b>				
1	<i>R.apiculata</i>	2mm - 6mm	3mm - 4mm	2mm - 6mm
2	<i>A. marina</i>	4mm - 10mm	4mm - 6mm	4mm-10mm
<b>II Methanol</b>				
1	<i>R.apiculata</i>	3mm - 8mm	3mm - 4mm	4mm - 8mm
2	<i>A. marina</i>	2mm -7mm	2mm -5 mm	1mm -7 mm
<b>III Aqueous</b>				
1	<i>R.apiculata</i>	Trace -1mm	Trace -1mm	Trace -1mm
2	<i>A. marina</i>	Trace - 2mm	Trace -2mm	Trace - 2mm

**Note:** All the tested MDRSA strains (100%) were found to be sensitive to the Positive control – Amikacin (Table 1)

**Figure 1 :**The better extractants to extract the anti-MDRSA bio-active compounds from the leaves of *Rhizophora apiculata* and *Avicennia marina* (maximum zone of inhibition produced).



Though the aqueous extracts of the *Rhizophora apiculata* and *Avicennia marina* acted against 60% and 70% tested MDRSA strains, the degree of their anti-MDRSA activity was varied, from trace to 1mm zone of inhibition with aqueous extract of *Rhizophora apiculata* and trace to 2 mm in case of *Avicennia marina* (Table 2). The maximum anti-MDRSA activity of the aqueous extracts of the *Rhizophora apiculata* and *Avicennia marina* was recorded as 1mm and 2mm respectively. The reason for the minimal anti-MDRSA activity could be due to the low level extracting ability of the water, in the extraction of the bio-active compounds (especially all those compounds acting on MDRSA strains) present in the leaves of *Rhizophora apiculata* and *Avicennia marina* (Table 2).

Comparatively, the ethanol and methanol seems to have the ability to extract the bio-active compounds at maximum level, especially the bio-active compounds active against MDRSA strains in better way. The zone of inhibition produced by ethanol extracts of *Rhizophora apiculata* and *Avicennia marina* were varied between 2 mm and 6mm, and 4mm - 10mm respectively. While the methanol extracts of *Rhizophora apiculata* and *Avicennia marina* produced the zone of inhibition which varied from 3mm to 8mm and 2mm to 7mm respectively. The study report of Dhayanithi et al. 2012, stating that the maximum antibacterial activity was shown by the methanolic extract of *A.marina* leaf, compared to the ethyl-acetate and chloroform. The maximum zone of inhibition produced by the methanolic leaf extract of *A.marina* against the multidrug resistant *S.aureus* was mentioned as 17mm. But we were able to record the maximum anti bacterial activity of the methanolic extract of *A.marina* as 7mm. The reason may be due to the

concentration and the quantity of the bioactive compounds present in the dried leaves may be more, when compared to the fresh leaves what we have used. It is well known fact that all the dried materials comparatively possess more quantity of its compounds though their various bioactivities are with uncertain in their own way.

From this we are able to understand that the ethanol and methanol are the better extractants (compared to water), to extract the anti-bacterial bio-active compounds from the leaves of *Rhizophora apiculata* and *Avicennia marina*. Still more, the study results proving that the ethanol is the best extractant (fig 1) for *Avicennia marina* and methanol is the best extractant for *Rhizophora apiculata* to get extracts from their leaves, to screen the in-vitro anti-bacterial activity (fig.1), and this point of our study made us to realize the fact about the extracting activity of different extractants that may vary with plant to plant as well as with species to species. The interesting point we were able to observe from our study was that, the oxacillin and vancomycin (both) resistant *S.aureus* strains had shown their susceptibility towards the ethanol and methanol leaf extracts of *R.apiculata* and *A. marina*. The reason could be, not only due to the presence of strong antibacterial action bioactive compounds of the *R.apiculata* and *A. marina* leaf extracts, but also due to the susceptible nature of the cell wall components of the tested multidrug resistant *S.aureus* strains. It was also very interesting to note the remarkable difference in the antibacterial activity of the selected mangrove plant extracts towards the MDRSA strains of different clinical specimens (Table 2). We were able to record the maximum antibacterial activity (anti MDRSA) expressed by the ethanol and methanol extracts of *R.apiculata* and *A. marina* towards the

MDRSA strains isolated from the pus specimens, and low level anti-MDRSA activity was expressed against the MDRSA strains isolated from the urine specimens (Table 2). The promising antibacterial activity of the mangrove plants opens the gate for the discovery of new antibacterials which are economically and medically valuable. Our study results

## CONCLUSION

From our one year research study, we conclude that the leaf part of the mangrove plant species *R.apiculata* and *A. marina* had shown their promising antibacterial activity against the MDRSA strains of the clinical source. The ethanol and methanol was found as good extractants to extract the leaf part of *R.apiculata* and *A.marina* and shown their difference in their ability to extract the maximum number of the bioactive compounds. The minimum (Trace to 1 mm) antibacterial activity was expressed by the aqueous extracts of *R.apiculata* and *A.marina*. Ethanol extract of *A.marina* leaves and the methanol extract of

gives hope for the solution to control the pyogenic infections associated with the MDRSA strains. Yet this field of study needs the attention from the researchers to step forward to have an elaborative study on the surveillance of MDRSA strains from the clinical source and about the epidemiology of related drug resistant infections with MDRSA strains. *R.apiculata* leaves had shown the maximum (10 mm and 8mm) antibacterial activity against MDRSA. From this we can suggest that the researchers those who intent to do the screening of antibacterial activity of the leaves of *R.apiculata* & *A.marina*, can select the ethanol and methanol as best extractants to get the best quality extracts, and can avoid testing with the aqueous extract by which one can save time energy and economy. The *Rhizophora apiculata* and *Avicennia marina* leaves may be considered as one of the major sources for the production of the effective drugs to control the multi-drug resistant infections with MDRSA strains. We welcome either support or criticism for our report.

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