Int.J.Curr.Microbiol.App.Sci (2017) *6*(7): 4305-4309

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 7 (2017) pp. 4305-4309**
Journal homepage: http://www.ijcmas.com

Short Communication https://doi.org/10.20546/ijcmas.2017.607.447

Effect of *Hemidesmus indicus* Extract against Methcillin Resistant *Staphylococcus aureus* from Various Clinical Specimens

B. Vignesh Kanna[1], E. Kavitha[2], M. Swapna[2] and Joshy M. Easow[3]*

[1]Department of Microbiology, Annapoorana medical College and Hospital, Salem, India
[2]Department of Microbiology, Sri Venkateshwaraa Medical College, Hospital and Research centre, Pondicherry, India
[3]Nirmal lab, Pondicherry, India
**Corresponding author*

Keywords

Hemidesmus indicus, MRSA, MIC.

Article Info

Accepted:
29 June 2017
Available Online:
10 July 2017

A B S T R A C T

Methicillin Resistant *Staphylococcus aureus* (MRSA) strains represent a worldwide threat because of their virulence and broad distribution in the hospital settings and community. MRSA strains are often resistant to beta lactam antibiotics and also to flouroquinolones, chloramphenicol, clindamycin, tetracycline and aminoglycosides. In addition, resistance is shown to recently develop anti-staphylococcus agents including the Oxazolidione and Streptogramin. Increasing in multi-drug resistant strains there is a need of alternative source. Bioactive compounds from natural products and traditional medicinal plants show promising role in treatment to control MRSA infection. We aimed to report the anti MRSA activity of ethanolic extract of *Hemidesmus indicus* root and its possible interaction with antibiotics. A total of 100 MRSA isolates from various clinical samples were included in the study. Ethanolic root extract of *Hemidesmus indicus* was done by using soxhlet apparatus. These isolates were subjected to perform antimicrobial susceptibility testing by Well-diffusion method, MIC and synergistic interaction with antibiotics. In this study for the concentration of 50µl shows 25% were sensitive and 75% were resistant. At 100µl 61% were sensitive and 39% were resistant. For MIC ranging from 6.20 to 10.65mg, 70% shows sensitive and 30% were resistant. Doxycycline, ciprofloxacin and amikacin 61%, 45%, 40% were showed synergism respectively.

Introduction

Human infections involving microorganisms i.e. bacteria, fungi, viruses, cause serious infections in tropical and subtropical countries of the world. In recent years, a multidrug resistance microorganism is on rise due to indiscriminate use of commercial drugs (Gutmann *et al*., 1988; Mohanasundari *et al*., 2007). *Staphylococcus aureus* is a Gram positive cocci arranged in clusters and is ubiquitous in nature. Penicillin was the drug of choice, to which *Staphylococcus aureus* developed resistance by producing enzyme beta lactamase. So, Methicillin was introduced in 1959. But, resistance to Methicillin emerged rapidly and in 1961 Methicillin resistant *Staphylococcus aureus* (MRSA) cases were reported (Poonam *et al*., 2008).

Methicillin Resistant *Staphylococcus aureus* (MRSA) strains represent a worldwide threat because of their virulence and broad distribution in the hospital settings and

community (Aqil *et al*., 2006). MRSA strains are often resistant to beta lactam antibiotics and also to fluoroquinolones, chloramphenicol, clindamycin, tetracycline and aminoglycosides. In addition, resistance is shown to recently develop anti-staphylococcus agents including the Oxazolidione and Streptogramin (Deresinski, 2005). Bioactive compounds from natural products and traditional medicinal plants show promising role in treatment to control MRSA infection. It show synergistic interaction with antibiotics or reduces the resistance level of MRSA strains to antibiotics. The increasing prevalence of multidrug resistant strains and recent appearance of strains with reduced susceptibility to antibiotics shows an urgent to need alternative approach for treatment (Rochon *et al*., 2000; Smith, 1999). According to WHO herbal medicines serves the health needs of about 80% of the world's population. Natural plant products are the source of most active ingredients of the medicine. The extract of many plants used in traditional medicine contain a wide range of curative agents that are used in many modern medicines.

Hemidesmus indicus is commonly known as Indian Sarsaparilla it belongs to family Asclepiadaceae. This is a common medicinal plant widely used in Indian System of Medicine. It is an official drug in Indian Pharmacopoeia and British Pharmacopoeia. It contains various Phytochemicals like glycosides, flavonoids, tannins, sterols and volatile oils. Roots, Leaves and bark of these plant has been reported useful in blood diseases, dysentery, diarrhoea, respiratory disorders, syphilis, leprosy, leucoderma, leucorrhoea, itching, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation ulcer and rheumatism. MRSA is an emerging threat to the community owing its antibiotic resistance. So, we intend to report the anti MRSA activity of ethanolic extract of *Hemidesmus indicus* root and its possible interaction with antibiotics.

Materials and Methods

It is a prospective cohort study conducted at Sri Venkateshwaraa Medical College, Hospital and Research center, from September 2014 to September 2015. A total of 100 consecutive MRSA isolates from various clinical specimens.

Preparation of plant extract

50g of *H.indicus* root powder is taken and 170 ml of solvent (Ethanol) added in Soxhlet apparatus and kept for 6 cycles. The extract was collected and kept for concentration using rotary vacuum evaporator and gravimetrically measured to determine the quantity of extract obtained. Then stored at 4ºC for further use. The crude extract was prepared by dissolving known amount of the dry extract in DMSO, to have a stock solution of 100mg/ml.

Antimicrobial assay

Sensitivity test was performed by agar well diffusion method. An inoculum size of 10^8 CFU/ml of bacterium, compared with 0.5 McFarland turbidity standards was used. It was spread on Muller Hinton agar plate. Wells of 10mm diameter were punched into the agar medium. About 50µl and 100µl of plant extract was added carefully in a well. Plates were kept in a refrigerator for 15 minutes for pre diffusion of extract. These plates were incubated at 37°C for 24 hours. Zone of inhibition of bacterial growth around each well was measured in mm.

Minimum inhibitory concentration of plant extract

MICs values were determined by the microtitre broth method in sterile flat bottom 96 well polystyrene plates. The *Hemidesmus indicus* root extract were in the range of 6.20 to 10.65mg/ml.

Synergism among plant extract and antibiotics

Synergistic effect of plant extract and antibiotics were done by using Agar well diffusion method. The antibiotics used are ciprofloxacin, amikacin and doxycycline. The wells were punched at predetermined distance so that the inhibitory circles of extract and antibiotics touch each other only tangentially. The wells were inoculated with 100µl of plant extract and the antibiotic disc placed at determined distance. Plates were the incubated at 37°C of 18 h.

Interpretation

Enlargement of inhibition zone indicates a positive interaction.
rC - Distance between centres of two wells.
rD - Combined radius of plant extract and antibiotics
rC > rD - Synergism (+)
rC = rD - Indifference (=)
rC ≤ rD – Antagonism (-)

Results and Discussion

A total of 100 MRSA were isolated from various clinical specimens. Out of 100 MRSA isolates 25% (n=25) were sensitive to 50µl ethanolic extract of *Hemidesmus indicus* root and 75% (n=75) were resistant. ATCC MRSA showed 16mm of Zone of Inhibition. For 100µl ethanolic extract of *Hemidesmus indicus* root 61% (n=61) isolates were sensitive to and 39% (n=39) were resistant. ATCC MRSA showed 21mm of Zone of Inhibition. *H. indicus* extracts exhibited moderate inhibition with the MIC ranging from 6.20 to 10.65mg against MRSA. In that 70% (n=70) were sensitive, 30% (n=30) were resistant. 8.5mg/ml is the break point of ATCC MRSA. Synergistic effect showed for Doxycycline 61% (n=61) synergism, 22% (n=22) indifference, 17% (n=17) antagonism, for Ciprofloxacin 45% (n=45) synergism, 21% (n=21) indifference, 34% (n=34) antagonism, for Amikacin 40% (n=40) synergism, 20% (n=20) indifference, and 40% (n=40) antagonism. The genus *Staphylococcus* includes pathogenic organisms in which *Staphylococcus aureus* is the most important. It has overcome most of the therapeutic agents that have been developed in the recent years and hence the antimicrobial chemotherapy for this species has always been empirical. The most notable example of this phenomenon was the emergence of MRSA.

The organism's tremendous potential to cause disease depends on the nature of the organism such as potential to colonize virulence, antibiotic resistance pattern, and genetic makeup and host susceptibility factors such as age, chronicity of disease, introduction of invasive device or procedures and impaired immunity.

According to the European Antimicrobial Surveillance System, MRSA represents currently a huge burden for many healthcare institutions and it is by far the most significant antibiotic resistant acquired pathogen worldwide.

MRSA has shown increasing endemic and epidemic spread in the last four decades causing serious medical and socio-economic difficulties. There is in need of newer antibiotics to treat the MRSA. Medicinal plants are known for its antimicrobial properties. Due to fewer side effects and cost effective, a plant based antibiotics is preferable for treating MRSA.

In a present study ethanolic extract of *Hemidesmus indicus* was prepared by cold method of extraction. Similar method was performed by Mamun *et al.,* in 2014 Bangladesh. Ahmad *et al.,* in 2001 performed agar well diffusion for Antimicrobial susceptibility testing against *Hemidesmus indicus* extract showed the zone of inhibition

range varied from 11mm to 44mm. These results were in accordance to the present study in which the zone of inhibition varied from 10mm to 44mm, similar results 19mm zone of inhibition were obtained by a study conducted by Riazunnisa *et al.,* (2013). Zone of inhibition about 12mm were obtained in a study conducted by Krishnan Kannabiran *et al.,* in 2008. Similar study was conducted by *et al.,* (2013) in Italy showed the zone of inhibition range varied from 8mm to 18mm.

In the present study we showed the Minimal inhibitory Concentration of *Hemidesmus indicus* root extract against MRSA were 6.20 to 10.65mg/ml. Similar study was conducted by Farrukh Aqil *et al.,* showed the MIC value in the range of 0.32 to 3.25 mg/ml. Another study conducted by Ali Mirzaei *et al.,* (2013) showe the MIC range of MRSA were 6.25 to 12.5 mg/ml. Eric Omwenga Omori *et al.,* (2012) conducted study, the MIC were performed by microdilution method in microtitre plate in that MIC value ranged from 18.75 to 37.5 mg/ml. Haque *et al.,* in 2014 conducted a study on MIC against MRSA and the results were 1 to 16µg/ml. In the study conducted by Ellof *et al.,* (1998) MIC of *Hemidesmus indicus* root extract against MRSA showed the range of 16 to 32 mg/ml.

In present study synergistic interaction between *Hemidesmus indicus* extract and some antibiotics like doxycycline, ciprofloxacin and amikacin the results were 61%, 45%, and 40% isolates of MRSA showed synergism respectively. Similar study conducted by Ghaleb Mohamma Adwan *et al.,* Combination of *Hemidesmus indicus* and antibiotics were showed synergism. *Farrukh* Aquil *et al.,* in 2006 conducted a study were the synergistic interaction between *Hemidesmus indicus* extract with tetracycline, chloramphenicol, ciprofloxacin, cefuroxime and ceftazidime the results were synergism. MRSA is very much prevalent in hospitals and in the community. Treatment options to these strains are narrowing down as vancomycin and linezolid resistance are being reported globally. *Hemidesmus indicus* is the one of the medicinal plant which possesses the anti MRSA effect.

The present study proves the antibacterial effect of *H. indicus* against MRSA. The Antimicrobial susceptibility testing and Minimal Inhibitory Concentration reveals it. The bioactive compounds in this plant with other drugs showed a synergism. This study probably suggests the possibility of concurrent use of these antimicrobial drugs and extracts in combination in treating infection caused by MRSA.

In conclusion, methicillin resistant *Staphylococcus aureus* has been recognized as an important and universal hospital acquired pathogen causing epidemic and endemic infections in the health care centres. These organisms became resistant to antibiotics because of unnecessary uses of antibiotics by humans, antibiotics in food and water and Germ mutation. MRSA is resistant to all the groups of antimicrobial agents, so we in need of newer antibiotics to treat the MRSA. The only hope full source is Traditional medicine. In ancient period Medicinal plants are used to cure most of the diseases. Generally these types of plants are easily available and the side effects are also less. *Hemidesmus indicus* is one of the medicinal plant which possess the antibacterial property. Our results support the use of these plants as traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as effective antimicrobial agents in the field of biomedical science.

References

Ahmed, L., Bej, A.Z. 2001. Antimicrobial and phytochemical studies on 45 Indian Medicinal Plants extract against multi drug

resistant human pathogen. *J. Ethnopharmacol.*, 74: 113-23.

Ali Mirzaei, Mehdi Akbartabar Toori, Nooshin Mirzaei, Reza Mirzaei. 2013. Antioxidant, Antimicrobial and Antimutagenic Potential of 4 Iranian Medicinal Plants. *Life Sci. J.*, 10(7): 689-710.

Anwarul Haque, Rashidul Alam and Akbar Hossain. 2014. Time – Kill assay Studies of Ethanolic Crude Extract of Bangladeshi *Streptomyces sp*. Isolated from the Mohasthangor, Bogra, Bangladesh. *J. SUB,* 5(1): 13-20.

Aqil, F., Ahmad, I., Owais, M. Evaluation of anti-methicillin resistant *Staphylococcus aureus* activity and synergy of some bioactive plant extract. *J. Biotechnol.*, 1: 1093-102.

Deresinski, S. 2005. Methicillin resistant *staphylococcus aureus*: an evolutionary, epidemiologic, and therapeutic odyssey. *J. Clin. Infect. Dis.*, 40: 562-73.

Eloff, J.N. 1998. Sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extract for bacteria. *Plant Med.*, 64: 711-13.

Eric Omwenga Omori, Ogol calistus, Paul Kamau Mbugua and Paul Owour Okemo. 2012. *Malaysian J. Microbiol.*, 8(2): 68-74.

Gutmann, L., Billot-Klein, D., Williamson, R., Goldstein, F.W., Mounier, J., Acar, J.F., *et al*. 1988. Mutation of *Salmonella* paratyphi A conferring cross-resistance to several groups of antibiotics by decreased permeability and loss of invasiveness. *Antimicrob. Agents Chemother.*, 32: 195-201.

Javad Sharifi, R., Seyedeh Mahsan, H.A., Majid Sharifi Rad, Marcello, I. 2013. In – Vitro Antioxidant and Antibacterial activities of *Xanthium strumarium* L. extract on Methicillin susceptible and Methicillin resistant *Staphylococcus aureus*. *Ancient Sci. Life*, 33(2): 90-102.

Krishna Kannabiran and Venkatesan Gopiesh Khanna. 2008. Antimicrobial activity of Saponin Fraction from the Roots of *Hemidesmus indicus*. *Res. J. Med. Plants*, 2(1): 39-42.

Mamun Or Rahid, Mohammad Ruhul Amin, Hassan Chowdhury MM, Arifur Rahman, Shamim, M. 2014. Comparative Study on the Antibacterial Activity of Four Medicinal Plants Leaves of Different Ages. *Intl. J. Pharma. Sci. Invention*, 3(3): 26-32.

Mohanasundari, C., Natarajan, D., Srinivasan, K., Umamaheswari, S.A. and Ramachandran, A. 2007. Antibacterial properties of *Passiflora foetida* (L) – A common exotic medicinal plant. *African J. Biotechnol.*, 6(23): 2650-3.

Poonam Soodlomba, Juhi Taneja, Bibhasati Mishre. 2008. Methicillin and Vancomycin resistant *Staphylococcus aureus* in hospitalized patients. *J. Global Infect. Dis.*, 2: 275-83.

Riazunnisa, K., Adilakshmamma, U., Habeeb khadri, C. 2013. Phytochemical analysis and In Vitro Antibacterial activity of *Soymida febrifuga* (Roxb.) Juss and *Hemidesmus indicus* (L.). *Indian J. Appl. Res.*, 3(12): 49-55.

Rochon Edouard, S., Pestel caron, M., Lemeland, J.P., Caron, F. 2000. In-vitro synergistic effects of double and triple combination of beta lactams, vancomycin and netilmycin against methicillin resistant *staphylococcus aureus* strains. *Antimicrob. Agents Chemother.*, 44: 3055-60.

Smith, D.W. 1999. Decreased antimicrobial resistance after changes in antibiotic use. *J. Pharmacother.*, 19: 1299-329.

How to cite this article:

Vignesh Kanna, B., E. Kavitha, M. Swapna and Joshy M. Easow. 2017. Effect of *Hemidesmus indicus* Extract against Methcillin Resistant *Staphylococcus aureus* from Various Clinical Specimens. *Int.J.Curr.Microbiol.App.Sci.* 6(7): 4305-4309.
doi: https://doi.org/10.20546/ijcmas.2017.607.447