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Original Article

Antimicrobial activity of *Rhipsalis baccifera* and *Drymoglossum piloselloides* against Methicillin Resistant *Staphylococcus Aureus* and Drug Resistant *Acinetobacter baumanii.*

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Abstract

Methicillin Resistant Staphylococcus aureus (MRSA) and multidrug-resistant Acinetobacter baumanii are known to cause delayed healing of infections in both acute and chronic wounds. Plants are a natural source of novel antimicrobials and many new drugs are derived from plants, as plants contain phytochemicals that have antimicrobial activity. Sri Lanka is a tropical country with a wide variety of plant species, many of which were identified as possessing medicinal qualities and have been used by traditional medicinal practitioners in the treatment of various diseases and ailments. Dressings made of Rhipsalis baccifera and Drymoglossum piloselloides have been used to treat wounds by Sri Lankan traditional medicine practitioners. This study determined the antibacterial activity of aqueous and methanol extracts of *R. baccifera* and *D. piloselloides* against MRSA and Multidrug-resistant *A. baumanii.* Aqueous and methanolic extractions of both plants were done by maceration. Their antibacterial properties were checked against MRSA and *A. baumanii* by the well diffusion method. The effectiveness of the extract was further tested against factors like temperature and storage time. *R. baccifera* (aqueous extract) exhibited antimicrobial properties against MRSA but no activity against *A. baumanii*. The antibiotic activity against MRSA was increased after two months of storage at 4°C. *D. piloselloides* exhibited no antibiotic activity against both MRSA and *A. baumanii.* The methanolic extracts did not demonstrate any antibacterial activity.

Keywords: Antibiotic activity, MRSA, *Acinetobactorbaumanii, Rhipsalisbaccifera, Drymoglossumpiloselloides.*

1. Introduction

The increase of antimicrobial resistance poses a very serious health threat worldwide, but an accurate net estimate of the global health burden due to antimicrobial resistance to antibiotics is unavailable (Laxminarayanan et al., 2016). New forms of antibiotic resistance can cross international boundaries and spread between continents, with ease and remarkable speed. Antibiotic-resistant microorganisms are described as “nightmare bacteria” that “pose a catastrophic threat” to people in every country in the world (WHO, 2013). When infections cannot be treated by first-line antibiotics, it is necessary to use more expensive medicines. An extended period of illness and treatment, often in hospitals, increases health care costs in addition to the economic burden on families and societies (Dijkshoorn, Nemec & Seifert, 2007). Antibiotic resistance is placing the achievements of modern medicine at risk. Chemotherapy, organ transplantations and surgeries have become much riskier without effective antibiotics for the prevention and treatment of infections (WHO, 2013).

Most bacteria can produce new strains against antibiotics and produce many virulence factors leading to antibiotic resistance which occurs when bacteria change in a way that reduces the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections (Kadurugamuwa & Beveridge, 1996).

Methicillin-resistant Staphylococcus aureus and Acinetobacter baumanii are resistant to most chemicals and drugs, such as methicilin and β-lactamase of Ampicillin (Bishburg & Bishburg, 2009) and they are frequently extremely resistant to antimicrobials, and treatment is complicated, especially for *A. baumanii*. Therefore, there is a great necessity to find new antibiotics against these two types of bacteria, which are common in wound infections (Gordon & Wareham, 2009).

Plants are a natural source of novel antimicrobials and according to the World Health Organization, 50% of new drugs are derived from phytochemicals. However, there are many plants that have not been yet investigated for antimicrobial activity. In traditional medicinal practices, plants are used in the treatment of wounds and infections. Sri Lanka, being a tropical country, has a rich diversity of plants and testing for the antimicrobial activity of the medicinal plants can lead to valuable discoveries.

Even though hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been sufficiently assessed (Sangameswaran et al., 2012). The recognition of traditional medicine as an alternate form of health care and the development of microbial resistance to the available antibiotics have led researchers to investigate the antimicrobial activity of medicinal plants (Sanogo et al., 1996).

Furthermore, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that, in order to find active compounds, a systematic study of medicinal plants is vital. Many ayurvedic medicinal plants in Sri Lanka are used against wound infections (Biswas & Mukherjee, 2003). There are also many herbal plants endemic to Sri Lanka having antibacterial properties (Rajakaruna, Harris & Towers, 2002). The use of plant materials against wound infections is a very common practice in Sri Lankan traditional medicine. Considering the massive potentiality of plants as sources for antimicrobial drugs this research aims to identify the antimicrobial, activity of Rhipsalis baccifera (Kasipethi) and Drymoglossum piloselloides (Navahandi) extracts against Multidrug-resistant *A. baumanii* and MRSA.

2. MATERIALS AND METHODS

2.1 Study setting

Microbiology laboratory of the Department of Biomedical Sciences, Faculty of Health Sciences, KIU.

2.2 Study design

Descriptive study.

2.3 Collection of plant material

The plant materials that were used in this experiment, *D. piloselloides* (leaves) and *R. baccifera* (whole plant) were collected from the 'Wedagedara' Beruwala, Sri Lanka and using sealable bags all parts of plant materials were transferred to the Microbiology laboratory, KIU. Then all the plant materials were washed with tap water to remove dust and dried in shade at room temperature.

2.4 Microbiological identification of clinical isolates used in the study

2.4.1 Culture conditions and storage

All organisms were cultured on Muller Hinton Agar (MHA) and were incubated at 37°C for 24 hours. Clinical isolates of multidrug-resistant *A. baumanii* and MRSA were obtained from the University of Sri Jayewardenepura, Sri Lanka.

2.4.2 Plant Authentication

Both plants were authenticated by the Botany Division, Bandaranayake Memorial Ayurvedic Research Institute, Navinna, Maharagama. (Acc. No 2039, 2040).

2.4.3. Preparation of plant extracts

Collected plant materials were washed and dried under the shade to obtain constant weight.
Aqueous and Methanolic extracts were prepared using the maceration method.

2.4.4. Testing the antibacterial activity of selected medicinal plants against MRSA and multidrug-resistant *A. baumanii*

Both MRSA and *A. baumanii* were inoculated on a MHA plate and incubated at 37°C for 24 hours. Inoculums of 24 hours old culture was prepared in sterile saline. The turbidity of the suspensions was adjusted to the 0.5 McFarland turbidity standard. A 3 ml of bacterial suspension was added to the surface of the solidified MHA plate and spread by swirling the plate. The remaining bacterial suspension was pipetted out from the plate. A sterile pipette tip of 9mm diameter was used to cut wells on each MHA plate. The bottom of the wells was sealed by adding a drop of molten agar in the wells. A volume of 150 microliter of each concentrated plant extracts x1 (0.25mg/ml), x2 (0.50mg/ml), x 5 (1.25mg/ml) and x10 (2.50mg/ml), positive control (Gentamicin) and negative control (sterile distilled water) were loaded into wells using a micropipette. The culture plates were incubated at 37°C for 24 hours and any resulting inhibitory zones were recorded. The procedure was carried out after storage of the extracts for 1 day, 1 week and 2 months respectively. Further, the storage of the extract was done in both 4°C and -20°C to check the temperature stability.

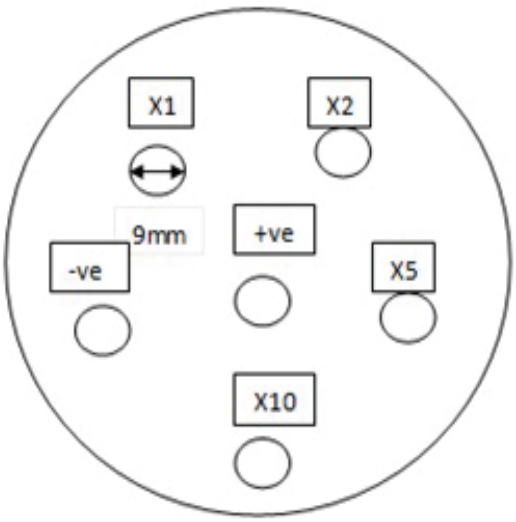


Fig 2.1; Partitioning the MHA plate for well diffusion assay.

2.5 Antibacterial sensitivity tests of bacterial strains.

In order to confirm that the collected *A. baumanii* strain is multidrug-resistant, the Antibacterial Sensitivity Test (ABST) was conducted using the disk diffusion method. *A. baumanii* was tested against cephalosporins; Ceftazidime, Cef - sulbactam - penicillin combination; ampicillin-sulbactam, Piperacillin -t azobactam -f luoroquinolones; Ciprofloxacin, Levofloxacin aminoglycosides; Gentamicin and Amikacin

2.6. Determination Minimum Inhibitory Concentration (MIC)

2.6.1 MIC by agar dilution method

For the agar dilution method, 2x concentrated aqueous extract was prepared by boiling down from 1440ml to 120ml. A concentration series of the extract was prepared by double diluting the stock solution of the aqueous extract. Molten MHA, (cooled 50°C) was added to each universal bottle to which the dilution series of the stock solution was previously added until the volume of the mixture reaches 20ml (plant extract and molten agar). Then the contents in the bottle were added to sterile Petri dishes. After the agar had solidified, the reverse sides of the plates were partitioned as shown in the figure, for the inoculation of organisms.

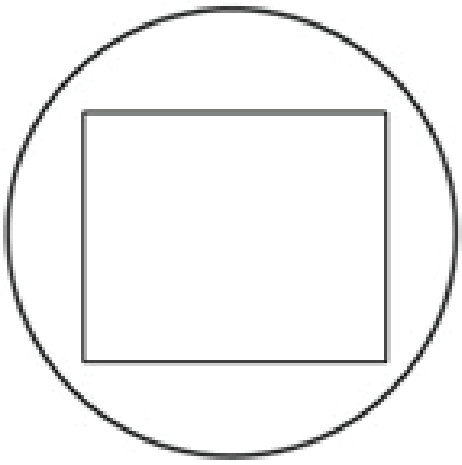


Fig 2.2: Partitioning the agar dilution plate for MIC

Next, the plates were dried at 44°C. The dried plates were inoculated with 0.5 McFarland suspension of each clinical isolate (MRSA and *A. baumanii*). The plates were allowed to stand for a few minutes to adhere to the suspension and were incubated at 37°C. The presence or absence of any growth of the organism was observed after 24 hours. The lowest concentration of extract that inhibited the visible growth of the microorganism after overnight incubation was determined as MIC. The experiment was done in triplicates and the average MIC was calculated.

RESULTS

3.1. Biochemical characterization and identification of micro-organisms.

The identity and purity of the test organisms were carried out using Gram's staining and biochemical tests. Gram's stain was carried out for both of the tested organisms at the biochemistry laboratory, KIU, Battaramulla, Sri Lanka. The test organisms considered under this research were multidrug-resistant, *A. baumanii* and MRSA (obtained from the culture collection of The Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura) which comprised of Gram-negative coccobacilli *A. baumani* and Gram-positive cocci *S. aureus*.

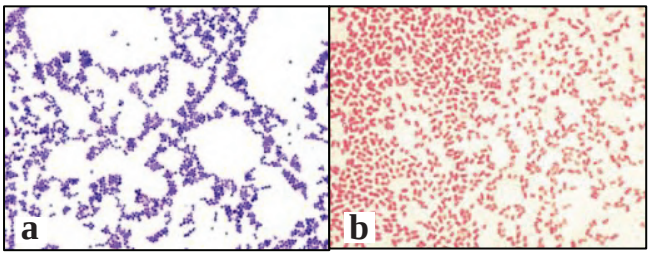


Figure 3.1: Gram characteristics of two test organisms.

(a) MRSA (b) *A. baumannii*

The biochemical characters of *A. baumanii* are shown in table 3.1. *A. baumanii* is a Gram-negative coccobacillus that is catalase positive, oxidase negative, indole, MR and VP negative and nonmotile. *A. baumanii* grew on Muller-Hinton agar, MacConkey agar, Nutrient

agar and Blood agar producing colonies of 2-3mm diameter, after 18-24 hours. The colonies produced in Muller Hinton agar were pale yellow to white greyish. Although it is known as a non-lactose fermenting organism, when grown on MacConkey agar, it fermented lactose partially with slight pink colour colonies (Figure 3.2). The colonies did not show hemolysis when grown on blood agar.

Biochemical test	Oxidase test	Catalase	Indole	Methyl Red	VP	Citrate	Urease	Haemolysis	Motility	KIA	OF
Results for *Acinetobacterbaumnnii*	-	+	-	-	-	+	-	-	Non motile	No change	Oxidative

Table 3.1; Results of the biochemical tests done for *A. baumanii*

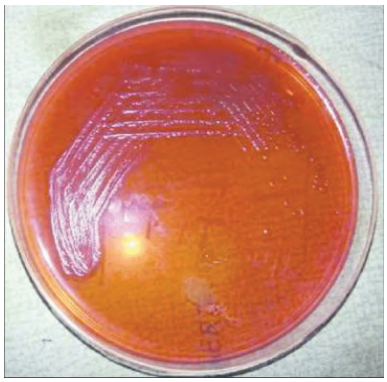


Figure 3.2; Appearance of *A. baumanii* on MacConkey agar.

Biochemical characters of MRSA (from the culture collection of Department of Microbiology, Faculty of Medical Sciences, Sri Jayewardenepura) were tested and the results were recorded. MRSA is a Gram-positive coccus that is catalase-positive and coagulase positive.

3.2 Antibacterial sensitivity tests of bacterial strains.

In order to confirm that the collected *A. baumanii* strain is multidrug - resistant, Antibacterial Sensitivity Test (ABST) was conducted. *A. baumanii* was resistant to cephalosporins; Ceftazidime, Cef - sulbactam - penicillin combination; ampicillin-sulbactam, Piperacillin - tazobactam - fluoroquinolones; Ciprofloxacin, Levofloxacin aminoglycosides; Gentamicin and Amikacin. Figure 3.3 shows some results of ABST test done for multidrug resistant *A. baumanii*.

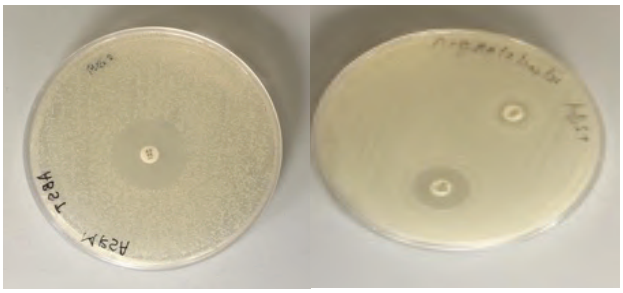


Figure 3.3; Antibiotic sensitivity tests for *A. baumanii* was done using Gentamicin, Ciprofloxacin and Ampicillin-sulbactam

3.3. Physical properties of prepared plant extracts

3.3.1 Aqueous extracts

D. piloselloides (Kasipethi) and *R. baccifera* (Navahandi) were selected to detect the antibacterial activity against *A. baumanii* and MRSA From each plant material, a concentration series was prepared as x1 (0.25mg/ml), x2 (0.50mg/ml), x5 (1.25mg/ml) and x10 (2.50mg/ml).

After preparing both plant extracts, they were filtered using a 0.22-micrometre filter. The appearance and pH of each extract were recorded (Table 3.2). The Colour of *R. baccifera* extract was yellowish-green and after boiling the clear filtered extract, a white precipitate was formed. The colour of *D. piloselloides* extract was a light green colour and no precipitate was formed upon boiling.

Concentration)	*Rhipsalis baccifera* (pH)		*Drymoglossum piloselloides*(pH)	
(mg/ml)	4^0C	-20^0C	4^0C	-20^0C
0.25 (x1)	7	7	6	7
0.50 (x2)	6	6	7	6
1.25 (x3)	7	7	6	7
2.50 (x10)	6	6	7	6

Table 3.2; pH values of the prepared concentrated extracts of each plant aqueous extracts.

3.4. Well diffusion assay

Well diffusion method was used to screen the aqueous extracts and methanolic extracts for antimicrobial activity against MRSA and *A. baumanii*. In this, any zone of inhibition observed around the well was considered as a positive response. The zones of inhibition of well diffusion assay (for both plant extracts) were obtained with respect to three variables, including concentration, storage temperature and storage time. Gentamicin was used as the positive control for both *A. baumanii* and MRSA and sterile distilled water was used as the negative control.

3.4.1. Well diffusion assay against MRSA

3.4.1.1. Rhipsalis baccifera aqueous extract against MRSA

Table 3.3 and 3.4 show the resulted mean of inhibitory zone diameter of three trial sessions conducted with respect to concentration, stored time and stored temperature.

The well diffusion against MRSA which was done one day after the preparation of the extract, (stored at 4^{o}C) showed an average zone of inhibition (ZOI) of 13.70mm for x1 concentration, 14.70mm for x2 concentration, 15.80mm for x5 concentration and 16.80mm for x10 concentration with *R. baccifera* aqueous extract (Table 3.3). Representative plates are shown in Figure 3.4; a.
The well diffusion against MRSA which was done one week after the preparation of the extract, (stored at 4^{o}C) showed an average zone of inhibition (ZOI) of 15.22 mm for x1 concentration, 14.44mm for x2 concentration, 15.67mm for x5 concentration and 16.67mm for x10 concentration with *R. baccifera* aqueous extract (Table 3.3). Representative plates are shown in Figure 3.3;b.

The well diffusion against MRSA which was done one week after the preparation of the extract, (stored at -20^{o}C) showed an average zone of inhibition (ZOI) of 15.33 mm for x1(0.25mg/ml) concentration, 14.22 mm for x2(0.50mg/ml) concentration, 15.44mm for x5(1.25mg/ml) concentration and 14.33mm for x10 (2.50mg/ml) concentration with *R. baccifera* aqueous extract (Table 3.4). Representative plates are shown in Figure 3.3;c.

The well diffusion against MRSA which was carried out two months after the preparation of the extract, (stored at 4^{0}C) showed an average zone of inhibition (ZOI) of 13.80 mm for x1 (0.25mg/ml) concentration, 14.70 mm for x2 (0.50mg/ml) concentration, 15.80 mm for x5 (1.25mg/ml) concentration and 16.80 mm for x10 (2.50mg/ml) concentration with *R. baccifera* aqueous extract (Table 3.3). Representative plates are shown in Figure 3.3;d.

Gentamicin which was used as the positive control showed an average zone of inhibition (ZOI) of 25.00mm. (diameter of a prepared well was 9 mm.)

Stored time	Concentration	Mean diameter of the inhibition zone (mm) (extracts stored at 4^{0}C)
One day after the preparation of the extract	Positive control	25.00
	0.25mg/ml (x1)	13.70
	0.50mg/ml (x2)	14.70
	125mg/ml (x5).	15.80
	2.50mg/ml (x10)	16.80
One week after the preparation of the extract	Positive control	25.00
	0.25mg/ml (x1)	15.22
	0.50mg/ml (x2)	14.44
	1.25mg/ml (x5)	15.67
	2.50mg/ml (x10)	16.67
Two months after the preparation of the extract	Positive control	25.00
	0.25mg/ml (x1)	13.80
	0.50mg/ml (x2)	14.70
	125mg/ml (x5)	15.80
	2.50mg/ml (x10)	16.80

Table 3.3; Average ZOI of three trial sessions of well diffusion assay *R. baccifera* aqueous extracts (stored at 4^{0}C) against MRSA) that were conducted with respect to concentration and stored time.

Concentration	Mean diameter of the inhibition zones (mm) (extracts stored for one week at -20^{0}C)
Positive control	25.00
0.25mg/ml (x1)	13.70
0.50mg/ml (x2)	14.70
125mg/ml (x5)	15.80
2.50mg/ml (x10)	16.80

Table 3.4; Average ZOI of well diffusion assay which was done in the presence of *R. baccifera* aqueous extracts (stored at -20^{0}C) against MRSA with respect to concentration

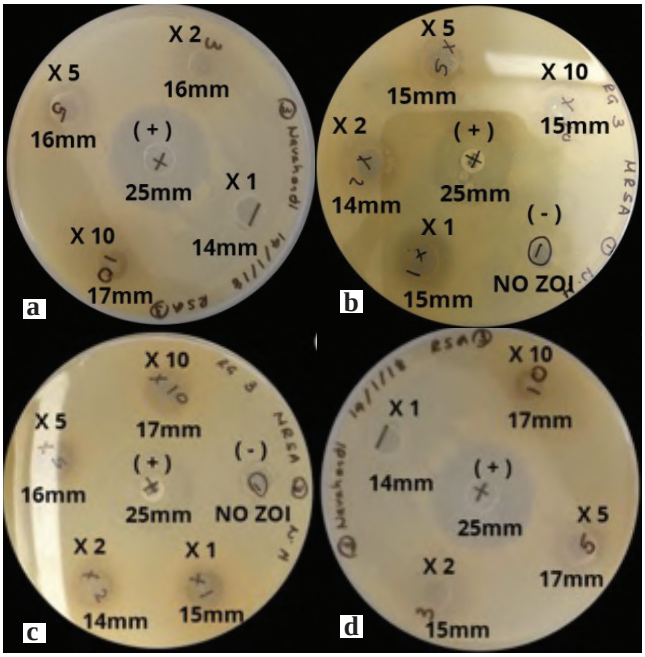
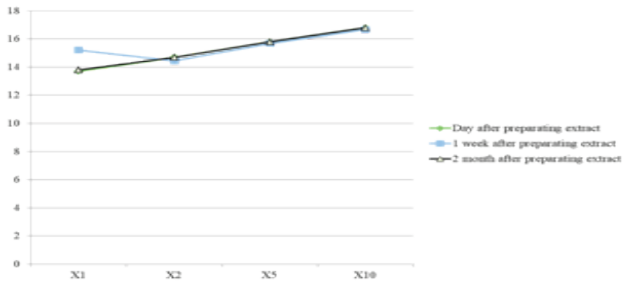


Figure 3.4; Well diffusion assay for the determination of the ZOI at different concentrations of (0.25mg/ml /x1, 0.50mg/ml /x2, 1.25mg/ml/ x5, 2.50mg/ml /x10) *R. baccifera* leaf extracts.

(a) One day after preparation (extracts stored at 4^0C) (b) one week after preparation (extracts stored at 4^0C) (c) one week after preparation (extracts stored at -20^0C) (d) Two months after preparation (extracts stored at 4^0C)



Graph 3.1 Concentration factor vs Inhibitory zones in accordance with the time stored at 4°C.

The results are shown in Figure 3.3, Table 3.3 and 3.4 depict that *R. baccifera* leaf extract (aqueous) has potential antimicrobial activity against MRSA. Furthermore, the diameter of the ZOI increases with increasing concentration. But as shown in Graph 3.1, there is no significant difference in zones of inhibition with the storage temperature and storage time in the well diffusion assay of *R. baccifera* aqueous extract against MRSA.

3.4.1.2. Drymoglossum piloselloides aqueous extract against MRSA

D. piloselloides leaf extract (aqueous) has not demonstrated any antimicrobial activity against MRSA.

3.4.1.3. Methanolic extracts of four selected medicinal plants against MRSA

Methanolic extracts of both medicinal plants have not demonstrated any antibacterial activity against MRSA.

3.4.2. Well diffusion assay against Multidrug-Resistant A. baumanii

Both aqueous and methanolic extracts of both medicinal plants *D. piloselloides* and *R. baccifera* extracts did not demonstrate any antimicrobial activity against Multidrug-Resistant *A. baumanii.*

3.5. The minimum inhibitory concentration (MIC) of tested compounds

Minimum inhibitory concentration assay was done only for *R. baccifera* against MRSA as inhibitory zones were not observed against Multidrug-Resistant *A. baumanii.* Minimum inhibitory concentration assay was not done for Drymoglossum piloselloides as no inhibitory zones were observed against both MRSA and Multidrug-Resistant *A. baumanii.*

3.5.1. MIC for Rhipsalis baccifera

The minimum inhibitory concentration of *R. baccifera* aqueous leaf extract was tested as described in the methodology and recorded as + = Growth, - = no Growth. Table 3.5 describes the MIC of *R. baccifera* aqueous leaf extract for MRSA. Representative plates are shown in Figure 4.4. The minimum inhibitory concentration of *R. baccifera* aqueous leaf extract against MRSA was x 0.5 (0.125mg/ml).

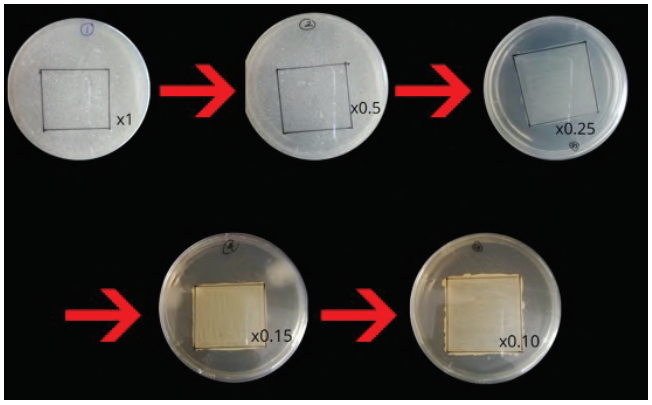


Figure 3.5; MIC assay of MRS Ain the presence of *R. baccifera* aqueous extract

Concentration	Growth of micro-organism
X1(0.25mg/ml)	-
X0.5(0.125mg/ml)	-
X0.25 (0.0625mg/ml)	+
X0.15 (0.0375mg/ml)	+
X0.10 (0.025mg/ml)	+

Table 3.5; MIC assay of MRSA in the presence of *R.baccifera* aqueous extract

4. DISCUSSION

R. baccifera is a plant widely used in traditional medicinal practices but has not been scientifically studied, so the scientific basis of its medicinal properties remain unknown.

There are many methods to prepare plant extractions to test the antimicrobial activity such as Percolation, Soxhlet extraction, Microwave - assisted extraction, Ultrasound - assisted extraction, Accelerated solvent extraction and Super rival fluid extraction. In this experiment, selected methods of extraction were aqueous and methanolic maceration extraction methods were based on the technique carried out by the traditional healer in his ayurvedic practice. These extraction methods are advantageous when considering factors like cost, and simplicity of technique.

Pre extraction techniques are performed to get a proper yield and to preserve the bio-molecules in the plants prior to extraction. The preservation methods used in the experiment were grinding and drying.

The positive control used in this experiment was Gentamicin. Even though Vancomycin is the standard antibiotic that is used against MRSA, the inability to obtain sufficient quantities of aqueous vials necessitated the selection of another option. Gentamicin was selected since it could be used against both MRSA and Acinetobactor baumanii. This plant was selected for this experiment as per the traditional healer's advice, as this is used in his practice for the healing of wounds. The results for the aqueous extract of *R. baccifera* against MRSA showed positive inhibition zones proving that it possesses antibacterial activity against MRSA. The inhibition zones increased with the increase in concentration. It is possible that this occurs due to the reason that the concentration of the active compound increases when the extract is concentrated. The initial solution produced by maceration was a clear solution, but upon boiling to prepare the concentration series, the formation of a precipitate was observed, and the amount of precipitate seen increased with the concentration. Further research is required to confirm if the formed precipitate is an active compound being crystallized. This might be advantageous for future researchers.

D. piloselloides is being used in traditional medicine for various ailments and remedies but its antimicrobial activities have also not been scientifically studied. *D. piloselloides* did not demonstrate any antimicrobial activity against both MRSA and *A. baumanii*. Further studies could be carried out testing their antimicrobial activity against other wound pathogens to determine any positive activity.

5. CONCLUSION

R. baccifera (aqueous extract) showed positive inhibition zones against MRSA which increased with the increase in concentration. It has not demonstrated any activity against Acinetobacter

baumanii.
D. piloselloides did not demonstrate any antimicrobial activity against both MRSA and *A. baumanii.*

Further studies should be carried out to identify the phytochemicals responsible for the activity of *R. baccifera* and the study could be extended to determine antimicrobial activity against other common wound pathogens. Further studies could be carried out testing the antimicrobial activity of *D. piloselloides* against other wound pathogens to determine any positive activity.

References

Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Røttingen, J.-A., Klugman, K., & Davies, S. (2016). Access to effective antimicrobials: a worldwide challenge. *The Lancet*, 387(10014), 168-175.https://doi.org/10.1016/s0140-6736(15)00474-2

World Health Organization (2013). Global action plan for the prevention and control of noncommunicable diseases 2013-2020: World Health Organization.

Dijkshoorn, L., Nemec, A., & Seifert, H. (2007). An increasing threat in hospitals: multidrug resistant Acinetobacter baumannii. *Nature Reviews Microbiology,* 5(12), 939-951. https://doi.org/10.1038/nrmicro1789

Kadurugamuwa, J. L., & Beveridge, T. J. (1996). Bacteriolytic effect of membrane vesicles from Pseudomonas aeruginosa on other bacteria including pathogens: conceptually new antibiotics. *Journal of bacteriology,* 178(10), 2767-2774. https://doi.org/10.1128/jb.178.10.2767-2774.1996

Bishburg, E., & Bishburg, K. (2009). Minocycline—an old drug for a new century: emphasis on methicillin-resistant Staphylococcus aureus (MRSA) and Acinetobacter baumannii. *International journal of antimicrobial agents,* 34(5), 395-401. https://doi.org/10.1016/j.ijantimicag.2009.06.021

Gordon, N., & Wareham, D. (2009). A review of clinical and microbiological outcomes following treatment of infections involving multidrug-resistant Acinetobacter baumannii with tigecycline. *Journal of antimicrobial chemotherapy,* 63(4), 775-780.https://doi.org/10.1093/jac/dkn555

Sangameswaran, B., Saluja, M. S., Sharma, A., & Manocha, N. (2012). Anti-tumor effect of acetone extract of Madhuca longifolia against Ehrlich ascites carcinoma (EAC) in mice. *Phytopharmacology,* 3(1), 130-136.

Sanogo, R., Germanò, M., De Pasquale, R., Keita, A., &Bisignano, G. (1996). Selective antimicrobial activities of MitracarpusscaberZucc. against Candida and Staphylococcus sp. *Phytomedicine,* 2(3), 265-268. https://doi.org/10.1016/S0944-7113(96)80053-5

Biswas, T. K., & Mukherjee, B. (2003). Plant medicines of Indian origin for wound healing activity: a review. *The international journal of lower extremity wounds,* 2(1), 25-39. https://doi.org/10.1177/153473460300200106

Rajakaruna, N., Harris, C. S., & Towers, G. (2002). Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. *Pharmaceutical Biology,* 40(3), 235-244https://doi.org/10.1076/phbi.40.3.235.5825

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Conflicts of Interest

None declared