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Research Article

# *In-Vitro* Evaluation of the Antibacterial Activity of the Methanol Leave Extract of *Lawsonia Inermis* Against Methicillin-Resistant Staphylococcus Aureus (MRSA)

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ABSTRACT

This study is to determine the antibacterial efficacy of *Lawsonia inermis* against methicillin-resistant Staphylococcus aureus (MRSA) which has become a leading cause of infections. The leaves of *Lawsonia inermis* also known as Henna or lalley leave were subjected to extraction with seventy percent (70%) methanol using the cold maceration technique after which phytochemical screening and partitioning were carried out following standard procedures, hence, three fractions of the methanol extract of *Lawsonia inermis* (crude extract, aqueous and chloroform fractions) were used against thirty strains of MRSA by using the agar well-diffusion and minimum inhibitory concentration as a determination method. Preliminary phytochemical screening revealed the presence of Alkaloids, Saponins, Tannins, Terpnoids, and steroids. A two old serial dilution was done for each of the fractions viz; 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. The various dilutions were used on the test organism and there was increase in zones of inhibitions with increase in extract concentrations. At 100mg/ml, the crude fraction had a maximum inhibition zone of 15.30mm which was higher than the inhibition zone of the standard antibiotic used, and a minimum inhibition zone of 9 mm as compared to the standard antibiotic used having a minimum inhibition zone of 4.33mm. Among all the fractions used, the crude fraction of the methanol extract of *Lawsonia inermis* showed the best activity against the test organism (MRSA) Methicilin Resistant Staphylococcus aureus. Others showed lesser activity as compared to the standard antibiotic used.

**Keywords:** Lawsonia inermis linn; MRSA; Crude extract

## INTRODUCTION

Plants play a major role on the earth, and human beings depend on plants due to its medicinal properties. Medicinal plant is any plant which in one or more of its organs contains compounds that possesses therapeutic activities or compounds which are precursors for the synthesis of useful drugs due to the presence of secondary metabolites.[1]

Treatment using medicinal plants is one of the oldest practices which are almost as old as mankind itself as almost about 80% of the world's population have made use of plants as a source of medicinal drugs even with the advent of technology due to the lesser side effects they possess in contrast to synthetic compounds and show synergistic effects unlike modern medicine.[2] In the past, the man in search of the cure for certain ailments before the development of contemporary science, discovered that some plants in nature when taken orally or applied to the surface of the skin had therapeutic effects. The beginning of the use of these plants was instinctive and during this period, there was little or no sufficient information concerning the causes of the illness or diseases also there were minute data on the plants used for cure and healing. In recent times, there has been a paradigm shift from the use of synthetic drugs to the use of medicinal plants for therapeutic purposes. Much research has been focused on medicinal plants which have been regarded as a reservoir for different types of bioactive compounds with a range of pharmacological and therapeutic activities.[3] According to a report by Gull et





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al., 2013, the emerging resistance of pathogens against currently available antimicrobial agents demands the search for new antimicrobial agents of plant origin. The use of medicinal plants as a natural substitute is the paramount area of research to overwhelm the drug resistance of infective agents.

Traditional medicines prepared from these plants are recognized in modern times as a preferred method for treatment in the health care system in many parts of the world because of their usefulness and affordability in the treatment of diseases due to the presence of bioactive compounds known as secondary metabolites or phytochemicals such as terpenes, alkaloids, saponins, and polyphenols.[3]

*Lawsonia inermis* also known as henna more commonly or Lalley leave is a shrub or small tree cultivated in many regions as an ornamental and commercial dye crop. It is mostly found in the tropic subtropic and semiarid zones of Africa, South Asia, and North Australia. As reported by Ahmed et al. [3], a wide range of chemical constituents has been isolated from Henna which includes naphthoquinone derivatives of which Lawsone is a chief constituent and the coloring matter of the leaves. Other constituents are phenolic derivatives, coumarins, xanthones, tannins, flavonoids, terpenes, and sterols as well as other chemical constituents such as amino acids contributing to its medicinal properties.[4]

According to Mohamed et al. [4], methanolic extracts of the plant showed considerable antibacterial activity almost on all tested microorganisms. The Henna plant is majorly used all over the world for its cosmetic values and also Pharmacological studies have shown that *Lawsonia inermis* exhibits antibacterial, antifungal, antidiabetic, antipyretic, and wound healing properties due to its bioactive components.[5] As a medicinal plant, Henna has been used in folk remedies as an astringent, and hypotensive agent as well as against leprosy and jaundice. The leaves were also used for skin diseases, smallpox, etc. Also, the seeds of the plant possess medicinal properties as well as roots.[4]

*Lawsonia inermis* has been suggested to display a noteworthy antimicrobial activity against both gram-positive and gram-negative bacterial strains. The Antimicrobial activity of the plant – Henna, as reported by Sharma & Bhalti[3] was generally more evident in the leaves of the plant rather than the seeds, the seeds demonstrated only a limited antibacterial activity and at higher concentrations. This is probably due to the inherent constituents of the fully grown plant and the maturity of its chemically active constituents.

To investigate the antimicrobial efficacy against common human pathogens such as Escherichia coli, Staphylococcus aureus, Candida albicans, etc, the extracts of the leaves of *Lawsonia inermis* were applied on woolen yarn. The active component of the plant was investigated to be Lawsone (2-Hydroxy-1,4–naphthoquinone), which is also the principal dye compound of the plant. Current research also suggests that Lawsone is non-problematic for external use because of its slow toxicity and genotoxicity. The bioactive compound of *Lawsonia inermis* with ampicillin (a known antibacterial drug) and fluconazole (a known antifungal drug) were found to considerably inhibit the growth of test microorganisms.[6]

Staphylococcus aureus is a Gram-positive, coagulase-positive pathogen from the Staphylococcaceae family. This is a spherical bacterium with a diameter of about 1 m that forms grape clusters.[7] Staphylococcus aureus (S. aureus) is a common microorganism that colonizes the nasal cavity of humans and other animal species.[8] This bacteria may also be found on external body surfaces as commensal or pathogenic bacteria that can cause a variety of infectious diseases.[9] Staphylococcus aureus has a wide range of virulence factors and toxins, and it is frequently implicated in many toxin-related diseases such as toxic shock syndrome, Staphylococcal foodborne diseases (SFDs), and scalded skin syndrome.[10] Staphylococcus has the potential to develop resistance to broad-spectrum antibiotics in a short period of time. Methicillin-resistant Staphylococcus aureus (MRSA) has arisen, spread worldwide, and become a prominent source of bacterial infections in both health-care and community settings since the 1960s.[11]

Methicillin-resistant Staphylococcus aureus (MRSA) strains, also known as multidrug-resistant *S. aureus*, were first identified in the 1960s and have since become a leading cause of nosocomial infections, including life-threatening pneumonia, necrotizing fasciitis, endocarditis, osteomyelitis, severe sepsis, and toxinoses like toxic shock syndrome. Immunosuppression, hemodialysis, peripheral mal perfusion, advanced age, extended in-hospital stays, residency in long-term care facilities (LTCFs), inadequacy of antimicrobial therapy, indwelling devices, insulin-requiring diabetes, and decubitus ulcers are just a few of the independent risk factors for MRSA that have been reported.[12] Due to the increase in resistance of MRSA to antibiotics used in modern times, this study hence focuses on the treatment of this bacterial infection by making use of *Lawsonia inermis* since research findings result have shown that the plant possesses antimicrobial medicinal properties because of their potential to tackle the problem of drug resistance in microorganisms, medicinal plants are increasingly being used as raw materials in the development of novel medications. Both developing and developed countries are seeing an increase in demand for medicinal plants.

## MATERIALS AND METHOD

- **Glassware and other Materials:** Retort stand, Separating-funnel, Spatula, Porcelain dish, Mortar and pestle, Pasteur pipette, Beakers, Measuring cylinder, Sterile Conical flasks, Sterile Petri-dishes, Sterile Test tubes, Sterile Syringes (5ml and 2ml), Test tube stand,

Sieve, Inoculating loop, Sterile swab sticks, Sterile bijou bottles, Sterile water, Distilled water, Methanol.

- **Media:** Nutrient broth, Mueller Hinton agar.
- **Disinfectant:** Methanol.
- **Instruments:** Autoclave, Hot air oven, Weighing balance, Refrigerator, Bunsen burner, 6mm Core borer, Incubator.

## *EXTRACTION PROCEDURE*

Already pulverized Lalley (*Lawsonia inermis*) leaves with a Lots number (PHC 12824) were obtained from a local outlet in Kaduna state on the 28th of February, 2022 and stored in air tight container. 600g of the pulverized leaves of *Lawsonia inermis* was weighed and extracted with 70% methanol using cold maceration technique of extraction for about 72 hours with occasional manual agitation. The resulting mixture was filtered and the filtrate was allowed to concentrate to dryness in an evaporating dish by making use of the rotary evaporator to remove the extracting solvent (methanol). The resulting extract (crude) was then weighed (101.17g) and 45.74g was weighed out of the crude extract and dissolved in equal amount of methanol and distilled water (100ml each), this was then transferred into a separating funnel after which 200ml of chloroform was added to the extract in the separating funnel and shaken properly and carefully. The funnel was left to stand for a while until the chloroform fraction and the aqueous fraction had visibly separated in the separating funnel. The fractions (chloroform and aqueous) were collected separately and allowed to concentrate using the rotary evaporator and hot air oven, the extracts were then weighed using the weighing balance. Chloroform extract was 1.55g and aqueous extract was 29.69g. The extracts where thereafter stored in a refrigerator at a temperature of 4°C.[13,14]

## *Phytochemical Screening*

The phytochemical screening was performed and analysed as described by Enwa et al.[14] The results obtained are presented in Table 1.

- **Preparation of Media:** Each of the media used was prepared according to the manufacturer's instructions. They are:
- **NUTRIENT BROTH:** 0.65g of nutrient broth was weighed out and dissolved in 50ml of distilled water in a conical flask, the medium was well mixed after which the conical flask was sealed and sterilized by autoclaving at 121°C for fifteen (15) minutes. It was allowed to cool to 50°C before dispensed aseptically into sterile test tubes.
- **MUELLER HINTON AGAR:** 21.2g of Mueller Hinton agar powder was weighed out and dissolved in 450ml of distilled water in a conical flask; the medium was well mixed and sterilized by autoclaving at 121°C for 15 minutes. It was then allowed to cool down to 50°C before aseptically dispensed into sterile Petri-dishes.

## *Antimicrobial Assay*

### *Microorganisms*

Thirty (30) pathogenic strains of methicillin resistant Staphylococcus aureus were obtained from the pharmaceutical microbiology and biotechnology laboratory, Faculty of Pharmacy, Delta state university, Abraka, Delta State. The organisms were maintained on mueller hilton slants prior to use following the method adopted by Enwa et al.[14]

### *Antibacterial Activity by Agar Well Diffusion Method*

0.4g (400mg) of each of the extracts i.e. the crude extract, aqueous and chloroform fraction were reconstituted in 70% methanol to obtain a stock solution of 100mg/ml. Serial dilution was then carried out on the stock solution to obtain various concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. Making use of a Pasteur pipette, the different concentrations of the plant extracts were introduced into each of the equidistant wells (6mm) bored on the mueller hilton agar plates surface previously inoculated with each of the strains of methicillin resistant staphylococcus aureus. A positive and negative control well containing ciprofloxacin and methanol respectively were placed in each of the plates seeded with methicillin resistant staphylococcus aureus. The agar plates were incubated at 37°C for 24 hours. Antimicrobial activity was expressed as diameter of the zones of inhibition calculated as the difference in diameter of the observed zones as those of the wells, comparing it with the corresponding standard antibacterial drug. The result of antimicrobial activities are presented in Table 2.

### *Determination*

The MIC of the different phases of leaf extract was determined by incorporating various concentrations of the reconstituted leaf extracts that possessed antimicrobial activity from the result of the Agar well diffusion method (1.625mg/ml to 200mg/ml) by serial dilution into test tubes containing the culture media (mueller Hilton agar). The culture media and plant extracts were mixed thoroughly together and introduced into petri dishes. They were swirled gently in a radial and longitudinal fashion on the work bench and allowed to solidify at room temperature. After solidification, the pathogenic strains of MRSA were inoculated into the agar plates using the wire loop and were then incubated at 37°C for 18-24 hours. The minimum inhibitory concentration was regarded as the lowest concentration of the extracts that did not yield a single bacterial growth on mueller Hilton Agar plates after 18-24 hours of incubation at 37°C. The results of MIC of crude extract are presented in Table 3. The results of the

antimicrobial activity of the aqueous fraction expressed as zone of inhibition are presented in Table 4. The results of MIC of the aqueous fraction are presented in Table 5. The Result of the antimicrobial activity of the chloroform fraction expressed as zone of inhibition are presented in Table 6. The Results of the MIC of chloroform fraction are presented in Table 7.

## RESULTS AND DISCUSSION

It is evident from Table 1 that the secondary metabolites present in the methanol leaf extract of *Lawsonia inermis* are responsible for the antibacterial activity.

Tables 2 and 4 show that the antimicrobial activity is concentration dependent or dose dependent. The susceptibility of the test organisms to the crude extract and the different methanol extract fractions were all concentration based.[15]

The methanol crude extract of the *Lawsonia inermis* leaves demonstrated highest antibacterial activity with maximum ZI of 15.30mm ± 0.47 and a minimum ZI of 9mm ± 0.82 in Table 2. Whereas, the standard antibiotic used as a positive control (Ciprofloxacin) showed a maximum zone of inhibition of 13mm and a minimum of 4.33mm. Comparing these two results, it can be deduced from this study that the 100mg/ml concentration of the methanol extract of *Lawsonia inermis* has a better antibacterial activity against MRSA as compared to ciprofloxacin. The other concentrations of the leaf extract of the crude extract (50mg/ml, 25mg/ml, 12.5mg/ml, and 6.25mg/ml) showed lesser effect against the test organism as compared to ciprofloxacin (Positive Control) which is closely related to the work done by Enwa et al.[16]

The results shown in Table 3, which is the MIC of the crude fraction, indicates that most of the strains of the test organisms where susceptible to the 100mg/ml, 50mg/ml and 25mg/ml concentration of the leave extract making 25mg/ml concentration the least concentration at which there was no growth of organism. Although, 15 of the strains of the test organism were susceptible to the 12.5mg/ml concentration of the leave extract, hence, it can be deduced that this concentration was the least concentration possessing antibacterial properties against the test organism while the last concentration of 6.25mg/ml possessed no antibacterial property against the test organism (MRSA).[17]

Table 4 shows that antimicrobial activity is concentration dependent or dose dependent. The susceptibility of the test organisms to the crude extract and the different methanol extract fractions were all concentration based.[15]

For the aqueous Fraction in Table 5, it can be seen from the result of the zone of inhibition that this fraction possessed a lower antibacterial property in comparison with both the standard antibiotic (ciprofloxacin) and the crude extract but a higher antibacterial property against the test organism when compared to the crude extract. It can be deduced from the MIC result that, the strains of the test organisms were resistant to all the concentrations of the aqueous fraction of the methanol leave extract of *Lawsonia inermis*. Only 12 strains of the organism were susceptible to the 100mg/ml and 50mg/ml concentrations of the aqueous fraction with 50mg/ml being the least concentration which inhibited the growth of the test organisms. One of the strains of the test organism was susceptible to 25mg/ml and 12.5mg/ml concentrations making 12.5mg/ml the least concentration that inhibited the growth of that particular strain of the organism.[16]

The least antibacterial activity was shown by the Chloroform fraction particularly the least concentration of 12.5 mg/ml and 6.25 mg/ml having no zone of inhibition on any of the strains of the test organism as seen in Table 6. The highest concentration for the chloroform phase (100mg/ml) had a maximum zone of inhibition of 4.67mm ± 0.47 and showed no zone of inhibition for some of the strains.

For the chloroform fraction of the methanol extract of *Lawsonia inermis*, most of the strains of the test organisms were resistant to all the concentrations of this fraction, only six strains of the test organisms were susceptible to the 200mg/ml and 100mg/ml concentrations with 100mg/ml being the least concentration of this fraction which inhibited the growth of the test organism.[17]

## CONCLUSION

According to previous studies, *Lawsonia inermis* L. is a plant that has a variety of phytochemicals that can be used to cure human disorders like arthritis, diabetes, ulcers, inflammation, bacterial infection and more. From this study, it can be extrapolated that the reasons behind these claims is due to the presence of very active secondary metabolites present in the plant such as alkaloids, steroids, saponins and terpenoids.

The methanol extract of *Lawsonia inermis* was found to have antibacterial activity against MRSA at varying degrees which is in supportive of the use of the plant in the treatment of several infections. The crude extract of the plant was most effective against MRSA compared to the aqueous and chloroform fraction and this could be, because these fractions work better together without fractionation. However, further research should be carried out in order to establish a safe dosage regimen.

**Table 1: Phytochemical screening of the methanol extract of the leaves of *Lawsonia inermis***

| Test | Observation | Inference |
|---|---|---|
| Saponins | Persistent frothing | Present |
| Tannins | Dark green colour formation | Present |
| Alkaloids | Reddish brown precipitate with Dragendorff"s reagent | Present |
| Flavonoids | No visible colour change | Absent |
| Terpenoids | Solution formed with a brown ring at the interface | Present |
| Steroids | Red colour on lower chloroform layer | Present |
| Cardiac glycoside | No visible colour change | Absent |
| Reducing sugar | No visible colour change | Absent |

**Table 2: Antimicrobial Activity of the Crude Extract of the Leaves of *Lawsonia inermis***

| Organism | 100mg/ ml (mm) | 50mg/ml (mm) | 25mg/ml (mm) | 12.5mg/ml (mm) | 6.25mg/ml (mm) | Positive Control (mm) | Negative Control (mm) |
|---|---|---|---|---|---|---|---|
| MRSA 1 | 10.00 ± 1.63 | 6.33 ± 0.47 | 4.33 ± 0.47 | - | - | 9.00 ± 0.82 | - |
| MRSA 2 | 10.33 ± 1.24 | 6.33 ± 0.47 | 3.00 ± 0.82 | - | - | 11.00 ± 0.82 | - |
| MRSA 3 | 11.00 ± 0.82 | 7.00 ± 1.41 | 6.00 ± 0.82 | 4.00 ± 0 | 3.00 ± 0.82 | 4.33 ± 0.47 | - |
| MRSA 4 | 9.00 ± 0.82 | 6.33 ± 0.47 | 4.33 ± 0.47 | - | - | 12.60 ± 1.88 | - |
| MRSA 5 | 15.30 ± 0.47 | 11.67± 1.30 | 8.66 ± 0.47 | 6.66 ± 1.69 | 1.33 ± 0.47 | 6.66 ± 1.41 | - |
| MRSA 6 | 11.60 ± 2.0 | 9.33 ± 0.98 | 8.33 ± 0.47 | 5.66 ± 0.47 | 4.33 ± 0.47 | 12.00 ± 0 | - |
| MRSA 7 | 10.83 ± 1.18 | 8.00 ± 1.41 | 7.00 ± 0.82 | 4.66 ± 0.94 | 1.33 ± 0.47 | 11.33 ± 0.82 | - |
| MRSA 8 | 9.00 ± 0.82 | 6.33 ± 0.47 | 4.67 ± 0.47 | - | - | 10.00 ± 0.82 | - |
| MRSA 9 | 9.33 ± 0.47 | 6.66 ± 0.47 | 3.33 ± 0.47 | - | - | 10.33 ± 1.69 | - |
| MRSA 10 | 10.67 ± 0.47 | 7.00 ± 1.41 | 7.00 ± 0.82 | - | - | 9.00 ± 1.63 | - |
| MRSA 11 | 11.00 ± 0.82 | 6.00 ± 0 | 4.00 ± 0 | - | - | 10.00 ± 0 | - |
| MRSA 12 | 9.33 ± 1.24 | 11.67± 1.30 | 7.67 ± 0.47 | - | - | 12.33 ± 0.94 | - |
| MRSA 13 | 10.33 ± 0.47 | 9.33 ± 0.98 | 7.33 ± 1.24 | 5.33 ± 0.47 | 4.33 ± 0.47 | 5.00 ± 0.47 | - |
| MRSA 14 | 11.66 ± 0.47 | 8.00 ± 1.41 | 4.33 ± 0.47 | 5.33 ± 1.24 | 5.00 ± 0.82 | 5.67 ± 1.41 | - |
| MRSA 15 | 13.33 ± 1.23 | 7.00 ± 0 | 3.33 ± 0.47 | 4.33 ± 0.47 | 4.00 ± 0 | 8.67 ± 1.69 | - |
| MRSA 16 | 11.66 ± 0.94 | 6.33 ± 0.47 | 7.67 ± 0.94 | 5.00 ± 0.82 | 3.67 ± 0.82 | 5.00 ± 0 | - |
| MRSA 17 | 11.00 ± 0.82 | 7.00 ± 1.41 | 4.67 ± 0.47 | - | - | 12.00 ± 0.94 | - |
| MRSA 18 | 9.00 ± 0.82 | 6.33 ± 0.47 | 7.33 ± 1.24 | 4.00 ± 0 | 2.67 ± 1.41 | 7.33 ± 0.47 | - |
| MRSA 19 | 11.33 ± 0.47 | 10.66± 0.94 | 5.67 ± 1.69 | 5.00 ± 0.82 | 1.66 ± 0.47 | 10.67 ± 0.47 | - |
| MRSA 20 | 10.00 ± 2.16 | 9.33 ± 0.98 | 4.00 ± 0 | - | - | 12.67 ± 0.94 | - |
| MRSA 21 | 10.33 ± 1.24 | 8.00 ± 1.41 | 4.33 ± 0.47 | - | - | 13.00 ± 0.82 | - |
| MRSA 22 | 11.00 ± 0.82 | 8.00 ± 1.41 | 5.33 ± 0.47 | - | - | 10.67 ± 0.94 | - |
| MRSA 23 | 10.66 ± 1.69 | 10.33± 1.69 | 4.67 ± 0.47 | - | - | 9.33 ± 1.24 | - |
| MRSA 24 | 12.33 ± 1.24 | 9.33 ± 0.86 | 5.00 ± 0.82 | - | - | 10.33 ± 1.24 | - |
| MRSA 25 | 10.00 ± 1.63 | 8.66 ± 0.47 | 5.33 ± 0.94 | 4.00 ± 0 | 3.00 ± 0.82 | 7.00 ± 0.82 | - |
| MRSA 26 | 11.00 ± 1.63 | 8.66 ± 0.94 | 8.00 ± 0.82 | 5.00 ± 0.82 | 1.33 ± 0.47 | 10.67 ± 1.24 | - |
| MRSA 27 | 11.00 ± 0.82 | 7.00 ± 0.82 | 6.33 ± 0.47 | 6.00 ± 0 | 5.00 ± 0.82 | 7.00 ± 1.63 | - |
| MRSA 28 | 10.00 ± 1.63 | 9.00 ± 0.82 | 7.00 ± 0.82 | 5.33 ± 0.47 | 4.33 ± 0.47 | 11.00 ± 0 | - |
| MRSA 29 | 11.66 ± 0.94 | 11.00± 1.69 | 8.33 ± 0.47 | - | - | 11.67 ± 1.24 | - |
| MRSA 30 | 11.33 ± 0.94 | 9.00 ± 0.82 | 6.33 ± 0.47 | - | - | 9.33 ± 1.24 | - |

Keys: (-) shows no zone of inhibition

**Table 3: Minimum inhibitory concentration (MIC) of thecrude extract**

| S/N | Organism | 100mg/ Ml | 50mg/ Ml | 25mg/ ml | 12.5mg/ ml | 6.25mg/ ml | 3.125mg/ Ml |
|---|---|---|---|---|---|---|---|
| 1 | MRSA 1 | - | - | - | + | + | + |
| 2 | MRSA 2 | - | - | - | + | + | + |
| 3 | MRSA 3 | - | - | - | - | + | + |
| 4 | MRSA 4 | - | - | - | + | + | + |
| 5 | MRSA 5 | - | - | - | - | + | + |
| 6 | MRSA 6 | - | - | - | - | + | + |
| 7 | MRSA 7 | - | - | - | - | + | + |
| 8 | MRSA 8 | - | - | - | + | + | + |
| 9 | MRSA 9 | - | - | - | + | + | + |
| 10 | MRSA 10 | - | - | - | + | + | + |
| 11 | MRSA 11 | - | - | - | + | + | + |
| 12 | MRSA 12 | - | - | - | + | + | + |
| 13 | MRSA 13 | - | - | - | - | + | + |
| 14 | MRSA 14 | - | - | - | - | + | + |
| 15 | MRSA 15 | - | - | - | - | + | + |
| 16 | MRSA 16 | - | - | - | - | + | + |
| 17 | MRSA 17 | - | - | - | + | + | + |
| 18 | MRSA 18 | - | - | - | - | + | + |
| 19 | MRSA 19 | - | - | - | - | + | + |
| 20 | MRSA 20 | - | - | - | - | + | + |
| 21 | MRSA 21 | - | - | - | + | + | + |
| 22 | MRSA 22 | - | - | - | + | + | + |
| 23 | MRSA 23 | - | - | - | + | + | + |
| 24 | MRSA 24 | - | - | - | + | + | + |
| 25 | MRSA 25 | - | - | - | - | + | + |
| 26 | MRSA 26 | - | - | - | - | + | + |
| 27 | MRSA 27 | - | - | - | - | + | + |
| 28 | MRSA 28 | - | - | - | - | + | + |
| 29 | MRSA 29 | - | - | - | + | + | + |
| 30 | MRSA 30 | - | - | - | + | + | + |

Keys: (-) shows no growth of organism (+) shows growth of organism.

**Table 4: Antimicrobial activity of the aqueous fraction expressed as zone of inhibition**

| S/N | Organism | 100mg/ ml (mm) | 50mg/ml (mm) | 25mg/ml (mm) | 12.5mg/ml (mm) | 6.25mg/ml (mm) | Positive Control (mm) | Negative Control (mm) |
|---|---|---|---|---|---|---|---|---|
| 1 | MRSA 1 | 7.67 ± 0.47 | 6.00 ± 0 | 1.33 ± 1.88 | 1.33 ± 1.88 | - | 9.00 ± 0.82 | - |
| 2 | MRSA 2 | - | - | - | - | - | 11.00 ± 0.82 | - |
| 3 | MRSA 3 | 7.67 ± 1.24 | 6.33 ± 1.24 | 5.33 ± 1.08 | 5.00 ± 0.82 | 3.00 ± 0 | 4.33 ± 0.47 | - |
| 4 | MRSA 4 | - | - | - | - | - | 12.60 ± 1.88 | - |
| 5 | MRSA 5 | - | - | - | - | - | 6.66 ± 1.41 | - |
| 6 | MRSA 6 | - | - | - | - | - | 12.00 ± 0 | - |
| 7 | MRSA 7 | 11.00 ± 0 | 9.33 ± 0.82 | 7.67 ± 0.47 | 7.33 ± 1.24 | 4.00 ± 0.82 | 11.33 ± 0.82 | - |
| 8 | MRSA 8 | - | - | - | - | - | 10.00 ± 0.82 | - |
| 9 | MRSA 9 | 7.00 ± 1.41 | 4.33 ± 0.47 | - | - | - | 10.33 ± 1.69 | - |
| 10 | MRSA 10 | - | - | - | - | - | 9.00 ± 1.63 | - |
| 11 | MRSA 11 | - | - | - | - | - | 10.00 ± 0 | - |
| 12 | MRSA 12 | 9.00 ± 1.63 | 7.00 ± 0.82 | 1.33 ± 1.88 | 1.33 ± 1.88 | - | 12.33± 0.94 | - |
| 13 | MRSA 13 | - | - | - | - | - | 5.00 ± 0.47 | - |
| 14 | MRSA 14 | 12.00 ± 1.63 | 8.67 ± 1.24 | 5.33 ± 0.94 | 3.00 ± 0 | 1.00 ± 0 | 5.67 ± 1.41 | - |
| 15 | MRSA 15 | - | - | - | - | - | 8.67 ± 1.69 | - |
| 16 | MRSA 16 | - | - | - | - | - | 5.00± 0 | - |
| 17 | MRSA 17 | - | - | - | - | - | 12.00 ± 0.94 | - |
| 18 | MRSA 18 | - | - | - | - | - | 7.33 ± 0.47 | - |
| 19 | MRSA 19 | 8.67 ± 0.47 | 4.67 ± 0.47 | 1.33± 1.88 | - | - | 10.67 ± 1.47 | - |
| 20 | MRSA 20 | 6.33 ± 1.24 | 4.00 ± 0 | - | - | - | 12.67 ± 0.94 | - |
| 21 | MRSA 21 | 6.33 ±0.47 | 5.33 ± 0.47 | - | - | - | 13.00 ± 0.82 | - |
| 22 | MRSA 22 | - | - | - | - | - | 10.67 ± 0.94 | - |
| 23 | MRSA 23 | - | - | - | - | - | 9.33 ± 1.24 | - |
| 24 | MRSA 24 | - | - | - | - | - | 10.33 ± 1.24 | - |
| 25 | MRSA 25 | 6.00 ± 1.41 | 4.67 ± 0.47 | - | - | - | 7.00 ± 0.82 | - |
| 26 | MRSA 26 | - | - | - | - | - | 10.67 ± 1.24 | - |
| 27 | MRSA 27 | 5.67 ± 0.47 | 4.00 ± 0 | - | - | - | 7.00 ± 1.63 | - |
| 28 | MRSA 28 | 7.00 ± 0.82 | 4.67 ± 0.47 | - | - | - | 11.00 ± 0 | - |
| 29 | MRSA 29 | - | - | - | - | - | 11.67 ± 1.24 | - |
| 30 | MRSA 30 | - | - | - | - | - | 9.33 ± 1.24 | - |

Keys**:** (-) shows no zone of inhibition.

**Table 5: MIC of the aqueous fraction**

| S/N | Organism | 100mg/ ml | 50mg/ Ml | 25mg/ ml | 12.5mg/ ml | 6.25mg/ ml | 3.125mg/ ml | 1.625mg/ ml |
|---|---|---|---|---|---|---|---|---|
| 1 | MRSA 1 | - | - | + | + | + | + | + |
| 2 | MRSA 3 | - | - | + | + | + | + | + |
| 3 | MRSA 7 | - | - | - | - | + | + | + |
| 4 | MRSA 9 | - | - | + | + | + | + | + |
| 5 | MRSA 12 | - | - | + | + | + | + | + |
| 6 | MRSA 14 | - | - | + | + | + | + | + |
| 7 | MRSA 19 | - | - | + | + | + | + | + |
| 8 | MRSA 20 | - | - | + | + | + | + | + |
| 9 | MRSA 21 | - | - | + | + | + | + | + |
| 10 | MRSA 25 | - | - | + | + | + | + | + |
| 11 | MRSA 27 | - | - | + | + | + | + | + |
| 12 | MRSA 28 | - | - | + | + | + | + | + |

Keys: (-) shows no growth of organism (+) shows growth of organism

**Table 6: Antimicrobial activity of chloroform fraction expressed as zone of inhibition**

| S/N | Organism | 100mg/ml (mm) | 50mg/ml (mm) | 25mg/ml (mm) | 12.5mg/ml (mm) | 6.25mg/ml (mm) | Positive Control (mm) | Negative Control (mm) |
|---|---|---|---|---|---|---|---|---|
| 1 | MRSA 1 | - | - | - | - | - | 9.00 ± 0.82 | - |
| 2 | MRSA 2 | - | - | - | - | - | 11.00 ±0.82 | - |
| 3 | MRSA 3 | - | - | - | - | - | 4.33 ±0.47 | - |
| 4 | MRSA 4 | 3.00 ±0 | 2.67 ±0.47 | 2.00 ±0 | - | - | 12.60 ±1.88 | - |
| 5 | MRSA 5 | 4.67 ±0.47 | 3.33 ±0.47 | - | - | - | 6.66 ±1.41 | - |
| 6 | MRSA 6 | - | - | - | - | - | 12.00 ±0 | - |
| 7 | MRSA 7 | - | - | - | - | - | 11.33 ±0.82 | - |
| 8 | MRSA 8 | - | - | - | - | - | 10.00 ±0.82 | - |
| 9 | MRSA 9 | - | - | - | - | - | 10.33 ±1.69 | - |
| 10 | MRSA 10 | - | - | - | - | - | 9.00 ±1.63 | - |
| 11 | MRSA 11 | - | - | - | - | - | 10.00 ±0 | - |
| 12 | MRSA 12 | - | - | - | - | - | 12.33 ±0.94 | - |
| 13 | MRSA 13 | - | - | - | - | - | 5.00 ±0.47 | - |
| 14 | MRSA 14 | 4.00 ±0.82 | 2.67 ±0.47 | 0.67 ±0.94 | - | - | 5.67 ±1.41 | - |
| 15 | MRSA 15 | 3.67 ±0.47 | 2.67 ±0.47 | - | - | - | 8.67 ±1.69 | - |
| 16 | MRSA 16 | - | - | - | - | - | 5.00 ±0 | - |
| 17 | MRSA 17 | - | - | - | - | - | 12.00 ±0.94 | - |
| 18 | MRSA 18 | - | - | - | - | - | 7.33 ±0.47 | - |
| 19 | MRSA 19 | - | - | - | - | - | 10.67 ±1.47 | - |
| 20 | MRSA 20 | 4.00 ±0.82 | 3.00 ±0.82 | - | - | - | 12.67 ±0.94 | - |
| 21 | MRSA 21 | - | - | - | - | - | 13.00 ±0.82 | - |
| 22 | MRSA 22 | - | - | - | - | - | 10.67 ±0.94 | - |
| 23 | MRSA 23 | 4.33 ±0.47 | 3.33 ±0.47 | 2.00 ±0 | - | - | 9.33 ±1.24 | - |
| 24 | MRSA 24 | - | - | - | - | - | 10.33 ±1.24 | - |
| 25 | MRSA 25 | - | - | - | - | - | 7.00 ±0.82 | - |
| 26 | MRSA 26 | - | - | - | - | - | 10.67 ±1.24 | - |
| 27 | MRSA 27 | - | - | - | - | - | 7.00 ±1.63 | - |
| 28 | MRSA 28 | - | - | - | - | - | 11.00 ±0 | - |
| 29 | MRSA 29 | - | - | - | - | - | 11.67 ±1.24 | - |
| 30 | MRSA 30 | - | - | - | - | - | 9.33 ±1.24 | - |

Keys: (-) shows no zone of inhibition

**Table 7: MIC of Chloroform Fraction**

| S/N | ORGANISM | 200mg/ml | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml |
|---|---|---|---|---|---|---|
| 1 | MRSA 4 | - | - | + | + | + |
| 2 | MRSA 5 | - | - | + | + | + |
| 3 | MRSA 14 | - | - | + | + | + |
| 4 | MRSA 15 | - | - | + | + | + |
| 5 | MRSA 20 | - | - | + | + | + |
| 6 | MRSA 23 | - | - | + | + | + |

Keys: (-) shows no growth of organism (+) shows growth of organism

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