

# Antibacterial Activities and Phytochemical Analysis of *Chromolaena odorata* Leaves on Methicillin Resistant *Staphylococcus aureus*

Mbah-Omeje Kelechi Nkechinyere<sup>1</sup>, Ossai-Oloto Mary Chioma<sup>2</sup>

<sup>1</sup>Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Enugu, Nigeria

<sup>2</sup>Department of Science Laboratory Technology, Institute of Management and Technology, Enugu, Nigeria

## Email address:

Kelechimbahomeje@gmail.com (Mbah-Omeje K. N.), Ossaimaryc@gmail.com (Ossai-Oloto M. C.)

## To cite this article:

Mbah-Omeje Kelechi Nkechinyere, Ossai-Oloto Mary Chioma. Antibacterial Activities and Phytochemical Analysis of *Chromolaena odorata* Leaves on Methicillin Resistant *Staphylococcus aureus*. *American Journal of Biomedical and Life Sciences*.

Vol. 8, No. 2, 2020, pp. 33-40. doi: 10.11648/j.ajbls.20200802.12

Received: February 3, 2020; Accepted: March 13, 2020; Published: March 31, 2020

**Abstract:** Plants contains secondary metabolites or phytochemicals which when consumed by humans have therapeutic effects. This study therefore analyzed the phytochemical composition of *Chromolaena odorata* so as to give an idea of its possible pharmacological potentials. The present study also evaluates the antibacterial activity of the crude extracts of the leaves of *Chromolaena odorata* against methicillin-resistant *Staphylococcus aureus*. A total of 40 samples were collected from food vendors and male students in Esut. These comprised of 10 samples each of soya milk, zobo, ugba and 10 urine samples. Cultures were done on Manitol salt agar. Methicillin resistant *Staphylococcus aureus* (MRSA) were determined using oxacillin 10ug sensitivity disc. Preliminary qualitative phytochemical analysis were done using standard methods to reveal the presence of the basic phytochemicals. The powder was also macerated in ethanol and chloroform to produce ethanol and chloroform crude extracts that were reconstituted with dimethyl sulfoxide to concentrations (mg/ml) of 200, 100, 50, 25, 12.5 and 6.25. MRSA isolates were screened for sensitivity to the extracts using the agar well diffusion method. MRSA isolates were highly prevalent at 80%. The leaves of *Chromolaena odorata* contained alkaloids, tannins, flavonoids, terpenoids, saponins, phenols and cardiac glycosides. The ethanol extracts had highest concentration of almost all phytochemicals present. The antibacterial activity of the plant was concentration dependent in both ethanol and chloroform extracts. The ethanol extracts showed higher zone of inhibition of 28mm at concentration of 200mg/ml while chloroform extracts showed lower zones of inhibition of 12mm at the same concentration. The substantial quantity of the basic phytochemicals in *Chromolaena odorata* could render it a utility plant in therapeutic use. Due to the profound antibacterial effect, the plant could be classified as a broad spectrum antibacterial agent for the management of MRSA diseases.

**Keywords:** Antibacterial Activity, Phytochemical Analysis, *Chromolaena odorata* Leaves, Methicillin Resistant *Staphylococcus aureus*

## 1. Introduction

Infectious diseases have been troubling mankind even before civilization and they are believed to be present from the very existence of man. The control of infectious diseases has encountered many problems, typical amongst them being the rise in the number of microorganisms that are resistant to antimicrobial agents. Thus, resistance are observed in cases of infections caused by some microorganisms; hence, the

failure to respond to conventional treatment. The primary cause of antibiotic resistance is mainly genetic mutation in bacteria with others including abuse of antimicrobial drugs. These problems provide conducive atmosphere for the growth, spread, emergence and persistence of resistant microorganisms. Typical illustration of such resistant microorganism is Methicillin Resistant *Staphylococcus aureus* (MRSA) which is a very infectious bacterium that is resistant to numerous antibiotics including methicillin, amoxillin, penicillin, and oxacillin. This resistant makes it

challenging to treat or manage. Over the years, it has developed resistance to beta-lactam antibiotics. The resistance might be due to a resistant gene, *MecA*, which prevents  $\beta$ -lactam anti-infection agents from inactivating the compound transpeptidases that are basic for the production of cell wall [21].

MRSA infection is one of the leading causes of hospital-acquired infections and community-associated infections usually associated with overcrowded community or environment such as schools and hospitals. It can cause a range of organ specific infections, the most common being the skin and subcutaneous tissues, followed by invasive infections like osteomyelitis, meningitis, pneumonia, lung abscess, and empyema.

Plants have been utilized for therapeutic purposes much sooner than written history and the medicinal uses were described by ancient Chinese and Egyptian as early as 3,000 [13]. The term herbal plant includes various types of plants used in herbal medicine. It is the use of plants for medicinal purposes. Traditionally, herbal medicine is used alongside conventional medicine and it is gradually becoming one of the major ways in advance clinical researches in preventing and treating diseases, which are otherwise considered difficult to cure [6].

*Chromolaena odorata* is a species of flowering shrub in the sunflower family, Asteraceae. Medicinal uses of *C. odorata*, are anti-bacterial activities [20, 23]; anti-cancer [1]; anti-fungal [13]; anti-biofilm activity [28]; anti-hepatotoxicity [5]; anti-malarial activity [20]; phytopathogenic activity [22]; wound healing activities [2]; and antioxidant activity [3]. Chemical compounds produced by this plant play a role in inhibiting the growth of pathogenic micro-organisms: Leaf, root and stem extracts of *Chromolaena odorata* contain tannins, saponins, terpenoids, flavonoids, alkaloids and cardiac glycosides [3, 19, 27].

Previous studies on the antibacterial activity of this plant extract have been limited to clinical diarrheal strains such as *B. Cereus*, *E. Coli*, *Kleb. Oxytoca*, *Salmonella enterica*, *Salmonella Typhimurium*, *ShigellaSonnei* and *Vibro Cholera* [4, 9, 13, 15, 27]. A few reports have investigated the effect of *C. odorata* leaf extracts on MRSA.

## 2. Materials and Methods

### 2.1. Collection and Identification of the Plant Materials

*Chromolaena odorata* leaves were harvested from Faculty of Natural Science, Agbani Enugu early in the morning. The collected plants materials were placed in clean celophane bags and transported immediately to the applied microbiology and Brewing laboratory, ESUT. It was authenticated in the department of applied biology ESUT.

### 2.2. Preparation of Crude Extract from the Plant Materials

The fresh plant materials were exposed to suitable preliminary processing by removal of unwanted materials

and contaminants. They were washed and air-dried in a shade at room temperature. They were pulverized and stored in air-tight bags for further use.

#### 2.2.1. Preparation of *Chromolaena Odorata*

##### *Ethanol extract.*

A total of 200g of air-dried powdered *Chromolaenaodorata* was weighed into conical flask and mixed with 500ml of ethanol. The mixture was left at room temperature for 3 days for maceration. The solution was filtered using muslin cloth and the filtrates evaporated to dryness at room temperature. They were stored at 4°C until use.

#### 2.2.2. Chloroform Extract

A total of 200g of the air dried powdered *Chromolaenaodorata* was weighed into a conical flask and mixed with 500ml of chloroform. The mixture was left at room temperature for 3 days for maceration. The solution was filtered using muslin cloth and the filtrates evaporated to dryness at room temperature. They were stored at 4°C until use.

### 2.3. Sample Collection

A total of 40 samples comprising of zobo, soya milk drink, ugba and urine were collected. 10 samples each of zobo, soya milk and ugba were collected from different food vendors in ESUT while ten urine samples were collected with sterile universal bottles from 10 male students in ESUT.

### 2.4. Isolation of Organism

A total of 1g of ugba will be pulverized with 9mls of distilled water, 0.1ml will be inoculated on manitol salt agar plates and incubated at 28°C for 24hr. With the aid of a sterile wire loop, a loopful of each samples (soya milk, zobo and urine) respectively were picked and inoculated on Manitol salt agar plates and incubated at 28°C for 24h. Pure cultures were obtained by subculturing on nutrient agar plates and incubated at 28°C for 24hr. They were kept on nutrient agar slants at 4°C for future use.

### 2.5. Phytochemical Screening of the Extracts

Phytochemical Screening test were carried out on the chloroform and ethanol extracts of *Chromolaenaodorata* according to the methods used by [12]: the test samples were subjected to phytochemical analysis in order to determine the presence of phytochemical constituents. The phytochemical tests employed were for tannins, saponins, cardiac glycosides, flavonoids, terpenoids, alkaloids, reducing sugar and phenols.

#### 2.5.1. Test for Flavonoids (Ferric Chloride Test)

A total of 20mg of each extract was dissolved in 1ml of distilled water. 0.5ml of dilute ammonia solution and concentrated sulphuric acid was added later. A yellow colour showed the presence of flavonoids. The yellow colour becomes colourless on allowing the solution to stand.

### 2.5.2. Test for Phenols

A total of 20mg of each extract was treated with aqueous 5% ferric chloride ( $\text{FeCl}_3$ ) and observed for the formation of deep blue or black colour indicating the presence of phenol.

### 2.5.3. Test for Saponin

A total of 40mg of each extract was dissolved in 5ml of distilled water and shaken vigorously till a stable persistent froth was obtained. The froth was mixed with 3 drops of olive oil and shaken vigorously. The presence of emulsion indicated the presence of saponin.

### 2.5.4. Test for Tannins

A total of 20mg of each extract was dissolved in 1ml of distilled water and heated on aburnsen burner flame. The mixture was then filtered and 3 drops of ferric chloride were added to the solution. A green colour indicated the presence of tannins in the extract.

### 2.5.5. Test for Terpenoids

A total of 20mg of each extract was dissolved in 1ml of chloroform and 1ml of concentrated sulphuric acid was added to it. A reddish brown discolouration at the interface showed the presence of terpenoid.

### 2.5.6. Test for Cardiac Glycosides

A total of 1g of each extract was dissolved in 9ml of distilled water and kept for further use. 1ml of concentrated  $\text{H}_2\text{SO}_4$  was dispensed in a test tube and 5ml of each extract was mixed with 2ml of glacial acetic acid ( $\text{CH}_3\text{CO}_2\text{H}$ ), containing 1 drop of iron chloride ( $\text{FeCl}_3$ ). Each mixture was carefully added to 1ml of concentrated hydrochloric acid so that it was underneath the mixture. If cardiac glycoside were present in the extract, a brown ring was observed indicating the presence of the cardiac glycolysis constituent.

### 2.5.7. Test for Reducing Sugar

A total of 20mg of each extract were heated with Fehlings solution A and B. The appearance of orange red precipitate showed the presence carbohydrates.

### 2.5.8. Test for Alkaloids

A total of 20mg of each extract was dissolved in 2ml of concentrated HCL and few drops of Mayer's reagent was added. Absence of a green or white precipitate indicated a negative result.

## 2.6. Isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA)

Methicillin resistant *Staphylococcus aureus* were determined by using oxacillin 10 $\mu\text{g}$  sensitivity disc. The pure isolates of each of the test organisms were inoculated in sterile slants containing nutrient agar and used for antibacterial assay.

## 2.7. Inoculum Preparation

A loopful of each of the MRSA pure isolates were transferred into sterile 5ml nutrient broth in a test tube and

incubated at 28°C for 24hr. Each of the cultures was then adjusted to 0.5 MacFaland turbidity standard..

## 2.8. Reconstitution of Extracts

Aliquots of 0.8g of ethanol and chloroform extracts of *Chromolaena odorata* leaves were transferred respectively into sterile test tubes, 4ml of Dimethyl Sulfoxide (DMSO) was added giving a concentration of 200mg/ml. 2 fold dilutions were further made by transferring 2ml from previous tube into five different test tubes containing 2ml of distilled water each giving a concentration of 100, 50, 25, 12.5 and 6.25mg/ml.

## 2.9. Assay for Antibacterial Activity

Antibacterial activity of chloroform and ethanol extracts of *Chromolaena odorata* was determined by agar well diffusion method as described by lino and Deogracious (2006), 0.1ml of the organism already matched with MacFarland's turbidity standard were inoculated onto Mueller hinton agar plates. A sterile cork borer was then used to make two wells (6mm diameter each) for different concentrations of the extracts on each of the plates containing cultures of the test isolates. 0.1ml of each concentrations of 6.25, 12.5, 25, 50, 100 and 200mg/ml of the extracts were then introduced into seven wells using sterile Pasteur pipettes. Ciprofloxacin was used as a positive control. The culture plates were allowed to stand on the working bench for 30mins for pre -diffusion and were then incubated at 28°C for 24h. After 24h, antibacterial activity was determined by measuring the diameter zones of inhibition in mm.

## 2.10. Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extract

Minimum Inhibitory Concentration was determined by agar well diffusion method as described by [14]. The concentrations used in the antimicrobial susceptibility test were diluted further (2 fold-dilution) to get different concentrations of 25, 12.5, 6.25, and 3.125mg/ml which were incorporated into various test tubes, 0.1ml of the test organism previously diluted to 0.5 MacFarland's turbidity standard was introduced and spread on each plate containing Mueller Hinton agar 6mm wells were cut using sterile cork borer and each filled with 0.1ml of extracts. The plates were incubated at 28°C for 24hr and the resultant zone of inhibitions was measured. The least concentration of the extracts that inhibits the growth of the test organisms were designated as the Minimum Inhibitory Concentrated (MIC).

## 2.11. Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal concentration (MBC) were deduced from those wells with lowest concentrations at which no growth was detected after culture for 24hr of incubation as described by Nester *et al* (2004). 0.1ml of test organism was transferred to sterile petridish and 0.1ml of the least concentrations that inhibited growth were also

transferred and molten nutrient agar were added and rocked and allowed to stand for 95min and incubated at 28°C for 24hr. The plates were subsequently examined for the presence or absence of living organisms. The minimum concentration at which plates showed no microbial growth was regarded as the MBC.

### 3. Result

#### 3.1. Phytochemical Analysis of Ethanol and Chloroform Extract of *Chromolaena Odorata* Leaves

The preliminary phytochemical analysis of ethanol and chloroform extracts of *Chromolaena odorata* showed presence of some important phytochemicals like alkaloids, tannins, flavonoids, saponins, and phenolic compounds. These phytoconstituents have important pharmacological activities like anti- mutagenic, anti-inflammatory, anti-protozoal, and antioxidant properties (Table 1).

#### 3.2. Occurrence of *Staphylococcus Aureus* from Different Samples

Prevalence of *Staphylococcus aureus* were highest from ugba samples at (9) 90%, while they occurred least in urine at 3 (30%) (Table 2)

#### 3.3. Cultural and Morphological Characteristics of *Staphylococcus Aureus*

The *Staphylococcus aureus* isolates showed yellowish

colonies characteristic of *S. aureus* on manitol salt agar (Plate I).

#### 3.4. Prevalence of MRSA from the Different Samples

MRSA isolates showed oxacillin resistance and there was high prevalence at 32 (80%) (Table 4).

#### 3.5. Antibacterial Activity of Chloroform and Ethanol Extract of *Chromolaena Odorata* on Methicillin Resistant Isolates

The ethanol and chloroform extracts of *Chromolaena odorata* exhibited various antibacterial activities against MRSA. At highest concentration of 200mg/ml the zones of inhibition ranged from 12.0-28mm respectively for chloroform and ethanol extracts (Table 5).

#### 3.6. Minimum Inhibitory Concentration (MIC) of *Chromolaenaodorata* Extracts

The least concentration that inhibited the growth of MRSA was (6.25mg/ml) for ethanol and chloroform extracts respectively (Table 6).

#### 3.7. Minimum Bactericidal Concentration (MBC) of *Chromolaena Odorata* Extracts

The ethanol and chloroform extracts showed similar results, the least concentration at which no growth occurred was (6.25mg/ml) (Table 7).

Table 1. Phytochemical analysis of chloroform and ethanol extract.

| Phytochemical Constituents | Test                | Observation                    | Ethanol | Chloroform |
|----------------------------|---------------------|--------------------------------|---------|------------|
| Saponins                   | Foam test           | Presence of emulsion           | +       | —          |
| Reducing sugar             | Fehling's test      | —                              | —       | —          |
| Flavonoids                 | Feric chloride test | White precipitate              | +       | +          |
| Tannins                    | Lead test           | Green colour                   | +       | +          |
| Terpenoids                 | Salkowaski's test   | Reddish-brown colour           | —       | +          |
| Cardiac Glycosides         | Brown ring test     | Formation of brown ring        | +       | +          |
| Alkaloids                  | Wagner's test       | Red precipitate                | +       | —          |
| Phenols                    | Phenol Test         | Greenish brown or black colour | +       | +          |

KEY:

+: indicates presence of phytochemical constituents.

—: indicates the absence of phytochemical constituents.

The preliminary phytochemical analysis of ethanol and chloroform extracts of *Chromolaenaodorata* showed presence of some important phytochemicals like alkaloids, tannins, flavonoids, saponins, and phenolic compounds. These phytoconstituents have important pharmacological activities like anti- mutagenic, anti-inflammatory, anti-protozoal, and antioxidant properties.

Table 2. Occurrence of *Staphylococcus aureus* from different samples.

| Samples   | Number Tested | Number Positive | Number Negative |
|-----------|---------------|-----------------|-----------------|
| Urine     | 10            | 7 (70%)         | 3 (30%)         |
| Zobo      | 10            | 8 (80%)         | 2 (20%)         |
| Soya Milk | 10            | 8 (80%)         | 2 (20%)         |
| Ugba      | 10            | 9 (90%)         | 1 (10%)         |
| Total     | 40            | 32 (80%)        | 8 (20%)         |

Prevalence of *Staphylococcus aureus* were highest from ugba samples at (9) 90%, while they occurred least in urine at 3 (30%).

**Table 3.** Prevalence of MRSA from the different samples.

| Samples   | Number Positive for <i>S. aureus</i> | Number positive for MRSA. |
|-----------|--------------------------------------|---------------------------|
| Urine     | 7 (70%)                              | 4 (40%)                   |
| Zobo      | 8 (80%)                              | 6 (60%)                   |
| Soya Milk | 8 (80%)                              | 7 (70%)                   |
| Ugba      | 9 (90%)                              | 8 (80%)                   |
| Total     | 32 (80%)                             | 25 (62.5%)                |

MRSA showed oxacillin resistance and there was high prevalence at 32 (80%) in ugba samples.

**Table 4.** Zones of inhibition (mm) of ethanol and chloroform extracts of *Chromolaena odorata* on MRSA isolates. Extracts Inhibitory Concentration (mg/ml).

| Test organism | Extracts (mg/ml) | 200 | 100 | 50 | 25 | 12.5 | 6.25 | Control CIP |
|---------------|------------------|-----|-----|----|----|------|------|-------------|
| MRSA          | Ethanol          | 28  | 24  | 20 | 16 | 8    | 4    | 20          |
|               | Chloroform       | 12  | 10  | 8  | 6  | 4    | -    | 20          |

Keys: += visible turbidity, significant growth.

\_ = No visible turbidity.

The ethanol and chloroform extracts of *Chromolaena odorata* exhibited various antibacterial activities against MRSA. At highest concentration of 200mg/ml the zones of inhibition ranged from 12.0-28mm respectively for chloroform and ethanol extracts.

**Table 5.** Minimum Inhibitory Concentration (MIC) of ethanol and chloroform of *Chromolaena odorata*. Extracts inhibitory concentration (mg/ml).

| Test Organism | Extract (mg/ml) | 25 | 12.5 | 6.25 | 3.125 | MIC (mg/ml) |
|---------------|-----------------|----|------|------|-------|-------------|
| MRSA          | Ethanol         | -  | -    | -    | +     | 6.25        |
|               | Chloroform      | -  | -    | -    | +     | 6.25        |

The least concentration that inhibited the growth of *Staphylococcus aureus* was (6.25mg/ml) for ethanol and chloroform extracts respectively.

**Table 6.** Minimum Bactericidal concentration (MBC) of ethanol and chloroform extracts of *Chromolaenaodorata*. Extract Bactericidalconcentration (mg/ml).

| Test Organism | Extract (mg/ml) | 6.25 | 3.125 | MBC (mg/ml) |
|---------------|-----------------|------|-------|-------------|
| MRSA          | Chloroform      | -    | +     | 6.25        |
|               | Ethanol         | -    | +     | 6.25        |

Keys: + visible turbidity, significant growth

No visible turbidity

The ethanol and chloroform extracts showed similar results, the least concentration at which no growth occurred was (6.25mg/ml).

**Table 7.** Pattern of AntibacteriaResistance/Susceptibility of the Isolate (Ethanol extract of *Chromolaenaodorata*).

| Ethanol extract of <i>Chronolaenaodorata</i> (mg/ml) | Number of MRSA samples (n = 25) |               |
|--|---------------------------------|---------------|
|  | Susceptibility                  | Resistance    |
| 200  | 25 (100%)                       | -             |
| 100  | 20 (80%)                        | 5 (20%)       |
| 50   | 16 (64%)                        | 9 (36%)       |
| 25   | 12 (48%)                        | 13 (%) 52 (%) |
| 12.5   | 8 (32%)                         | 1 (68%)       |
| 6.25   | 4 (16%)                         | 21 (84%) 7    |

At 200mg/ml the MRSA isolate were completely susceptible to ethanol extract of *Chromolaena odorata* extract.

**Table 8.** Pattern of Antibacteria Resistance/ Susceptibility of the Isolate (Chloroform extract of *Chromolaenaodorata*).

| Chloroform extract of <i>Chronolaena odorata</i> (mg/ml) | Number of MRSA samples (n = 25) |            |
|--|---------------------------------|------------|
|  | Susceptibility                  | Resistance |
| 200  | 22 (88%)                        | 3 (12%)    |
| 100  | 18 (72%)                        | 7 (28%)    |
| 50   | 12 (48%)                        | 13 (52%)   |
| 25   | 8 (32%)                         | 17 (68%)   |
| 12.5   | 4 (16%)                         | 21 (84%) 7 |
| 6.25   | 2 (8%)                          | 23 (92%)   |

Varied susceptibility was obtained in MRSA isolates to the chloroform extract of *Chronolaena odorata*.

## 4. Discussion

Phytochemicals are non-nutritive plant compounds, which have protective, curative or disease preventive properties. Plants produce these chemicals to protect themselves, but recent research demonstrates that many phytochemicals can protect humans against diseases. [18]. In the present study, the ethanol and chloroform extracts showed varied results of phytochemical analysis. (Table I). The ethanol extracts of *Chromolaena odorata* leaves showed the presence of alkaloids, saponin, flavonoids, tannin, cardiac glycosides and phenol while reducing sugar and terpenoids were absent. The chloroform extracts of *Chromolaena odorata* leaves showed the presence of flavonoids, tannins, terpenoids, cardiac glycosides and phenols with reducing sugar, saponin and alkaloids being absent. Ethanol exerted more of the active constituents than the corresponding chloroform. These may be attributed to the nature of biological active components (saponin, tannins, alkaloids) and the stronger extract ion capacity of ethanol could have produced greater number of active constituents responsible for antibacterial activity [3]. The presence of phytochemical constituents in *Chromolaena odorata* leaves indicate that they can be used for antimicrobial application. This is supported by [10] who purported that the medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. The presence of alkaloids in the leaves could suggest its efficacy in inhibiting microorganisms and this is in agreement with [24] who suggests that alkaloids are major plant components responsible for antimicrobial activity as it intercalates with DNA and interferes with the cell division. From the study, occurrence of *Staphylococcus aureus* was high in various samples, urine (70%), zobo (80%), soya milk (80%) and ugba (90%) with ugba having the highest prevalence at 90%. (Table 4). The Presence of *Staphylococcus aureus* in food indicates poor or improper handling which is of public health importance. This is in agreement with the works of Barber (2014) and Jacques-Antoine *et al.* (2012) who implicated the presence of *S. aureus* in food capable of causing food poisoning. Methicillin resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to treatment with a common class of antibiotics and the leading causes of hospital-acquired infections and community associated infections. (Abdul *et al.*, 2018). The burden of MRSA colonization and infection has recently expanded to further ecological niches. Since the 1990s, an increasing incidence of MRSA infections arising in the community (CA-MRSA) has been reported from many countries worldwide [8]. MRSA is an important cause of bacterial endocarditis which can cause mortality in about a third of infected patient [7]. MRSA disease have as of late turned into the major concentration concern of healthcare professionals and has risen as a

noteworthy medical issue that is never again restricted to just doctors and nurses (Chambers *et al.*, 2009). Reports of MRSA contaminations happening in group settings (for example daycares, institutions, sports groups) also resulting in deaths of children and grown-ups have made it a cause of concern in all fields and increased its global awareness [25]. From the study, MRSA showed high prevalence in the various samples, urine (40%), zobo (60%), soya milk (70%) and ugba having the highest prevalence at (80%). This is in line with the work of Abdul *et al.* (2018) who also reported high prevalence of HA- MRSA and CA-MRSA which is also considered a significant risk factor for MRSA colonization. In the study, ethanol extracts of *Chromolaena odorata* leaves showed higher antibacterial activity than the chloroform extracts (Table 5). At 200mg/ml ethanol extracts showed the highest zone of inhibition at 28mm while chloroform extract showed lower zone of inhibition at 12mm on isolates at 200mg/ml. This is in line with the work of [17] who observed that fractions of *Chromolaena odorata* had anti-MRSA activities. The anti-microbial ability of *Chromolaena odorata* extracts can be supported with the works of [26] who found *Chromolaena odorata* leaves effective against *S. aureus*, *S. typhi*, *E. coli*, *Candida albicans* and *Aspergillus niger*. There was statistical difference at  $p < 0.05$  between the control (ciprofloxacin) and ethanol extract of *Chromolaena odorata* leaves. The ethanol extract of *Chromolaena odorata* leaves compared well with ciprofloxacin (control). The MIC of the plant extracts against the test organism was also determined. The MIC of ethanol and chloroform extracts of *C. odorata* leaves were found to be at 6.25mg/mls. The results of MIC showed that *C. odorata* extracts are active against the test organism even at low concentration as shown in Table 7.

From this study, it was observed that ethanol extracts exhibited higher inhibitory activity on the test organism than the chloroform extracts. This can be as a result of the ability of ethanol to extract more of essential oils and secondary plants metabolites which are believed to exert antibacterial activity on the test organism [16]. This study however, justifies the scientific use of these plants in traditional medicine in the treatment of infections caused by the test organisms.

## 5. Conclusion

From the study, the presence of *Staphylococcus aureus* in the samples were high and MRSA also showed high prevalence at 80%. The rapid emergence of MRSA in the community underpins the fact that MRSA transmission can occur in everyday life, in home care, during travel, leisure activities, cross-border commuting, exposure to contaminated food samples. It was also observed that the ethanol extracts exhibited higher inhibitory activity on the test organism than the chloroform extracts and compared well with the standard drug ciprofloxacin. This is significant because of the possibility of incorporating its use against

multi-drug resistant organism such as MRSA.

## References

- [1] A. A. Adedapo, O. J. Aremu, and A. Oyagbemi. Anti-oxidant, anti-inflammatory and antinociceptive properties of the acetone leaf extract of *Vernonia amygdalina* in some laboratory animals. *Adv. Pharm. Bull.* 4 (2) (2016): 591-598.
- [2] G. N. Anyasor, D. A. Ama, M. Olushola, and A. F. Aniyikaye. Phytochemical Constituent, Proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extracts of *Chromolaena odorata*. *Annal of Biological Research*, 2 (2) (2011). 441-451.
- [3] A. C. Akinmoladun, E. O. Ibukun, and O. I. A. Dan. Phytochemical Constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. *Scientific Research and Essay*, 2 (6) (2007). 191-194.
- [4] M. Atindehou, L. Lagnuca, B. Guerold, J. M Strub, M. Zhao, A. V. M. Dorsselaer and M. H. Boutigue. Isolation and identification of two antibacterial agents from *Chromolaena odorata* L. activity against four diarrheal strains. *Advances in microbiology*, 3 (2013). 115-121.
- [5] C. S. Alisi, G. O. C. Onyeze, O. A. Ojiak, and C. G. Osuagwu. Evaluation of the Protective Potential of *Chromolaena odorata* Leaf extract on carbon tetrachloride induced oxidative liver damage. *International journal of Biochemistry Research and Review*, 1 (3) (2014). 69-81.
- [6] G. Bordeker, and F. Kronenberg. A Public health agenda for traditional, complementary and alternative medicine. *American Journal of Public Health*. Vol. 12 (10) (2002). 1582-1591
- [7] S. E. Cosgrove, G. Sakoulas, Perencevich, E. N. Schwaber, A. W. Karchmer, Y. Carmeli. Comparison of mortality associated with methicillin-resistant and methicillin –susceptible *Staphylococcus aureus*. *Clin Infect Dis*. 36 (1) (2003). 53-59.
- [8] R. H. Deurenberg, C. Vink, S. Kalenic, A. W Friedrich, C. A Bruggeman, E. E. Stobbering. The molecular evolution of methicillin- resistant *Staphylococcus aureus*. *ClinMicrobiol Infect*. 13 (3) (2007). 222-235.
- [9] E. A. Eze, N. E. Oruche, V. C. Onuora, and C. N. Eze. Antibacterial screening of crude ethalolic leaf extracts of four medicinal plants. *Journal of Asian Scientific Research*, 3 (5) (2013). 431-439.
- [10] H. O. Edeoga, D. E. Okwu. and B. O. Mbaebie. Phytochemical Constituents of some Nigerian medicinal plants. *African journal of Biotechnology* Vol. 4 (7) (2005), pp. 685-688.
- [11] K. K. Naidoo, and G. Naidoo. Screening of *Chromolaena odorata* (L.) King and Robinson for anti-bacterial and anti-fungal properties. *Journal of Medicine and plant research*. 5 (19) (2011): 4859-4862.
- [12] K. Uthayara, K. Pathmanathan, J. P. Jeyadevanand E. C. Jeyadevanand E. C. Jeeyaseelan. Antibacterial activity and qualitative, phytochemical analysis of medicinal plant extracts obtained by sequential extraction method. *International Journal of Universal Pharmacy and Biosciences*. 10 (2) (2010) 76-81.
- [13] K. K. Naidoo, R. M. Cooposamy, and G. Naidoo, G. Screening of *Chromolaena odorata* (L.) King and Robinson for antibacterial and antifungal properties. *Journal of medicinal plants Research*, 5 (19) (2011). 4859-4862.
- [14] M. Ogata, M. Hoshi, S. Urano and T. Endo. Antioxidant activity of cugenol and related monomeric and dimertic compounds. *Chem. Pharm. Bull*, 48 (2002) 1467-1469.
- [15] S. Maji, P. Danlopat, D. Ojha, C. Maity, S. K. Halder, P. K. Mohapatra, and K. C. Mondal. In vitro antimicrobial potentialities of different solvent extracts of ethnomedicinal plants against chemically isolated human pathogens. *Journal of phytotherapy*. 2 (4) (2010). 57-64.
- [16] O. C. Nwinyi, N. S. Chinedu, O. O. Ajani, C. O. Ikpo and K. O. Ogunniran. Antibacterial effects of extracts of *ocimumgratissimum* and Piper guineense on Escherichia coli and *Staphylococcus aureus*, *Afri. J. Food Science*. 3 (3) (2009).81-85.
- [17] D. E. Okwu. Phytochemical and Vitamin content of indigenous species of South Eastern Nigeria *J. sustain. Agric. Envirion*; 6: (2015) 30-34.
- [18] D. Pathak, K. Alam, H. Rohilla. And A. Agarwal. Phytochemical Investigation of Boerhaviadiffusa and andrographisPaniculata: A Comparative study. *Int. Journal of Pharm Sci*, 4 (2012) 250-262.
- [19] T. T. Phan, L. Wang, P. See, R. J. Grayer, S. Y. Chan and S. T. Lee. Phenolic Compounds of *ChromolaenaOdorata* protect cultural skin cells from oxidative damage: implication for cutaneous wound healing. *Biological and Pharmaceutical Bulletin*, 24 (12) (2001). 1373-1379.
- [20] N. Pisuthanan, B. Liawruangrath, S. Liawruang, A. Baramée, A. Apsisariyakul, J. Korth, and J. B. Bremner. Constituents of the essential oil from aerial parts of *Chromolaenaodorata* from Thailand. *Natural Product Research*, 20 (6) (2005) 636-640.
- [21] F. Shahkarani, A. Rashki, and Z. Ghalehnoo. Microbial susceptibility and plasmid profiles of methicillin resistant *Stapylococcusaureus* and methicillin-susceptible *S. aureus*.. *J. Microbial* (7) (2014) 169-172.
- [22] S. L. Sukanya, J. Sudisha, M. Hariprasad, S. R. Niranjana, H. S. Prakash and S. K. Faithima. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *African Journal of Biotechnology*, 8 (23) (2009). 6677-6682.
- [23] A. Suksamra, A. Chotipong, T. Suavansri, S. Boongird, P. Timsuksai, S. Vimultipong, and A. Chuaynugul. Antimycobacterial activity and cytotoxicity of flavonoids from the flowers of *Chromolaenaodorata*. *Archieves of Pharmacal Research*, 27 (5) (2004) 507-511.
- [24] H. Tsuchiya, M. Sato, T. Miyazaki, S. Fujiwara, S. Tanigaki, M. Obayama, T. Tanaka and M. Linuma. Comparative study to the Antibacterial activity of thePhytochemical flavones against Methicillin – resistant *Staphylococcus aureus*. *Journal of Ethanopharmacology*, 50 (2006). 27–34.
- [25] E. E. Udo, J. W. Pearman, W. B. Grubb. (2003) Genetic analysis of community isolates of methicillin-resistance *Staphylococcus aureus* in WesterAustralia. *J Hosp infections* 25 (2003). 97-108.

- [26] C. E. Ugwoke, J. Orgi, S. P. Anze, and V. C. Ulodibia. Quantitative Phytochemical analysis and Antimicrobial potential of the ethanol and aqueous extracts of the leaf, stem and root of *Chromolaena odorata*; *International Journal of Pharmacognosy and Phytochemical Research*, 9 (2) (2017). 207-214.
- [27] P. G. Vital, and W. L. Rivera. Antimicrobial Activity and cytotoxicity of *Chromolaena odorata* (L. F.) King and Robinson and *Uncaria Tomensis*. *Extracts Journal of medicinal plants research* 3 (7) (2009). 511-518.
- [28] M. F. Z. R. Yahya, M. S. A. Ibrahim, W. H. W. M. Zawawi, and U. M. A. Hamid. Biofilm Killing effects of *Chromolaena odorata* extracts against *Pseudomonas aeruginosa*. *Research Journal of Phytochemistry*, 8 (3) (2014). 64-73.