European Journal of Botany Plant Sciences and Phytology
Vol.2,No.4, pp.1-7, December 2014
Published by European Centre for Research Training and Development UK (www.eajournals.org)

# ANTIBACTERIAL ACTIVITIES OF THE PLANT EXTRACT OF *ALTERNANTHERA REPENS*

**Ogundare, A. O. & *Oladejo, B. O.**
Department of Microbiology, Federal University of Technology,
P.M.B. 704, Akure, Nigeria

**ABSTRACT:** *Methanolic extract of the plant Alternanthera repens was obtained using the cold method of extraction. The bioactivity of the extracts was tested against bacterial isolates namely: Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Bacillus subtilis, Bacillus cereus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, and Proteus mirabilis. The agar well diffusion method was used for the in-vitro antibacterial bioassay and it revealed that the extract was able to inhibit the growth of the test organisms at a concentration of 25.0 mg/ml except Escherichia coli, Streptococcus faecalis, and Salmonella typhi which were resistant. The extract had the highest zone of inhibition (30mm) on Staphylococcus aureus, followed by Klebsiella pneumonia and Bacillus subtilis with 26.0 mm and 25.0 mm respectively. The minimum inhibitory concentration (MIC) of the plant extract ranged from 25.0 to 3.125 mg/ml. The antibacterial activity of the methanolic plant extract compared favourably well with commercial antibiotics. The rate of killing of the plant extract on the isolates showed a decrease in the bacterial count with an increase in the exposure time to the extract. Phytochemical screening tests showed the presence of saponins, alkaloids, salkowski, and keller killianie in the plant extract.*

**KEYWORDS**: Antibacterial, Zone of inhibition, Phytochemical screening, Rate of killing.

## INTRODUCTION

Medicinal plants are plants in which one or more of its organs contain substances that could be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Sofowora, 1993). Plant compounds have been reported to be of interest as a source of safer or more effective substitutes than synthetically produced antimicrobial agents (Banso, 2009). Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity (Chang, 1995). More recently, medicinal plant extracts were developed and proposed for use in food as natural antimicrobials. In recent years, pharmaceutical companies have spent considerable time and money in developing therapeutics based upon natural products extracted from plants (Coruh *et al*., 2007). Therefore, the rising incidence of multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources (Veronica *et al*., 2006). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can thus be of great significance in therapeutic treatments.

Several researches demonstrated that many strains of Gram-positive and Gram-negative bacteria currently developed outstanding drug resistance marking the search of new, safe, non toxic and effective antibacterial agents become strictly a necessity. Many antibacterial agents are available in the nature for the treatment of systemic infections. Plants therefore constitute good source active



ISSN 2055-8139(Print), ISSN 2055-8147(Online)

agents for this purpose and many plants extracts have been reported to possess various antimicrobial activities (Nawel *et al*., 2005).

**Description of plant**

*Alternanthera repens* (family Amaranthaceae) is usually a burgundy foliage plant that spreads on the ground and works well for edging, annual groundcover or in a formal knot garden. It performs well in high heat where its colour becomes deeper and richer. It's a dense mat forming plant with annual tops, a fleshy, perennial rootstock, reddish, and hairy stems.The plant has been used as medication for gastrointestinal disease (Adela *et al*. 2008), and traditional Mexican medicine for the treatment of diarrhoea and dysentery (Osuna *et al*., 2005). *A. repens* is commonly used in pig feeding. They can also be used as fresh forage or cooked. They withstand some drought and also grazing. In the wet season, the crude protein content of *A. repens* is high (22.6%), but the fibre content is also rather high.

## MATERIALS AND METHODS

**Collection and preparation of *Alternanthera repens***

The whole plant sample of *Alternanthera repens* was collected from their natural habitat on the ground of Federal university of Technology, Akure, Nigeria. They were identified at the Department of Crop Science and Production of the Federal University of Technology, Akure. The fresh plants were spread and air dried at room temperature and ground into fine powder using milling machine. Exactly 400 g of the pulverized plant was soaked in methanol to saturation for 72 hours. The mixture was agitated after the addition of the solvent. It was sieved with muslin cloth into a clean beaker and then filtered using No. 1 Whatman filter paper. The filtrate was dried using rotary evaporator.

**Test organisms**

Pure cultures of the bacteria used in this research included *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus mirabilis* were collected from Don Bosco Catholic Medical Centre, Araromi Street, Ondo State, Nigeria.

**Antibacterial activities of plant extract**

This was done using the agar well diffusion method as described by Olutiola *et al*., (1991). A 25mg/ml of the plant extract was prepared and introduced into different holes bored on the sterile medium containing the test organism. The plates were incubated uninvertedly at $37^0$C for 24 hours. Areas that showed clear zones around the bored holes indicate the susceptibility of the test organisms to the extracts and this was measured and recorded.

**Minimum Inhibitory Concentration**

Four concentrations (25.0 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml) of the methanol plant extract of *Alternanthera repens* were introduced into different wells bored on the sterile medium containing the test organism. The plates were incubated at $37^0$C for 24 hours. The lowest concentration of the crude plant extract that showed zone of inhibition was recorded.



ISSN 2055-8139(Print), ISSN 2055-8147(Online)

**Antibiotics sensitivity test**
The disc diffusion method as described as Khan *et al*., (2002) was used to determine the antibacterial activities of standard or commercially produced antibiotics against the test isolates.

**Rate of killing of the organism by the extract**
The cells of the organisms (18 hours old broth culture) were prepared by centrifuging at 2000 rpm for 10 minutes, the supernatant was discarded and the cells were washed 3 to 4 times with sterile distilled water. The washed cells (5 ml) and 5 ml of 25 mg/ml of the methanol plant extract was mixed together in a clean sterile test tube and allowed to stand for 24 hours. At intervals of 1 hour each, 1 ml of the mixture was pour plated using nutrient agar medium and incubated at $37^0$C for 24 hours. The microbial load was thereafter determined.

**Phytochemical screening**
The plant extract was screened for alkaloids, saponins and cardiac glycoside as described by Trease and Evans (2004).

## RESULTS AND DISCUSSION

Table 1 shows the antibacterial activity of the methanolic extract of *Alternanthera repens*, the highest inhibitory effect to the extract was observed on *Staphylococcus aureus* with a zone of inhibition of 30 mm while *Pseudomonas aeruginosa* was the least inhibited with a zone of inhibition of 16 mm. The extract had no effect on *Escherichia coli*, *Streptococcus faecalis*, and *Salmonella typhi* as there was no zone of inhibition. Plants of the genus *Alternanthera* are thought to possess antimicrobial and antiviral properties Sunil *et al*. (2008) reported that the wound healing property of *Alternanthera sessilis* might be due to the inhibitorty effect of the plant extract observed in *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Escherichia coli*, *Salmonella typhi*, and *Streptococcus faecalis* were not susceptible to the extract. This might be due to the inability of the active components in the plant extract to inhibit these organisms. In the findings of Adela *et al*. (2008) where aqueous and ethanolic extract of *Alternanthera repens* and *Bidens odorata* were used as medication for gastrointestinal diseases mainly in relation to diarrhoea, the validity of the medicinal use of these extracts were however confirmed contributing to the use of these plants as antidiarrheal agents in Mexican traditional medicine.

**Table 1:** Antibacterial activity of the methanol extract of *Alternanthera repens*

| Organisms | Zone of inhibition (mm) at 25.0 mg/ml of plant extract |
|---|---|
| *Staphylococcus aureus* | 30.0 |
| *Streptococcus faecalis* | - |
| *Escherichia coli* | - |
| *Bacillus subtilis* | 25.0 |
| *Bacillus cereus* | 17.0 |
| *Klebsiella pneumonia* | 26.0 |
| *Pseudomonas aeruginosa* | 16.0 |
| *Salmonella typhi* | - |
| *Proteus mirabilis* | 20.0 |


ISSN 2055-8139(Print), ISSN 2055-8147(Online)

The antibacterial effects of this plant extract on *Proteus mirabilis*, with a zone of inhibition of 20 mm showed that the plants can be used in the treatment of urinary tract infection associated with *Proteus sp*, as reported by Madigan *et al*. (2000).

**Table 2:** Minimum Inhibitory Concentration of the methanol extract of *Alternanthera repens*

| Organisms | Concentration of methanol extract (mg/ml) |
|---|---|
| *Staphylococcus aureus* | 3.125 |
| *Bacillus subtilis* | 3.125 |
| *Bacillus cereus* | 6.250 |
| *Klebsiella pneumonia* | 3.125 |
| *Pseudomonas aeruginosa* | 12.50 |
| *Proteus mirabilis* | 6.250 |

The minimum inhibitory concentration (MIC) assay of the plant extract in table 2 revealed that *Pseudomonas aeruginosa* exhibited the highest inhibitory effect at 12.50 mg/ml, followed by *Bacillus cereus* and *Proteus mirabilis* at 6.250 mg/ml. The low inhibitory values obtained in the other test organisms explains the wide use of this extract in treating wound and as antimicrobial agent as earlier discussed.

**Table 3:** Phytochemical screening of methanol extract of *Alternanthera repens*

| **Phytochemical tests** | **Presence/absence** |
|---|---|
| Saponins | +ve |
| Tannins | -ve |
| Phlobatannin | -ve |
| Alkaloids | +ve |
| Anthraquinone | -ve |
| | |
| **Cardiac glucoside** | |
| Legals Test | -ve |
| Salkowski Test | +ve |
| Keller Killian Test | +ve |
| Liebermans | -ve |

**Keys:** + = presence, - = absence

Phytochemical test of the plant extracts revealed the presence of some bioactive components like saponins, and alkaloids which might be responsible for the antibacterial activity of the extract. These are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and other herbivores (Marjorie, 1999).



ISSN 2055-8139(Print), ISSN 2055-8147(Online)

**Table 4:** Antibiotic sensitivity test

| **Organisms** | **Zones of inhibition (mm)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Gram positive** | **GEN** | **PEN** | **STR** | **TET** | **AMP** | **CHL** | **CXC** | **ERY** |
| *Staphylococcus aureus* | 25.0 | - | 20.0 | 13.0 | - | 13.0 | - | 26.0 |
| *Streptococcus faecalis* | 15.0 | - | - | 15.0 | - | - | - | - |
| *Klebsiella pneumonia* | 14.0 | - | - | 8.0 | - | - | - | - |
| *Bacillus subtilis* | 13.0 | - | 11.0 | - | - | 8.0 | - | - |
| *Bacillus cereus* | 15.0 | - | 13.0 | - | - | 10.0 | - | - |
| | | | | | | | | |
| **Gram negative** | **GEN** | **NAL** | **NIT** | **COL** | **STR** | **TET** | **AMP** | **COT** |
| *Escherichia coli* | 17.0 | 22.0 | 15.0 | 10.0 | 10.0 | - | - | - |
| *Pseudomonas aeruginosa* | 10.0 | - | - | 10.0 | 9.0 | - | - | - |
| *Salmonella typhi* | 14.0 | 19.0 | 14.0 | 10.0 | 13.0 | - | - | - |
| *Proteus mirabilis* | 12.0 | - | - | - | 10.0 | 18.0 | - | 16.0 |

**Keys:** GEN – Gentamycin, PEN – Penicillin, STR – Streptomycin, TET – Tetracycline, AMP – Ampicillin, CHL – Chloramphenicol, CXC – Coxacillin, ERY – Erythromycin, NAL – Nalidixic acid, NIT – Nitrofurantion, COL – Colistine, COT – Cotrimazole, (-) = no inhibition

The demonstration of antibiotic sensitivity test against both Gram-positive and Gram-negative bacteria as shown on table 4 indicates the broad spectrum activity of Gentamycin. *Escherichia coli* and *Salmonella typhi*, were susceptible to all the commercial antibiotics except tetracycline, ampicillin, and cotrimazole, while *Streptococcus faecalis* was susceptible to only gentamycin and tetracycline. However, these bacteria: *Escherichia coli*, *Salmonella typhi* and *Streptococcus faecalis* were resistant to the plant extract. This might be due to the more purified nature of the commercial antibiotics compared to the crude plant extract (Hugo and Russell, 1977).

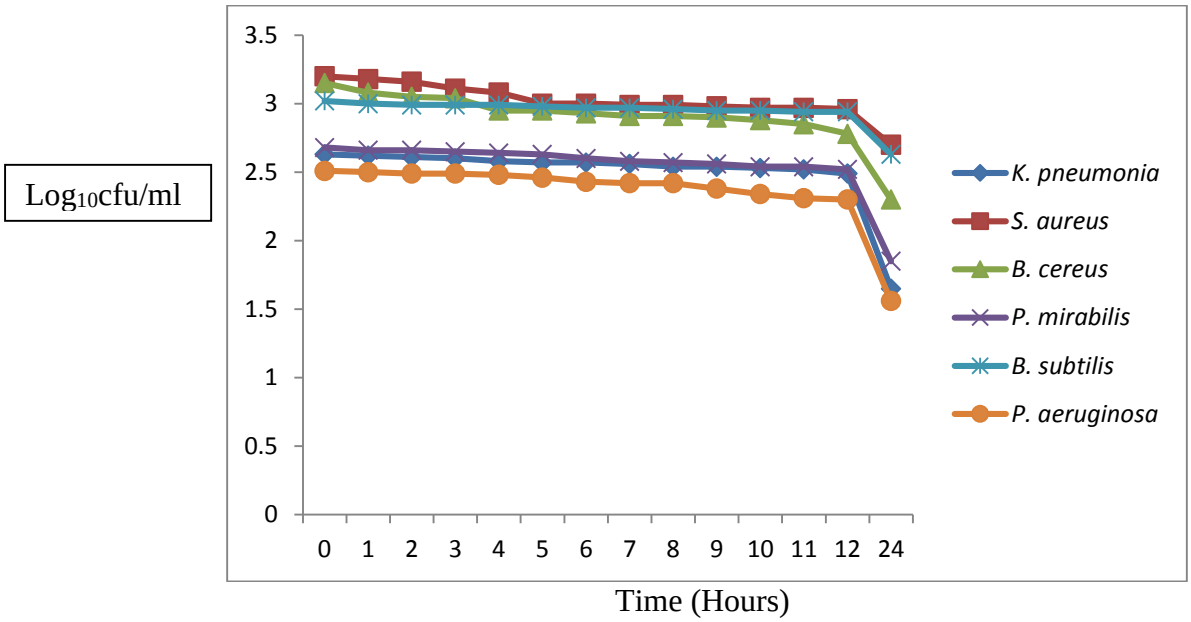


**Figure 1**: Rate of killing of bacterial isolates by the test extract



ISSN 2055-8139(Print), ISSN 2055-8147(Online)

There was gradual reduction in the number of colonies from 0 hour to 24 hours in all the test isolates, however at 24 hours, there was no total inhibition of any of the isolates. An increase in the exposure time beyond 24 hours might cause a total cidal effect on the organisms.

This study showed that *Alternanthera repens* has antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* as verified by the *in vitro* experiments. This is an indication that *Alternanthera repens* can be possibly used for the treatment of wound and urinary infections. Antibacterial activity of methanol extract of *Alternanthera repens* showed broad inhibitory effects on the test isolates. Further purification may enhance greater antibacterial potency. This work has however not included the toxicological analysis, which if done may reveal the tolerance statistics of the extract by mammalian body. However, there is the need to subject the crude extract to further purification for more antibacterial effectivity as in the case of commercial antibiotics, and more research should be conducted on other medicinal plants that can act synergistically with *Alternanthera repens*.

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