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SHORT COMMUNICATION

Antibacterial activities of the plant extract of *Alternanthera repens*

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ABSTRACT

Aims: Plants have been described as gift of nature and they have been utilized as a therapeutic agent against various infectious diseases affecting both human and animals. This present study is aimed at determining the antibacterial efficacy of the methanol plant extract of *Alternanthera repens* against some clinical bacterial isolates.
Methodology and results: Methanolic extract of the plant *A. 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 *E. coli*, *S. faecalis* and *S. typhi*, with the highest zone of inhibition (30 mm) observed on *S. aureus*. 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. Phytochemical screening tests showed the presence of saponins, alkaloids, salkowski, and keller killianie in the plant extract.
Conclusion, significance and impact study: *A. repens* has wound and urinary tract infection healing property as observed from its antibacterial effect against some of the implicating organisms. Its usage can thus serve as an alternative means of cost effective treatment of infections in covering the basic health needs of people in developing countries.

Keywords: Antibacterial, therapeutic agent, 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 (Veronika *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 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

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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 *A. 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 h. 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 25 mg/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 °C for 24 h. 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 *A. repens* were introduced into different wells bored on the sterile medium containing the test organism. The plates were incubated at 37 °C for 24 h. The lowest concentration of the crude plant extract that showed zone of inhibition was recorded.

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 h old broth culture) were prepared by centrifuging at 2000 rpm for 10 min, 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 h. At intervals of 1 h each, 1 mL of the mixture was pour plated using nutrient agar medium and incubated at 37 °C for 24 h. The microbial load was thereafter determined.

Phytochemical screening

The plant extract was screened for alkaloids, saponins and cardiac glycoside as described by Evans (2002).

RESULTS AND DISCUSSION

Table 1 shows the antibacterial activity of the methanolic extract of *A. repens*, the highest inhibitory effect to the extract was observed on *S. 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 *E. coli*, *S. faecalis* and *S. typhi* as there was no zone of inhibition.

Table 1: Antibacterial activity of the methanol extract of *A. repens.*

Organisms	Zone of inhibition (mm) at 25.0 mg/mL of plant extract
S. aureus	30.0
S. faecalis	-
E. coli	-
B. subtilis	25.0
B. cereus	17.0
K. pneumonia	26.0
P. aeruginosa	16.0
S. typhi	-
P. mirabilis	20.0

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 inhibitory effect

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of the plant extract observed in *S. aureus* and *P. aeruginosa*.

E. coli, *S. typhi,* and *S. 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 *A. 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.

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).

The minimum inhibitory concentration (MIC) assay of the plant extract in Table 2 revealed that *P. aeruginosa* exhibited the highest inhibitory effect at 12.50 mg/mL, followed by *B. cereus* and *P. 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.

Phytochemical test of the plant extracts in Table 3 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).

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. *E. coli* and *S. typhi* were susceptible to all the commercial antibiotics except tetracycline, ampicillin, and cotrimazole, while *S. faecalis* was susceptible to only gentamycin and tetracycline. However, these bacteria: *E. coli*, *S. typhi* and *S. 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).

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

Organisms	Concentration of methanol extract (mg/mL)
S. aureus	3.125
B. subtilis	3.125
B. cereus	6.250
K. pneumonia	3.125
P. aeruginosa	12.50
P. mirabilis	6.250

Table 3: Phytochemical screening of methanol extract of *A. repens.*

Phytochemical tests	Presence/absence
Saponins	+
Tannins	–
Phlobatannin	–
Alkaloids	+
Anthraquinone	–
Cardiac glucoside	
Legals Test	–
Salkowski Test	+
Keller Killian Test	+
Liebermans	–

+, presence; –, absence.

Table 4: Antibiotic sensitivity test.

Organisms	Zones of inhibition (mm)							
Gram positive	GEN	PEN	STR	TET	AMP	CHL	CXC	ERY
S. aureus	25.0	-	20.0	13. 0	-	13.0	-	26.0
S. faecalis	15.0	-	-	15.0	-	-	-	-
K. pneumonia	14.0	-	-	8.0	-	-	-	-
B. subtilis	13.0	-	11.0	-	-	8.0	-	-
B. cereus	15.0	-	13.0	-	-	10.0	-	-
Gram negative	GEN	NAL	NIT	COL	STR	TET	AMP	COT
E. coli	17.0	22.0	15.0	10.0	10.0	-	-	-
P. aeruginosa	10.0	-	-	10.0	9.0	-	-	-
S. typhi	14.0	19.0	14.0	10.0	13.0	-	-	-
P. mirabilis	12.0	-	-	-	10.0	18.0	-	16.0

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.

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There was gradual reduction in the number of colonies from 0 h to 24 h in all the test isolates, however at 24 h, there was no total inhibition of any of the isolates as shown in Figure 1. An increase in the exposure time beyond 24 h might cause a total cidal effect on the organisms.

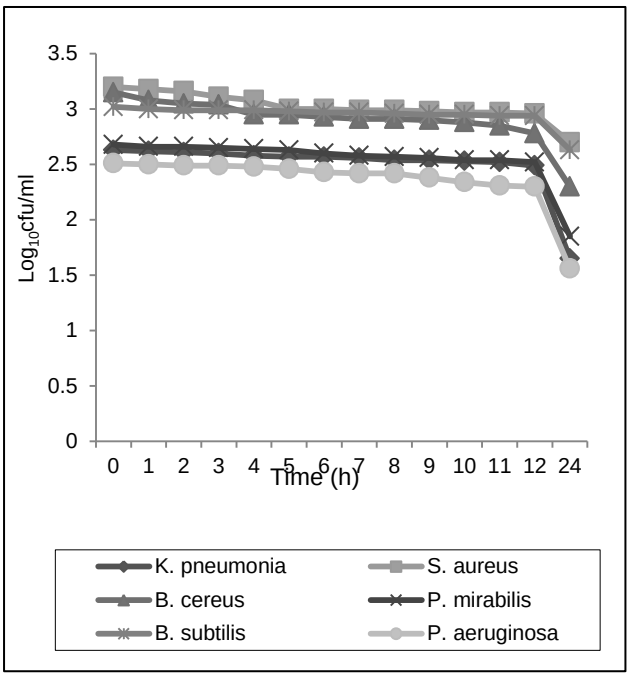

Figure 1: Rate of killing of bacterial isolates by the test extract.

This study showed that *A. repens* has antibacterial activity against *S. aureus*, *P. aeruginosa* and *P. mirabilis* as verified by the *in vitro* experiments. This is an indication that *A. repens* can be possibly used for the treatment of wound and urinary infections. Antibacterial activity of methanol extract of *A. 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 *A. repens*.

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