African Journal of Microbiology Research Vol. 5(12), pp. 1467-1472, 18 June, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR10.866
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Antimicrobial activity of *Vernonia amygdalina* on selected urinary tract pathogens

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Accepted 10 March, 2011

The aqueous ethanol and acetone-ethanolic extracts of *Vernonia amygdalina* leaves and stems were investigated for their antimicrobial activities compared to ciprofloxacin, against *Escherichia coli, Klebsiella* sp and *Staphylococcus* sp. The antimicrobial activity of each extract on the isolates was determined by agar well diffusion and minimum inhibitory concentration methods. The acetone-ethanolic extract of the leaf showed the highest antibacterial activity on *Klebsiella* sp in both methods used, followed by the ethanol leaf extracts on the same organism. *E. coli* and *Staphylococcus* sp were not susceptible to any of the extracts even at higher concentrations. The aqueous extracts of both leaf and stem were ineffective on all the test isolates at all concentration. Comparatively, the test isolates were sensitive to ciprofloxacin in both methods used but the antibacterial effect of acetone-ethanolic leaf extract on *Klebsiella* sp was higher than that of ciprofloxacin on *Klebsiella* sp. The results from this research showed that *V. amygdalina* could be used as raw material in the synthesis of antimicrobial drugs, especially against *Klebsiella* sp which have been attributed with high level drug resistance in urinary tract infections.

Key words: Ciprofloxacin, acetone, urinary tract infections, ethanol.

INTRODUCTION

Urinary tract infection is a bacterial infection that affects any part of the urinary tract. The important pathogens include; *Escherichia coli*, *Proteus, Klebsiella, Pseudomonas* and *Staphylococcus.* It is one of the major diseases that affect people of all age groups and sexes (Ojo et al., 2004). The prevalence of urinary tract infection is quite alarming and the effectiveness of antimicrobial chemo-therapeutic agents is being compromised by several alarming trends; inappropriate prescription, use of broad-spectrum drugs instead of narrow spectrum drugs, sale of over-the -counter anti-microbial drugs in some countries and lack of sufficient tests before prescription (Talaro, 2005). The conse-quences of the aforementioned trends produced more or less, foreseeable and important side effects. These side effects include toxicity, allergic reactions, disruption of normal flora and acquisition of drug resistance by formerly sensitive microbes. Ciprofloxacin, among other antibiotics, are frequently used to treat urinary tract infection. It belongs to a class of antibiotics called, quinolones. The most frequent side effects of cipro-floxacin includes; nausea, vomiting, diarrhea, abdominal pain, rash, headache, restlessness and rare allergic reactions such as hives and anaphylaxis. Ciprofloxacin has been associated with tendonitis and has the tendency to alter the normal flora in the colon as well as encourage the growth of bacteria, responsible for the development of inflammation of the colon (Simplice et al., 2009). It reported that one out of ten medical cases is related to a medicine's adverse effect. Thus, the future effectiveness of antimicrobial therapy is in doubt. Hence, there is a need for an alternative means to treat infections. A great interest has developed in searching for antimicrobial drugs from natural plant products and this interest arises from the belief that, drugs derived from plants are safe and dependable, compared to synthetic drugs.

Vernonia amygdalina, a member of the Asteraceae family, is a woody shrub or small tree of 2.5 mm with petriolate leaf of about 6 mm in diameter and elliptic in

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shape. The leaves are green with characteristic odour and bitter taste (Bonsi et al., 1995a). *V. amygdalina* is a valuable medical plant that is widespread in East and West Africa (Burkill, 1985), it is known as bitter leaf, due to its characteristic bitter taste and flavour, and may be used as an active anticancer (Izevbigie, 2003), antibacterial, antimalaria and antiparasitic agent (Tadesse et al., 1993). This plant contains complex active components that are pharmacologically useful. The roots and the leaves are used in ethno-medicine to treat fever, hiccups, kidney problems and stomach discomfort. The stem and root divested of the bark are used as chew-sticks in many West African countries like Cameroon, Ghana and Nigeria (Burkill, 1985; Hamowia, 1994). It is also documented that *V. amygdalina* has been used traditionally in blood clotting and has elicited a significant reduction in blood glucose levels at post-prandial time point (Uchenna et al., 2008). Fasola et al. (2010) reported that *V. amydalina* has hypoglycaemic activity. They observed a dose-dependent reduction in fasting blood sugar level in alloxan-induced diabetic rats after treatment with different concentrations of the aqueous leaf extracts. Yedjou et al. (2008) also demonstrated *V. amygdalina* leaf extracts as a DNA - damaging, anticancer agent in the management of breast cancer. However, much work have not been documented on the antimicrobial effectiveness of the stem extracts of this plant. This research is designed to screen the anti-microbial effectiveness of *V. amygdalina* leaf and stem extracts on selected urinary tract pathogens, compared to ciprofloxacin antibiotics.

MATERIALS AND METHODS

Sample collection and extraction

The leaves and stems of *V. amygdalina* were collected on 15th May 2009, in a garden at Choba, in University of Port-Harcourt, Rivers state, Nigeria and identified by comparing their morphological and anatomical characteristics with the standard description.

Extraction process

The freshly collected plant samples were thoroughly washed with sterile distilled water and sodium hypochlorite for surface sterile-zation. The leaves and stems were separately pounded in a clean mortar. The pastes were collected in a foil and used for extraction. The automatic soxhlet extraction method was employed in preparation of the acetone- ethanol and ethanol extracts of both stem and leaves while the water soluble extraction method was used for preparing the aqueous extracts of both leaves and stems.

Isolation of test isolates

Samples of urine from patients implicated with urinary tract infection were collected from the University of Port Harcourt teaching hospital, and analyzed microbiologically in the laboratory. The urine samples were cultured in Cysteine lactose-electrolyte deficient agar (CLED) and MacConkey agar plates and incubated at 37°C for 24 h. After incubation, discreet colonies were inoculated on nutrient agar slants and preserved in refrigerator at 4°C prior to biochemical identification.

Screening of *Vernonia amygdalina* extract (leaf and stem) and ciprofloxacin for antimicrobial activity

The direction adopted for screening was as described by Andrew (2004).

Preparation of inoculum

An inoculum size that corresponds to the 0.5 Mcfarland standard was used. The 0.5 McFarland standard was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of distilled water to make 1% v/v solution of sulphuric acid. Similarly, 0.5 g of dehydrated barium chloride ($Bacl_2$ $2H_20$) was dissolved in 50 ml of distilled water to make 1% w/v solution of barium chloride. The 0.5 ml barium chloride was added to 99.5 ml sulphuric acid solution and mixed well. The test isolates were inoculated into peptone water and their densities were adjusted to 0.5 Mcfarland standard by adding sterile distilled water to the suspensions.

Preparation of different concentrations of extracts and ciprofloxacin

A concentration of 400 mg/10 ml was prepared for the extracts (leaf/stem) and ciprofloxacin, as stock solutions. A two fold serial dilution of the extracts and ciprofloxacin was carried out from their stock solutions to obtain varying concentrations. The varying concentrations obtained were 20, 10, 5 and 2.5 mg/ml respectively.

Inoculation of test isolates in iso-sensitivity agar - plates (ISA)

The iso sensitivity agar medium was prepared following the manufacturer's directions. 0.1 ml aliquot of each test organism suspension was transferred onto the well-dried agar plates in duplicate and was spread evenly with a sterilized hockey slick. The plates were allowed to dry, followed by boring of well in the ISA plates. Five wells were bored and the centre well served as the control. Each of the wells was appropriately labeled on the reverse side of the media plates.

Inoculation of extracts / ciprofloxacin in well-in-agar plates

A sterile pipette was used to transfer 0.2 ml of the extracts (leaf and stem) and ciprofloxacin into respective wells in the agar plates. Water was used as control in centre wells. The plates were allowed to stand for 30 min at 37°C for 24 to 48 h, after which, the incubation zone sizes were measured in millimeters and recorded.

Determination of the minimum inhibitory concentration (MIC) of extracts (stem/leaf) and ciprofloxacin

The broth dilution method was used. A stock solution of 20 mg /10 ml was prepared for each extract and ciprofloxacin separately. 1 ml of nutrient broth was dispensed into test tubes and sterilized by autoclaving at 121°C at 15 psi for 15 min. The different extracts and ciprofloxacin were serially diluted from their stock solutions to obtain varying concentrations. The concentrations were; 1, 0.5, 0.25 and 0.125 mg/ml. 0.1 ml of each test isolate was inoculated into the various test tubes containing varying concentrations and then, incubated at 37°C for 24 h. After incubation, the presence or

Table 1. Inhibition zone sizes of extracts and ciprofloxacillin on test isolates.

Extracts/ Antibiotic	Bacterial isolates	Concentrations (mg/ ml)				
		20	10	5.0	2.5	Control
Acetone ethanol (leaf)	*Klebsiella*	30	27	25	20	0
	E. coli	0	0	0	0	0
	Staphylococcus	0	0	0	0	0
Acetone ethanol (stem)	*Klebsiella*	28	21	15	0	0
	E. coli	0	0	0	0	0
	Staphylococcus	0	0	0	0	0
Ethanol (leaf)	*Klebsiella*	26	24	23	20	0
	E. coli	0	0	0	0	0
	Staphylococcus	0	0	0	0	0
Ethanol (stem)	*Klebsiella*	14	13	12	10	0
	E. coli	0	0	0	0	0
	Staphylococcus	0	0	0	0	0
Aqueous (Leaf)	*Klebsiella*	0	0	0	0	0
	E. coli	0	0	0	0	0
	Staphylococcus	0	0	0	0	0
Aqueous	*Klebsiella*	0	0	0	0	0
	E. coli	0	0	0	0	0
	Staphylococcus	0	0	0	0	0
Ciprofloxacin	*Klebsiella*	27	24	21	19	0
	E. coli	33	31	29	27	0
	Staphylococcus	16	14.6	13.1	11	0

absence of growth on each tube was rated using the following scale:

= no growth, + = scanty growth, ++ = moderate growth, +++ = heavy growth.

Identification of isolates

The cultural, morphological and biochemical characteristics of the respective isolates were compared with criteria in Bergey's manual of Determinative Bacteriology (Bergey and Holt, 1993). The biochemical tests performed includes; indole production test, coagulase test, catalase, MR -VP, sugar fermentation and motility tests (Cheesbrough, 2002). The test isolates in this research *are Escherichia coli, Staphylococcus* sp and *Klebsiella* sp.

RESULTS

Seven isolates were identified from the cultured urine samples with reference to Bergey's manual of determinative bacteriology. These isolates are; *Klebsiella* sp, *Proteus* sp. *Staphylococcus* sp. *E. coli*, *Citrobacter* sp, *Pseudomonas* sp and *Enterobacter* sp. The results of inhibition zone sizes of the different bitter leaf extracts and ciprofloxacin measured in millimeters are shown in Table 1. The result showed that the acetone - ethanol and ethanol leaf extracts had higher zones of inhibition than the stem extracts but acetone-ethanol leaf extracts showed the highest inhibition zone size. These extracts were effective only on *Klebsiella* sp while *E. coli* and *Staphylococcus* sp showed no susceptibility to any of the leaf or stem extracts.

The result also showed that higher zones of inhibition were recorded at the higher concentrations of the extracts. The aqueous solution of both leaf and stem extracts were not effective on any test Isolate even at higher concentrations. Comparatively, all the test isolates were susceptible to ciprofloxacin antibiotics at all concentration, but the acetone-ethanol extract of the leaf and stem of *V. amygdalina* showed a higher zone of inhibition for *Klebsiella* sp at 20 mg/ml than ciprofloxacin at the same concentrations. However, the turkey's multiple comparison statistical test showed that there is no significant difference between the acetone-ethanol leaf/stem extracts and ciprofloxacin on *Klebsiella.*

Table 2. MIC of test extracts and ciprofloxacillin of the test isolates.

Extracts / Antibiotics	Concentration (mg/ml)	Bacterial *Klebsiella*	Isolates *E. coli*	*Staphylococcus*
Acetone ethanol (stem)	1.0	-	++	++
	0.5	-	++	++
	0.25	+	++	++
	0.125	+	++	++
Acetone ethanol (leaf)	1.0	-	++	++
	0.5	-	++	++
	0.25	-	++	++
	0.125	+	++	++
Ethanol (stem)	1.0	-	++	++
	0.5	+	++	++
	0.25	++	++	++
	0.125	++	++	++
Ethanol (leaf)	1.0	-	++	++
	0.5	-	++	++
	0.25	+	++	++
	0.125	+	++	++
Aqueous	1.0	+++	+++	+++
	0.5	+++	+++	+++
	0.25	+++	+++	+++
	0.125	+++	+++	+++
Ciprofloxacin	1.0	-	-	-
	0.5	-	-	-
	0.25	-	-	+
	0.125	0	+	+

Table 2 shows the minimum inhibitory concentration (MIC) result of the test isolates in the presence of the extracts and ciprofloxacin. The acetone-ethanol stem and leaf extracts showed MIC against *Klebsiella* sp at 0.5 and 0.25 mg/ml respectively while the ethanol stem and leaf extract showed MIC against *Klebsiella* sp at 1.0 and 0.5 mg/ml respectively. The growth of *E coli* and *Staphylococcus* sp were not inhibited by any of the extracts at any concentrations but ciprofloxacin showed MIC against *E. coli* at 0.25 and at 0.5 mg/ml for both *Staphylococcus* sp and *Klebsiella* sp.

DISCUSSION

The bacterial isolate that showed different levels of susceptibility to both leaf and stem extracts of *V. amygdalina* was *Klebsiella* sp while S*taphylococcus* and *E. coli* were not susceptible to either of the extracts. However, in previous studies by Iwalokun (2003), he reported the antimicrobial activity of *V. amygdalina* leaf extracts on standard strains of *E. coli* ATCC25922 and *Staphylococcus aureus* ATCC 25923. The result obtained in this study on *Staphylococcus* sp and *E. coli* may be as a result of strain differences from standard strains used in the above research.

The effects of different solvents have been demonstrated in this study. Statistically, the mean value of acetone - ethanol leaf and stem extracts were 2.55 and 1.60 respectively, compared to the mean value of 2.35 and 1.23 exhibited by ethanol leaf and stem extracts respectively. Using the ANOVA analysis, there is a significant difference between the means of the different extracts at $P< 0.05$. The turkey's multiple comparison test showed that there was no significant difference between acetone-ethanol (I) and ethanol (II) leaf extracts at the concentrations used. There was also no significant difference between acetone-ethanol (III) and ethanol (IV) stem extracts at the different concentrations.

Eloff (1998) examined a variety of extractants for their ability to solubilize antimicrobial agents from plants and observed that acetone is not one of the most frequently

used extracts but is the most effective. The most commonly used solvent (ethanol and methanol) may not demonstrate the greater sensitivity in yielding anti-microbial chemicals on an initial screening. The results obtained in this work agree with the observation by Eloff (1998), he reported that the acetonic extracts of *Acacia pennate* were very active against *Aspergillus fumigatus*, indicating a strong antifungal activity against the pathogen. Chinakwu (2003) showed the antifungal effect of acetone and chloroform extracts of bitter leaf on *Fusarium moniliforme*. The aqueous extracts showed no inhibition on all three isolates. Eloff (1998) also reported that most active components are not water soluble. Akinpelu (1999) reported that, *V. amygdalina* leaves, 60% methanol extract was found to be active at 25 mg/ml against bacterial isolates. According to Ibrahim et al. (2009), The antibacterial susceptibility test of *V. amygdalina and Occimum gratissimum* showed that the ethanolic extracts of the plants has higher inhibition on food borne pathogens(*E. coli*, *Bacillus cereus, Shigella dysentriae and Salmonella typhimurium*) compared to the aqueous extract with low inhibition. The high activity of the ethanolic extracts verifies the use of the ethanolic extraction method by local herbalists (Allero and Afolayan, 2006).

The leaf extracts of *V. amygdalina* was observed in this present study to be more effective against *Klebsiella* sp than the stem extracts of the same solvent. This observation agrees with the work of Iwalokun (2003), who reported on the effectiveness of *V. amygdalina* leaf extract. In his study, *V. amygdalina* leaf extract dose restored efficacy of chloroquine against chloroquine-resistant *Plasmodium beiglei* strains. According to Abosi and Raserok (2003), *V. amygdalina* suppressed parasitaemia during early infection when the parasites had not taken a strong hold. Leaf and root-bark extracts produced large decreases in parasitaemia, resulting in chemosuppression ranging between 41.5 and 67.0% for the leaf extract and 38.5and 53.5% for the root-bark extract.

High levels of chemosuppression were produced at high doses of the leaf and root-bark extracts, indicating a dose-dependent effect. At 125 mg/kg per day, the leaf extract reduced parasitaemia by approximately 50% (80.4% with chloroquine), while the root-bark extract reduced parasitaemia by 50% at 250 mg/kg per day. This suggests that the concentration of active compounds is higher in the leaf than in the root. However, in both cases, chemosuppression was less than that produced by chloroquine.

The three bacterial isolates were susceptible to the ciprofloxacin antibiotics compared to the extracts that showed their effectiveness against *Klebsialla* sp only, but this work reveals that, the acetone-ethanol leaf and stem extracts were more bactericidal than ciprofloxacin at higher concentrations (20 and 10 mg/ml). *Klebsiella* sp have been attributed with high level of resistance in urinary tract infections due to its resistance to a wide range of antibiotics (Akortha and Aromorse, 2003; Akorta and Aluyi, 2002). According to Simplice et al. (2009), four gram negative bacteria; *E. coli, K. pneumoniae, E. cloacae* and *Acinetobacter* sp were tested for their susceptibility to some antibiotics, the highest resistance rates were recorded with *E. coli* (76.64%) and *K. pneumoniae* (85.57%) for ampicillin, amoxicillin, trimethoprin sulfamethoxazole and chloramphenicol. It is quite relieving to know that these widely available vegetable can be used in the synthesis of drugs against the organism. This agrees with the observation of Mukhar and Huda (2005) that, plant extracts are more potent than source antibiotics. According to Sharma and Sharma (2010), the acetonic, methanolic and ethanolic extracts of *V. amygdalina* showed a higher antifungal activity against *S. cerevisiae* than the control ciprofloxacin and gentamycin. *V. amygdalina* possesses good antibacterial and antifungal activity. The excellent activity of the plant on bacteria and fungal species shows a very good potential of *V. amygdalina* to treat diseases caused by pathogens. Okigbo and Mmeka (2008) also reported the antibacterial and antifungal activity of *V. amygdalina* extracts on *S. aureus, E. coli* and *Candida albicans.*

These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and disseminated properly. Furthermore, the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phyto-chemistry, ethnobotany and other biological actions for drug recovery. Cos et al. (2002) reported the antifungal, antiviral and antibacterial activity of 45 Rwandan plant extracts. These extracts showed antimicrobial activity on dermatophytes, DNA and RNA viruses, and gram positive bacterial isolates. Thus, traditional plants may represent new sources of antimicrobials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine.

In conclusion, this research shows the antimicrobial activity of *V. amygdalina* using different solvents. This work suggests the possible exploration of *V. amygdalina* as a source of natural product for future use in the management of multi-drug resistant urinary tract infections especially caused by *Klebsiella* sp.

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