Sjournals
Scientific Journals
Scientific Journal of Biological Sciences (2012) 1(4) 91-100
ISSN 2322-1968

Contents lists available at Sjournals
Scientific Journal of
Biological Sciences
Journal homepage: www.Sjournals.com

Original article

In vitro antibacterial efficacy of crude ethanolic leaf extract and alkaloidal fractions of Phyllanthus amarus on uro-pathogens

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ARTICLE INFO

Article history:
Received 03 September 2012
Accepted 18 October 2012
Available online 30 October 2012

Keywords:
Phyllanthus amarus
Uro-pathogens
Alkaloids
Urine
Antibacterial

ABSTRACT

The phytochemistry and *in vitro* antibacterial efficacy of crude ethanolic leaf extract and alkaloidal fractions of *Phyllanthus amarus* on the Gram positive (*Streptococcus* sp, *Staphylococcus aureus*; Coagulase negative *Staphylococcus* sp and *Enterococcus faecalis*) and Gram negative bacteria (*Escherichia coli, Enterobacter* sp.; *Serratia marcescens*; *Klebsiella* sp and *Pseudomonas aeruginosa*) isolated from mid stream urine were carried out using standard microbiological and disc diffusion techniques. The preliminary phytochemical analysis of the ethanolic leaf extracts of *P. amarus* revealed the presence of phyto-constituents such as alkaloids (+++), tannins (+++), saponins (++), flavonoids (++), cardiac glycoside (+), free anthraquinones (++), deoxy-Sugar test (+) and phlobatanins (+), while combined anthraquinones was not detected. The results showed that *P. amarus* extracts exhibited varying degrees of inhibitory effects against both Gram positive and Gram negative bacteria isolated from urine samples. Highest mean zones of inhibition (14.3±1.0 mm) and activity index (0.97) were obtained at 40.0mgml^{-1} among the Gram positive bacteria, while the highest mean zones of inhibition (14.5 ± 0.5mm) and activity index (1.12) at 40.0mgml^{-1} were obtained among the Gram negative. The results also showed that Coagulase negative *Staphylococcus* sp (CS03) was

not sensitive to both 20.0mg/ml^{-1} of crude extracts of *P. amarus* and alkaloidal fractions. Among the Gram negative, *Enterobacter* sp (ES03) was not sensitive to 20.0mg/ml^{-1} of crude extracts of *P. amarus*, alkaloidal fractions and Streptomycin. Therefore, there is a need to consider the use of this potent ethanolic leaf extracts and the alkaloids for developing synthetic drugs against uro-pathogens.

1. Introduction

Urinary tract infections (UTIs) are caused by pathogenic invasion of the urinary tract, which lead to an inflammation of the urothelium (Akinjogunla, 2010a)**.** Urinary tract infections are the most frequently diagnosed cases, having an estimated figure of 150 million per annum worldwide (Stamm and Norrby, 2001). Urinary tract infections are the most commonly observed infection in many hospitals, and accounts for approximately 35% of all hospital acquired diseases (Manges et al., 2006). The clinical manifestations of UTI depend on the portion of the urinary tract involved, the etiologic organisms, the severity of the infection, and the patient's ability to mount an immune response to it (Akinjogunla, 2010a). The common uro-pathogens identified in patients with urinary tract infections were Gram negative such as *Escherichia coli, Proteus* sp*, Klebsiella* sp and *Pseudomonas aeruginosa (*Manges et al., 2006; Akram, 2007; Saonuam et al., 2008) while the Gram positive bacteria were *Staphylococcus aureus* and coagulase *Staphylococcus* sp (Stamm and Norrby, 2001). *Escherichia coli* causes 85% of community acquired urinary tract infections and more than 80% of cases of uncomplicated pyelonephritis (Noor et al., 2004). The prevalence and incidence of urinary tract infection is higher in women than in men, which is likely the result of several clinical factors including anatomic differences, hormonal effects, and behavioural patterns (Akinjogunla, 2010a; Harding and Ronald, 1994). In spite of the availability and use of the antimicrobial drugs, urinary tract pathogens caused by bacteria have shown increasing trends in recent years. Much of the increase has been related to emerging antibiotic resistance in urinary tract pathogens and the emergence of multi-drug resistant bacteria is of great concern to both clinicians and the pharmaceutical industry, since it is a major cause of treatment failure in many infectious diseases (Davies, 1994; Martino et al., 2002). Thus, it is necessary to search for alternative antimicrobial agents.

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (Sofowora, 2006; Iwu, 1993). Traditional medicine using plant extracts continues to provide health coverage for approximately 80% of the world's population and is still much practiced by a large proportion of the rural populace especially those in low income world population because of its availability and affordability (Iwu, 1993, Bharatiya, 1992). *Phyllanthus amarus,* an erect annual herb of not more than one and half feet tall and has small leaves and yellow flowers belongs to the family Euphorbiaceae. It was first identified in central and Southern India in 18th century. It is commonly called "carry me seed", or "gulf leaf flower", "hurricane weed", "windbreaker", (Bharatiya, 1992). There are over 300 genera with over 5000 species in the Euphorbiaceae worldwide. *Phyllanthus amarus* has reportedly been used to treat jaundice, otitis, diarrhoea, skin ulcer, diabetes, hypertension and gastrointestinal disturbances and block DNA polymerase in the case of hepatitis B virus during reproduction (Odetola and Akojenu, 2000; Kassuyaa et al., 2003; Oluwafemi and Debiri, 2008). Consequently, the aim of this study is to determine the in-vitro efficacy of crude ethanolic leaf extract and alkaloidal fractions of *P. amarus* on uro-pathogens.

2. Materials and methods

2.1. Source and extraction of plant

The leaves of *P. amarus* were collected in Uyo, Akwa Ibom State. The taxonomic identification of the plants was done at the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State. The plant was washed under running tap water and with distilled water in order to remove extraneous matters and air-dried at room temperature for one month. The dried plant part was pulverized in a mill and stored in polythene bag until

required. The powdered *P. amarus* was exhaustively extracted by Soxhlet Apparatus using 95% ethanol. The filtrate was evaporated using a rotary evaporator attached to a vacuum pump. After complete evaporation, the extract was thereafter transferred into weighed bottle which was properly labelled with dates and stored in the refrigerator at 4^0C until required for used. The graded concentrations (20 $mgml^{-1}$ and 40 $mgml^{-1}$) of the extract were prepared using Dimethyl sulphoxide (DMSO) and then subjected to antibacterial assays.

2.2. Sterilization of materials

The Petri dishes and pipettes packed into metal canisters were appropriately sterilized in the hot air oven at 180°C for 2hrs at each occasion. The culture media were autoclaved at 121°C for 15mins.

2.3. Source of bacterial isolates

The bacterial isolates used were: Staphylococcus aureus, Coagulase negative Staphylococcus spp, Streptococcus pyogenes, Enterococcus faecalis, Serratia marcescens, Pseudomonas aeruginosa, Escherichia coli. Proteus mirabilis, Klebsiella spp. and Enterobacter spp. These species were freshly isolated from clinical specimens (midstream-urine) and identified using routine conventional laboratory techniques including Gram staining, motility, coagulase, catalase, oxidase, indole, urease production, citrate utilization, methyl red, Vogues Proskauer. Carbohydrate fermentation tests such as mannitol, sucrose, glucose and lactose were also carried out using the methods described by (Holt, 1994; Cowan and Steel, 1985; Cheesbrough, 2006). Stock cultures were maintained on nutrient agar slant at 4^oC until needed.

2.4. Phytochemical screening (quantitative)

2.4.1. Test for alkaloids

Half a gram (0.5g) of the plant extracts was stirred with 5ml in 5% Hydrochloric Acid (HCl) on a steam bath and then filtered 1ml of the filtrate was treated with a few drop of Mayer's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent, turbidity or precipitation of cream and pink or red colouration respectively indicated in positive test. The their 1ml portion was treated with a few drops of picric acid while yellow precipitate was taken as preliminary evidence for the presence of alkaloids

2.4.2. Test for saponins

(a) Frothing test: Half a gram (0.5g) of each extract was shaken vigorously with distilled water in a test tube, frothing that persisted on warning was taken as preliminary evidence for the presence of saponins.

(b) Sodium bicarbonate test: Half a gram (0.5g) of each extract was added with 5% sodium bicarbonate and Fehling's solution A and B and boiled. Presence of brown precipitate was termed as a positive test.

2.4.3. Test for tannins

(a) Ferric chloride test: Half a gram (0.5g) of each plant extract was stirred with 10mls of distilled water and filtered; to the filtrate 5% Ferric chloride reagent was added. A blue-black green or blue-green precipitate was taken as evidence for the presence of tannins.

(b) Bromine water test: Five (5) drops of each plant extract solution as mixed with 10ml distilled water and bromide water. Decolourization of bromine water indicated the presence of tannins.

2.4.4. Test for anthraquinones

(a) Borntrager's test for the free hydroxyanthraquinones.

Half a gram (0.5g) of plant extract was shaken with 10ml benzene and filtered. To the filtrate were added 10% ammonia solution and the mixture shaken. The presence of a pink, red or violet colour in the ammonical (lower) phase indicated the presence of free anthraquinones.

(b) Borntrager's test for combine anthraquinones

Half a gram (0.5g) of plant extract was boiled with 10ml dilute sulphuric acid and filtered. The filtrate was shaken with 5ml benzene and 10% ammonia solution was added to the separate benzene layer. A pink red or violet colouration in the ammonia (lower) layer indicated the presence of anthraquinones derivative.

2.4.5. Test for flavonoids

Shinoda Reduction Test: Few pieces of magnesium metal were added to 5ml of each plant extract solution. The solution was obtained by using concentrated hydrochloric acid to dissolve the extract. The formation of orange, red crimson or magenta colouration was taken as evidence of preliminary presence of flavonoids.

2.4.6. Test for cardiac glycosides

(a) Salkowski's test: Half a gram (0.5g) of extract was dissolved in 2ml of chloroform. Concentrated sulphuric acid was carefully added by running it down the side of the test tube. A reddish brown colour at the interphase indicated the presence of glycon portion of cardiac.

(b) Keller-Killiani's test: Half a gram (0.5g) of plant extract was dissolved in 2ml glacial acetic acid. This was then underplayed with 1ml concentrated sulphuric acid. A brown ring obtained at the interphase, indicates the presence of deoxy-sugar, characteristic of cardiac glycoside. A violet ring appeared below the brown ring while in the acetic acid layer a greenish, ring formed just above the brown ring and gradually spread throughout this layer.

(c) Lieberman's test: Half a gram (0.5g) of plant extract was added to 3mls of chloroform and filtered; 10 drops of acetic anhydride was added to the filtrate along with 2 drops of concentrated sulphuric acid. A pink colour at the interphase was a positive for terpenes while a bluish colour was the steroids.

2.4.7. Test for phlobatanins

Half a gram (0.5g) of the plant extract is added with 3 drops of 40% formaldehyde, 6 drops of dilute HCl was also added to boiling and cool. A precipitate was formed, if positive and washed with hot water; this left a colourless residue after washing indicated the presence of phlobatanins.

2.4.8. Test for deoxy-sugar

Half a gram (0.5g) of the filtered plant extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride. It was then underplayed with 1ml of concentrated sulphuric acid (H_2SO_4). Violet ring observed which settled after few minutes was an indication of a positive test.

2.5. Alkaloidal partitioning (qualitative test)

The methods of (Harborne, 1998; Trease and Evans, 2002) were used for the alkaloidal partitioning. The ethanolic extract of *P. amaru*s was dissolved in 5% Hcl and partitioned successively with 50ml of chloroform ($CHCL_3$) with the aid of separating funnel to give aqueous fraction and chloroform fraction, respectively. The aqueous fraction was then made alkaline with ammonium hydroxide (NH_4OH) solution of pH 12 and subsequently partitioned with chloroform ($CHCL_3$) to give alkaloidal fractions.

2.6. Sensitivity testing of crude ethanolic leaf extracts of *phyllanthus amarus* on uro-pathogens

The crude ethanolic extracts of *Phyllanthus amarus* were tested for antibacterial potency by the disc diffusion method (NCCLS, 2004; Nair et al., 2005) using Oxoid- Mueller Hinton agar (Difco Laboratories, Detroit, Mich) supplemented with 2% NaCl. The Mueller – Hinton agar (MHA) was sterilized in flasks cooled to 45 – 50^{o}C and then poured into sterilized Petri dishes. Sterile filter paper discs of 6 mm diameter were impregnated with extract solution of graded concentrations (20 mg/ml and 40 mg/ml) and then placed on to the agar plates which had previously been inoculated with the tested uropathogenic bacteria and the plates were incubated at 37^{o}C for 24 hrs. Control experiments comprising streptomycin and Dimethyl sulphoxide (DMSO) were also set up. The diameters of the inhibitory zones were measured in millimeters. Assays was performed in triplicate and the data are shown as the mean ± standard deviation (SD).

2.7. Sensitivity testing of alkaloidal fractions of *phyllanthus amarus* on uro-pathogens

The alkaloidal fractions of *Phyllanthus amarus* was also tested for antibacterial potency by the disc diffusion method using Oxoid- Mueller Hinton agar supplemented with 2% NaCl. The Mueller – Hinton agar was sterilized in flasks cooled to 45 – 50^{o}C and then poured into sterilized Petri dishes. Sterile filter paper discs of 6 mm diameter were impregnated with 20 mg/ml of the alkaloidal fractions and then placed on to the agar plates which had previously been inoculated with the tested uropathogenic bacteria. The plates were then incubated at 37^{o}C for 24

hrs and the diameters of the inhibitory zones were measured in millimeters. Assays were performed in triplicate and the data are shown as the mean ± standard deviation (SD).

2.8. Determination of activity index (A.I)

Activity Index (A.I) was calculated as the mean inhibition zone of sample (*P. amarus*) divided by the mean inhibition zone of the standard drug used (streptomycin).

$$\text{Activity Index} = \frac{\text{Mean inhibition zone of sample (}P.\ amarus\text{)}}{\text{Mean inhibition zone of the standard drug (Streptomycin)}}$$

2.9. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The Minimum Inhibitory Concentration (MIC) of crude ethanolic extracts of *Phyllanthus amarus* was determined for each of the uro-pathogens using micro broth method. Zero point five (0.5) ml each of the isolate was added to the following varying concentrations of the extracts: 5 mg/ml, 10 mg/ml, 20 mg/ml, 40 mg/ml and 80 mg/ml containing 2ml of nutrient broth. The cultures were then incubated at 37^{o}C for 24 hrs. After incubation, the microbial growth was examined by observing for turbidity. The tube containing the least concentration of extract showing no visible sign of growth was considered as the minimum inhibitory concentration. To determine the MBC for each of the test isolate, 1ml of the broth was collected from the tubes that showed no growth and inoculated onto sterile nutrient agar. The plates were then incubated at 37^{o}C for 24 hrs. After incubation the concentration that showed no visible growth was considered as the Minimum Bactericidal Concentration (MBC).

Fig. 1. Phyllanthus amarus.

3. Results

The results of preliminary phytochemical analysis of the ethanolic leaf extracts of *Phyllanthus amarus* revealed the presence of phyto-constituents such as alkaloids (+++), tannins (+++), saponins (++), flavonoids (++), cardiac glycoside (+), free anthraquinones (++), deoxy-Sugar test (+) and phlobatanins (+), while combined anthraquinones was not detected (Table 1). The results of the morphological and biochemical characteristics of the Gram positive (*Streptococcus* sp *Staphylococcus aureus*; Coagulase negative *Staphylococcus* sp and *Enterococcus faecalis*) and Gram negative bacteria (*Escherichia coli, Enterobacter* sp.; *Serratia marcescens*; *Klebsiella* sp and *Pseudomonas aeruginosa*) isolated from mid-stream urine samples are shown in Table 2.

There was no bacterial growth when ethanolic leave crude extracts of *Phyllanthus amarus* were plated out on Petri dishes containing nutrient agar and incubated at 37^{o}C for 24hrs. Thus, indicated and confirmed the purity of the ethanolic leave crude extracts of *Phyllanthus amarus*. The results showed that ethanolic leave crude extracts of *Phyllanthus amarus* exhibited varying degrees of inhibitory effects against both Gram positive and Gram negative bacteria isolated from mid-stream urine samples and the zones of inhibition increased with the increase in

concentrations of the ethanolic leave crude extracts of *Phyllanthus amarus*, thus, exhibiting concentration dependent activity. Ethanolic leave crude extracts of *Phyllanthus amarus* showed the highest mean zones of Inhibition (MM) ±SD of 14.3 ± 1.0mm and activity index 0.97 at 40.0 mgm1^{-1} among the Gram positive bacteria, while the highest mean zones of Inhibition (MM) ±SD of 14.5 ±0.5mm and activity index of 1.12 at 40.0 mgml^{-1} among the Gram negative (Tables 3 and 4). The results also showed that Coagulase negative *Staphylococcus* sp (CS03) was not sensitive to both 20.0 mgml^{-1} of crude extracts of *Phyllanthus amarus* and alkaloidal fractions. Among the Gram negative, *Enterobacter* sp (ES03) was not sensitive to 20.0 mgml^{-1} of crude extract of *Phyllanthus amarus*, alkaloidal fractions and streptomycin. The results showed that di-methyl sulphoxide (DMSO) exhibited no inhibition on all the isolates tested (Tables 3 and 4). Duncan's multiple range tests showed significant difference ($P<0.05$) among the mean diameter inhibition zones for the ethanolic leave crude extracts of *Phyllanthus amarus*, flavonoids fraction and streptomycin.

Table 1
The preliminary phytochemical screening of ethanolic leaf of *phyllanthus amarus.*

Bioactive compounds	**Inferences**
Alkaloid	+++
Tannin test	+++
Saponins	
A. Frothing test	++
B. Sodium bicarbonates test	++
Flavonoid	++
Cardiac glycoside	
A. Salkowski's test	+
B. Lieberman's test	+
C. Keller-killiani's test	+
Deoxy-sugar test	+
Phlobatanins	+
Anthraquinones test	
A. Free anthraquinones	++
B. Combined anthraquinones	-

Keys: + = Present in small concentrations; ++ =Present in moderately high concentration; +++ = present in very high concentration; - = Not detected

Table 2
Morphological and biochemical characteristics of bacteria isolated from mid-stream urine samples.

Parameters	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Grams reaction	+/cocci	+/cocci	+/cocci	-/rod	-/rod	+/cocci	-/rod	-r/rod	-/rod
Catalase test	-	+	+	-	-	-	-	-	-
Citrate test	-	-	-	-	-	-	+	+	-
Coagulase test	-	+	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	-	+
Indole test	-	-	-	+	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	+
Urease activity	-	-	-	-	-	-	-	+	-
Glucose	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	+	+	-	-	+	-
Sucrose	-	-	-	-	-	-	+	+	-
Mannitol	+	+	+	+	+	+	+	+	+
Vogues proskauer	-	+	+	-	+	-	+	+	-

Keys - = negative; + = positive; a: streptococcus sp; b: staphylococcus aureus; c: con-staphylococcus sp d: escherichia coli; e: enterobacter sp; f: enterococcus faecalis; g: serratia marcescens; h: klebsiella sp; i: pseudomonas aeruginosa

The minimum inhibitory concentration and minimum bactericidal concentration concentrations of *Phyllanthus amarus* are shown in Figs 2and 3. The results showed that minimum inhibitory concentration of 5mg/ml was observed with Coagulase negative *Staphylococcus* spp (CS01), *Enterococcus faecalis* (EF02) and *Escherichia coli* (EC03), while in *Streptococcus pyogenes* (SP02) and CON-*Staphylococcus* sp (CS03), the minimum bactericidal concentration of *Phyllanthus amarus* was 80 mg/ml (Figs 2 and 3).

Table 3

Antibacterial Activity of Crude Ethanolic Extracts of *Phyllanthus amarus* and Alkaloidal Fractions on Gram negative Uro- pathogens

		Crude Extracts				Alkaloid fractions			
Bacterial spp.	**Code**	**20mg/ml**	**AI**	**40 mg/ml**	**AI**	**20 mg/ml**	**AI**	**Streptomycin**	**DMSO**
Escherichia coli	EC01	10.2 ± 0.5^{b}	0.68	14.0 ± 1.0^{b}	0.93	8.0 ± 2.0^{a}	5.33	15.0 ± 1.3^{a}	NZ
Escherichia coli	EC02	10.0 ± 1.2^{b}	0.81	10.0 ± 2.0^{a}	0.81	7.7 ± 1.0^{a}	0.62	12.4 ± 2.0^{a}	NZ
Escherichia coli	EC03	12.0 ± 10^{b}	0.93	14.5 ± 0.5^{b}	1.12	8.9 ± 2.5^{a}	0.68	12.9 ± 1.6^{a}	NZ
Klebsiella sp	KS01	10.0 ± 1.7^{b}	0.61	13.2 ± 0.5^{b}	0.80	8.1 ± 1.0^{a}	0.49	16.4 ± 2.0^{b}	NZ
Klebsiella sp	KS02	7.5 ± 1.3^{a}	0.47	9.9 ± 1.0^{a}	0.62	7.5 ± 0.1^{a}	0.47	16.0 ± 1.5^{b}	NZ
Klebsiella sp	KS03	8.3 ± 0.5^{a}	0.54	11.5 ± 1.5^{a}	0.74	7.9 ± 1.2^{a}	0.51	15.6 ± 0.4^{a}	NZ
Pseudomonas aeroginosa	PA01	9.2 ± 1.2^{a}	0.61	12.5 ± 2.5^{a}	0.82	8.1 ± 0.1^{a}	0.54	15.1 ± 2.0^{a}	NZ
Pseudomonas aeruginosa	PA02	10.2 ± 1.5^{b}	0.62	12.3 ± 0.5^{a}	0.75	9.1 ± 1.0^{a}	0.55	16.4 ± 2.2^{b}	NZ
Pseudomonas aeruginosa	PA03	9.0 ± 1.1^{a}	0.48	12.8 ± 1.1^{a}	0.68	7.2 ± 2.2^{a}	0.38	18.8 ± 1.0^{b}	NZ
Serratia marcescens	SM01	10.8 ± 1.5^{b}	0.65	14.2 ± 0.2^{b}	0.83	7.4 ± 1.5^{a}	0.43	17.1 ± 0.5^{b}	NZ
Serratia marcescens	SM02	10.3 ± 1.5^{b}	0.65	11.0 ± 1.5^{a}	0.65	8.2 ± 0.5^{a}	0.49	16.7 ± 1.0^{b}	NZ
Serratia marcescens	SM03	9.2 ± 1.8^{a}	0.66	11.5 ± 2.0^{a}	0.82	7.7 ± 1.0^{a}	0.55	14.0 ± 0.5^{a}	NZ
Enterobacter sp	ES01	$7.5 \pm 1.^{5a}$	0.61	9.9 ± 1.0^{a}	0.81	7.1 ± 0.5^{a}	0.58	12.2 ± 1.6^{a}	NZ
Enterobacter sp	ES02	10.0 ± 1.5^{b}	-	12.0 ± 0.5^{a}	-	8.3 ± 1.2^{a}	-	NZ	NZ
Enterobacter sp	ES03	NZ	-	9.9 ± 1.0^{a}	-	NZ	-	NZ	NZ

AI: Activity Index; DMSO: Dimethyl Sulphoxide; NZ: No Zone of Inhibition. Each value represents the mean of three experiments and standard deviation. Means within the column followed by the same letter do not differ significantly as determined by Duncan's multiple range test ($P<0.05$) among the treatment

Table 4

Antibacterial activity of ethanolic extract of *phyllanthus amarus* and alkaloidal fractions of gram positive uro-pathogens

		Crude extracts				Alkaloidal fractions			
Bacterial sp	**Code**	**20 mg/ml**	**Ai**	**40 mg/ml**	**Ai**	**20 mg/ml**	**Ai**	**Streptomycin**	**Dmso**
Staphylococcus aureus	Sa01	8.0 ± 0.5^{a}	0.57	10.5 ± 1.5^{a}	0.75	7.0 ± 1.0^{a}	0.5	14.0 ± 2.0^{a}	Nz
Staphylococcus aureus	Sa02	9.2 ± 1.0^{a}	0.56	$12.5 \ 2.0^{b}$	0.76	6.9 ± 2.1^{a}	0.42	16.4 ± 1.5^{b}	Nz
Staphylococcus aureus	Sa03	9.0 ± 1.5^{a}	0.56	12.3 ± 0.1^{b}	0.77	8.4 ± 1.2^{a}	0.53	16.0 ± 0.2^{a}	Nz
Streptococcus pyogenes	Sp01	7.0 ± 0.8^{a}	-	10.5 ± 0.5^{a}	-	Nz	-	Nz	Nz
Streptococcus pyogenes	Sp02	9.8 ± 1.5^{a}	0.68	12.0 ± 0.1^{b}	0.83	7.6 ± 2.0^{a}	0.53	14.4 ± 2.6^{a}	Nz
Streptococcus pyogenes	Sp03	9.4 ± 0.4^{a}	0.59	12.1 ± 0.2^{b}	0.76	9.2 ± 1.1^{a}	0.58	15.9 ± 1.0^{b}	Nz
Con-*staphylococcus* sp	Cs01	12.2 ± 0.5^{b}	0.59	14.0 ± 0.1^{b}	0.69	9.0 ± 0.5^{a}	0.44	20.4 ± 2.1^{b}	Nz
Con-*staphylococcus* sp	Cs02	10.8 ± 0.5^{a}	0.65	13.6 ± 1.0^{b}	0.82	8.6 ± 1.1^{a}	0.52	16.6 ± 1.0^{b}	Nz
Con-*staphylococcus* sp	Cs03	Nz	-	8.2 ± 0.2^{a}	0.79	Nz	-	10.4 ± 1.2^{a}	Nz
Enterococcus faecalis	Ef01	10.2 ± 0.4^{a}	0.64	13.8 ± 1.2^{b}	0.86	7.4 ± 2.0^{a}	0.46	16.0 ± 2.1^{b}	Nz
Enterococcus faecalis	Ef02	12.0 ± 1.1^{b}	0.82	14.3 ± 1.0^{b}	0.97	8.9 ± 0.5^{a}	0.61	14.7 ± 1.5^{a}	Nz

ai: activity index; dmso: dimethyl sulphoxide; nz: no zone of inhibition. Each value represents the mean of three experiments and standard deviation. Means within the column followed by the same letter do not differ significantly as determined by duncan's multiple range test ($p<0.05$) among the treatment.

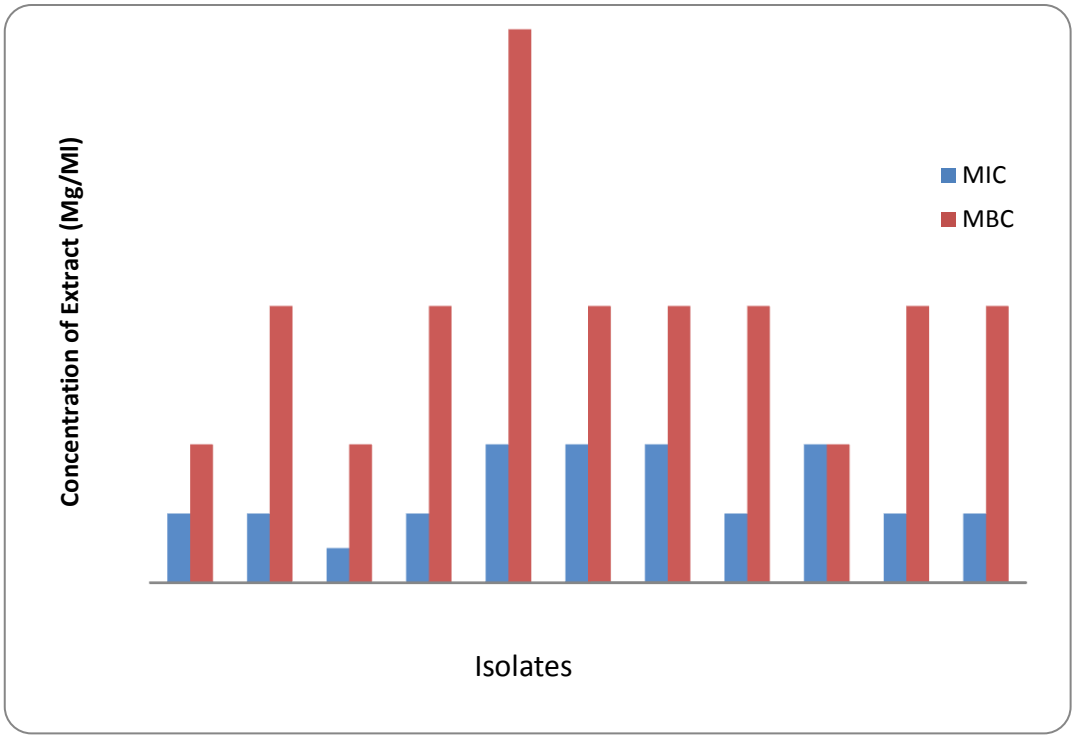

Fig. 2. Graphical representation of MIC and MBC of Gram positive uropathogenic bacteria.

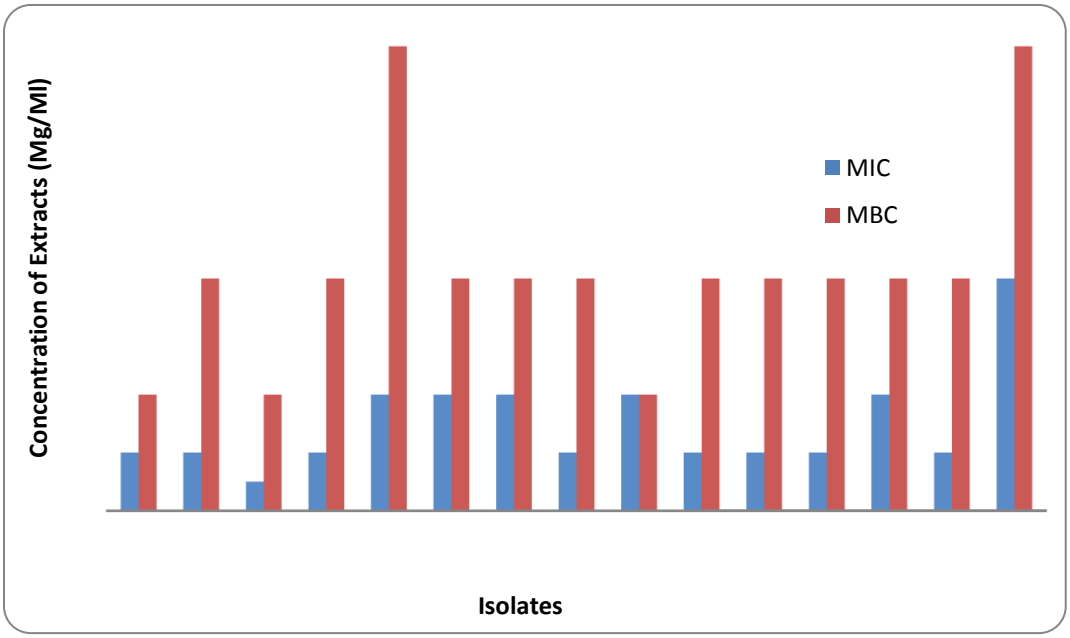

Fig. 3. Graphical representation of MIC and MBC of Gram negative uropathogenic bacteria.

4. Discussion

Urinary tract infection is the largest single group of hospital-acquired infection and account for about 40–50% of the total nosocomial infections (Hassan et al., 2011). The occurrence of *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis and Staphylococcus aureus* in the mid stream urine is in agreement with (Saleem and Daniel, 2011). The defeat suffered by antibiotics such as penicillin, methicillin, vancomycin and other

antibiotics has necessitated the search for valuable compounds that potentiate antibacterial activity on multidrug bacteria especially those that cause urinary tract infections. Preliminary phytochemical tests of ethanolic leaf extracts of *P. amarus* revealed the presence of anthraquinones, cardiac glycosides, saponins, tannins, alkaloids, flavonoids, which is in conformity with the earlier reports on *P. amarus* (Oluwafemi and Debiri, 2008; Akinjogunla, 2010a). The antimicrobial effect of plant extracts could be due to the presence of some of these phyto-constituents (Ebana et al., 2005; Cushnie and Lamb, 2005). The antibacterial activities of alkaloids have been reported by Ebana et al., (2005).

The secondary metabolites of plants exert antimicrobial activity through different mechanisms. Tannins form irreversible complexes with proline rich protein, resulting in the inhibition of cell protein synthesis and the flavonoids complex with extracellular- soluble proteins and with bacterial cell wall proteins while the lipophilic flavonoids exert antimicrobial activity by disrupting microbial cells membranes (Tsuchiya et al., 1996; Olowusulu and Ibrahim, 2006). *P. amarus* ethanolic extracts tested were active against both Gram-positive bacteria and Gram-negative bacteria, suggesting a broader spectrum activity of the plant. The degree of susceptibilities of the uro-pathogenic Gram positive and Gram negative bacteria to *P. amarus* ethanolic extracts may be as a result of the physical and chemical compositions of their cell walls.

5. Conclusion

There is a need to consider the use of *P. amarus* ethanolic extracts and its alkaloidal fractions that have shown some measures of antibacterial potency, judging by the antibacterial activity, activity index, minimum inhibitory concentration and minimum bactericidal concentration in the treatment of urinary tract infection caused by bacteria.

Acknowledgements

The authors greatly acknowledge the assistance of Dr. Felix Ogbemudia of Department of Botany and Ecological Studies Akwa Ibom State.

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