PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL SCREENING OF THE BARK EXTRACTS OF Myrianthus arboreus

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ABSTRACT

The inhibitory effects of the bark extract of Myrianthus arboreus was studied with chloroform, ethanol, methanol, acetone, aqueous hot and cold extracts on Staphylococcus aureus, Escherichia coli, Proteus sp., Klebsiella sp., Aspergillus sp., and Candida albicans, using disc diffusion and agar diffusion method. All the solvents showed inhibitory effect with varying degree of susceptibility between 10.00-18.00mm except aqueous cold that did not show any activity to any of the test organism. Ethanol, acetone and methanol extract had highest zones of inhibition on most of the isolates between 12.00- 18.00mm, while aqueous hot extracts showed the least zones of inhibition at 1.00mm. The zones of inhibitions of the extract coincided well with some of the standard antibiotics and antifungal drugs used which showed that the plant can be used for phytomedicinal purposes because of their related properties. The bark of Myrianthus arboreus can be developed as a chemotherapeutic agent due to its antimicrobial properties.

Keywords: Agar diffusion, Antimicrobial properties, Bark extracts, Disc diffusion, Inhibitory effects

Introduction

Medicinal plants are nature’s gift for a disease free healthy life and play vital role in preserving our health. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem (Nascimento et al., 2000) and hence new prototype antimicrobial agents are needed to address the situation. Several medicinal plants traditionally contain large amount of antioxidants that can delay and prevent oxidative reactions and have potential antimicrobial properties which can be used to treat chronic as well as infectious diseases (Biapa et al., 2007). Recently, there has been increasing interest in discovering new natural antimicrobials; over 25% of prescribed medicine in industrialized countries is derived directly or indirectly from plants (Newman et al., 2000). These medicinal plants are potential drugs that are always available, inexpensive, easily accessible, no adverse effects, unlike most antibiotics of microbial origin within sub-saharan Africa and this makes them more attractive (Agbor and Ngogang, 2005; Agbor et al., 2004). Synthetic antibacterial and antifungal drugs employed for the treatment of bacterial and fungal disease that are expensive, causes undesirable side effects and affects the body metabolism. The development of drug resistance in microorganisms to most of these drugs calls for the use of medicinal plants.
Phytochemical are biological chemical compounds formed during the plants normal metabolically processes which are referred to as secondary metabolites with bioactive constituent which serves as precursors for synthesizing useful drugs for curing diseases (Okwu and Okwu, 2004; Okigbo et al., 2009). *Myrianthus arboreus* (Moraceae) bark is fairly smooth, grayish, thin slash white part with annular scars possessing enormous antimicrobial properties as well as other functional uses. The sap from the bark of *Myrianthus arboreus* has anti-diarrheic and antigenorrheal properties due to myrianthinic acid isolated from the plant (Papajewski et al., 2001; Akujobi et al., 2004). Okafor (2004) reported that euscaphic acid, myrianthic acid, tormentic acid, ursolic acid, ursenoic acid and myrianthinic acids was isolated from the bark. The bark shows invitro antiplasmodial, antymycobacterial and antityranosomal effects which supports some of its uses in traditional medicine and can be used for treating diarrhea, dysentery and diabetes (Okafor, 2004; Omotayo and Borokini, 2012). This study investigates the phytochemical properties for bioprospecting as medicinal plant, and its effectiveness against some selected clinical isolates using appropriate standard methods which authenticates its use as a therapeutic agent.

**Materials and Methods**

**Plant material**

The fresh bark of *Myrianthus arboreus* were collected from Ogbakiri in Emohua Local Govt. Area, Rivers State, Nigeria, was brought to the laboratory and sundried to crisp. The plant was identified at the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria.

**Preparation and extraction**

The barks were cut into smaller pieces, sun dried for few weeks, pulverized to get coarse powder which was subjected to extraction using a modified method of Ogueke et al. (2006). The samples were treated separately and successfully with aqueous (hot/cold), methanol, ethanol, and acetone and chloroform. The details of the extraction and sterilization of materials were reported in our previous research (Agwa et al., 2011).

**Phytochemical analysis**

Qualitative phytochemical analyses were done using the methods described by AOAC (1990) and Trease and Evans (2002). The presence of tannins, phytic acids, saponin, terpene, cyanogenic glycosides, alkaloids, oxalates and flavonoids were analyzed.

**Microbial cultures**

The clinical isolate (*Staphylococcus aureus, Escherichia coli, Proteus sp, Klebsiella sp, Aspergillus sp. and Candida albicans*) was collected from Medical laboratory of Microbiology and Parasitology unit of the University of Port Harcourt Teaching Hospital. The organisms were cultured on nutrient and potatoes dextrose agar, incubated at 37°C for 24h for bacteria, the fungi was incubated at room temperature for 5-7days, after sub-cultured on the appropriate media and kept until when required. Their identity was confirmed using morphological and biochemical test as described (Agwa and Wokoma, 2011). Colonies of the isolates were picked from the slant and suspended in about 9ml of sterile nutrient and potatoes dextrose agar, the turbidity of the isolate suspension was corresponded to 0.5 McFarland standards before used.

**Antimicrobial activity assay**

Antimicrobial susceptibility test was carried out using disc diffusion (Bauer, 1966) and well in agar diffusion (Osadebe and Ukwueze, 2004) method. The disc diffusion was carried out using filter paper disc measuring about 6mm in diameter. The plates were prepared by spread plate technique using nutrient agar for bacterial strains and potato dextrose agar for fungal strains with the proper concentration of the inoculums. The sterilized filter paper discs impregnated with various concentrations of the bark extracts of *Myrianthus arboreus* were placed at suitable distance on the plate. The plates were incubated at 37°C for 24h and were examined after 24h. The zones of inhibition were measured in millimeter (mm) and recorded (Agwa et al., 2011). Widely used five antibiotics such as Ampicillin, Chloramphenicol, Amoxicillin, Ampiclox and Griseofulvin were used as reference to determine the sensitivity of each bacterial species tested.
About 0.1ml of the organism containing 1x10^5 cfu/ml was taken from the broth culture, introduced into sterile Petri dish of about 15ml of the molten nutrient and potato dextrose agar respectively, mixed and allowed to solidify. Using a sterile cup borer, four holes were bored (6mm each) and equal volume of the different concentrations of the plant extract was transferred into the holes using a sterile syringe, plated out in triplicate for each organism and allowed to stand for about one hour pre diffusion of the extract to occur (Esimone et al., 1998), incubated at 37°C for 24hrs, zones of inhibition was measured in millimeters (mm).

Minimum Inhibitory Concentration (MIC) was determined by inoculating about 0.1ml broth cultures of the test isolates into sterile Petri dish of molten nutrient and potato dextrose agar. The content was thoroughly mixed, allowed to solidify and incubated at 37°C for 24h. Controls were used with the test organisms, using distilled water instead of the plant extract. The lowest concentration with no visible growth was taken as the MIC. Minimum Bactericidal and Fungicidal Concentration (MBC and MFC) were determined by transferring inocula from tubes showing no visible growth unto nutrient and potato dextrose agar using the spread plate technique, incubated at 37°C for 24h. The highest dilution of the extract that showed no growth was recorded as the MBC and MFC.

Results

Phytochemical screening of the plant extracts of *Myrianthus arboreus* revealed the presence of tannins, flavonoids, alkaloids, saponin, terpene and phytic acid but glycosides and oxalate were absent as shown in Table 1. The antimicrobial activity of the bark extracts of *Myrianthus arboreus* against six test organisms are illustrated in Figures 1-6 below. From the disc diffusion method, highest activity was conferred by the extracts of methanol with zones of inhibition against *Proteus* sp. (17.34mm, 14.66mm and 11.64mm) at concentrations of 500mg/ml, 375mg/ml and 250mg/ml respectively, followed by ethanol extracts on *Klebsiella* sp., and acetone on *Candida albicans*, while aqueous hot extract showed the least zone of inhibition on all test isolates (Fig.1 and 2). The agar diffusion method revealed chloroform extract with the highest zone of inhibition of 18.00mm at a concentration of 500mg/ml on *E. coli*, *Klebsiella* sp. and *Proteus* sp followed by acetone on *Aspergillus* sp and ethanol on *Candida albicans*, but the least zone of inhibition was conferred on all test isolates by aqueous hot extract (Figs.3 and 4). The effect of commercial antibiotics were monitored on the test isolates and chloramphenicol was effective against *Klebsiella* sp, followed by ampiclox (*Proteus* sp), ampicillin (*Klebsiella* sp), amoxicillin (*Klebsiella* sp) and griseofulvin on *Candida albicans* with the disc diffusion method (Fig. 5). As can be seen, the well in agar diffusion method showed similar trend with chloramphenicol effective against *E. coli*, ampicillin (*Proteus* sp), ampiclox (*Klebsiella* sp), amoxicillin (*Klebsiella* sp) and the least was exerted on *Candida albicans* by griseofulvin (Fig. 6). The aqueous cold extract did not exert activity on the test isolates, due to failure of the active ingredient to dissolve in it. All the sensitive extracts were more at higher concentrations than lower concentrations. The MIC and MBC of the different extracts on the test organisms is given in Table 2 and 3 and shows that the 125mg/ml and 250mg/ml shows inhibition on all isolates.

<table>
<thead>
<tr>
<th>Table 1: Phytochemical constituents of <em>Myrianthus arboreus</em></th>
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<tr>
<td><strong>Test</strong></td>
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<tr>
<td>Tannin</td>
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<tr>
<td>Cyanogenic glycosides</td>
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<td>Phytic acid</td>
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<tr>
<td>Flavonoids</td>
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<td>Alkaloids</td>
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<td>Oxalate</td>
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<td>Saponin</td>
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<td>Terpene</td>
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<td>Steroid</td>
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Figure 1. Zone of inhibition (mm) of the different concentrations of the extracts on the isolates using disc diffusion method.

Figure 2: Average Zones of Inhibition of the Extracts on the Isolates using Disc Diffusion Method.
Figure 3: Zone of inhibition of the different concentration of the extract on the clinical isolates using well in agar diffusion

Figure 4: Average zone of inhibition (mm) of the extracts on the isolates using well in agar diffusion method
Figure 5: Average zone of inhibition of antibiotics/antifungal drug on the isolates using disc diffusion method.

Figure 6: Average zone of inhibition of the antibiotics/antifungal drugs using well in agar diffusion.
Table 2: Minimum Inhibitory (mg/ml) Concentration of the bark Extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Staph aureus</th>
<th>E. coli sp.</th>
<th>Proteus sp.</th>
<th>Klebsiella sp.</th>
<th>Aspergillus sp.</th>
<th>Candida albicans</th>
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<tr>
<td>Chloroform</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>-</td>
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<tr>
<td>Ethanol</td>
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<td>125</td>
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<tr>
<td>Acetone</td>
<td>125</td>
<td>125</td>
<td>125</td>
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<td>125</td>
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<tr>
<td>Aqueous hot</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
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<tr>
<td>Aqueous cold</td>
<td>500</td>
<td>500</td>
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Table 3: Minimum Bactericidal (Fungicidal) Concentrations

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Staph aureus</th>
<th>E. coli sp.</th>
<th>Proteus sp.</th>
<th>Klebsiella sp.</th>
<th>Aspergillus sp.</th>
<th>Candida albicans</th>
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<tbody>
<tr>
<td>Chloroform</td>
<td>500</td>
<td>500</td>
<td>500</td>
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<td>Aqueous hot</td>
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<tr>
<td>Aqueous cold</td>
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Discussion

*Myrianthus arboreus* has been previously known as one of the medicinal plants commonly used in Africa with different ethnomedical properties (Biapa et al., 2007). This could be as a result of the presence of various phytochemical substances, in contrast to synthetic pharmaceuticals which exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process (Papajewski et al., 2001; Okigbo et al., 2009). Today, in modern medicine, plants are used as sources of direct therapeutic agents, as models for new synthetic compounds, and as a taxonomic marker for discovery of new compounds. They serve as a raw material base for the elaboration of more complex semi synthetic chemical compounds because most plant extracts owe their potency to the presence of phytochemicals (Aboaba et al., 2006; Omotayo and Borokini, 2012). Most of these phytochemical constituents are potent bioactive compounds found in medicinal plant parts which are precursors for the synthesis of useful drugs. These phytochemicals are used to cure the disease in herbal and homeopathic medicine and known for their various physiological effects like anti-irritant, anti-oxidant, antisecretolytic, anti-inflammatory, antiphlogistic, antiparasitic and anti-microbial properties, soothing relief, skin regeneration, and diuretics properties which are associated with tannins (Okwu and Okwu, 2004). Phytotherapeutically containing plants are used to treat nonspecific diarrhea, inflammations of mouth and throat and slightly injured skin (Westendarp, 2006). Flavonoids are known to be synthesized by plants in response to microbial infection for their anticarcinogenic, antitumor, antimicrobial and antioxidant properties (Manikandan et al., 2006). The action of flavonoid in-vitro is probably due to their ability to complex with extracellular and suitable proteins and to complex with bacterial cell walls, often leading to inactivation of the proteins and loss of functions (Thaakur et al., 2009). The most pharmacologically active phytochemical-alkaloids are utilized because of their actions in the promotion of diuresis, respiratory system, autonomic nervous system, digestive system, transport system, reproductive system, malignant diseases, infections and malaria with potent
antispasmodiac, analgesic and bactericidal effects (Trease and Evans, 2002; Okwu and Okwu, 2004). Saponins are of wide interest because of their medicinal, antimicrobial, anti-diabetic and anti-carcinogenic properties have been known to lower the cholesterol level, serves as expectorants, cough suppressants and for haemolytic activities and their likely role in determinants of plant disease resistance (Sofowora, 1993; Haralampidis et al., 2002; Trease and Evans, 2002; Okwu, 2005). Terpenes helps to prevent liver damage (cirrhosis), have antimicrobial, anti-hepatoxic and anti-septic properties (Omotayo and Borokini, 2012).

The antimicrobial activity of the solvent extracts were found to be higher for methanol, followed by ethanol and acetone, similarly chloroform was found to be higher followed by acetone and subsequently ethanol extract. The maximum inhibitory activity of methanol extract of Myrianthus arboreus obtained against Proteus sp., Klebsiella sp., E. coli, Aspergillus sp., C. albican and S. aureus (Babu and Ammani, 2008; Eazhsaiavallabi et al., 2012). It can be seen that the chloroform extract maximum inhibitory concentration was higher in E. coli, Klebsiella sp., Proteus sp., C. albican, Aspergillus sp. and S. aureus. The chloroform and methanol extract was found to have highly significant antimicrobial activity against all the test isolates compared to the other solvent extracts (Kamble and Deshmukh, 2008). Nweze et al. (2004) and Ogueke et al. (2006) found that various extracts of plants inhibited the growth of some clinical isolates. Prakash (2006) obtained similar results with extracts of Ocimum sanctum. Some inferior activity was also detected in the aqueous hot extract. Singh and Karnwal (2006) studied antifungal activity of Cassia fistula extracts against Candida albicans. On the other hand, aqueous cold extracts did not show any activity against any of the test isolates, while aqueous hot did not show any appreciable activity against the test isolates used in the investigation. This is in agreement with the findings of Biapa et al. (2007) and Anibijuwon et al. (2010). These researchers reported that the aqueous extracts did not exert any antimicrobial effect on the isolate and even if there were sensitivity, it was at lower concentrations and not at higher concentrations. Proteus sp. showed the highest zones of inhibition, which means it can be used in the treatment of urinary tract infections, wounds infections, and sepsis commonly associated with the organism. Klebsiella sp. at 500mg/ml concentration of ethanol and methanol extracts shows zones of inhibition, thus can be used to treat pneumonia associated with the organism. Ethanol, methanol and acetone extracts were sensitive to E. coli and Staph aureus which are responsible for a number of food related illness that manifest themselves as diarrhea. Staph aureus causes impetigo (a superficial skin infection) common in children and wound infections (Prescott, et al., 2008). Methanol and acetone extracts were sensitive only on the fungal isolates and can be used to treat opportunistic infections associated with them. Comparison of the inhibition zones of Myrianthus arboreus with those of standard antibiotics, (Figs. 5and 6) indicated that the extracts possess good antimicrobial activity. The different concentrations of the extracts were not pure as compared to standard antibiotics, the inhibition zone although smaller than standard antibiotics are clearlyindicative of good antimicrobial action (Parmar et al., 2008). The MIC of the extract on the isolates revealed that 250mg/ml concentration of Chloroform shows inhibition on Staph aureus, Proteus sp. and Klebsiella sp. 250mg/ml of Ethanol on Staph aureus and E. coli. 125 mg/ml of Methanol on Staph aureus. E. coli, Klebsiella sp., Aspergillus sp. and Candida albican. Acetone shows 125 mg/ml inhibition on Staph aureus, Proteus sp., Klebsiella sp., Aspergillus sp. and Candida albican. 250mg/ml of aqueous hot was inhibitory on all the isolates, while 500mg/ml of aqueous cold was inhibitory on all the isolates. The lower MIC values indicates plant extracts are potent enough in causing inhibitory effect. The concentrations of the extracts that are bactericidal or fungicidal (MBC/MFC) on the isolates shows 500mg/ml of chloroform on Staph aureus, E. coli, Proteus sp. and Klebsiella sp. 250mg/ml of Methanol on all isolates except Proteus sp. 500mg/ml of Acetone shows 250mg/ml inhibition on all isolates except E. coli.
Proteus sp. and 375mg/ml on C. albican. Ethanol and aqueous extracts did not show any bacteriocidal or fungicidal inhibition on all the isolates. From results obtained, it shows that chloroform, methanol, ethanol and acetone are good extractors of the active ingredients of the bark of Myrianthus arboreus.

Conclusion
This result suggests that the presence of these phytochemicals is good, active and principle antimicrobial potency in the bark extracts Myrianthus arboreus. The high concentration of the solvent extract confirms the therapy of infections and diseases therapeutic claims of this plant. The antimicrobial action has shown that the bark of Myrianthus arboreus is a potential source of antimicrobial agent against E. coli, Proteus sp., Klebsiella sp., and Candida albican and could be used in the treatment of infections caused by the test isolates.

References


