In vitro antiproliferative and antimicrobial evaluation of *Kigelia pinnata*

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Abstract

Kigelia pinnata (Lam.) Benth is a multi-medicinal plant with highly valued root extracts that are often applied in folk medicine for the management of various types of cancers and microbial infections. This study evaluates the anti-proliferative and antimicrobial potential of *K. pinnata* extracts. Oil and extracts from the root were obtained by cold extraction using hexane, ethylacetate and methanol and were concentrated *in vacuo* and examined for their antimicrobial activities and anti-proliferative activities on human breast cancer cell lines using agar diffusion method and tetrazolium dye assay, respectively. The oil and ethylacetate extract showed little or no antimicrobial activities for all organisms tested except for moderate activity on *Aspergillus* sp., but the methanol extract showed significant activity against *Salmonella typhi, Proteus* sp., *Rhizopus* sp. and *Aspergillus* sp. Most importantly, the three extracts significantly inhibited the growth of tumour cells *in vitro* with high cytotoxicity indices, while the ethylacetate extract had the highest potency, with IC_{50} values of 10.53 ± 1.6 on human breast cancer cell lines. The root extracts showed significant anti-proliferative activity, which supports the folk claims of use of the plant as an anticancer repertoire.

Key words: antimicrobial activity; antiproliferative; cancer cell line; cytotoxicity; *Kigelia pinnata*; MTT assay.
Abbreviations: DMSO, dimethylsulphoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MIC, minimum inhibition concentration.

Introduction

Kigelia pinnata (Lam.) Benth is a member of Bignoniaceae family. It is a multi-purpose medicinal plant, and its root extracts are commonly used in folk medicine for the treatment of various types of cancer (Houghton et al. 1994; Olatunji, Atolani 2009; Atolani et al. 2009). The chemical composition of the plant has been described and *in vitro* studies of the leaf phytochemicals showed anticancer potential on various cell lines (Atolani et al. 2013). The plant has local history of its use as antimicrobial agent. The biochemical analysis and antimicrobial activity of stem bark and tissue-culture-raised callus extract of the *K. pinnata* has been reported (Sachan et al. 2013). The study indicated that methanol extract was more potent as antimicrobial agent. Aqueous and methanol extracts of fruit have also been found to possess non-fatal toxicity effects in an *in vivo* study (Shama, Marwa 2013). The entire plant has been reported to be a non hyper-accumulator of metal, thereby predicting its relative bio-safety due to metal accumulation from the environment (Atolani et al. 2013b).

As a sequel to our previous work on *K. pinnata* root extracts (Atolani et al. 2011), non-polar (oil), semipolar (ethylacetate extract) and polar extracts (methanol extract) were further evaluated for antimicrobial and toxic activities in mammalian cell cultures. This was done to determine if the traditional use of the plants as anticancer and/or anti-microbial agents has scientific merit.

Materials and methods

Chemicals
Cell viability reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma, USA) and solvents were obtained in analytical grade.

Microbial strains
Four gram negative bacterial species (*Escherichia coli, Proteus* sp., *Pseudomonas aeruginosa, Salmonella typhi*), three gram positive bacterial species (*Bacillus cereus, Staphylococcus aureus, Micrococcus* sp.), and three fungi (*Candida albicans, Rhizopus* sp., *Aspergillus* sp.) were used in the study. The microbial strains were obtained from the culture collection of the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria, where they had been maintained on agar slant at 4 °C. The strains were activated at 37 °C for 24 h on peptone broth medium before use.

Sample preparation
Oil was obtained by cold extraction of dried and pulverized root material (420 g) with n-hexane followed by successive extraction with ethylacetate and methanol, respectively for five days each. The three extracts were filtered and concentrated under reduced pressure using a rotary evaporator to obtain solvent-free hexane extract (oil, 344 mg), ethylacetate extract (1.55 g) and methanol extract (20.50 g).

Antimicrobial bioassay
Antibacterial and antifungal activities were examined by the disc agar diffusion method as peviously reported (NCCLS 2006). The concentrated extracts were re-constituted in 1% dimethylsulphoxide (DMSO) for antimicrobial assay. Mueller Hinton agar (Torlak, Serbia) was prepared as per the manufacturer's protocol. The sterile Mueller Hinton agar was poured into sterile Petri dishes and seeded with test microorganisms of Mcfarland standard. Sterile filter paper discs (Whatman No. 1) of 6 mm diameter were impregnated with approximately 20 μL of the sample solutions each. The impregnated discs were air-dried at room temperature and thereafter placed on the surface of the inoculated agar plates.

Fungi were inoculated into mycological peptone and then incubated for one hour and this was then used to swab Sabouraud's Dextrose agar. Then, the plates were incubated for 24 h (for bacteria strains) at 37 °C and 48 h at 27 °C for fungi strains. Streptomycin and ketoconazole were used as the positive control for bacterial and fungi respectively while negative controls included disks impregnated with 1% DMSO. The antimicrobial activity of the extracts and compounds tested were evaluated at the end of the inoculated period by measuring the inhibition zone diameter in millimeters. The presence of zones of inhibition around each of well after the period of incubation was regarded as the presence of anti-microbial action while the absence of any measurable zone of inhibition was interpreted as absence of antimicrobial action.

Minimum inhibitory concentration
The minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited visible bacterial growth (NCCLS 2006). The MIC values were evaluated according to published procedures (Moussa et al. 2012). MIC was determined only with micro-organisms that displayed inhibitory zones. MIC was determined by dilution of the extracts in medium and application on a dish using the disc diffusion method. Dilutions of the extracts and isolates were carried out. The bacterial plates were incubated at 37 °C and fungi at 27 °C and the zone of inhibition was measured in mm after 24 or 48 h of growth, respectively. A control experiment was carried out by using an equal amount of sterile medium (only) in place of different extract concentrations. The lowest concentration of the sample solutions that caused complete inhibition of the bacteria was taken as the MIC.

Cell viability assay
The evaluation of toxicity to MDA cells was determined using a commercial 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit (Roche Manheim, Germany) following standard procedure (Delamare et al. 2012). Briefly, the cells were grown to confluent and seeded at density of 1.0×10^5 mL^{-1} in a 96-well plate at a volume of 50 μL. The cells were incubated for 24 h before dosing with the extracts at different concentrations. The concentrated extracts were dissolved in 1% DMSO and various concentrations of the extracts were prepared in cell media and 50 μL added to each well. The dosed cells were further incubated for 96 h in humidified condition (5% CO_2). The plates also contained cells to which only 50 μL of media was added to serve as controls. Cells treated with 1% DMSO were also included in order to account for activity due to DMSO. At the end of 96 h, 10 μL of the MTT reagent was added to each well and incubated for 4 h. Subsequently, 100 μL of the solubilizing reagent was added with another incubation for 24 h. The absorbances were read at 550 – 690 nm using a microplate reader (Epoch, USA).

The IC_{50} was defined as the cytotoxic concentration of each sample that reduced the absorbance of treated cells by 50%. By using optical density, the percentage cytotoxicity of cells were calculated as $[(A - B) / A] \times 100$, where A and B are the OD_{nm} of untreated and of treated cells respectively. Using the inhibition response curve for the cell line, the IC_{50} was determined on GraphPad Prism 5 software through a non-linear regression analysis.

Data analysis
Data were analysed using analysis of variance (ANOVA) on Graphpad Prism 3.0 software (USA). Differences among the mean values are captured by using the Dunnett Test. Differences at $p < 0.05$ are considered significant. The 50% cytotoxicity (IC_{50}) was estimated from dose-response-inhibition curves using non-linear regression analysis. Results are presented as mean ± standard error of the mean for the cytotoxicity or mean ± standard deviation for the antimicrobial activities.

Results

The data presented in Table 1 and 2 revealed the antibacterial and antifungi potential as well as the MIC of the extracts against the tested organisms. All extracts showed significant activities either against the bacteria or fungi relative to streptomycin. Oil had the least activity against both bacteria and fungi compared to the ethylacetate and methanol extracts. The highest antibacterial activity was recorded for the methanol extract against *Proteus* sp. and *Salmonella typhi*. The methanol extract was also active

Table 1. Antimicrobial activity of root oil of *Kigelia pinnata*. DIZ, diameter of inhibition zone (mm); NA, not applicable; IA, insignificant activity. Experiments were performed in triplicate and results are mean values ± standard deviation. Concentration used was 1mg mL^{-1} of each sample dissolved in DMSO/H$_2$O, 20 μL per disc; 20 μg per disc of reference compounds. Values in the same row bearing superscript * are significantly different relative to streptomycin at $p < 0.05$

Tested organisms	DIZ of Streptomycin (1 mg mL^{-1})	DIZ of Ketoconazole (1 mg mL^{-1})	DIZ of root oil (1 mg mL^{-1})	DIZ of ethyl-acetate (1 mg mL^{-1})	DIZ of methanol extract (1 mg mL^{-1})
Bacillus cereus	14.67 ± 1.15	NA	8.00 ± 0.00*	8.67 ± 1.15*	9.30 ± 1.15*
Salmonella typhi	19.33 ± 4.16	NA	IA	9.33 ± 1.15*	12.67 ± 1.15*
Staphylococcus aureus	16.67 ± 1.15	NA	8.00 ± 0.00*	IA	IA
Escherichia coli	17.33 ± 1.15	NA	IA	8.00 ± 0.00*	IA
Pseudomonas aeruginosa	14.00 ± 0.00	NA	IA	IA	IA
Micrococcus sp.	13.33 ± 1.15	NA	IA	IA	10.00 ± 0.00*
Proteus sp.	27.33 ± 2.31	NA	8.00 ± 0.00*	8.00 ± 0.00*	14.67 ± 2.31*
Candida albicans	NA	12.00 ± 2.00*	8.00 ± 0.00*	IA	IA
Rhizopus sp.	NA	16.00 ± 0.00*	IA	10.00 ± 0.00*	10.67 ± 1.15*
Aspergillus sp.	NA	16.67 ± 2.31*	9.33 ± 1.15*	IA	10.67 ± 2.31*

Table 2. Minimum inhibitory concentration (MIC) of the oil and extracts of *Kigelia pinnata* root. Experiments were performed in triplicate and results are mean values

Tested organisms	MIC of root oil (μg mL^{-1})	MIC of ethylacetate extract (μg mL^{-1})	MIC of methanol extract (μg L^{-1})
Bacillus cereus	>500	>500	>500
Salmonella typhi	>500	>500	250
Staphylococcus aureus	>500	>500	>500
Escherichia coli	>500	>500	>500
Pseudomonas aeruginosa	>500	>500	>500
Micrococcus sp.	>500	>500	>500
Proteus sp.	>500	>500	125
Candida albicans	>500	>500	>500
Rhizopus sp.	>500	500	250
Aspergillus sp.	500	>500	>500

against all fungi while ethylacetate extract had medium activity among the three. The methanol extract also had the most significant MIC against three organisms *Proteus* sp. (125 μg mL^{-1}), *Salmonella typhi* (250 μg mL^{-1}) and *Rhizopus* sp. (250 μg mL^{-1}). Most other extracts had a MIC greater than 500 μg mL^{-1}. The antiproliferative activity of the oil, ethylacetate and methanol extracts of the root of Kigelia pinnata on MDA cell cultures evaluated using MTT reagent assay are shown in Table 3. Ethyacetate extract had the most significant activity (IC$_{50}$ of 05.01 ± 0.01 μg mL^{-1}) while methanol extract had the least significant activity (IC$_{50}$ of 33.01 ± 9.20 μg mL^{-1}).

Discussion

A previous study has demonstrated that the main fatty acid present in root oil of *Kigelia pinnata* is elaidic acid (56.10%; Atolani et al. 2011; Fabiyi et al. 2012). The root oil did not show significant activity against any of the organisms tested except *Aspergillus* sp. However, ethylacetate extract had moderate activities against *Bacillus cereus, Salmonella typhi* and fungus *Rhizopus* sp. relative to the reference compound, streptomycin. Conversely, the methanolic extract significantly inhibited the growth of gram negative bacteria: *Salmonella typhi* and *Proteus* sp., as well as two fungi: *Rhizopus* sp. and *Aspergillus* sp. at markedly low MIC values (Table 1 and 2). However, no activity was recorded against *Escherichia coli* and *Pseudomonas aeruginosa* as well as all gram positive bacteria tested.

K. pinnata extracts were highly active on the cancer cell line (Table 3). The cells were more sensitive to ethylacetate extract (IC$_{50}$ value of 5.01 μg mL^{-1}), compared to root oil or methanol extracts (IC$_{50}$ of 10.53 and 33. 01 μg mL^{-1}, respectively). The high percentage of elaidic acid (fatty acid) present in root oil, which has been previously reported to be a possible antitumour agent in *in vivo* studies (Breistøl et al. 1999), could have contributed to the observed antiproliferative activity. The biological

Table 3. Cytotoxicity of root oil on MDA cell lines. IC$_{50}$, 50% inhibition concentration. Results represent mean ± standard error of triplicate determinations

Samples	Cytotoxicity IC$_{50}$ (µg mL^{-1})
Root oil	10.53 ± 1.60
Ethylacetate extract	05.01 ± 0.01
Methanol extract	33.01 ± 9.20

importance of elaidic acid, a 18:1 fatty acid has remained controversial. Many studies have indicated that unsaturated fatty acids have less side effects in *in vivo* model, but other emerging studies have revealed that trans-fatty acids that are monounsaturated could aggravate cardiovascular disorders, as is common for saturated fatty acids (Lanser et al. 1986; Breistøl et al. 1999; French et al. 2002; Malik et al. 2001). Elaidic acid, which was the main compound detected in oil from *K. pinnata*, has been reported in other studies to increase the cholesterol low density lipoprotein in an equivalent amount comparable to saturated fatty acid (Judd et al. 2002). It is interesting to observe that the most potent antimicrobial extract had the least potent cytotoxic effect on the cell line used. Careful evaluation of the MDA cells incubated with sample extracts suggested cell death via apoptosis, since the cells shriveled as it contracted.

In conclusion, the root oil and extracts were not active against most of the tested gram positive and gram negative bacteria and fungus. The methanol extract, due to the polar nature of its compounds, showed a significant level of growth inhibition against *Salmonella typhi, Proteus* sp. and *Rhizopus* sp. The high anti-proliferative activity, especially of the ethylacetate extract, is an indicator of future possible application in drug development.

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