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Research Article

Antimicrobial activity and cytotoxicity of selected medicinal plants found in Nandi County, Kenya

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Background: Medicinal plants are widely used by the local people to treat various human diseases cause by drug resistant microorganisms. For instance, *Kigelia Africana* fruits and barks are boiled in water and taken orally as a laxative in treating stomach ailments, *Ekebergia capensis* bark is boiled in water and use for the control of gonorrhea and tuberculosis while *Fagaropsis angolensis* stem bark is used to treat pneumonia, back ache and joins. The efficacy and safety of most of these plants has not been determined.

Objective: The present study seeks to determine antimicrobial activities and cytotoxicity of the selected medicinal plants indicated above, that are commonly used to treat infectious diseases.

Materials and Methods: Fresh plants were collected from the field; air dried ground and extracted using acetone and water. The extracts were screened for antibacterial and antifungal activities using *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Shigella dysentriae, Candida albicans, Cryptococcus neoformans*, *Microsporum gypseum* and *Trychophyton mentagrophytes.* The methods were disc diffusion and broth dilution methods while *in vitro* cytotoxicity test was carried out following a modified rapid calorimetric assay, using actively dividing sub-confluent Vero E6 cells.

Results: In disc diffusion assay, water extracts of *E. capensis* were the most active (14.7 mm) while those of *Fagaropsis angolensis* were the least (6.0 mm) against *S. aureus*. Acetone extracts of *E. capensis* and *K. Africana* had a Minimum Inhibitory Concentration of 3.125 mg/ml and 6.25 mg/ml respectively and were bactericidal. Cytotoxicity showed that *K. africana* was not cytotoxic against Vero cell lines while acetone extracts of *E. capensis* was moderately toxic with a CC_{50}(µg/ml) of 12.5.

Conclusion: These results support the use of the plants in the traditional medicine as antimicrobials and they can be exploited for novel drugs.

Key words: Antimicrobial activity, cytotoxicity, *Kigelia africana, Ekebergia capensis, Fagaropsis angolensis,*

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1. Introduction

The usage of herbal healthcare preparations is the most popular practice to majority of people in the world. For example, it is estimated that 80% of the population in Asia, Latin America and Africa use such remedies as they are reported to have minimal side effects (Doughari, 2006). The same percentage of individuals from developed countries also makes use of traditional medicines. However, such plants should be investigated

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to understand their properties, safety and efficacy (Arunkumar and Muthuselvam, 2009).In Africa, it is estimated that 75% of the population still relies on traditional healing practices and medicinal plants for their healthcare needs (Beentje, 1994). This is because of their ancient and rich tradition in the field of herbal medicine.

In Kenya, according to (Kokwaro, 2003), 10,000 plant species have been documented as medicinal according to various communities in Kenya. The major ingredients known to occur in most medicinal plants are the alkaloids, terpenoids and flavonoids, whose presences is attributed to the antimicrobial activity in plants (Nostros et al*,* 2000). An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, parasites or viruses (Davey, 2000).

Ethnobotanical study carried out in Aldai division and South Nandi Sub-County indicated the usefulness of medicinal plants in the treatment of various ailments including; skin conditions, gastrointestinal worms, rheumatism and HIV/Aids caused by viruses, bacteria and fungi (Jeruto et al, 2008). Conventional treatment is inadequate in Nandi South Sub-County as there is only one government hospital and a few health centers; furthermore they are costly, often inaccessible and unaffordable. This explains why several plant species from the wild are still being used for treating a variety of medical and other conditions, (Jeruto et al*,* 2008). There is also an increasing failure of chemotherapeutics and antimicrobial resistance of pathogens isolated from humans and animals, necessitates research for new antimicrobial that lacks side-effects on humans. This has led to the screening of several medicinal plants for their potential antimicrobial activities, (Njenga et al, 2005)1

Kigelia africana Lam and Benth (Bignoniaceae). The vernacular name among the Nandi people is "Ratinwet". It's fruits and barks are boiled in water and taken orally as a laxative in treating stomach ailments while thebark ethanolic extract possess antibacterial and antifungal activity against *S. aureus* and *C. albicans*, (Davey, 2000). *Ekebergia capensis* Sparrm (Meliaceae)). Is commonly known as "Teldet" by the Nandi community, the bark is boil in water and use for the control of gonorrhea, tuberculosis as well as diarrhea, (Jeruto et al, 2008). *Fagaropsis angolensis* (Rutaceae) is referred to as "Noiwet", by the Nandis and use the stem bark to treat pneumonia while the Embu people boil the bark and use it for the treatment of back ache and joins (Kareru et al, 2007).

2. Methodology

2.1 Collection and Identification of plant materials

The information on the medicinal plants were gathered from the traditional practitioners, herbalists using structured questionnaires in order to obtain information on the medicinal plants that are traditionally used for management of infectious diseases in the Kaptumo Division. The criteria that was used to decide the medicinal plants to be collected in the study include the disease it cures, their effectiveness and how common is it used in the region as well as available literature.

The parts that are commonly used were the stem barks that were collected and prepared. The plant parts collected for identification consisting of flowers, roots, stems and leaves that were obtain from their natural habitats in Kaptumo Division and identified by a taxonomist at the Department of Botany herbarium University of Eldoret where the Voucher specimens were deposited. The voucher specimens numbers were assigned as follows*; Kigelia africana* (Kim/Kap/12/11/003), *Ekebergia capensis* (Kim/Kap/12/11/005) and *Fagaropsis angolensis* (Kim/Kap/12/11/ 002).

2.2 Preparation of extracts

The collected plant materials were transported to Kenya Medical Research Institute (KEMRI) Phytochemistry laboratory and washed thoroughly with running tap water. They were then chopped into small pieces and air-dried for two weeks at room temperature by spreading evenly in the open drying area. Later, the dry samples were ground into fine powder using a Willy mill and labeled appropriately using their voucher numbers. Fifty grams of the ground plant material were exhaustively extracted with acetone to obtain the organic extracts for preliminarily investigation of the antimicrobial activities. The extracts were filtered through Whatman No. 1 filter paper and the solvents were removed using a rotary evaporator and stored in sterile airtight vials at 4 °C in readiness for bioassay tests.

2.3 Microorganisms used

The microbial organisms used in the study were the standard reference strains and clinical isolates that were obtained from the Centre for Microbiology Research (CMR) for bio-assay. The gram positive bacteria were *Staphylococcus aureus* (ATCC 25923) and clinical isolate of Methicillin resistant *Staphylococcus aureus* (MR*SA*) while the Gram negative were *Escherichia coli (*ATCC 25922), *Pseudomonas aeruginosa (*ATCC 27853) and clinical isolate of *Shigella dysentriae.* Fungi consisted of yeast comprising *Candida albicans (*ATCC 90028) and *Cryptococcus neoformans* (ATCC 32602), and clinical isolates of *Microsporum gypseum* and *Trychophyton mentagrophytes* which are dermatophytes.

2.4 Antimicrobial assay

The antimicrobial activity of the extracts was determined based on the zones of inhibition using disc diffusion method described by (Bauer et al, 1996). 100 mg of each extract was dissolved in 1ml to come up with a concentration of 100 mg /ml. Ten microliters of each prepared plant extracts was measured and impregnated onto 6 mm sterile filter paper disk with a diameter of 6 mm and air dried. The disk was then placed aseptically onto the inoculated plates and incubated for 18 hours at 37 °C for bacteria (Bauer et al, 1996) while molds and yeast cultures were incubated at 30 °C for 72 hours and 35 °C for 24 hours respectively. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and Cytotoxicity test for *Kigelia africana* and *Ekebergia capensis* extracts were determined.

MIC was determined using broth micro dilution method. The reference was the 0.5 McFarland turbidometry to achievement of inoculums approximately $1x10^6$ colony forming units. Plant extracts were transferred into micro-titer plate, using micro-titre pipette, starting with 200 µl of plant extracts in the first well that was left empty. Half of the solution in the first well was transferred to the second well containing 100 µl of water and mixed thoroughly. Half of the solution in the second well was transferred to the third and mixed thoroughly and this was continued up to the 10^{th}. to make serial dilutions ranging from 10^{-1}, 10^{-2}, and 10^{-3} up to 10^{-10}. The wells were then inoculated with 20 µl of microbial suspension. The bacteria were then incubated at 37° C for 24 hours in ambient air. The MIC value was determined as the lowest concentration of the crude extract in broth medium that inhibited the visible growth of the test microorganism as compared to the control.

The MBC was determined by collecting a loop full of broth from those wells which did not show any growth in MIC, two wells above and two below the lowest MIC value and inoculated on sterile Muller-Hinton agar by streaking. The plates were incubated at 37 °C for 18-24 hours. The highest dilution that yielded no colony fraction on a solid medium was considered as MBC.

2.5 Cytotoxicity Assay

The cytotoxic concentration causing 50% cell lysis and death (CC_{50}) was determined for the active extracts by following a modified rapid calorimetric assay, (Mosmann, 1983). The extracts of the active plants were tested for *in vitro* cytotoxicity, using actively dividing sub-confluent Vero E6 cells that were obtained from the kidney cells of the African green monkey and stocked at cytotoxicity laboratory at KEMRI. Preparation of plant extracts involved 100 µg of each extract was dissolved in 1ml of Dimethyl sulfoxide (DMSO) to come up with a concentration of 100 µg /ml. They were poured into a 75ml culture flasks containing minimum essential medium (MEM) supplemented with 10% Fetal bovine serum (FBS) and incubated at 37 °C and 5% CO_2.

Upon attainment of confluence, cells were pooled in a 50 ml centrifuge tube to aid in cell density count to attain 2×10^5 using tryphan blue exclusion test. One hundred (100) µl of the cell suspension at 2 x 10^5 cells per ml were seeded into each well of a 96- well plate and incubated at 37 °C in 5% CO_2 for 24 hours to attach. The test sample extracts diluted with MEM at a ratio of 1:99 to a starting concentration of 100 µg/ml and a volume of (10+990) 1000 µl were seeded in duplicate in columns in a 96 well plate.

The plates were then incubated for 48 hours at 37° C in a 5 % CO_2 incubator. 10 µl of Thiazolyl blue tetrazolium bromide (MTT) dye was added into each well and cells were incubated for another 4 hours, (with 0.8 mg/ml of MTT), dissolved in phosphate buffered saline (PBS) and observed for dye intake, then read on a scanning multiwall spectrophotometer (Mullikan Ex labs systems) at 562 nm and 620 nm as reference and analyzed.

3. Results

3.1 Disc diffusion

Kigelia africana and *Ekebergia capensis* extracts demonstrated antimicrobial activity withthe water extracts of *E. capensis* being the most active and those of *Fagaropsis angolensis* were the least against *S. aureus* with inhibition zone diameters of 14.7 mm and 6.0 mm respectively as shown in **Table 1**.

Table 1: Inhibition zones in millimeters of three selected medicinal plants against five selected bacterial test microorganisms. The values are an average of three replicates for each**.**

Plants	Extracts/Drug	Conc. (mg/ml)	Average inhibition Zone diameters (mm)				
			S. aureus	MR *S. aureus*	*P. aeruginosa*	*E. coli*	*S. dysentriae*
E. capensis	Acetone	100.0	12.3	11.0	9.3	7.0	6.0
	Water	100.0	14.7	11.6	10.3	7.0	6.0
+ Control	Gentamicin	*30.0	21.0	19.3	21.7	26.3	22.3
- Control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0
- Control	Water	100.0	6.0	6.0	6.0	6.0	6.0
K. africana	Acetone	100.0	11.3	10.0	7.3	7.0	6.0
	Water	100.0	7.7	6.0	6.0	6.0	6.0
+ Control	Gentamicin	*30.0	19.0	9.0	21.0	19.0	17.0
F. angolensis	Acetone	100.0	6.0	6.0	6.0	6.0	6.0
	Water	100.0	6.0	6.0	6.0	6.0	6.0
+ Control	Gentamicin	*30.0	21.0	19.3	21.7	26.3	22.3

**The concentration of the control standards drug Gentamycin in ug/ml.*

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Table 2: Minimum Inhibitory Concentration (MIC) in mg/ml, Minimum Bactericidal Concentration (MBC) in mg/ml and Cytotoxicity in µg/ml of active plant extracts.

Plants	**Plant part**	**Type of extract**	**CC_{50} µg/ml**	**Test organism**	**MIC&MBC (mg/ml)**
Kigelia africana	Stem bark	Acetone	≥100	*S. aureus*	6.25
Kigelia africana	Stem bark	Acetone	≥100	*MR S. aureus*	100.0
Ekebergia capensis	Stem bark	Acetone	12.5	*S. aureus*	3.13
Ekebergia capensis	Stem bark	Acetone	12.5	MR *S. aureus*	12.25
Ekebergia capensis	Stem bark	Water	≥100	*P. aeruginosa*	6.25
Standard (Gentamicin)		Water		All tested bacteria	0.5

Key: MR. - Methicillin resistant

3.2 Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Cytotoxicity.

The active plant extracts that had a zone of inhibition of 10.0 mm and above in the disc diffusion assay were considered active and MIC and Brand Cytotoxicity were carried out. The MIC values of acetone extracts of *E. capensis* and *K. africana* was 3.125 mg/ml and 6.25 mg/ml against MRS. *aureus* respectively and were bactericidal at different concentrations against different strains of bacteria. The extracts of *K. Africana* (Acetone) and water extracts of *E. capensis* were considered to be safe because it recorded a CC_{50} which was greater than 90 µg/ml while the acetone extracts of *E. capensis* was moderately cytotoxic with a CC_{50} of 12.5 µg/ml (**Table 2**).

4. Discussion

The extracts from *E. capensis* and *K. africana* were active against *Staphylococcus aureus* which is a common opportunistic pathogen that causes skin infections. This agrees with the ethnobotanical uses of the plants for the treatment of skin diseases among the Nandi community of Nandi County. Plant extracts tested were active against gram positive bacteria than gram negative. This is because the outer membrane of gram negative bacteria is rich in a molecule called lipopolysaccharide which exclude certain drugs and antibiotics from penetrating the cell, partially accounting for why gram-negative bacteria are generally more resistant to antibiotics than are gram positive bacteria (Kaplan, 2012).

The high MIC values ranging from 3.125 mg/ml as the most potent to 100 mg/ml as the least potent (Table 2)could be due to high resistance rates of the test isolates. The most potent plant extracts with MIC 3.125 against *S. aureus* proved to be *E. capensis* and 6.25 mg/ml for *K. africana* against the same microorganism, compared with the drug of choice Gentamycin (0.5µg/ml) the antibacterial potency of the plants extracts were encouraging since they were crude extracts. The concentrations and proportions of the active compounds in plant extracts components depend on the plant variety, origin, time of harvest, solvent used, conditions of processing and storage (Deans and Ritchie, 1987).

The MBC showed that *E. capensis* water and acetone extract were bactericidal against *S. aureus* and *P. aeruginosa* while *Kigelia africana* was bactericidal against *S. aureus*. The antibacterial compounds may target bacterial cell wall, cell membrane and bacterial enzymes this was shown by the extracts of *E. capensis*and *Kigelia africana* while those which target protein synthesis such as the aminoglycosides, macrolides and tetracyclines are usually bacteriostatic (Finberg et al, 2004).

The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would previously be lethal (Cowen, 2008). Some bacteria may develop their own defense against the drugs by producing enzymes that can destroy a drug hence becoming resistant to the antibiotic. The cell wall may also become resistant to being broken by the action of the antibiotic. This usually happens when an antibiotic is used most incorrectly during the treatment eventually making the treatment more inefficient. For example, some strains of *Staphylococcus aureus* that cause pneumonia may become resistant to all antibiotics (Mark et al, 2003).This could be the reason why MRS. *aureus* and some Gram- negative bacteria; for instance *E. coli* and *S. dysentria* observed in this study. *E. capensis* and *Kigelia africana* showed significant activity against MRS. *aureus* with a Zone of inhibition of 11.6 and 10.0mm respectively this is in conformity with the studies done by (Lall and Meyer,1999) in south Africa who found *E. capensis* bark extracts being active against resistant strain of *M. tuberculosis* at 0.1mg/ml. This therefore, shows that the two plants can be exploited for the treatment of infections associated with resistant pathogens. However, the extracts from *Fagaropsis angolensis* did not show any activity against bacteria and fungi tested at 100 mg/ml. This does not mean that the plant extracts are not medicinal because they phytochemicals present may be active in combination with other plant extracts due to synergistic effect of several compounds that are active singly (Gessler et al, 1995).This is the reason why herbalist mix several plants when preparing concoctions. It is also possible that some of the compounds found in *Fagaropsis angolensis* could exhibit activity *in vivo* due to enzyme catalyzed transformation into potent derivatives and

therefore are playing the role of prodrugs, (Omulokoli and Chhabra, 1997).

Moreover, the crude extracts were not active against the fungal pathogens selected with an inhibition zone of 6.0 mms at 100 mg/ml. Fungi are eukaryotes thus the cell is difficult to be penetrated by the plant extracts due to the presence of cell wall made of cellulose. Bacterial infections are prevalent due to various factors such as the HIV/AIDS pandemic, poor hygiene, overcrowding and resistance to conventional antimicrobials but natural products obtained from higher plants may provide a new source of antimicrobial agents with possibly novel mechanisms of action, (Adenisa et al, 2000). Infections associated with bacterial pathogens are among some of the indications treated using traditional remedies in Kenya (Njoroge and Bussman, 2007). This is the reason why the medicinal plants under investigation found effective on bacteria than the fungi.

Toxicity studies are very important during the screening of medicinal plants in order to determine their safety. Two plant extracts that had a zone inhibition of 10.0 mm and above in disk diffusion were considered potent and their cell toxicity were determined which were plant extracts were categorized into three: cytotoxic at CC_{50}<2 µg/ml, moderately toxic at CC_{50} between 2-89 µg/ml and not toxic at CC_{50}>90 µg/ml (Rukunga and Simons, 2006). The extracts of *K. Africana* (Acetone) and water extracts of *E. capensis* were considered to be safe because it recorded a CC_{50} which was greater than 90 µg/ml while the acetone extracts of *E. capensis* was moderately cytotoxic with a CC_{50} of 12.5 µg/ml. In vitro cytotoxicity does not mean that an extract cannot be used in humans (Kokwaro, 2003) as there is potential for isolation of save non-toxic compounds. For instance, *Galega officinalis* is a plant that has proved too toxic for widespread agricultural use, with the potential to induce tracheal frothing, hypertension, paralysis and even death and yet Metroformin the current gold standard for management of Type II diabetes was isolated from it. (Bailey et al, 2007). Water extracts of *E. capensis* were moderately toxic which are in agreement with other work done by (Muthaura et al, 2007) on antimalarial properties of *Boscia angustifolia* water extracts, which he found to have no cytotoxicity. These also seem to confirm the validity of their traditional uses, since traditionally herbs are boiled in water (Gessler et al, 1995), suggesting that they may be safe as antimicrobials.

Conflict of Interest declaration

The authors declare no conflict of interest.

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