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Research Article

Chemotherapeutic Activities of Selected Medicinal Plant Extracts Used in Management of AIDS-Related Opportunistic Infections in The Lake Victoria Basin of Kenya

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Abstract

Background: The current management of HIV is highly active antiretroviral therapy (HAART), which has challenges such as toxicity, emergence of drug resistance, and mutations. This has led to quest for alternative medicine from plants which have been explored for their antimicrobial activities, cytotoxicity, and anti-proliferative properties. The chemotherapeutic components of plant preparations in Lake Victoria Basin (LVB) of Kenya has not been fully determined. This the study determined the potential antimicrobial properties, cytotoxicity and anti-proliferative effects of *Piptadeniastrum africanum* (bark), *Chaemacrista nigricans* (leaves), *Kigelia africana* (fruit), and *Centella asiatica* (leaves) extracts used in the management of HIV/AIDS opportunistic infections in LVB.

Methods: Twenty-four hours old cultures of S. aureus, E. coli, and C. albicans, were used to investigate the antimicrobial properties of the plant extracts. Morphological changes in CCRF-CEM cell lines and use of MTT assay were used to investigate the cytotoxicity and antiproliferative level of plant extracts.

Results: The plant concentrations ≥ 500µg/mL had antibacterial effect on *S. aureus* (df = 3, P = 0.019), *E. coli* (df = 3, P = 0.017) and *C. albicans* (df = 3, P = 0.017). The plant extracts showed antiproliferative effect on CCRF-CEM cells within 72 hours treatment period (*P. africanum* R^2=0.9962, P=0.0019, *C. nigricans* R^2=0.9935, P = 0.0032, *K. africana* R^2 = 0.9706, P = 0.0148, *C. asiatica* R^2 = 0.9954, P = 0.0023).

Conclusion: These findings demonstrate that the plant extracts are effective in management of HIV/AIDs-related secondary infections caused by bacteria and fungi, even at high concentrations without cytotoxicity. Therefore, this study provides evidence-based information on the chemotherapeutic value of traditional plants in the management of HIV/AIDS associated infections.

Keywords: HIV; HAART; AIDS

Abbreviations

AIDS: Acquired Immuno-Deficiency Syndrome; CLSI: Clinical and Laboratory Standards Institute; IC50: Half Maximal Inhibitory Concentration; LD50: Lethal Dose; CCFR-CEM: Human CD4+ T-cell line; HAART: Highly Active Antiretroviral Therapy; HIV: Human Immunodeficiency Virus; HCMV: Human Cytomegalovrius; MBC: Minimum Bactericidal Concentration; MIC: Minimum Inhibitory Concentration; THP: Traditional Health Practitioner; MTT: [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide].

Introduction

Human Immuno-deficiency Virus (HIV) is associated with depletion of primary immune cells that include $CD4^+$-T cells, macrophages and Natural Killer (NK) cells, which are critical to the

normal functioning of the human immune system that defends the body against all types of illnesses [1]. Many approaches are sought by patients to control symptoms of HIV and Acquired Immune Deficiency Syndrome (AIDS). Highly Active Anti-Retroviral Therapy (HAART) is the current widely used remedy and has dramatically improved the survival and quality of life among the people living with HIV and AIDS [2]. Nonetheless, the HAART treatment faces some challenges to successful clinical outcomes such as lack of adherence to dosage compliance [3,4], which result in development of resistance [5,6]. Other challenges include high cost of HAART in the absence of global support from developed country partners, drug toxicity, mutagenicity, carcinogenicity, low nutrients in the diet, lack of early diagnosis of secondary infections that suppress immune and development of resistance to $CD4^+$-T cells due to over-expression of P-glycoprotein, hence the need for alternative medicine. P-glycoprotein is a pump mechanism found on $CD4^+$-T cells that pumps out foreign bodies from the cell; HAART is not exempted [7]. It has been noted that about 80% of the world use natural products or their extracts as sources of drugs [8]. Many traditional healers (TH) residents of LVB are concerned with identification of medicinal plants used in management of gonorrhea, coughing, syphilis, diarrhea, headache, eczema, and wounds; which are HIV and AIDS-related illness. It is a major concern that the bacteria have the genetic ability to transmit and acquire resistance to therapeutic drugs, which has led to multi-resistance [9]. Studies have shown that the problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the management of HIV and AIDS-related illnesses is still uncertain [10,11]. Plant extracts of *P. africanum, C. nigricans, K. africana* and *C. asiatica* have been investigated in Europe and Asia, where they have been shown to have antimicrobial properties that can be of great significance in therapeutic treatments [12,13].

In-vitro studies using continuous cell lines have documented that *P. africanum, C. nigricans, K. africana,* and *C. asiatica* plants contain pomolic acid, futokadsurin, acridone alkaloid, and flavonoid glycoside that induce cytotoxicity in cells [14-16]. AIDS patients in LVB are using concoctions of crude plant extracts of *P. africanum, C. nigricans, K. africana*, and *C. asiatica* in the management of HIV and AIDS-related opportunistic infections [17,18]. However, cytotoxicity of *P. africanum, C. nigricans, K. africana*, and *C. asiatica* plant extracts found in LVB in lymphoid cell lines has not been determined. Anti-proliferative screening provides important exploratory data in selection of plant extracts with minimal anti-proliferative effect *in vitro* and *in vivo* [19]. Cells exposed to extreme physiochemical or mechanical stimuli die in an uncontrollable manner, as a result of their immediate structural breakdown [20]. Previous in vitro studies have reported superior antiproliferative potential on fractionated extracts of *Mesua ferra, Centella erecta, P. africanum* and *Solumum torvum* which are closely related species to the plants in this study [21,22]. Traditional healers in LVB are managing HIV and AIDS-related infections using *P. africanum, C. nigricans, K. africana*, and *C. asiatica* plant extracts [17], yet these plant extracts inhibits human cell proliferation [23]. However, antiproliferative properties of *P. africanum, C. nigricans, K. africana,* and *C. asiatica* found in LVB on lymphoid cell lines have not been determined. Therefore, this study investigated the antimicrobial, cytotoxicity, and anti-proliferative properties of *P. africanum, C. nigricans, K. africana*, and *C. asiatica* plant extracts used in the management of HIV and AIDS related opportunistic infections along the LVB of Kenya.

Materials and Methods

Collection of plant materials

The plants used in the study were obtained from Suba-Luo region of Homa Bay, Siaya, Kisumu and Migori counties in the LVB of Kenya and analyzed in Prof. P. Aduma's Laboratory at Maseno University. The study used *P. africanum* (bark), *C. nigricans* (leaves), *K. africana* (fruit), and *C. asiatica* (leaves). The plant materials were authenticated by herbarium staff in the department of botany at Maseno University and air dried away from direct sunlight.

Extraction and purification of plant extracts

The dried plant samples were ground using laboratory mills. The ground plant samples were weighed and the amount recorded [8]. The plants underwent total extraction using ethanol in a warring blender for 2-5 minutes. Blending involved mixing the crushed plant sample (≈1Kg) with absolute ethanol for 5 minutes. The amount of absolute ethanol added (≈2L) covered completely the plant sample. The extract was collected through filtration with an aid of Whatman filter paper (Whatman Inc, Piscataway, NJ) and bruchner funnel [9]. The blending process was repeated until the extract became colorless. The extracted plants were subjected to Rotary evaporator to remove the solvent used during extraction process, hence concentrating the extracts [35]. The concentrated crude extracts collected were stored in bijou bottles to be used for antimicrobial susceptibility assay, cytotoxicity, and antiproliferative assay.

Antimicrobial assay

Antimicrobial susceptibility test assays involved culturing of bacteria and fungi that have high pathogenicity in the HIV infected individuals. The microorganisms used in microbiology assay were Gram positive bacteria - *Staphylococcus aureus*, Gram negative bacteria - *Escherichia col*i, and *Candida albicans* – fungi. Gram staining technique was used to identify pure colonies of Gram positive and Gram negative bacteria. Culturing of *Candida albicans* was done on Potato Dextrose Agar (PDA) for colony isolation. Sensitivity testing of the microbes was conducted on Mueller-Hinton agar by the Kirby-Bauer disk diffusion technique in accordance to [24]. Varying concentrations (500mg/mL, 250mg/mL, 125mg/mL and 62.5mg/mL) of the selected medicinal plant extracts (*P. africanum, C. nigricans, K. africana* and *C. asiatica*) were impregnated on the 6mm diameter filter paper disks and gently placed on the Muller Hinton agar plate that was swabbed with the pathogenic organism. The test was conducted in triplicate and a negative control was included, which was a disk impregnated with dimethyl sulfoxide (DMSO). The plates were incubated at 37^0C for 24 hours then observed for the presence of zone of inhibition. The diameter of the zone of inhibition was measured in millimeters using a ruler. In addition, the test organisms were subjected to disks impregnated with the standard antibiotics (positive control) and incubated for 24 hours at 37^0C then observed for the presence of zone of inhibition. The diameter of the zone of inhibition was measured in millimeters using a ruler. The size of the inhibition of the plant extracts was compared with the standard antibiotics zone of inhibition to determine the sensitivity of the microorganism to the medicinal plant extracts.

Cell Culture Assays

A vial of CCRF-CEM lymphoid cells was imported from the American Type Culture Collection (ATCC, Manassas, VA). Vial was frozen upon receipt in liquid nitrogen vapor and resuscitated afterwards from the original frozen stock. Cells were maintained in a total volume of 20mL RPMI 1640 medium (ATCC 30-2001) supplemented with 10% FBS (ATCC 30-2020), 100µg/mL gentamicin, and 2.5µg/mL fungizone at 37^0C and 5% CO2 in 75cm3 cell culture flasks. The CCRF-CEM cells were observed every 2-3 days to maintain the density of 1.0 x 10^6 viable cells/mL. Daily monitoring was done for growth, contamination, or death. The cells were split at a ratio of 1:3 every 2-3 days. A third of the split cells was re-seeded in the flask to maintain the minimum density. Freshly prepared supplemented RPMI 1640 media was added to the growing cells every 2 to 3 days and the volume maintained to 20% through replacement of medium. Two-thirds of the split cells were frozen in freezing media that contains 5% DMSO and 95% FBS; then dispensed in 1.5mL cryovials. The cryovials were placed in dry ice and transferred to -196^0C liquid nitrogen tank. Morphological features of the cells were examined daily using the inverted light microscope (LB-341, Los Angeles, CA) and photomicrographs recorded. Cell density and viability of cultured cells was calculated at every sub-culturing procedure using Hemocytometer and Trypan blue exclusion dye.

Cytotoxicity and anti-proliferative measurements

The cytotoxicity effects of CCRF-CEM at concentrations of 125µg/mL to 1g/mL of the four plant extracts were quantified using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay reagents (ATCC® 3010K) according to the instructions of the manufacturing company. The assay was designed to test a total of forty eight assays of four plant extract concentrations. About 100µL of the plant extract of a defined concentration and CCRF-CEM cells were added into the wells of a micro-titer plate. The control wells in the assay contained RPMI 1640 media alone to provide the blanks for absorbance readings. The cells were incubated overnight at 37^0C in a humidified atmosphere containing 5% CO_2. 10µL of 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) reagent was then added to each well including control and the plate returned to cell culture incubator for 2 to 4 hours. Periodically, the cells were viewed under inverted light microscope (LB-341, Los Angeles, CA) for the presence of intracellular punctate purple precipitate. When the purple precipitate was clearly visible under the microscope, 100µL of detergent reagent was added to all wells including controls. The plate was swirled gently without shaking. The plate was covered and left in the dark for 2 hours at room temperature. The plate cover was removed and absorbance measured in each well including the blanks at 570nm using an ELISA plate reader (Labomed Inc® EMR-500 ELISA microplate reader). The average values was determined from the readings and subtracted from the average values for the blank. The IC50 value (concentration at which 50% of the cells are killed) was determined from the dose-response curve. The assay was performed in triplicate. Antiproliferative measurement was also conducted using trypan blue exclusion method. CCRF-CEM cells were seeded into 100mm x 15mm petri-dishes at a density of 5.0 x 10^5 per mL and treated with 500mg/mL and 1g/mL of the plant extracts

(*P. africanum, C. nigricans, K. africana* and *C. asiatica*). Controls were also included which contained CCRF-CEM cells at a density of 5.0 X 10^5 without treatment. The cells were incubated at 37^0C and 5% CO_2. Cell growth was monitored, counted and photomicrographs were recorded after 24 hours, 48 hours and 72 hours. Cell viability was determined by Trypan blue exclusion method, whereby, counting of viable cells for each concentration was done using a Hemacytometer. Each petri-dish had three repeats of counting and the average concentration was recorded.

Data analysis

Pearson correlation and chi-square (χ^2) test were used to determine the relationship between the plant extract concentrations and the resistance level expressed by the test microorganisms. Nonlinear regression analysis was used to compare the cytotoxicity level of the plant extracts through determination of IC_{50} values. Data are presents as means and standard error of the mean (SEM). Probability value was considered statistically significant at $P \leq 0.05$.

Results

Antimicrobial potential of plant extracts

Antimicrobial potential of the plant extracts was determined through testing the sensitivity or resistance of the *S. aureus, E. coli* and *C. albicans* pathogenic microorganism through measurement of the zones of inhibition. Zones of inhibition varied among the plant species and concentrations used in the study. The plant concentrations range was 65.5 mg/mL - 500 mg/mL, which gave a mean zone of growth of inhibition of 7mm – 19 mm (Table 1). The antibacterial and anticandidal activities of the plant extracts increased linearly with increase in concentration of extracts in mg/mL. In comparison with the reference drugs, *P. africanum, C. nigricans*, and *C. asiatica* were active against *S. aureus*, while *K. africana* was active against *E. coli*. High concentrations of *C. asiatica* (500mg/mL) was active against *S. aureus* and *C. albicans*. The plant extracts induced important inhibitory activities on tested Gram positive, Gram negative and fungi microorganisms with inhibition zone diameters varying from 7 to 19mm. The highest inhibition zone diameter (19mm) was obtained with *S. aureus*, which is a Gram positive strain. Moreover, *S. aureus* was the most sensitive microorganism (MIC 125 mg/mL) against *P. africanum. C. nigricans* and *C. asiatica* were less active when compared to the *P. africanum* (with inhibition zone diameters = 10mm and 7mm, MIC = 500mg/mL respectively) on *S. aureus*. In general, *P. africanum* and *C. nigricans* extracts were found to be active against gram positive bacteria, *K. africana* extract was effective against gram negative bacteria and *C. asiatica* was effective against gram positive and fungi microbes tested Table 1.

Antibacterial activity zone of inhibition (mm)				
Plant extract	**Plant concentration (mg/mL)**	**S. aureus ATCC 25923**	**E. coli EPEC-STG**	**C. albicans ATCC 10231**
P. africanum				
	65.5	0.0	0.0	0.0
	125	7.0	0.0	0.0
	250	10.0	0.0	0.0
	500	19.0	0.0	0.0
C. nigricans				
	65.5	0.0	0.0	0.0
	125	0.0	0.0	0.0
	250	0.0	0.0	0.0
	500	10	0.0	0.0
Kigelia africana				
	65.5	0.0	0.0	0.0
	125	0.0	0.0	0.0
	250	0.0	7.0	0.0
	500	0.0	8.0	0.0
C. asiatica				
	65.5	0.0	0.0	0.0
	125	0.0	0.0	0.0
	250	0.0	0.0	7.0
	500	7.0	0.0	10.0

Table 1: Antimicrobial activity of plant extracts of *P. africanum*, *C. nigricans*, *K. africana*, and *C. asiatica*

Antibacterial activity was determined by disk diffusion method. Mean zones of growth inhibition (plus diameter of well) are mean (mm) of 3 independent experiments, diameter of well = 6mm.

In vitro cytotoxicity assessment in CCRF-CEM leukemia cell lines

The in vitro cytotoxicity of *P. africanum, C. nigricans, K. africana*, and *C. asciatica* plant extracts is presented in Figure 1. The non-linear regression analysis shows that the number of live CCRF-CEM

cells decreases with increase in the plant concentration. The variations in the absorbance gave the likelihood of determining the concentration that would cause *in vitro* cytotoxicity after calculating their IC_{50} values as shown in Table 2. According to MTT assay results (Table 2), it is evident that *K. africana* has a likelihood of causing toxicity to the cells since its non-linear regression analysis deduced its IC_{50} value to be 1096µg/mL. On the other hand, the other three plant extracts (*C. nigricans. P. africanum*, and *C. asiatica*)

	P. africanum	***C. nigricans***	***K. africana***	***C. asiatica***
Log (inhibitor) vs. response (three parameters)				
Best-fit values				
Bottom	-1.45	0.69	1.07	-1.65
Top	3.74	3.81	2.59	2.73
$Log_{10}IC_{50}$	3.52	3.45	3.04	3.47
Span	5.19	3.12	1.52	4.38
95% Confidence Intervals				
Bottom	-6.57 to 3.67	-16.38 to 17.77	-7.98 to 10.12	-3.15 to -0.16
Top	3.64 to 3.84	3.35 to 4.27	1.23 to 3.95	2.69 to 2.76
$Log_{10}IC_{50}$	2.93 to 4.09	0.09 to 6.81	-2.28 to 8.36	3.26 to 3.67
Span	0.16 to 10.22	-13.57 to 19.80	-6.44 to 9.48	2.92 to 5.83
Number of points analyzed	4	4	4	4

Table 2: Summaries of IC_{50} values of the plant extracts.
Cytotoxicity of the plant extracts against the CCRF-CEM were determined using the IC_{50} values under the Best-Fit value category.

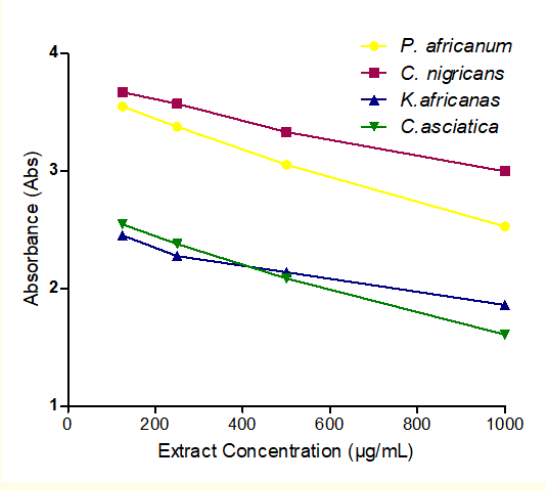


Figure 1: *In vitro* cytotoxicity assessment in CCRF-CEM leukemia cell lines: The *in vitro* cytotoxicity of *P. africanum, C. nigricans, K. africana*, and *C. asciatica* plant extracts. The graph represents the non-linear regression of live CCRF-CEM cells decreases with increase in the plant concentration.

would not cause toxicity to the CCRF-CEM cells considering their IC_{50} best fit values being 2828µg/mL, 3281µg/mL, and 2920µg/mL respectively. The results gave very high IC_{50} values of these plant extracts compared to the highest concentration used in the study, hence eliminating the likelihood of causing plant extracts causing cytotoxicity.

Cell proliferation and viability assessment

Observations under inverted microscope of the co-culture system showed that the CCRF-CEM cells maintained their morphology form from 24 hours to 72 hours. The cell morphology against each consecration is shown in the micrographs in Figures 2A-H. Cell viability at 24 hours, 48 hours and 78 hours, was determined for each treatment compared to the control (untreated cells). The plants extract however lowered the cell viability compared to the control (Figures 3 A-H). Notably, the number of CCRF-CEM cells at 72 hours was slightly higher as compared to the number of cells at 24 hours.

FIGURE 2A

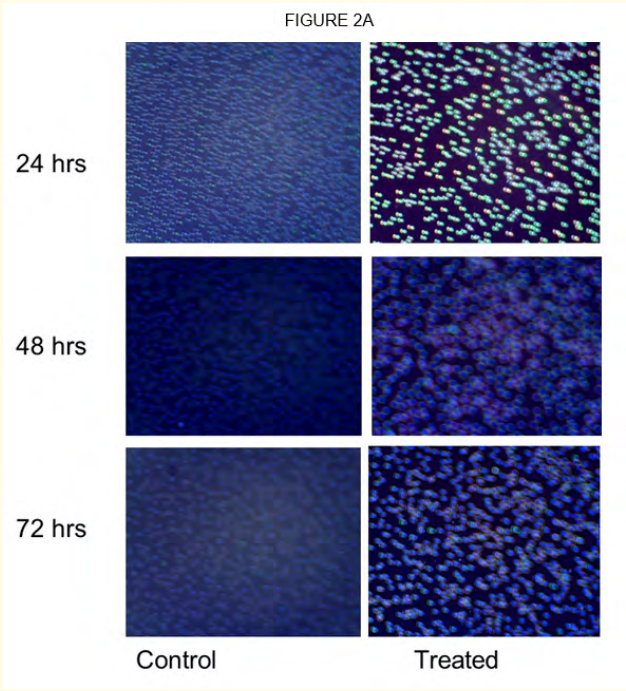


FIGURE 2B

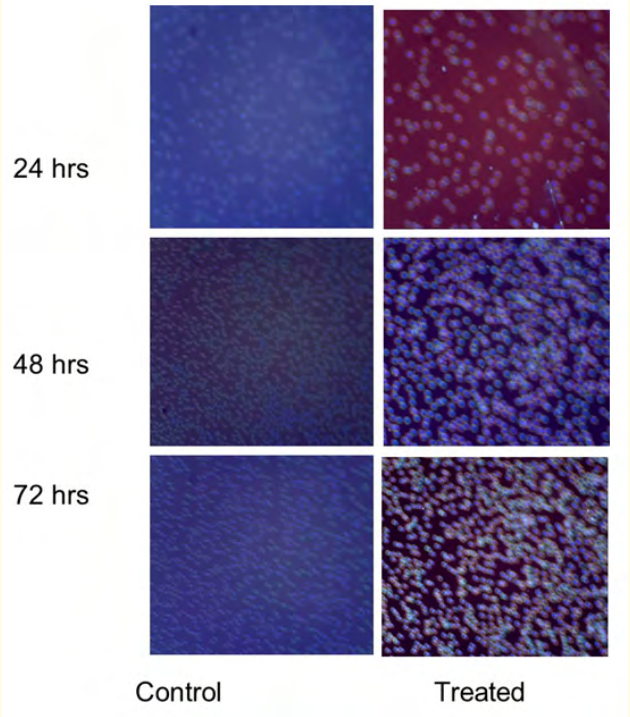


FIGURE 2C

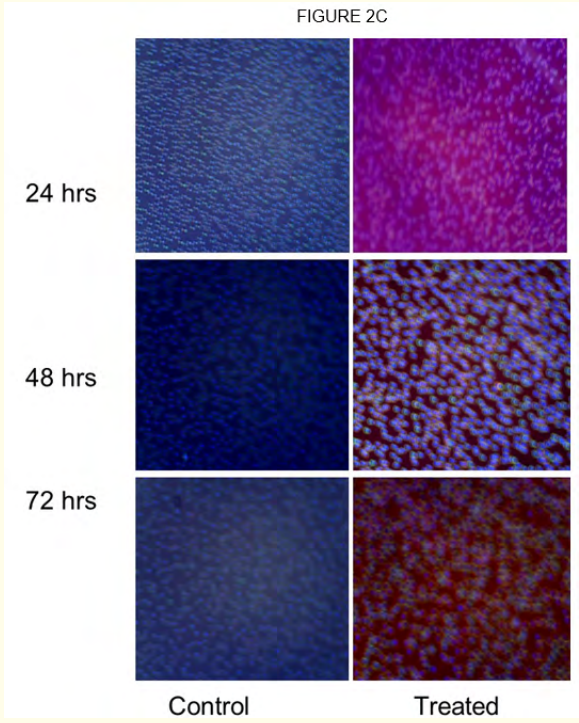
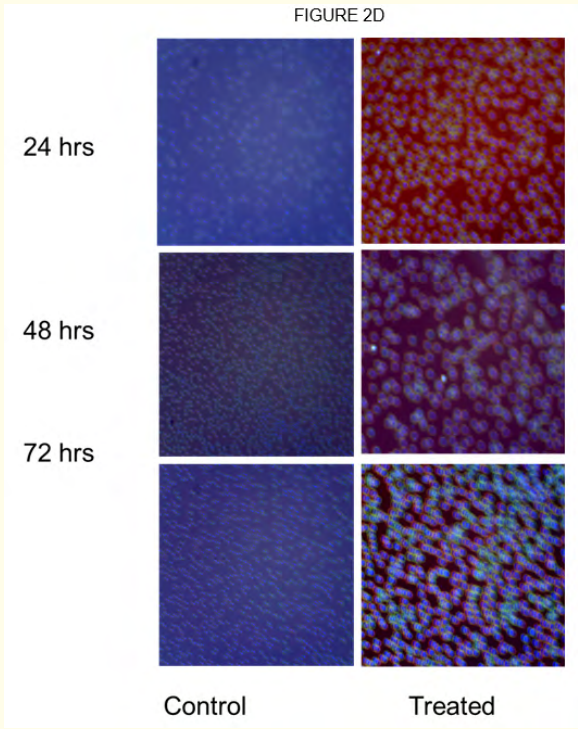
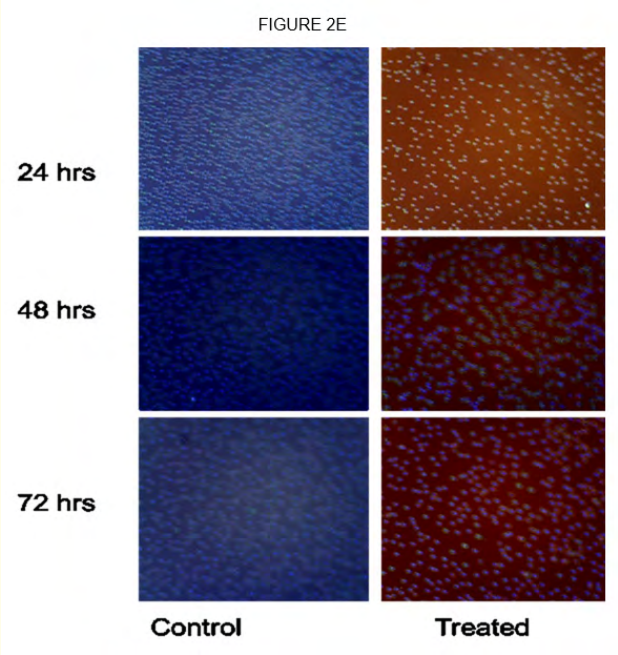
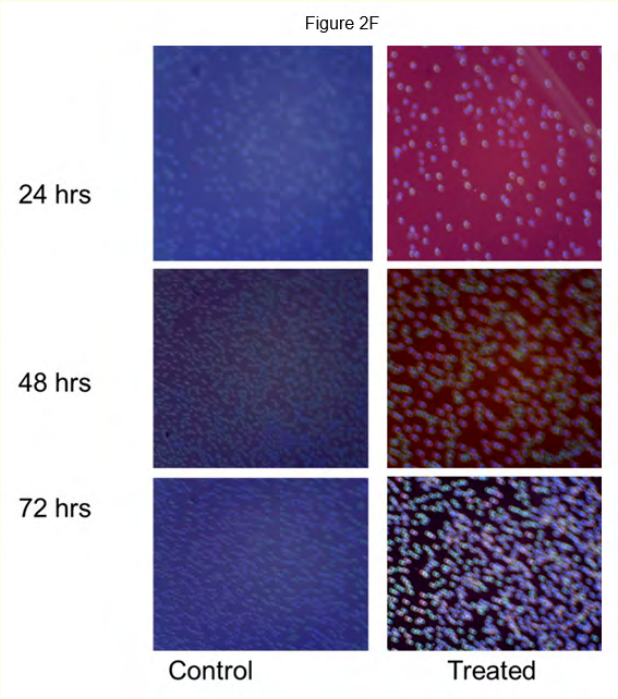
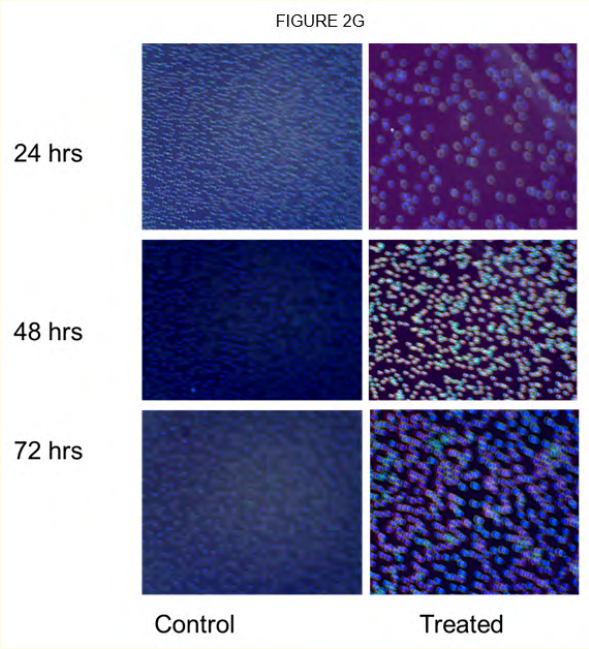


FIGURE 2D









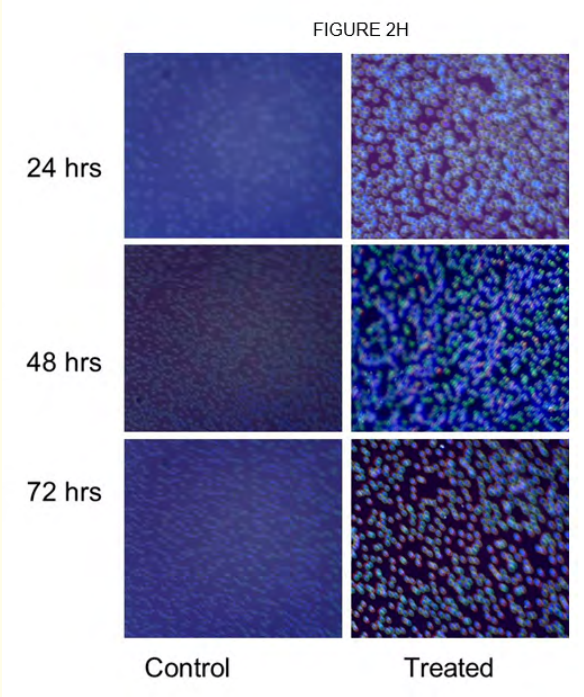
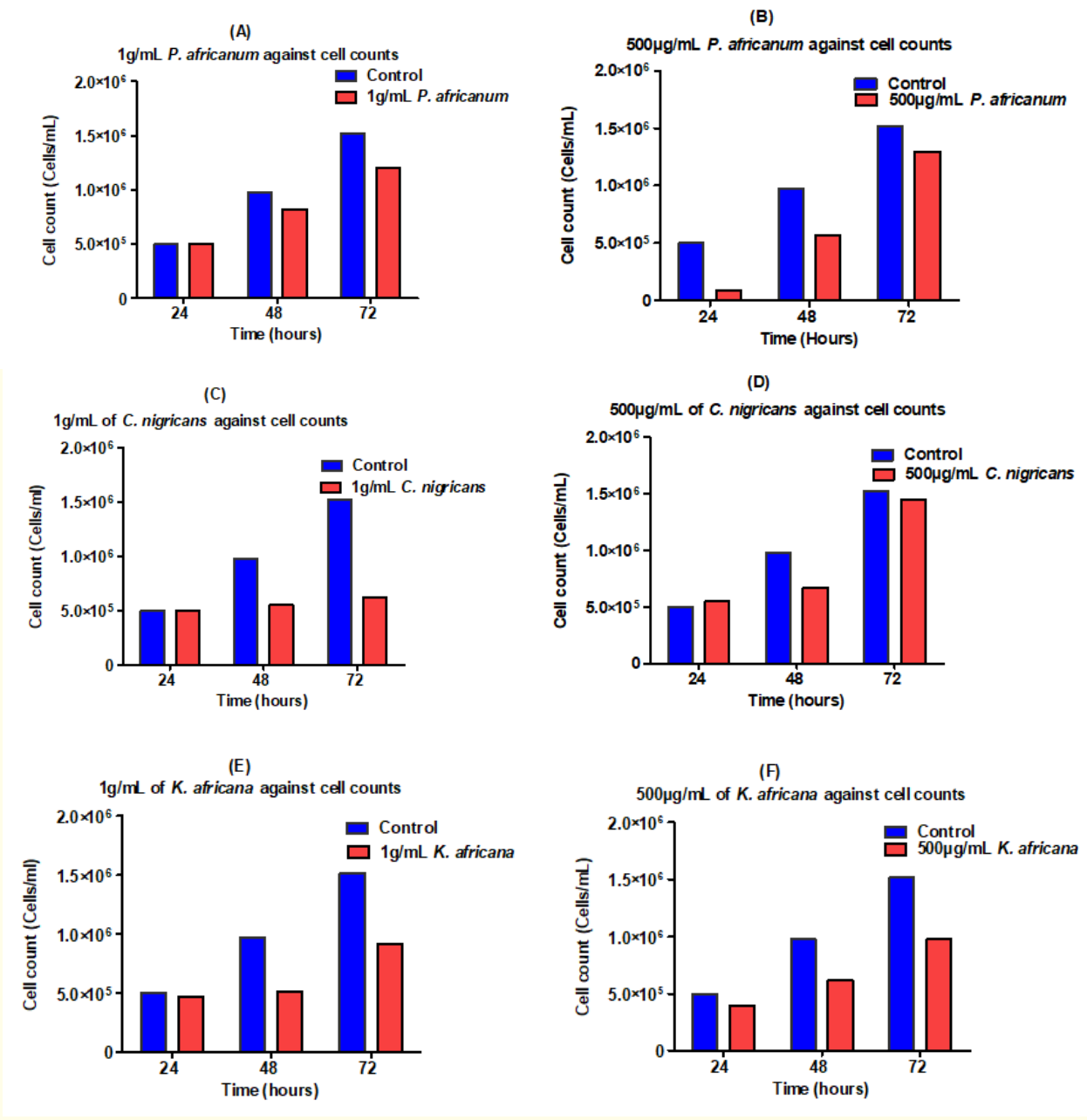
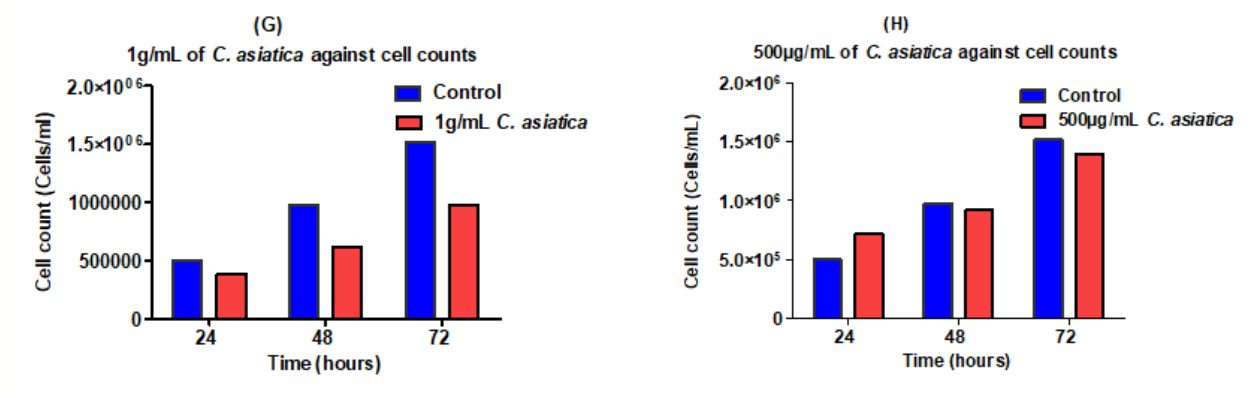


Figure 2: The cell morphology against each consecration is shown in the micrographs observations under inverted microscope of the co-culture system showed that the CCRF-CEM cells maintained their morphology form from 24 hours to 72 hours. .


(A)
1g/mL P. africanum against cell counts
Control
1g/mL P. africanum
Cell count (Cells/mL)
Time (hours)
(B)
500µg/mL P. africanum against cell counts
Control
500µg/mL P. africanum
Cell count (Cells/mL)
Time (Hours)
(C)
1g/mL of C. nigricans against cell counts
Control
1g/mL C. nigricans
Cell count (Cells/ml)
Time (hours)
(D)
500µg/mL of C. nigricans against cell counts
Control
500µg/mL C. nigricans
Cell count (Cells/mL)
Time (hours)
(E)
1g/mL of K. africana against cell counts
Control
1g/mL K. africana
Cell count (Cells/ml)
Time (hours)
(F)
500µg/mL of K. africana against cell counts
Control
500µg/mL K. africana
Cell count (Cells/mL)
Time (hours)



3A: Effect of 500µg/mL of *P. africanum* bark extract on the proliferation of CEM-SS cell line under inverted microscope. The cell viability on the treated plates on day 0 was 8.5 X 10^4, day 1 was 5.71 X10^5, and on day 2 was 1.3 X 10^6 cell per mL.

3B: Effect of 1g/mL of *P. africanum* bark extract on the proliferation of CEM-SS cell line under inverted microscope. The cell viability on the treated plates on day 0 was 5.0 X 10^5, day 1 was 8.2 X 10^5, and on day 2 was 1.2 X 10^6 cells/mL.

3C: Effect of 500µg/mL of *C. nigricans* leaves extract on the proliferation of CEM-SS cell lines under inverted microscope. The cell viability on the treated plates on day 0 was 5.5 X 10^5, day 1 was 6.7 X 10^5 and on day 2 was 1.45 X 10^6 cells per mL.

3D: Effect of 1g/mL of *C. nigricans* leaves extract on the proliferation of CEM-SS cell line under inverted microscope. The cell viability on the treated plates on day 0 was 5.0 X 10^5, day 1 was 5.5 X 10^5 and on day 2 was 9.2 X 10^5 cells per mL.

3E: Effect of 500µg/mL of *K. africana* fruit extract on the proliferation of CEM-SS cell lines under inverted microscope. The cell viability on the treated plates on day 0 was 3.9 X 10^5, day 1 was 6.2 X 10^5 and on day 2 was 9.75 X 105 cells per mL.

3F: Effect of 1g/mL of *K. africana* fruit extract on the proliferation of CEM-SS cell lines under inverted microscope. The cell viability on the treated plates on day 0 was 4.7 X 10^5, day 1 was 5.1 X 10^5 and on day 2 was 9.21 X 10^5 cells per mL.

3G: Effect of 500µg/mL of *C. asiatica* leaves extract on the proliferation of CEM-SS cell lines under inverted microscope. The cell viability on the treated plates on day 0 was 7.12 X 10^5, day 1 was 9.2 X 10^5 and on day 2 was 1.39 X 10^6 cells per mL.

3H: Effect of 1g/mL of *C. asiatica* leaves extract on the proliferation of CEM-SS cell lines under inverted microscope. The cell viability on the treated plates on day 0 was 5.0 X 10^5, day 1 was 8.4 X 10^5 and on day 2 was 1.1 X 10^6 cells per mL.

Discussion

In the present study, the selection of the microbial pathogens is based on the potential causes of the major opportunistic infections in HIV/AIDS patients and the safety issues toward cultivating the pathogens in a level 2 laboratory setting. The strains were selected on the basis of their virulence in causing secondary opportunistic infections in HIV/AIDS patients. All the plant extracts tested showed promising antibacterial and antifungal activity against the test strains. Moreover, the results of disks antibacterial and antifungal susceptibility testing showed that *S. aureus* and *E. coli* were highly susceptible to penicillin – a standard drug with average diameter zone of inhibition of 20mm and 17mm respectively, whereas, *C. albicans* was highly susceptible to fungizone – a standard drug with average diameter zone of inhibition of 15mm. The acceptable standard diameter zone of inhibition for sensitive organism for antibiotic and antifungal are >16mm and >14mm respectively [25]. However, *P. africanum* extract showed the greatest antimicrobial activity (inhibition zone 7mm, MIC 125 mg/ml, MBC 320mg/ml).

It is known that *P. africanum* is rich in saponin and tannins compounds, which have remarkable antimicrobial activity [12]. For the other plant extracts (*C. nigricans, K. africana*, and *C. asiatica*), the average inhibition zones observed against these pathogens ranged from 7mm to 10mm (Table 1). These values fall within the range of resistant and intermediate sensitive when compared with control antibiotic or antifungal [26]. Several studies have reported bioactivity of crude extracts of medicinal plants within such range of inhibition zones [27-29].

The relationship between zone of inhibition and MIC value may or may not be related, since the crude extracts have mixture of phytoconstitutents that may influence the diffusion power of the active constituents [30]. The use of plants to heal infectious diseases has been extensively applied by people considering the widespread resistance to conventional antibiotics. MIC values of *P. africanum* and *C. asiatica* were lower than the MBC values, suggesting that the plant extracts were bacteriostatic at lower concentration and bactericidal at higher concentration [31-33]. Data from the previous studies as well as this study reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated. The MBC and MIC results of the tested extracts reveal that the extracts inhibit bacteria growth without killing the bacteria. The possibility of the extracts being bacteriostatic instead of bactericidal would be that the majority of traditional preparations lack specific concentrations, this accounts for the use of large quantity of the extracts by TM for the treatment of their patients. Drugs used by TM are mostly prepared with water, the TM lack access to other more lipophilic solvents. It is possible that the TM do not extract all the active compounds that might be present in the plant. Dosage is important with regard to which solvent is being used. If water is used, the dosage would be higher, whereas the same dosage using a lipophilic solvent may be toxic. In this study, Ethanol was most efficient considering its safety toward conducting antimicrobial assays after extraction [26]. Determining the antibacterial and antifungal properties of medicinal plants is helpful to the rural communities and informal settlements [34]. This study offers scientific rationale for use of these four ethanol extracts of medicinal plants for treatment of HIV related opportunistic infections.

Concentration measurements have demonstrated the toxicity of different medicinal plants that are substitutes toward curing diseases like malaria, cancer, AIDS among others [16]. The potential of the plant extracts to be active against resistant pathogens minimizes the possible toxic effects as a result of high dosage of conventional drugs [35]. An assessment of the plants' cytotoxic potential is necessary to ensure antiproliferative property [36]. The recent study by Tiwary and colleagues, showed that organic extracts of the *p. africanum* and *K. africana* plant species had low cytotoxicity levels at a single dose of 250µg/ml against MCF7 and Vero cell lines [22]. Earlier studies had also showed that organic extracts of K. africana had low cytotoxicity levels with a potent cytotoxic activity of <50% at a single dose of 300µg/ml against cultured KB cells [22]. Teixeira and colleagues [37] have reported that infusions prepared from the medicinal plants *Solanum torvum, Oscbekia nepalensis, Kigelia africana, Selaginella monospora*, and *Mesua ferrea*, showed cytotoxic levels of < 50% at a single dose of 450 µg/mL and no activity for *Piptadeniastrum africanum, Centella erecta, Chamaecrista fasciculata* which are comparatively similar to this study. In this study, *P. africanum* (R^2=0.9962, P=0.0019), *C. nigricans* (R^2=0.9935, P=0.0032) and *C. asiatica* (R^2=0.9954, P=0.0023) had no significant observable cytotoxic effect against CCRF-CEM cells from the microscopic results and IC_{50} (µg/mL) values from MTT assay, although it is evident that, 1096µg/mL of K. africana (R^2=0.9706, P=0.0148) damages < 25% of the total CCRF-CEM cells used in the study. The morphological changes of CCRF-CEM cells as a result of cytotoxicity of K. africana was confirmed through microscopic observations. The CCRF-CEM cells indicated signs of cell apoptosis after administering 1g/mL of K. africana plant extract and incubation for 72 hours.

Cytotoxicity assays such as MTT measure DNA damage and apoptosis [38]. This study determined cell cytotoxicity through apoptosis measurement although the novel sign of toxicity was necrosis [21]. However, high concentration of *K. africana* resulted to slight morphological changes to CCRF-CEM, which is a novel sign of toxicity. The necrosis in this case is evidenced by cytoplasmic and plasma membrane swelling [39].

Cell growth and morphological alterations of CCRF-CEM cells with the tested plant extracts were compared with the control cells (untreated) for 72 hours, which maintained their original morphology form throughout the experiment. After recovery of the cell growth, it was observed that the antiproliferative effect was dose dependent. The variations in the absorbance between the plant extracts was likely due to the presence of active molecules and compounds that caused varying interactions with the cultured cells.

The increase of the tested compound had slight increase on anti-proliferative effect. All the plant extracts did not absolutely alter the morphology of the CCRF-CEM cells, but slightly decreased cell growth and proliferation. The results are in tandem with previous studies [22,37,40], whereby after the cell growth recovered from 24 hours treatment, the MCF-7, and HCT-116 cells had no observable morphological changes [40]. These findings confirmed that the tested plant extracts were not capable of causing cytotoxicity in CCRF-CEM cells [22].

Assessment of the tested plant extracts for their IC50 (dose that inhibits cell growth by 50%) values was at the concentration range of 500µg/mL and 1000 µg/mL. The selection of the concentration range depended on the microbial concentration that would inhibit growth of microbial pathogens [41]. The MTT assay applied the principle of formazan crystal formation through dehydrogenesae mitochondria activities in living cells. The rate of formation of MTT crystal formazan was directly proportional to the number of living cells. According to [17] *C. asiatica* and *K. africana* aqueous extracts suppressed the proliferation of keritinocytes and human dermal fibroblasts cell lines. The ethanolic extracts of the roots of *C. nigricans* had the lowest antiproliferative activities, which have similar results to this study [17,36]. The plant extracts did not alter the actively proliferating CCRF-CEM cells. There is a likelihood that the presence of secondary metabolites such as saponins and polyphenols of the plant extracts used in this study increased the metabolic activity of the CCRF-CEM cells [22,42]. This is evident since the cells did not decrease their proliferation rate even after subjecting the highest concentration of the extract used in this study. It is also evident that the plant extracts did not release toxic molecules that may result to diminishing activity of the CCRF-CEM cells [39].

The study results indicated that *K. africana* need be used with caution in treatment of secondary opportunistic infections that occur as a result of contracting HIV/AIDS. The other plants used in the study (*C. nigricans. K. africana*, and *C. asiatica*) showed no signs of toxicity at the tested levels of concentrations [43]. According to the results of the study, the IC_{50} values are higher than the concentrations used in the study. The results indicate that the plant extracts did not cause cell death (not toxic) even at high concentrations such as 1g/mL, although there was decrease in cell multiplication with increase in concentration. It is evident that the tested plant extracts have a high efficacy and have less noxious side effects, in dealing with the disease [38, 43].

The *in vitro* assay is an indicator of mammalian cell survival and growth [38]. Cell viability assay was conducted through counting the remaining viable cells after every 24 hours up to 78 hours and viability graphs obtained. It is evident that the treated cells continue to proliferate likewise to the control (untreated) cells, although the plant extracts lowered the rate of cell proliferation. Inclusion of control wells in the MTT viability assays was essential to compare possibilities of cell impairment, membrane integrity and changes in cell morphology which elicit strong likelihood of cell growth inhibition [38,39]. According to this study there were no signs of cell growth inhibition during the 72 hours of incubation. Basically, the assessment of cytotoxicity and proliferation in study was accomplished through MTT assay while the cell viability and survival capacity of the CCRF-CEM cells was accomplished through cell assays that involved cell counting. This suggest that the tested plant extracts had the sheer potency of treating HIV/AIDS opportunistic infections and were relatively nontoxic and safe for traditional or complementary medicine.

Conclusion

The four plant extracts (*P. africanum, C. nigricans, K. africana*, and *C. asiatica*) can be important sources of antibacterial and antifungal properties, which are essential in management of HIV and AIDS related opportunistic infections. The compounds extracted from the *P. africanum*, and *C. nigricans* have high affinity against gram positive bacteria, compounds extracted from *K. africana* have high affinity against gram negative bacteria, and compounds extracted from *C. asiatica* have high affinity against gram positive bacteria and Candida albicans. The effectiveness of the antimicrobial activity increases with increase in the plant extract concentration. The plant extracts are not toxic to the leukemia cell lines (CCRF-CEM) at concentration lesser than 1g/ml, hence making it safe for use as traditional medicine at low concentrated doses. The cytotoxicity effect vary with the species of the plant. *K. africana* has a high likelihood of causing toxicity to the leukemia cells at a concentration of 1g/mL, while the other plant extracts (*P. africanum, C. nigricans*, and *C. asiatica*) have a low likelihood of causing toxicity at the concentration of 1g/mL. Only *K. africana* that exerted a < 25% apoptosis on CCRF-CEM cells. More studies need to be conducted to isolate the active compounds using fractional distillation with antibacterial and antifungal activity in *P. africanum, C. nigricans, K. africana* and *C. asiatica*, which will be helpful in lowering the required dose concentration and quantities from the crude extracts.

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Authors' contributions

SNK, EOM, BG, SOW, and PRA designed, carried out the study in the rural population. SNK and EOM performed the statistical analyses and participated in the drafting of the manuscript. All authors read and approved the final manuscript.

Competing Interests

All authors have declared no competing interests in the submitted work

Ethics

The study was approved by the Maseno University Ethics review Committee (MUREC).

Consent for Publication

Not applicable

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