Research article

# *In vitro* anti-cancer efficacy and phyto-chemical screening of solvent extracts of *Kigelia africana* (Lam.) Benth

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## ARTICLE INFO

## ABSTRACT

*Background and objectives: Kigelia africana* is a medicinal plant growing naturally in many parts of Africa. In Kenya, a water concoction of the plant is used to treat breast and prostate cancers. Laboratory data on its anti-cancer activity and active principles is limited, hence no scientific rationale for its medicinal use. This study reports on *in-vitro* toxic activities of dichloromethane and methanol extracts of the plant against human breast cancer cells and phytochemical screening of the two extracts.

*Methodology:* Plant extracts were obtained by sequential solvent extraction of dry plant material (stem bark) using analytical grade dichloromethane: methanol (1:1) and methanol (Sigma Aldrich). *In-vitro* anti-cancer activities of the extracts were determined using the suphorhodamine (SRB) assay against a human breast cancer cell line (HCC 1937). Preliminary Thin layer chromatography of plant extracts was done using POLYGRAM® SIL G/UV$_{254}$ plates (Merck) to establish presence of different classes of secondary metabolites.

*Results: In-vitro* cytotoxic activities of the two extracts were significantly different ($P = 0.05$). The methanol extract exhibited higher activity (IC$_{50}$ = 26.02 µg/ml) compared to that of dichloromethane: methanol (1:1) (IC$_{50}$ = 55.01 µg/ml). Phyto-chemical screening of the two extracts revealed the presence of terpenoids, phenols, steroids and flavonoids.

*Conclusion:* The high *in-vitro* anti-cancer activities of solvent extracts of *Kigelia africana* justify its use in traditional medicine to manage breast cancer. Phytochemical analysis of the extracts reveal similar profiles hence the differences in their anti-cancer activities can be attributed to quantitative variations of various classes of secondary metabolites.

## 1. Introduction

Cancer is a disease characterized by abnormal cell division and proliferation that result from disruption of molecular signals that control these processes [1]. In the year 2000, lung (12.3%), breast (10.4%) and colorectum (9.4%) were the most prevalent forms of cancer worldwide [2]. By the year 2015, cancer was the second leading cause of death globally resulting to 8.8 million deaths [3]. One in six deaths globally is caused by cancer with 70% prevalence in low and middle income countries [3]. Incidences in these countries account for more than half of all new cancer cases globally and will represent more than 80% of global cancer burden by 2030 [4,5].

80% of the human population depends on plants for their primary health care [6]. As many as 35000 plant species have been screened for anti-cancer activities previously, leading to the discovery of clinically important anti-cancer drugs such as Vincristine, Vinblastine, Taxol, Indicine–*N*-oxide, Etoposides and Camptothecin, with ability to inhibit growth of cancer cells by controlling apoptosis and autophagic pathways [7]. However, due to multidrug resistance and toxic effects of current chemotherapeutic drugs to other non-target tissues, development of new bioactive molecules with fewer side effects and greater efficacy is essential [8].

*Kigelia africana* is a medicinal plant characterized with pendulous racemes of colored flowers and a long stalked large gourd-like fruit [9]. Geographically, it is predominantly distributed in tropical regions and India [9]. The plant is used in many traditional medicine systems to control various diseases including cancer. In western Kenya, stem bark of *K. africana* is boiled and taken orally one glass (300 ml) twice a day for

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**Table 1.** Mean absorbance at 510nm of treated wells containing HCC1937 cells.

| Concentration µg/ml | 0.1 | 12.5 | 25 | Mean absorbance P value $< 0.0001$ | | | |
| | | | | 37.5 | 50 | 75 | 100 |
|---|---|---|---|---|---|---|---|
| Methanol | 0.198 | 0.192 | 0.183 | 0.135 | 0.12 | 0.116 | 0.069 |
| Dichloromethane: Methanol (1:1) | 0.281 | 0.238 | 0.231 | 0.225 | 0.207 | 0.164 | 0.078 |
| Control | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 |

three months to cure breast, lung and skin cancers [10]. In Malawi and Nigeria, boiling water extract of *K. africana* is used to treat stomach cancer and urinary tract infections [11,12]. In Namibia and Zambia, the stem bark and leaf decoctions of this plant are used in folk medicine as a remedy for HIV/AIDS and opportunistic diseases in HIV/AIDS [13]. In this study, we demonstrate *in-vitro* activities of dichloromethane and methanol extracts of *K. africana* against the breast cancer cell line (HCC-1937) and determine the chemical profiles of the two extracts.

## 2. Materials and methods

### 2.1. Plant collection

Stem bark of *Kigelia africana* was collected from Kakamega tropical rain forest, in Kakamega county (0.2919° N 34.8561° E), Kenya in January 2018. Plant identification and authentication was done by Simon Njiru, a botanist at Kenya Institute of Organic Farming and a sample specimen deposited at the University of Embu herbarium (voucher number UoE-KA-2018).

### 2.2. Plant preparation and extraction

The plant was cut into sizable pieces and air dried away from sun light until dry. The dry plant material was ground into powder using model 4-E grinding mill (Thomas Scientific). 500 g of dry ground plant material was soaked in 3.0 L of dichloromethane: methanol (1:1) (Sigma Aldrich) and extracted several times until complete extraction. The residue was then extracted with 100% methanol and the two extracts were filtered using Whatman's NO.1 filter paper and concentrated separately under vacuum using an mrc rotary evaporator (ROVA-2L).

### 2.3. Anti-cancer activity of plant extracts against HCC1937 cell line

Anti-cancer activities of the two plant extracts were determined using the suphorhodamine (SRB) assay as previously described [14]. The protocol relies on the stoichiometric binding of the SRB dye to proteins and the bound dye as measured by absorbance is extrapolated to depict cell proliferation [15]. HCC1937 cells from ATCC (CRL-2336) were seeded in triplicate in a 96 well plate at a cell density of $1 \times 10^4$ cells per ml in RPMI1640 medium supplemented with 10% (V/V) fetal bovine serum (FBS) and 2 mM glutamine and allowed to adhere overnight at 37 °C in 9% $CO_2$. Cells were treated with different concentrations of extracts (between 0.1 to 100 µg/ml) diluted in 1% (v/v) dimethyl sulfoxide (DMSO) and incubated for 72 h. Wells containing cells without extracts (DMSO only) were included as a negative control. After 72 h, the cells were fixed with 50% (W/V) trichloroacetic acid and incubated at 4 °C for one hour. The plates were washed 4 times by gentle immersion in water

and dried on paper towel at room temperature. The plates were dyed with 50 µl of 0.04% (W/V) of suphorhodamine (SRB) and incubated for an hour at room temperature followed by four washes with 1% (V/V) acetic acid and air dried. 50 µl of 10 mM Tris base solution (pH 10.5) was added to each well and the mixture incubated for 10 min at room temperature with shaking on an orbital shaker (Star lab smart instruments) to solubilize the bound dye. Absorbance was read at 510 nm in a Synergy mx microplate reader (BioTek®) and the half-maximal inhibitory concentration ($IC_{50}$) determined by non-linear regression in Graphpad prism 4. % inhibition of cell growth was calculated using the formula: $\% \ cell \ growth = \frac{Absorbance \ of \ sample}{Absorbance \ of \ negative \ control} \times 100\%; \% \ Inhibition = 100 - \% \ cell \ growth$

### 2.4. Thin layer chromatography analysis of plant extracts

Thin layer chromatography analysis of crude extracts was carried out on POLYGRAM® SIL G/UV$_{254}$ plates (Merck). 1.0 g of each extract was dissolved in 2ml of dichloromethane and shaken for complete dissolution. Using a micropipette, 5µl of each extract was spotted on TLC plate (4 cm × 8 cm). The plate was air dried and developed with a mixture of dichloromethane and ethanol (95:5) as a mobile phase. After development, the plate was air dried, sprayed with a solution of 10ml Tin (IV) Chloride in 160 ml equal volumes of chloroform and Glacial acetic acid and heated at 100 °C for 10 min. Visualization of plates was done using a transilluminator UV lamp fitted with a cannon camera (Syngene, UK).

### 2.5. Statistical analysis

Data are presented as mean ± standard deviation from 3 independent experiments. Variance analysis was determined by one way Anova at a P value of <0.05 and non linear regression analysis of the dose dependent activity of the drug extracts performed in Graph pad prism 4.0 at 95% confidence interval.

## 3. Results and discussion

The mean absorbance of wells incubated with plant extracts and the control at 510nm is shown in Table 1.

At all tested concentrations, wells treated with methanol extract exhibited low absorbance values (low cell survival) compared to the dichloromethane: methanol (1:1) extract. The analysis of variance was done using a one way ANOVA at a P value of <0.05 and the means were found to be significantly different with a P < 0.0001. Percentage inhibition of cell growth by the two extracts was determined from respective absorbance values at all concentrations (Table 2).

At all tested concentrations, the methanol extract of *K. africana* showed higher inhibition of the growth of the HCC 1937 breast cancer

**Table 2.** % cell growth inhibition of HCC 1937 cells by methanol and dichloromethane extracts of *K. africana*.

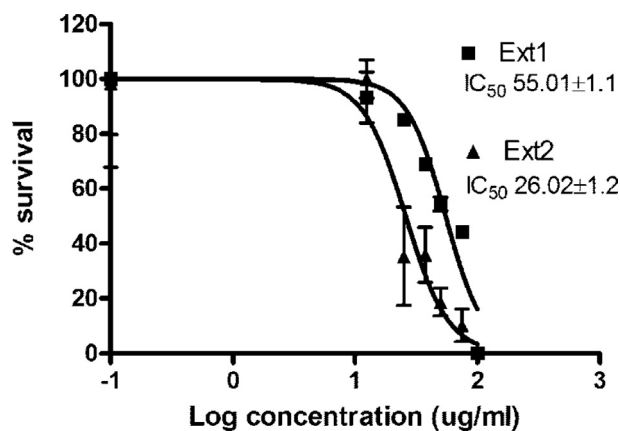| Concentration µg/ml | 0.1 | 12.5 | 25 | % inhibition | | | |
| | | | | 37.5 | 50 | 75 | 100 |
|---|---|---|---|---|---|---|---|
| Methanol | 40.0 | 41.82 | 44.55 | 59.09 | 63.64 | 64.85 | 79.10 |
| Dichloromethane: Methanol (1:1) | 14.85 | 27.88 | 30.0 | 31.82 | 37.27 | 50.30 | 76.40 |

**Figure 1.** Cytotoxicity of extracts in HCC1937 triple negative breast cancer cell line. The methanol extract exhibited higher activity (IC$_{50}$ = 26.02 μg/ml) compared to that of the dichloromethane: methanol (1:1) (IC$_{50}$ = 55.01 μg/ml).
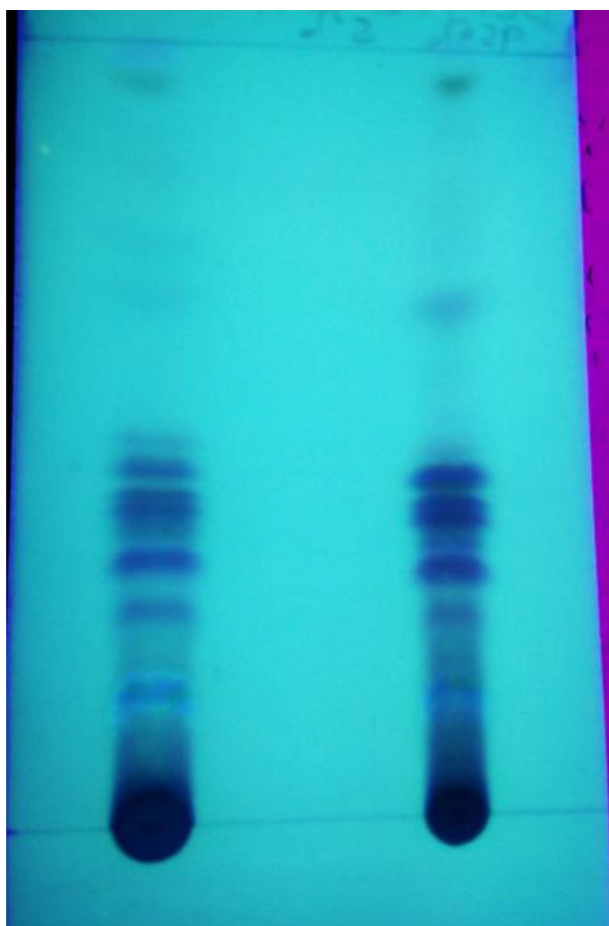


**Figure 2.** Thin layer chromatographic separation of methanol and methanol: dichloromethane (1:1) extracts. Solvent: dichloromethane: ethanol (95:5 vol/vol). Detection: Tin (IV) Chloride reagent (see text). The blue-violet color revealed presense of terpenoids, phenols, steroids and flavonoids.

cell line compared to that of 50% methanol in dichloromethane as depicted by the observed trend. For instance, at 100 μg/ml, the % inhibition of cell growth by methanol and dichloromethane: methanol (1:1) extracts was 79.10% and 76.40% respectively. However, the difference in inhibition of cell growth was more pronounced at low concentrations of extract. For example, at 0.1 μg/ml, inhibition of cell growth by methanol extract (40.0%) was considerably higher than that of

dichloromethane: methanol (1:1) extract (14.85%). Inhibition of cell growth was dose dependent for both extracts, with the level of inhibitory activity decreasing at lower concentrations. Our findings are in agreement with previous studies which have also reported potent anticancer properties for *K. africana* extracts against other cell lines [16, 17, 18]. The half-maximal inhibitory concentrations (IC$_{50}$) of the two extracts were determined by non-linear regression in Graphpad prism 4 (Figure 1).

## 4. Phytochemical screening of plant extracts

Phytochemical screening of the plant extracts revealed the presence of terpenoids, phenols, steroids and flavonoids. This corroborates to the previous studies, which in addition have also reported presence of furanonaphthoquinoids, fatty acids, coumarins, iridoids, caffeic acid and norviburtinal [19,20]. The two extracts displayed a similar profile of chemical constituents on thin layer chromatography plate (TLC). This finding suggested that the differences in the anti-cancer activities of the extracts can be attributed to quantitative variations of the major constituents (see Figure 2).

## 5. Conclusions

*Kigelia africana* dichloromethane and methanol extracts exhibits high *in-vitro* anti-cancer activity against the human breast cancer cell line HCC 1937. In general, the anti-cancer activities of the methanol and dichloromethane extracts can be attributed to the presence of flavonoids, triterpenes and phenolic constituents of the two extracts revealed on the TLC, as well as other constituents reported in literature (furanonaphthoquinones and norviburtinal). The findings for this study provide a scientific rationale of using the plant to control breast cancer in traditional medicine. More validation studies are important to determine cytotoxicity of the extracts and optimal dosage of application in traditional medicine.

## Declarations

### Author contribution statement

S. N. Kituyi and J. W. Mukavi: Performed the experiments; Analyzed and interpreted the data.

P. W. Mayeku: Conceived and designed the experiments; Wrote the paper.

J. M. Nyaga: Analyzed and interpreted the data.

### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information available for this paper.

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