Trop J Nat Prod Res, March 2021; 5(3):570-575

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antioxidant and Anti-Proliferative Studies on *Kigelia africana* (Lam.) Benth. and its Constituents

Akingbolabo D. Ogunlakin[1,2,3], Mubo A. Sonibare[1]*, Almas Jabeen[3], Syeda F. Shah[3], Farzana Shaheen[2]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan. Nigeria*
[2]*H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan*
[3]*Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan*

ARTICLE INFO

Article history:
Received 13 December 2020
Revised 22 February 2021
Accepted 10 March 2021
Published online 01 April 2021

ABSTRACT

Cancer is one of the leading causes of death worldwide. The prevalence of cervical and ovarian cancer among reproductive women is increasing. Therefore, this study intended to examine the effect of methanol extract, solvent fractions and isolated compounds from *Kigelia africana* (Lam.) Benth. on proliferation of cervical cancer (HeLa) and ovarian cancer (CHO-1) cells. The antioxidant activity, total flavonoid and phenolic contents of methanol extract and solvent fractions were evaluated by standard methods. The isolated compounds were identified by spectroscopic methods (1D-NMR, 2D-NMR and Mass Spectroscopy). Compounds 3-(3, 4-dimethoxyphenyl) acrylic acid **(1)**, sitosterol **(2)**, methyl 3-(3,4-dihydroxyphenyl) acrylate **(3)**, 2, 3-dihydro-5-(hydroxymethyl) furan-2, 3, 4-triol **(4),** 2, 3-(4-hydroxyphenyl) acrylic acid (*p*-Coumaric acid) **(5)**, 3-(3,4-dihydroxyphenyl) acrylic acid (caffeic acid) **(6)** were isolated. The MTT test was carried out on methanol extracts, solvent fractions and isolated compounds on CHO 1 and HeLa cell lines (ATCC, Manassas, USA), respectively. The methanol extract and solvent fractions of *K. africana* displayed high antioxidant activity and considerable amounts of phenolics and flavonoids. Hexane (IC_{50} = 5.3 ± 0.1 µg/mL), ethyl acetate (IC_{50} = 5.3 ± 1.10 µg/mL) fractions and compound **3** (IC_{50} = 17.7 ± 2.60 µg/mL) showed inhibitory effect on CHO 1 cell line while Compound **1** (IC_{50} = 33.5 ± 0.60 µg/mL) inhibited HeLa cell line proliferation. Furthermore, this is the first time of isolating compound **1** from the fruit of *K. africana*. These results revealed the anticancer potential of *Kigelia africana* fruit, justifying its usage among reproductive women.

***Keywords*:** Ovarian cancer, Cervical cancer, *Kigelia africana* fruit, Phenylpropanoids

Introduction

One of the leading causes of death worldwide is cancer. It is responsible for 8.2 million (about 22%) death in 2012, therefore, it has become a global burden.[1] According to IARC (International Agency for Research on Cancer) statistics, gynaecological cancer is one of the cancer affecting women with prevalence of about 1 million in 5.1 million new cases of cancer globally.[2] Gynaecological cancer associated with chronic hormone stimulation in reproductive women are cervical, endometrial and ovarian cancer.[3] Cervical cancer is common in developing country while endometrial cancer is more rampant among women in developed country.[2] The deceptive nature of its symptoms and challenge of effective diagnostic tests has positioned ovarian cancer as the most lethal gynaecological cancer among women worldwide.[4] Black women are at lower risk of being diagnosed with ovarian cancer compared with white women, however, ovarian cancer in black women is lethal.[5]

Oxidative stress is among numerous factors contributing to pathogenesis of cancers, especially ovarian and cervical cancers, among women of reproductive age.[6] Reactive Oxygen Species trigger genetic changes by damaging DNA, leading to DNA strand damage, point mutations, abnormal DNA cross-linking as well as DNA-protein cross-linking.[7] The mutations in protooncogenes and tumour suppressor genes pedal uncontrollable cell proliferation. This uncontrollable cell proliferation is also favoured by disrupted DNA repair mechanism.[8] Recently, aberrant *p53* tumour suppressor gene have been observed in the endometrium of polycystic ovary syndrome (PCOS) victims,[9,10] justifying the high occurrence of gynaecological malignancy among premenopausal women.[3,11]

Factors such as inaccessible health service delivery and good health services are the major setbacks in cancer management. Many patients have no access to adequate health services, proper diagnostic tests and funds for preventive, diagnostic and therapy services. These factors favour high prevalence of cancer.[12] Therefore, there is increasing need for cheaper and safer treatment options, such as the use of medicinal plants, in cancer management.

Kigelia africana (Lam.) Benth. (Bignoniaceae), a unique tree with therapeutic prominence, is found in the southern, central and western regions of Africa.[13] The leaves of Kigelia are used for treatment of stomach discomfort, kidney disease and snakebite, stem and twigs for wounds, snake bite, rheumatism, stomach and kidney diseases, while fruit is used for constipation, gynaecological problems and haemorrhoids. This plant is also used in cosmetics as sun block creams and sun lotions.[14] *Kigelia africana* is a natural source of about 150 bioactive compounds.[15] The compounds isolated include iridoids, naphthoquinones, flavonoids, terpenes and phenylethanoglycosides. Recently, novel and effective antimicrobial, antioxidant and anticancer compounds named verminoside, verbascoside and pinnatal were isolated from *K. africana*.[15]

The aim of this study was to examine the effect of *K. africana* fruit crude, solvent fractions and isolated compounds on proliferation of Chinese Hamster Ovarian (CHO 1) cell, tumour-forming cell with

*Corresponding author. E mail: sonibaredeola@yahoo.com
Tel: +234 813 490 1273

Citation: Ogunlakin AD, Sonibare MA, Jabeen A, Shah SF, Shaheen F. Antioxidant and Anti-Proliferative Studies on *Kigelia africana* (Lam.) Benth. and its Constituents. Trop J Nat Prod Res. 2021; 5(3):570-575. doi.org/10.26538/tjnpr/v5i3.25

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

characteristic irregular *p*53 function - observed in the endometrium of victims of polycystic ovary syndrome (PCOS), [9,10,16] and HeLa (Human Cervical Cancer) cells.

Materials and Methods

General experimental procedure

All solvents used were purchased from BDH Ltd, England while reagents such as Folin–Ciocaltaeu reagent, Ferric chloride, Sodium bicarbonate, Aluminium chloride, Potassium acetate, Gallic acid, Ascorbic acid, Quercetin, 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) and Rutin were manufactured by Sigma Aldrich Chemicals, Germany. Nuclear magnetic resonance (NMR) spectra were recorded using *Bruker AV (Avance)* spectrometer with the nondeuterated solvent peaks as internal standard. Proton NMR was recorded at 500 MHz while ^{13}C-NMR was recorded at 400 MHz and 800 MHz. Low resolution electron impact mass spectra were recorded using finnigan *MAT 312* and *MAT 312* spectrometer with *PDP 11/34* computer system. High resolution Mass was recorded using *Jeol JMS HX110* mass spectrometer, while IR absorbance was measured with *FTIR Bruker Vector 22*.

Plant material

Kigelia africana fruit was collected in Oluponna, Osun state of Nigeria on 20th of February, 2017. The plant was identified and authenticated at Forest Herbarium (FHI), Forest Research Institute of Nigeria, Ibadan. A voucher specimen (FHI No: 111350) was deposited at Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI), Ibadan.

Plant extraction and solvent partitioning

The air-dried fruit was pulverized into coarse powder using an electric grinding machine. The pulverised sample (3.50 kg) was macerated in 16.80 L of methanol for 72 h at room temperature with intermittent stirring. The extract was filtered through a fresh cotton plug. The filtrate was further filtered through Whatman (Number 1) filter paper. The brownish filtrate was concentrated *in vacuo* yielding dark-brown sticky extract with percentage yield of 6.06%. Solvent-solvent partitioning was done according to standard procedure.[17] The *n*-hexane, DCM and ethyl acetate fractions obtained were concentrated *in vacuo* and the percentage yields obtained were 3.96, 5.94 and 11.88 %, respectively.

Total phenolic content, Total flavonoid content and antioxidant activity

Estimation of Total phenolic content (TPC)

Total phenolic contents of the methanol extracts and fractions were measured using Folin-Ciocalteu spectrophotometric method.[18] Five mL of Folin–Ciocalteu's phenol reagent (diluted tenfold) was added into l mL aliquots of each test samples (l00 µg/mL). Afterward, 4 mL of Na2CO3 solution in distilled water (7.5 g/l00 mL) was added to mixture in each vial and thereafter incubated at 27 °C for 30 min in the dark. Blank was set up with l mL methanol. Each sample was analysed in triplicate. Absorbance of mixture after 30 min of incubation was read at 765 nm with UV–VIS spectrophotometer (Spectrumlab 752S). Total phenolic content was calculated using linear dose response regression curve generated from the absorbance of Gallic acid. Result of TPC was expressed as mg Gallic acid equivalent/ g of the dry weight of extracts.

Estimation of Total flavonoid contents (TFC)

The aluminium chloride colorimetry method used in this study was slightly modified.[19] Quercetin was used as standard to plot calibration curve prepared by diluting quercetin in ethanol (l00–6.25 µg/mL). Test samples were also prepared by reconstituting 30 mg of all test materials in 30 mL methanol. One mL of each diluted quercetin solutions or test samples (l.0 mL) were mixed separately with l.0 mL of methanol, 0.l mL of l% aluminium chloride, 0.l mL of l M potassium acetate and 2.8 mL. The amount of l% aluminium chloride was substituted by the same amount of distilled water in the blank. Each sample was analysed in triplicate. Incubation was done at 27°C for 30 min and the absorbance of the reaction was measured at 4l5 nm using UV–VIS spectrophotometer (Spectrumlab 752S). Total flavonoids content for each test samples were deduced from the equation obtained from the quercetin calibration curve and expressed in terms of quercetin equivalent (mg Quercetin equivalent/g of extract).

In vitro antioxidant capacity

The free radical scavenging effect of the methanol extract and the fractions was assessed according to the standard procedure with slight modifications. [20] For DPPH assay, 2 mL methanol solution of *K. africana* crude extract, solvents fractions and standards (ascorbic acid and rutin) at varying concentrations (200, l00, 50, 25, l2.5, 6.25 and 3.l25 µg/mL) were added separately to 3 mL (0.004%) of l, l-diphenyl-2-picryl-hydrazyl-hydrate (DPPH). Two mL of methanol was added as test sample in the control. The reaction mixtures were shaken vigorously and kept warm at 27 °C for 30 min in the dark. Absorbance of each vial was measured at 5l7 nm using Spectrumlab 752S UV–VIS spectrophotometer and expressed in percentage inhibition using [l-absorbance of the solution with sample and DPPH/absorbance of solution with DPPH] × l00. Percentage inhibition concentration (IC50) value was calculated using the plotted graph of scavenging activity against the concentration of the test samples (using linear regression analysis).

Isolation of compounds

Green coloured *n*-hexane fraction (0.39 g) was subjected to column chromatography (silica gel) and eluted with Hexane-DCM (50:50) and 100% DCM. Two hundred and twenty fractions were collected and pooled to 15 fractions (A-O). Fraction B, C and D (based on similar TLC chromatogram) were pooled to give A^1 (60 mg). Fraction A^1 was rechromatographed (silica gel) using Hex: DCM (50:50) and 100% DCM which yielded 24 fractions. Sub-fractions 14 to 18 were pooled to give $A^1_{(14\text{-}18)}$ of weight 14.19 mg. This was subjected to Preparative TLC and developed with Hexane-DCM-ethyl acetate (10:9:1) to yield **1** (8.85 mg, yellow amorphous solid) while sub-fraction A^1_{13} crystalized to give **2** (22.43 mg, white amorphous solid). Yellow coloured DCM fraction (3.03 g) was subjected to column chromatography (silica gel) and eluted with Hexane, Hexane-DCM, DCM-Ethyl acetate and Ethyl acetate-methanol with increasing polarity yielding 572 fractions. These fractions were pooled to 20 fractions (A-U). Fraction L (fractions 208-247, brown coloured, 630 mg) was subjected to prep-TLC, developed with DCM-ethyl acetate (50:50) to yield **3** (21.40 mg, light brown amorphous solid) and **4** (8.72 mg, greenish-yellow amorphous solid). Brownish-yellow coloured ethyl acetate fraction (7.48 g) was subjected to column chromatography (silica gel) and eluted with hexane, hexane-DCM, DCM-Ethyl acetate and Ethyl acetate-Methanol with increasing polarity to give 151 fractions. These fractions were pooled to 14 fractions (A-N). Pooled fractions C (fractions 20-25, greenish, 60.02 mg) and D (fractions 26-36, light green, 50.03 mg) were pooled and **2** (27.24 mg, white amorphous solid) crystalized out of this pooled fraction. Pooled fraction I (86-106, light brownish, 150.60 mg) vial prep-TLC technique (developed with DCM-ethyl acetate (70:30) with few drops of acetic acid) yielded **5** (24.23 mg, pink coloured needle-like crystal**)**. Furthermore, J (fractions 107-116, light brownish-yellow, 200.23 mg) was also subjected to prep-TLC (DCM-ethyl acetate (70:30) with few drops of acetic acid) and **6** (44.76 mg, yellow amorphous solid**)** was isolated.

Anti-proliferative activity

Antiproliferative effect of crude and solvent fractions of *K. africana* and compounds **1-6** on HeLa and CHO cell lines (ATCC, Manassas, USA) were evaluated by standard MTT colorimetric assay.[21] One hundred microliter (l00 µL) of 5 × l04 cells/mL of HeLa (Human cervical cancer cell lines) and 6 × l04 cells/mL of CHO l (Chinese Hamster Ovarian cancer cells) proliferating cells in DMEM (Dubecco's modified Eagle's medium) supplemented with l0% FBS were seeded into 96-wells flat bottom plate and incubated overnight at 37 ºC in 5% CO2. Three different concentrations of test samples (l, l0 and l00 µg/mL) were added to the plate in triplicates and incubated for

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

2 days. Fifty microliter (50 µL) of 0.5 mg/mL MTT was added to each well and plate was then further incubated for 4 hours. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was aspirated and l00 µL of DMSO was then added to all test and control wells. The magnitude of enzymatic MTT reduction to its formazan by NADH of the living cells was calculated by measuring the absorbance at 540 nm, using a spectrophotometer (Spectra Max plus, Molecular Devices, CA, USA). The antiproliferative activity was recorded as concentration causing 50% growth inhibition (IC50) for test samples.

Statistical analysis

Values were presented as Mean ± Standard Error of Mean (SEM). Data were analysed using one-way analysis of variance (ANOVA) and group means were compared using Dunnett's Multiple Comparison test and Bonferroni tests using GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego California USA. The *p* values of < 0.05 were considered significant.

Results and Discussion

Total phenolic content, Total flavonoid content and antioxidant activity

In order to provide justification for these medicinal plants antioxidant potential, the scavenging activity are measured as their ability to scavenge free radicals generated by DPPH reagent. This revealed varying strength of different plant extracts and fractions as radical scavengers (Table 1). These outcomes matched with the reported findings on antioxidant effect of several medicinal plants.[22] Phenolics are significant constituent of medicinal plant which exerts numerous therapeutic purposes including free radical scavenging ability due to the presence of -OH functional group. The hydroxyl functional groups in the skeleton of phenolic compound's structure might be responsible for high scavenging property of extract and solvent fractions. Various studies have shown the comparative relationship in-between phenolics and anti-oxidant potential.[22,23,24] The result of total phenolic contents showed that ethyl acetate fraction had the highest phenolics followed by DCM fraction (Table 1). The antioxidant activity of the three solvent fractions increases as TPC increases in the trend of $TPC_{Hexane} < TPC_{DCM} < TPC_{Ethyl\ acetate}$. Total flavonoid content values of ethyl acetate, DCM and hexane fractions had a well-defined trend ($TFC_{Ethyl\ acetate} < TFC_{DCM} < TFC_{Hexane}$). This suggest that the active constituents responsible for antioxidant activity could be the phenolics not flavonoids. The results obtained support other research outcomes in which a parallel association between phenolics and antioxidant activity have been established.[25]

Isolation of compounds

The column chromatography of *n*-hexane, DCM and ethyl acetate fractions yielded six compounds (**1-6**) as shown in Figure 1. The structure of these compounds were elucidated and established with detailed spectral analysis (NMR, Low and High resolution EIMS). Compound **1** was isolated as yellow amorphous solid with molecular formula of $C_{11}H_{12}O_4$ and molecular ion peak of 208 (M^+, 100) in LREIMS. The theoretical mass and observed mass obtained from HREIMS are 208.0736 and 208.0728, respectively. The ^{1}H-NMR spectroscopic data displayed signals for allylic protons at 7.61 ppm (IH, d, J = 16Hz, H-2^1) and 6.28 ppm (IH, d, J = 16Hz, H-3^1), aromatic protons (three methane groups) at 7.06 (IH, dd, J = 8.0Hz, J = 1.5Hz, H-6), 7.01 (1-H, d, J = 2.0Hz, H-2) and 6.90 (IH, d, J = 8.5Hz, H-5), and methoxyl group at 3.91 ppm (3H, $-OCH_3$) and 3.77 ppm (s, 3H, $-OCH_3$) as singlet. The ^{13}C-NMR spectra showed that the compound has nine carbon atoms excluding the two methoxyl groups. The two methoxyl groups are attached to para, C-3 (δ_c 150.2 ppm), and ortho, C-4 (δ_c 127.4 ppm), positions while the functional group of the compound (carboxylic acid functional group) is at positon C-1' (δ_c 170.8 ppm). This information showed that compound 1 is a cinnamic acid derivative with two substituted methoxyl groups on the aromatic ring system. Compound **1** was elucidated as 3-(3, 4-dimethoxyphenyl) acrylic acid**.**

Compound **3** is light brown amorphous solid with molecular ion peak of 194 (M^+, 100) and molecular formula of $C_{10}H_{10}O_4$ while theoretical mass and observed mass obtained from HREIMS are 194.0579 and 194.0572, respectively. This compound is a cinnamic acid derivative with characteristic olefinic protons at 7.57 (IH, d, J = 15.5 Hz, H-2) and 6.26 (IH, d, J = 16Hz, H-3) and three aromatic methine protons at 7.05 (1H, d, J = 2.0Hz, H-2'), 7.00 (IH, dd, J = 8.0Hz, J = 1.5Hz, H-6') and 6.85 ppm (IH, d, J = 8.0 Hz, H-5'). The ^{13}C-NMR data showed carbon of ester at C-1 (δ_c 167.7) and carbons with hydroxyl groups at C-3' (δ_c 143.7 ppm) and C-4' (δ_c 144.6 ppm). Compound **3** was elucidated as methyl 3-(3, 4-dihydroxyphenyl) acrylate. The LREIMS spectrum of compound **4** showed molecular ion peak of 148 (M^+, 100) suggesting a molecular formula of $C_5H_8O_5$. It was isolated as greenish-yellow amorphous solid. The hydroxyl group at position C-2 was observed as singlet at 9.59 ppm while proton attaching to C-2 (δ_c 121.2 ppm) and C-3 (δ_c 109.9 ppm) were observed as doublet at 7.20 and 6.50 ppm, respectively. Methylene protons which showed at 4.71 ppm as singlet is attached to C-1' (δ_c 57.7 ppm). Compound 4 was elucidated as 2, 3-dihydro-5-(hydroxymethyl) furan-2, 3, 4-triol. The data obtained for compounds **2**, **5** and **6** are similar with those reported in literatures.[26-28]

3-(3, 4-dimethoxyphenyl) acrylic acid (1):

^{1}HNMR (500 MHz, $CDCl_3$):δ (ppm)7.61 (IH, d, J = 16Hz, H-2^1), 7.06 (IH, dd, J = 8.0Hz, J = 1.5Hz, H-6), 7.01 (1H, d, J = 2.0 Hz, H-2), 6.90 (IH, d, J = 8.5 Hz, H-5), 6.28 (IH, d, J = 16Hz, H-3^1), 3.91(3H, s, $-OCH_3$), 3.77 (3H, s, $-OCH_3$).^{13}C-NMR (800 MHz, $CDCl_3$): δ (ppm) 170.8 (C-1^1, -COOH), 150.2 (C-3), 112.6 (C-2), 149.2 (C-1), 127.4 (C-4), 123.0 (C-6), 114.8 (C-5), 115.1 (C-3'), 112.2 (C-3), 55.9 ($-OCH_3$), 51.5 ($-OCH_3$). EI-MS *m/z* (% rel. abund.): 208 [M^+, 100]; l77 [M-($-OCH_3$); l45 [M-($-OCH_3$)-($-OCH_3$)]. HREIMS: m/z 208.0728 (calc. for $C_{11}H_{12}O_4$, 208.0736).

Methyl 3-(3, 4-dihydroxyphenyl) acrylate (3):

^{1}H-NMR (500 MHz, $CDCl_3$): δ(ppm)7.57 (IH, d, J = 15.5 Hz, H-2), 7.05 (1H, d, J = 2.0 Hz, H-2'), 7.00 (IH, dd, J = 8.0Hz, J = 1.5Hz, H-6'), 6.85 (IH, d, J = 8.0 Hz, H-5'), 6.26 (IH, d, J = 16Hz, H-3), 3.77 (s, 3H, $-OCH_3$-), 5.63 (s, broad, -OH), 5.49(s, broad, OH).^{13}C-NMR (800 MHz, $CDCl_3$): δ(ppm) 167.7 (C-3, $-COOCH_3$), 114.2 (C-2'), 144.6 (C–4', -OH), 143.7 (C-1'), 115.5 (C-5'), 122.4 (C-6'), 143.7 (C-3'), 115.5 (C-5'), 51.6 ($-OCH_3$). EI-MS *m/z* (% rel. abund.): l94 [M^+, 100]; l63 [M-($-CH_3$)]; l45 [M-($-CH_3$)-(-OH)]; l34 [M-($-COOCH_3$)]. HREIMS: m/z 194.0572 (calc. for $C_{10}H_{10}O_4$, 194.0579).

2, 3-dihydro-5-(hydroxymethyl) furan-2, 3, 4-triol (4):

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 9.59 (1H, s), 7.20 (d, J = 3.5 Hz, IH, H-2), 6.50 (d, J = 3.5 Hz, IH, H-3), 4.71 (s, 2H, H-1'). ^{13}C-NMR (800 MHz, $CDCl_3$): δ (ppm) 160.6 (C-4, -OH), 152.4 (C-5), 121.2 (C-2, -OH), 109.9 (C-3, -OH), 57.7 (C-1', -OH). EI-MS *m/z* (% rel. abund.): l48 [M^+, 100]; ll3 [M^+-(-OH)-(-OH)].

Anti-proliferative activity

In general, Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidoreductase in the mitochondria of living cells reduce colourless tetrazolium salts solutions to formazan of intense purple colour. The intensity of this colour is a function of amount of living cells that are not affected by the sample tested.[29] *Kigelia africana n*-hexane and ethyl acetate fractions displayed highest anti-proliferative activity on CHO-1 cell line having IC_{50} (µg/mL) values of 5.3 ± 0.1 and 5.3 ± 1.1 µg/mL, respectively (Table 2). This may be due to high concentration of flavonoids in hexane fraction and high concentration of phenolics in ethyl acetate fraction. The IC_{50} (µg/mL) values for crude extract and DCM fraction are 18.2 ± 8.2 and 13.2 ± 2.8 µg/mL, respectively. However, only DCM fraction had inhibitory effect on the HeLa cell line (IC_{50} = 79.0 ± 8.7 µg/mL). Doxorubicin (the standard) had IC_{50} (µg/mL) of 0.8 ± 0.01 and 3.1 ± 0.2 µg/mL with CHO-1 cell line and HeLa cell line, respectively**.**

According to American National Cancer Institute criteria for selection of plant extract for further analysis,[30] the *K. africana* extract and fractions had IC_{50} below 30 µg/mL in the preliminary assay, therefore,

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

all solvent fractions were subjected to isolation and MTT assay was repeated for six compounds isolated. These phenylpropanoids isolated from *K. africana*, compounds **1**, **3** and **5** had inhibitory effect on CHO 1 cell line with IC_{50} (µg/mL) values of 62.4 ± 1.6, 17.7 ± 2.6 and 31.9 ± 0.2 µg/mL, respectively. The mechanism of action for compounds **l**, **3** and **5** on the proliferation of CHO-l cells could be compared with activity of isotretinoin and metformin which have been reported to inhibit proliferation of cells with abnormal *p53*, *FoxOl* and *FoxO3* functions in PCOS patients.[9,10,31,32] Compound **2, 4** and **6** displayed no inhibition against CHO-1 cancer cell line while all the compounds isolated, except compound 1, had no significant inhibitory activity against HeLa cell line. The IC_{50} (µg/mL) value of compound 1 is 33.5 ± 0.6 µg/mL compared to IC_{50} (3.1 ± 0.2 µg/mL) of Doxorubicin on HeLa cell line. The activity of isolated compounds **1-6** on CHO-1 and HeLa cell lines is presented in Table 2.

Phenylpropanoids are responsible for synthesis of biochemicals needed for survival and reproduction in plants.[33] The metabolism of phenylpropanoid is the genesis of many secondary metabolites via shikimate pathway.[34] Enzymes, such as reductases, oxygenases, and transferases convert resulting hydroxycinnamic acids and esters into several secondary metabolites in different plant species.[34] The intermediate, *p*-Coumaroyl CoA, from cinnamate is responsible for biogenesis of several compounds such as flavonoids, coumarins, isoflavones, anthocyanidines, stilbenes, aurones and lignins.[34] Several phenylpropanoids have significant cytotoxic effect on human cancer cell lines when administered in combination with other phenylpropanoids or drugs such as cisplatin or fluorouracil.[35-37]

Structure-Anti-proliferative activity Relationships (SARs) for compounds **1**, **3**, **5** and **6** presented in Figure 2 revealed that substitution of specific functional groups, including hydroxyl group and methoxyl group, on the benzene ring and esterification of acid functional group enhanced the activity of these compounds against CHO-1 cell line. The highest activity was found in compound **3** with hydroxyl groups at R_3 and R_4 and methoxyl group at R_1. However, compound **6** with hydroxyl groups at R_1, R_3 and R_4 had no significant activity. The electrostatic points for substitutions in the benzene ring system of these compounds might be responsible for the varying anticancer activity.[35] Compound **1**, with methoxyl groups on benzene system, displayed moderate anti-proliferative effect on HeLa cell line. Few phenylpropanoids (Podocarioside A, Schizandrin and Dehydrodiconiferyl alcohol) isolated from *Podocarpium podocarpum*, with methoxyl groups on the benzene systems have also been reported to showed moderate cytotoxicity against HeLa cells.[38]

Table 1: DPPH (IC_{50}), TPC and TFC values of *Kigelia africana* methanol extract and its solvent fractions

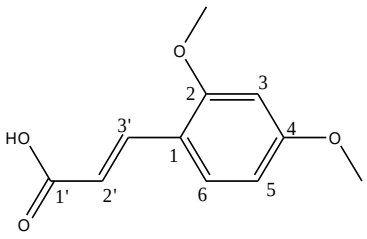
Extracts/standards	Solvents	DPPH (IC_{50}) (µg/mL)	TPC (µg GAE/g)	TFC (mg QE/g)
K. Africana	Crude	63.34 ± 3.47***/***	465.00 ± 0.94	272.12 ± 12.36
	Hexane	112.95 ± 0.47***/***	1643.33 ± 7.07	220.79 ± 0.18
	DCM	17.23 ± 0.34 $^{NS/NS}$	5125.00 ± 1.18	176.18 ± 0.99
	Ethyl acetate	12.72 ± 0.04$^{NS/NS}$	5138.34 ± 8.25	182.40 ± 0.59
Ascorbic acid		2.76 ± 0.01		
Rutin		20.60 ± 9.26		

Data are represented as mean ± (SEM) (n = 3). One-way ANOVA followed by Dunnett's Multiple Comparison Test at $p = 0.05$. IC_{50} DPPH of each extract was compared with standards (Ascorbic acid and rutin) with level of significant difference denoted by *** or asterisk separated by (/) indicating order of significance from Ascorbic acid and Rutin, respectively. *NS* means no significant difference from the standards.

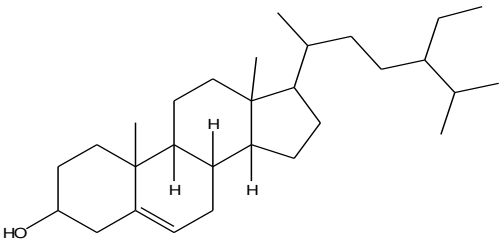
Table 2: The IC_{50} (µg/mL) of crude, fractions of *K. africana* and isolated compounds (**1-6**) on Chinese Hamster Ovarian (CHO1) and Human cervical (HeLa) cancer cell lines.

Extract, solvent fractions and compounds (1-6)	IC_{50} (µg/mL)	
	CHO 1 cell line	HeLa cell line.
K. africana crude	18.20 ± 8.20	>100
K. africana hexane fraction	5.30 ± 0.10	>100
K. africana DCM fraction	13.20 ± 2.80	79.00 ± 8.70
K. africana ethyl acetate fraction	5.30 ± 1.10	>100
3-(3, 4-dimethoxyphenyl) acrylic acid (1)	62.40 ± 1.60	33.50 ± 0.60
Sitosterol (2)	>100	>100
Methyl 3-(3, 4-dihydroxyphenyl) acrylate (3)	17.70 ± 2.60	>100
2, 3-dihydro-5-(hydroxymethyl) furan-2, 3, 4-triol (4)	>100	>100
3-(4-hydroxyphenyl) acrylic acid (p-Coumaric acid) (5)	31.90 ± 0.20	>100
3-(3,4-dihydroxyphenyl) acrylic acid (caffeic acid) (6)	>100	>100
Standard (Doxorubicin)	0.80 ± 0.01	3.1 ± 0.20

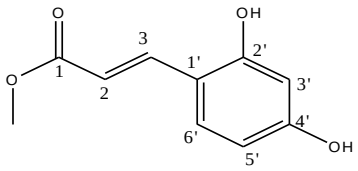
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)



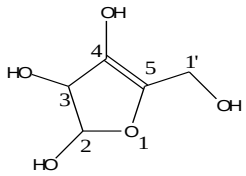
3-(2, 4-dimethoxyphenyl) acrylic acid (1)



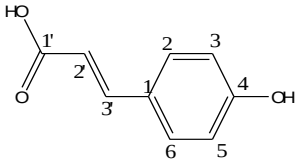
Sitosterol (2)



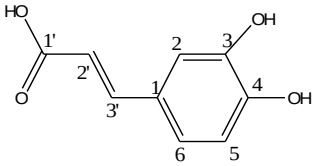
Methyl 3-(2, 4-dihydroxyphenyl) acrylate (3)



2, 3-dihydro-5-(hydroxymethyl) furan-2, 3, 4-triol (4)



3-(4-hydroxyphenyl) acrylic acid (p-Coumaric acid) (5)



3-(3,4-dihydroxyphenyl) acrylic acid (caffeic acid) (6):

Figure 1: Compounds (**1-6**) isolated from *K. africana* frui

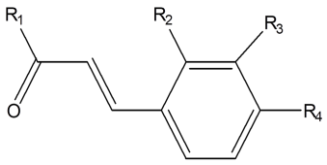


Figure 2: Structure-Anti-proliferative activity Relationships (SARs) for compounds **1, 3, 5** and **6**

Compounds					IC $_{50}$ (µg/mL)	
	R_1	R_2	R_3	R_4	CHO 1 cell lines	HeLa cell lines
1	OH	-OCH_3	H	-OCH_3	62.4 ± 1.6	33.5 ± 0.6
3	-OCH_3	-OH	H	-OH	17.7 ± 2.6	>100
5	OH	H	H	-OH	31.9 ± 0.2	>100
6	OH	H	-OH	-OH	>100	>100

Conclusion

This study revealed the effects of *Kigelia africana* crude, fractions and isolated phenylpropanoids on ovarian and cervical cancers. The antioxidant effect of *K. africana* extract and antiproliferative effect of compounds **1** (3-(3, 4-dimethoxyphenyl) acrylic acid), **3** (methyl 3-(3, 4-dihydroxyphenyl) acrylate) and **5** (*p*-Coumaric acid), which are abundant in the fruit of *K. africana*, on CHO-1 and HeLa cells are promising. To the best of our knowledge, compound **1** is new from *K. africana* fruit. These results justify the use of *K. africana* fruit among reproductive women for gynaecological purposes. This is the first time of examining the antiproliferative effect of these compounds on CHO-1 cell line.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This work was partly funded by The World Academy of Science (TWAS), Italy under TWAS-ICCBS Sandwich Postgraduate Fellowship awarded to *ADO* in 2017. We are thankful for the support of Higher Education Commission (HEC) Pakistan, (Project No. 8263, NRPU 2017-18) for *in vitro* biological study

References

1. Hung M, Lai W, Chen HHW, Lee J, Lin Y, Hsiao J, Cheng Y, Shan Y, Su W, Wang J. Cost effectiveness of cancer treatment in Taiwan. J Formos Med Assoc., 2016; 115(8):609-618.
2. Weiderpass E, Hashim D, Labrèche F. Malignant tumors of the female reproductive system. InOccupational Cancers, Springer, Cham. 2020; 439-453p.
3. Kim J, Mersereau JE, Khankari N, Bradshaw PT, McCullough LE, Cleveland R, Shantakumar S, Teitelbuam SL, Neugut AI, Senie RT, Gammon MD. Polycystic ovarian syndrome (PCOS), related symptoms/sequelae, and breast cancer risk in a population-based case–control study. Cancer Causes and Control. 2016; 27(3):403-14.
4. Henderson JT, Webber EM, Sawaya GF. Screening for ovarian cancer: updated evidence report and systematic review for the US preventive services task force. JAMA 2018. 319(6):595-606.
5. Siegel RL, Miller KD, Jemal A. Cancer statistics, CA. CA Cancer J Clin 2018; 68(1):7-30.
6. Calaf GM, Urzua U, Termini L, Aguayo F. Oxidative stress in female cancers. Oncotarget. 2018; 9(34):23824.
7. Nishida N and Kudo M. Clinical significance of epigenetic alterations in human hepatocellular carcinoma and its association with genetic mutations. Digestive Dis. 2016; 34(6):708-13.
8. Park JH, Zhuang J, Li J, Hwang PM. p53 as guardian of the mitochondrial genome. FEBS letters. 2016; 590(7):924-34.
9. Shafiee MN, Malik DA, Yunos RIM, Atiomo W, Omar MH, Ghani NAA., Hatta AZ, Seedhouse C, Chapman C, Mokhtar NM. The effect of Metformin on endometrial tumor-regulatory genes and systemic metabolic parameters in polycystic ovarian syndrome–a proof-of-concept study. Gynecol Endocrinol 2015; 31(4):286-290.
10. Gadducci A, Biglia N, Tana R, Cosio S, Gallo M. Metformin use and gynecological cancers: a novel treatment option emerging from drug repositioning. Crit Rev Oncol Hematol. 20l6; l05(1):73-83.
11. Ding DC, Chen W, Wang JH, Lin SZ. Association between polycystic ovarian syndrome and endometrial, ovarian, and breast cancer: A population-based cohort study in Taiwan. Medicine. 2018; 97(39):e12608.
12. Wu S, Zhu W, Thompson P, Hannun YA. Evaluating intrinsic and non-intrinsic cancer risk factors. Nat comm. 2018; 9(1):1-2.
13. Burkill, HM. The Useful Plants of West Tropical Africa, 2 ed. Royal Botanic Gardens, Kew. 1985.
14. Jackson S and Beckette K. Sausage tree *Kigelia pinnata*: An ethnobotanical and scientific review. HerbalGram 2012; 1(1):48-59.
15. Bello I, Shehu MW, Musa M, Asmawi MZ, Mahmudb R. Kigelia africana (Lam.) Benth. (Sausage tree): Phytochemistry and pharmacological review of aquintessential African traditional medicinal plant. J Ethnopharmacol. 2016: 189(1):253-276.
16. Hu T, Miller CM, Ridder GM, Aardema MJ. Characterization of p53 in Chinese hamster cell lines CHO-K1, CHO-WBL, and CHL: implications for genotoxicity testing. Mutat Res 1999; 426(1):51-62.
17. Ajayi OS, Aderogba MA, Obuotor EM, Majinda RR. Acetylcholinesterase inhibitor from *Anthocleista vogelii* leaf extracts. J Ethnopharmacol. 2019; 231:503-6.
18. Pengkumsri N, Kaewdoo K, Leeprechanon W. Influence of Extraction Methods on Total Phenolic Content and Antioxidant Properties of Some of the Commonly Used Plants in Thailand. Pakistan J bio sci PJBS. 2019; 22(3):117-26.
19. Woisky R and Salatino A. Analysis of Propolis: some parameter and procedures for chemical quality control. J Apic Res 1988; 37(2): 99-105.
20. Bursal E and Gülçin I. Polyphenol content and in vitro antioxidant activities of lyophilized aqueous extract of kiwifruit (*Actinidia deliciosa*). Food Res. Int 2011; 44(5):1482-1489.
21. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65(1-2):55-63.
22. Hussain T, Fatima I, Rafay M, Shabir S, Akram M, Bano S. Evaluation of antibacterial and antioxidant activity of leaves, fruit and bark of *Kigelia africana*. Pak J Bot. 2016; 48(1):277-283
23. Huyut Z, Beydemir Ş, Gülçin İ. Antioxidant and antiradical properties of selected flavonoids and phenolic compounds. Biochemistry research international. 2017; 2017:7616791.
24. Koleva II, van Beek TA, Linssen JPH, de Groot A, Evstatieva LN. Screening of Plant Extracts for Antioxidant Activity: a Comparative Study on Three Testing Methods. Phytochem Anal. 2002; 13(1):8-17.
25. Zhang C, Feng S, Wang Q, Wang P, Xu J, Chen T. Flavonoids and phenolic compounds from Smilax scobinicaulis. Chem Nat Compd. 2014; 50(2):254-257.
26. Khana MR, Jackson S, Beckette K. Sausage tree *Kigelia pinnata*: An ethnobotanical and scientific review. HerbalGram. 2012; 94(1):48 -59.
27. An SM, Lee SI, Choi SW, Moon SW, Boo YC. p-Coumaric acid, a constituent of Sasa quelpaertensis Nakai, inhibits cellular melanogenesis stimulated by a-melanocyte stimulating hormone. Br. J. Dermatol 2008; 159(2):292-299.
28. Park H, Nam M, Lee H, Jun W, Hendrich S, Lee K. Isolation of caffeic acid from *Perilla frutescens* and its role in enhancing γ-glutamylcysteine synthetase activity and glutathione level. Food Chem. 2010; 119(2):724-730.
29. Berridge MV, Herst MP, Tan AS. Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. Biotech. Ann. Rev 2005; 11(1):127-152.
30. Suffness M and Pezzuto JM. Assays related to cancer drug discovery. In: Hostettmann K. editor. Methods Plant Biochemistry: Assays for Bioactivity. London: Academic Press 1990; 71-133p.
31. Melnik BC. Acne vulgaris: The metabolic syndrome of the pilosebaceous follicle. Clin Dermatol. 20l8; 36(1):29-40.
32. Agamia NF, Hussein OM, Abdelmaksoud RE, Abdalla DM, Talaat IM, Zaki EI, El Tawdy A, Melnik BC. Effect of oral isotretinoin on the nucleo-cytoplasmic distribution of FoxOl and FoxO3 proteins in sebaceous glands of patients with acne vulgaris. Exp Dermatol. 20l8; 27(18):l344-l35l.
33. Dudareva N, Pichersky E, Gershenzon J. Biochemistry of plant volatiles. Plant Physiol. 2004; 135(4):1893-1902.
34. Deng Y and Lu S. Biosynthesis and regulation of phenylpropanoids in plants. Critical Reviews in Plant Sci. 2017; 36(4):257-90.
35. Hemaiswarya S and Doble M. Combination of phenylpropanoids with 5-fluorouracil as anti-cancer agents against human cervical cancer (HeLa) cell line. Phytomed. 2013; 20(2):151-158.
36. Yi J, Shi S, Shen Y, Wang L, Chen H, Zhu J, Ding Y. Myricetin and methyl eugenol combination enhances the anticancer activity, cell cycle arrest and apoptosis induction of cis-platin against HeLa cervical cancer cell lines. Int J Clin Exp Pathol. 2015; 8(2):1116-1127.
37. Koraneekit A, Limpaiboon T, Sangka A, Boonsiri P, Daduang S, Daduang J. Synergistic effects of cisplatin-caffeic acid induces apoptosis in human cervical cancer cells via the mitochondrial pathways. Oncol Lett 2018; 15(5):7397-7402.
38. Ma X, Liang J, Zheng C, Hu C, Zhao X, Rahman K, Qin L. Phenylpropanoids from *Podocarpium podocarpum*. Pharm. Biol 2013; 51(8):1021-1025.